HAWASSA UNIVERSITY

FACULTY OF VETERINARY MEDICINE

SEASONAL PREVALENCE OF BOVINE TRYPANOSOMOSIS AND VECTOR DYNAMICS IN SELECTED SITES OF WESTERN SIDAMA, SOUTHERN ETHIOPIA

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STATEMENT OF AUTHOR IN THE MSc THESIS

I declare that this thesis is my bonafied work and all sources of materials used for this thesis have been duly acknowledged. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma or certificate.

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ABBREVIATIONS

AWIPM	Area Wide Integrated Pest Management
BCS	Body Condition Score
BW	Body Weight
CI	Confidence Interval
GPS	Geographical Positioning System
GSBS	Geimsa Stained Blood Smear
NGU	Nguruman
OR	Odd Ratio

ABSTRACT

Trypanosomosis is a serious and often fatal disease of livestocks mainly in the rural poor community and rightfully considered as a root cause of poverty in the country. The temporal information about the prevalences of trypanosomosis and apparent density of flies are crucial for the design and implementation of the appropraite prevention and control measures. Therefore, the objectives of this repeated cross-sectional study conducted from December 2022 to October 2023 in selected sites of Western Sidama, Southern Ethiopia was to estimate the seasonal prevalence of bovine trypanosomosis and the apparent density of flies in the study area. A total of 480 systematically selected cattle from *Chirecha*, *Fokabadelecha*, and *Tulito* Kebeles were sampled in the dry and wet seasons of the study period. To estimate Trypanosoma infection, the buffy coat technique was used on blood samples collected from the marginal ear vein of the cattle. Out of the 480 cattle, 30 were found positive for trypanosomosis, resulting an overall prevalence of 6.3% (95% CI: 4.4-8.8; OR: 1.54). The wet season showed a relatively higher prevalence (7.5%, 95% CI: 4.8-11.6) than the dry season (5.0%, 95% CI: 2.9-8.6), although the difference was not statistically significant. The prevalence was significantly higher in Chirecha Kebele (12.0%, 95% CI: 8.1-17.4; OR: 6.4) and in black-coated cattle (19.4%, 95% CI: 9.4-35.8; OR: 6.84) ($p<0.05$). Two species of Trypanosoma, *T. congolense* (80%) and *T. vivax* (20%), were identified. The hematological finding found significantly lower mean PCV values in parasitemic animals (21.1±3.39) than aparasitemic animals (26.30±5.23). Out of 256 total flies caught by using 40NGU traps in the dry and wet seasons, 79(30.9%) were *G. pallidipes*, 56(21.9%) were Tabanus, and 121(47.2%) were stomoxys. The overall apparent density of *G. pallidipes* was 0.99 f/t/d with relatively higher density in the wet (1.95f/t/d) than the dry (0.025f/t/d) season. In conclusion, the study revealed bovine trypanosomosis and its vectors are widespread in the study area. To reduce the prevalence of trypanosomosis, particularly in high-risk areas such as Chirecha Kebele and black-coated cattles, control measures such as the use of traps, targets, deltamethrin, and trypanocidal drugs should be consolidated.

Keywords: *Black*, *Bovine Trypanosomosis*, *Chirecha*, *G. pallidipes*, *Prevalence*.

1. INTRODUCTION

African Animal Trypanosomosis affects all domestic animals, including dogs, cats as well as a wide range of wildlife species that serve as reservoirs of infection for both humans and domestic animals (Anderson *et al*., 2011). Trypanosomosis is a major constraint that contributes to direct and indirect economic losses in crop and livestock production, with a significant negative impact on economic growth in many parts of the world, particularly in sub-Saharan Africa (O'Gorman *et al*., 2006; Sachs, 2010).

In sub-Saharan Africa, trypanosomosis control and prevention are crucial for sustainable agricultural development and poverty reduction. Trypanosomas are flagellate protozoans that inhabit the blood, the lymph and various tissues of their hosts. These are obligate parasites that multiply in their definitive hosts and sometimes in an intermediate host (Desquesnes, 2004).

With an exception of *T. equiperdum*, which causes a venereal disease in equines, all have arthropod vectors in which the transmission is either cyclically by tsetse flies of the Glossina species or non-cyclical by many other flies. 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).

Ethiopia is known to have the largest livestock population in Africa and this sector has been contributing significantly to the country's economy. Livestock products such as meat, milk, eggs, cheese and butter supply animal protein that contributes to the improvement of the nutritional status of the people. Additionally, livestock provides export commodities such as live animals, hides and skins that earn foreign exchange for the country. Draught animals, such as oxen, provide power for smallholdings and crop threshing throughout the country. They also serve as essential mode of transport for farmers and their families to travel long distances, convey agricultural products to markets and bring back domestic necessities. Livestock provides a certain degree of security in times of crop failure, as they are a "near-cash" capital stock. Moreover, livestock provides farmyard manure that is commonly used to improve soil fertility and as a source of energy (CSA, 2020/21).

Despite of its huge number and function; the livestock production system is limited by several factors such as:- declining availability of grazing lands due to human population growth and increasing crop cultivation areas, poor production system and reproductive performance of animals, inadequate access to technologies and diseases (Duressa *et al*., 2014).

Trypanosomosis is one of the major diseases that impediments to livestock development and agricultural production in the Country (NTTICC, 2004). It has been existed in Ethiopia since time immemorial, but was first recorded in a report by Donaldson Smith in 1894-1895 (Langridge, 1976) while exploring in the southern Ethiopia in which his pack animals died of the same disease. However, it started emerging as a serious problem to livestock (and also humans) only since 1960's with the steady increase of human population and the need of expanding agricultural production into the unexploited lowland areas, in the south-west (FITCA, 1999).

Trypanosomosis is a serious and often fatal disease of livestocks mainly in the rural poor community and rightfully considered as a root cause of poverty in the country. Since more than 90% of crop production in Ethiopia is dependent on animal draught power mainly on ploughing oxen, many large fields lie fallow due to a lack of these animals in trypanosomosis infested area, which worsens the food supply and living conditions in affected areas (MoARD, 2007).

Ethiopia is one of the countries suffering from trypanosomosis with approximately 220,000 Km^2 of arable land is infested with five species of tsetse flies: namely *G. pallidipes*, *G. m. submorsitans*, *G. fuscipes*, *G. tachinoides* and *G. longipennis* which are confined to the southern, southwestern and north western regions and the associated river systems (Abay/Dedessa, Ghibe/Omo and Baro/Akobo and rift valley). Among these species the first four are widespread and most important while *G. longipennis* is of minor economic importance (NTTICC, 2004).

Darara and Loko Abaya are districts in the Sidama Regional State of Ethiopia which have a large number of livestock resources. Their location in tsetse and trypanosomosis belt of rift valley, proximity to the River Bilate, Gidabo and Lake Abaya as well as their diversified agro-ecology, offered a potential habitat for tsetse fly (Glossina) as well as other biting flies, which are the cyclical and mechanical vectors of animal trypanosomosis (Constable *et al*., 2017). Although a study had been conducted on the prevalence of the disease and its vectors in the area (Abebe *et al*., 2021), there is no research work done on the seasonal distribution of Glossina species apparent density and the prevalence of bovine trypanosomosis in the study area.

Therefore, the objectives of this study were:

- To estimate the seasonal prevalence of bovine trypanosomosis and identify the dominant Trypanosoma species in the study area.
- To associate the risk factors contributing to the occurrence of trypanosomosis.
- To assess the apparent density of vectors and identify the species prevailing in the study area.

`

2. LITERATURE REVIEW

2.1. Definition

Trypanosomosis also known as "Nagana" is a disease complex caused by several species of protozoan parasites of the genus Trypanosoma, which are transmitted by tsetse flies (Glossina) and other biting flies. Trypanosomosis affects a wide range of mammals, but from an economic perspective, it is particularly important in cattle. The disease causes a range of symptoms, including weight loss, anemia and reduced productivity and can be fatal if left untreated. Control and prevention of trypanosomosis require a multi-pronged approach, including vector control, use of trypanocidal drugs and deployment of trypanotolerant breeds of livestock (Holmes and Eisler, 2003; Shaw and Torr, 2019).

2.2. Etiology

T. vivax, T. congolense, T. b.brucei and *T. simiae* are the four main species responsible for African trypanosomosis affecting virtually all domestic mammals. *T. vivax* and *T. congolense* are the main pathogens of cattle. The four species are members of the Salivaria group of trypanosomas and are transmitted cyclically via the mouthparts of tsetse flies, hence the name salivarian trypanosomas (Constable *et al*., 2017). Trypanosomosis outside "tsetse belt" is caused by mechanically biting flies; the main etiological agent of mechanically transmitted trypanosomosis is *T.vivax* (Desquesnes and Davila, 2002).

2.3. Morphology

A sound knowledge of the basic features of the various Trypanosoma species enables the identification of each species and exact cause of diseases. The most structure are suspended in the cytoplasm, most prominent being the nucleus. For specific identification, a number of trypanosomas should be examined systematically for the presence or absence, size and position of a number of features: such as presence or absence of trypanosomas of different appearance and free flagellum, the size and position of the kinetoplast the degree of development of the undulating membrane and the shape of the parasite, particularly the shape of its posterior part (Uilenberg, 1998).

Duttonella: *T. vivax* is 20–27 µm long, undulating membrane is medium or not obvious, free flagellum present at the anterior end, posterior end rounded, kinetoplast large and terminal. *T. uniforme* presents the same characteristics although it is smaller, 12–20 µm long (Stephen, 1986; Uilenberg, 1998).

Nanomonnas: *T. congolense* is 8–25 µm (small species), undulating membrane not obvious, free flagellum absent, posterior end rounded, kinetoplast is medium sized and terminal, often laterally positioned. Although *T. congolense* is considered to be monomorphous, a degree of morphological variation is sometimes observed. In Nanomonnas, a number of morphotypes have been described so far; from the slender to the stumpiest: hyperleptomorph (rodhaini-form, very long and slender, with a free flagellum), leptomorph (this is *T. simiae*-form, slender, with a free flagellum), isomorph (congolense-form, short, without a free flagellum), pachymorph (montgomeryi-form, short and stout without a free flagellum) and hyperpachymorph ('hyper-montgomeryi-form', short and very stout without a free flagellum) (Molyneux and Ashford, 1983; Uilenberg, 1998).

Additionally, sphaeromorph and rosettes have also been described. Within *T. congolense*, different types or subgroups exist (savannah, forest, kilifi or Kenya coast) that have a different pathogenicity (Bengaly *et al*., 2002; Morrison *et al*., 2008); also there is a large variation in pathogenicity within the savannah subgroup. These types can only be distinguished using PCR. Finally, the pig and monkey parasite, *T. simiae*, is pleomorphic, appearing from hyperleptomorph to pachymorph, most often like a long parasite (leptomorph), with well developed undulating membrane, occasionally exhibiting a free flagellum; it may also appear like a classical *T. congolense* (Desquesnes *et al*., 2013).

Trypanozoon: *T. brucei* is large like *T. vivax*, but its rapid movement is in confined areas of the wet film. In stained smears, it is pleomorphic and may occur as long and slender forms, intermediate forms, or short and stumpy forms. The slender and intermediate forms have a long free flagellum, pointed posterior end, subterminal kinetoplast and a prominent undulating membrane, whereas the stumpy forms resemble *T congolense*, but are bigger and have a prominent undulating membrane (Desquesnes *et al*., 2013; Constable *et al*., 2017).

2.4. Life cycle

Trypanosomas undergo a series of morphological changes as they progress through their life cycle, which may involve more than one of the following configurations:

a) Promastigote: elongated form with antenuclear kinetoplast and flagellum emerging from the anterior end of the body.
b) Epimastigote: elongated form with a juxtanuclear kinetoplast and flagellum emerging from the side of the body as a short undulating membrane.
c) Trypomastigote: postnuclear kinetoplast, flagellum arising near it to run along a long undulating membrane.
d) Amastigote: rounded or oval forms devoid of external flagellum (Hunt, 2010).

The life cycle of trypanosomas typically involves the trypomastigote form in the vertebrate host and the trypomastigote or promastigote form in the gut of the invertebrate host. The trypomastigote morphology is unique to species in the genus Trypanosoma (Uilenberg, 1998).

2.5. Epidemiology

The distribution of trypanosomas of veterinary importance varies with locality and depends on the interaction between tsetse flies, the parasite, domestic and wild animals (Moore *et al*., 2012).

2.5.1. The Parasite

AAT is caused by different Trypanosoma species with *T. congolense* and *T. vivax* being the major pathogens for livestock, especially cattle and other ruminants. Other species that infect livestock include *T. b. brucei*, *T. simiae*, *T. godfreyi*, *T. uniforme* and *T. suis* (Rotureau and Van Den Abbeele, 2013). The epidemiology of animal trypanosomosis is determined mainly by the ecology of the tsetse fly which is found only in tropical Africa (NTTICC, 2004).

Parasite virulence is also an important factor influencing the epidemiology of AAT. The pathogenicity appears to vary depending on which type or strain of Trypanosoma is involved (Bengaly *et al*., 2002). Within *T. congolense*, different types exist (savannah, forest, kilifi and Tsavo) that have a different pathogenicity (Desquesnes *et al*., 2012).

T.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. Godfreyi* (Van Den Bossche *et al*., 2011).

2.5.2. The Vector

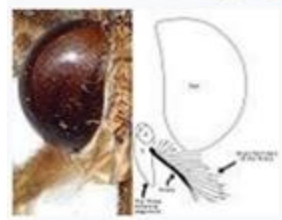
The distribution of the trypanosomosis is parallels the distribution of tsetse flies and comprises of an area approximately 10 million Km^2 in 37 sub-Saharan countries, extending on both sides of the equator from 15 ^{0}N to 30 ^{0}S (Ilemobade, 2009; Sachs, 2010). They belong to the class Insecta, Order Diptera and the family Glossinidae, which is close to Muscoidae, from which they differ mainly by the adaptation of their mouthparts to blood sucking. Their single genus Glossina includes three subgenera and 31 species and subspecies (Simarro *et al*., 2010).

Tsetse species can be grouped according to their preferred habitats as savannah species, riverine species and forest species. The savannah species (including *G. morsitans*, *G.austeni*, *G. pallidipes*, *G. swynnertoni* and *G. longipalpis*) pose the greatest threat to livestock because they inhabit grasslands where cattle are traditionally reared, they can easily adapt to other ecological niches, they feed primarily on cattle and pigs and they are efficient vectors of trypanosomas (Pollock, 1982).

The riverine species (*G. palpalis*, *G. tachinoides* and *G. fuscipes*) are important as vectors of bovine and porcine trypanosomosis as well as of Gambian sleeping sickness due to *T. b. gambiense*. On the other hand, forest species (including *G. fusca*, *G. bwuipalpis* and *G. longipennis*) are not frequently incriminated vectors of trypanosomas even though their preferred food hosts are ruminants and suids (Constable *et al*., 2017).

Tsetse can be distinguished from other large flies by two easily observable features. Tsetse folds their wings completely when they are resting so that one wing rests directly on top of the other over their-abdomens in a scissor like configuration. Tsetse also has a long-proboscis, which extends directly forward and is attached by a distinct bulb to the bottom of their heads (Hordofa and Haile, 2017). The characteristics that definitively differentiate adult tsetse from other kinds of flies (Pollock, 1982; Desquesnes *et al*., 2017).

Table 1: Four basic characterisitics to differentiate tsetsefly from other flies

Structures	Discriptions	
Proboscis	It has distinict proboscis, a long structure attached to the bottom of the head and pointing forward.	
Wings	When at rest, tsetse fold their wings completely one on the top of the other.	
Hatchet cell	The discal medial(middle) cell of the wing has a characteristic hatchet shape resembling meat cleaver or a hachet.	
Branched arista hair	The antenna have arista with hairs which are themselves branched	

(Source: Desquesnes *et al*., 2017)

The tsetse flies are nearly always some shade of brown or grey-brown; sometimes there is a slight pink or sandy-red tinge. Several species are very dark. The body usually has darker and lighter patches, making the insect difficult to see when it is settled on bark, rock or soil. At rest, the tsetse normally appears quite slim because the wings are placed one over the other on the back not projecting outwards at an angle to the body as in house flies or most blowflies (Pollock, 1982).

Both sexes of the tsetse are blood feeders. They depend only on vertebrate bloods for their survival (Wall and Shearer, 1997). When they bite an animal, they create a pool of blood at the site of the bite and drain saliva into this blood pool through the a long tube like structure known as “hypopharynx”. The salivary secretion contains a powerful anti-coagulant which keeps the blood not to coagulate and fluid so the fly can continue feeding (Okhoya, 2004).

When a tsetse fly feeds on an animal infected with trypanosomas, bloodstream forms of the parasite are ingested along with the bloodmeal and infection may become established in the fly. The saliva of an infected fly contains metacyclic trypanosomas, highly infective forms of the parasite, which are injected into the blood pool every time the fly feeds. Once infected, tsetse continues to transmit trypanosomas for the rest of their lives (Wall and Shearer, 1997).

2.5.3. The Host

Trypanosomas can infect a wide variety of domestic animals and more than 30 wild animal species. *T.congolense* infects cattle, pigs, goats, sheep and horses. Dogs occasionally become chronically infected carriers of this species. *T. b.brucei* can infect cattle, horses, dogs, cats, camels, sheep, goats and pigs. *T. vivax* primarily infects cattle, sheep and goats. Monkeys, rats, mice, guinea pigs and rabbits can also be experimentally infected by trypanosomas (CFSPH, 2015).

2.5.4. The Climate and Environment

Temperature has a strong influence on tsetse population dynamics and can be one of the strongest abiotic determinants of tsetse distributions. The tsetse flies only live in regions where the average annual temperature is above 20°C of which 25°C is the optimum temperature for their survival. Unlike high temperatures, low temperatures slow down tsetse activity and general physiology. Under extreme temperatures of below 10^{o}C, tsetse will die within 3 hours of exposure (Rogers and Robinson, 2004; Terblanche, 2008).

2.6. Mode of Transmission

Trypanosomosis is a disease, which is cyclically and a non-cyclically transmitted by different species of tsetse flies and other flies (Mulligan, 2006).

2.6.1. Cyclical

When the infected tsetse fly takes blood meal, it injects the metatrypanosomas into the mammalian host's bloodstream, where they continue their life cycle and cause disease. When a tsetse fly hatches from its pupal case it is free from trypanosomas. Until its first blood meal, it is called a teneral fly. It acquires a trypanosomal infection when feeding on a parasitaemic (having parasites in the circulating blood) mammalian host. The trypanosomas undergo a cycle of development and multiplication in the digestive tract of the fly until the infective metacyclic trypanosomas (metatrypanosomas) are produced, different trypanosoma species develop in different regions of the digestive tract of the fly and the metatrypanosomas occur either in the biting mouthparts or the salivary glands (Maudlin and Holmes, 1994; Uilenberg, 1998).

2.6.2. Non-cyclical

After trypanosomas have been introduced into a herd, transmission is possible even in the absence of Glossina. Biting flies such as Tabanidae, Stomoxyinae and Hippoboscidae are capable of mechanically transmitting trypanosomas in their mouth parts if they feed on more than one host within a short interval (Constable *et al*., 2017).

A mechanical insect vector is defined as any haematophagous insect that is liable to bite several hosts in succession within a few minutes or hours; the residual blood and/or lymph that remains in the mouthpart possibly contains pathogenic agents (although these do not develop or multiply in the vector) and is inoculated through the saliva (Rodhin and Perez, 1985)

a) Stomoxys

Stomoxys (stable flies) are biting haematophagous insects, regardless of gender. They are small and resemble the housefly apart from the appearance of the mouthpart which is of the biting type and comparable to that of Glossina, comprising a labium, labrum and hypopharynx. These are busy insects that are liable to switch hosts during a single bloodmeal and hence are excellent mechanical vectors (Kaufman and Nayduch, 2017).

Both the male and female adults need to take a bloodmeal before reproduction, which takes place three to five days after they emerge; the females lay some hundred eggs in small packets and require one meal every time they lay. Adult life expectancy is estimated to be between two and four weeks and the total number of eggs produced is from 60 to 800. Stable flies are permanently active although there are clear seasonal peaks in the wet or dry periods, depending on the species (Kangwagye, 1974; Foil and Hogsette, 1994).

b) Haematobia

Their overall morphology is similar to that of Stomoxys but they are smaller. Haemotobia are biting insects that feed mainly on livestock, and both males and females are haematophagous. Their English name ‘hornfly’ refers to their predilection for the base of cattle horns. Males and females take bloodmeals before reproduction, which occurs three to four days after the adults emerge; the female lays several dozen eggs either in small packets or spread out. They feed 20 to 30 times a day, which works in favour of mechanical transmission of pathogens, but do not often switch host which on the other hand works against this mode of transmission (Kaufman and Nayduch, 2017).

c) Tabanidae

Tabanids (Diptera: Tabanidae) belong to the order of the Dipterans, suborder Brachycera. There are more than 4,000 species of horseflies that are found on all ecological sites with considerable harmful effects. Adult tabanids have a general appearance similar to that of a typical fly, with a stout and compact body. Their mouth parts are adapted for telmophagous feeding, which involves biting and lacerating the skin of the host to feed on blood. The size of adult tabanids varies from 5 to 25 mm, depending on the species and environmental conditions. Tabanids come in a variety of colors and patterns, but they generally have dull colors except for their large and bright compound eyes, which are often iridescent. The eyes of tabanids are well developed and allow these insects to locate potential hosts from a distance (Mihok, 2002).

2.7. Pathogenesis

After being transmitted by tsetse flies, trypanosomas initially replicate at the site of inoculation in the skin, which can cause a swelling and a sore known as a chancre. From there, the trypanosomas can spread to the lymph nodes and bloodstream, where they continue to replicate and cause systemic infection. *T. congolense* can localize in the endothelial cells of small blood vessels and capillaries, where they can cause damage to the vascular system. On the other hand, *T. brucei* and *T. vivax* localize in various tissues, including the spleen, liver and lymph nodes. Antibodies developed against the glycoprotein coat of the trypanosoma can help to kill the parasite and may lead to the formation of immune complexes. These immune complexes can contribute to the development of various pathological conditions associated with trypanosomosis (Uilenberg, 1998; Hunt, 2010; Giddings *et al*., 2006).

Antibody however, does not clear the infection for the trypanosoma has genes that can code for many different surface-coat glycoproteins and change its surface glycoprotein to evade the antibody. Thus, there is a persistent infection that results in a continuing cycle of trypanosoma replication, antibody production, immune complex development and changing surface-coat glycoproteins. Immunologic lesions are significant in trypanosomosis and it has been suggested that many of the lesions (e.g., anemia and glomerulo-nephritis) in these diseases may be the result of the deposition of immune complexes that interfere with, or prevent, normal organ function. This marked immune-suppression lowers the host's resistance to other infections and thus results in secondary disease, which greatly complicates both the clinical and pathological features of trypanosomosis (Mare, 2004).

T. vivax usually multiplies rapidly in the blood of cattle, sheep and goats and is evenly dispersed throughout the cardiovascular system, whereas *T. congolense* tends to be aggregated in small blood vessels and capillaries of the heart, brain and skeletal muscle and rarely causes heavy parasitemias in ruminants. *T. congolonse* and *T. vivax* exert their effect mainly by causing severe anemia and mild to moderate organ damage. The anemia has a complex pathogenesis involving mainly increased erythro-phagocytosis, some hemolysis and dys-hemopoiesis (Constable *et al*., 2017).

2.8. Clinical Manifestation

Bovine trypanosomosis causes severe anaemia, oedema, immunosuppression and various neurological disorders, which may eventually produce the death of the affected animals (Gonzatti *et al*., 2014). 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, loss of condition and nervous signs (Batista *et al*., 2009).

Superficial lymph nodes become visibly swollen; mucous membranes are pale, diarrhoea occasionally occurs and some animals have oedema of the throat and the underline (Mary and David, 2009), anaemia appears with progressing parasitaemia and there is lysis of large numbers of red blood cell resulting in drop in PCV (Gonzatti *et al*., 2014). 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 (Constable *et al*., 2017).

2.9. Diagnosis

Accurate diagnosis of trypanosomosis in domestic livestock and tsetse flies is essential for epidemiological studies, planning and implementing chemotherapy and monitoring vector control operations. In addition to clinical diagnosis, several methods are available for the diagnosis of trypanosoma infections in livestock. For example, direct (parasitological) methods such as Wet blood film, thick blood film and thin blood film, in which the trypanosomas are visualized by Microscope in blood, lymph, or other tissues. Indirect (serological) methods such as the detection of specific antibodies in serum or other body fluids and animal inoculation methods, in which the trypanosomas are inoculated into a susceptible animal and monitored for the development of the disease, are also available. Molecular diagnostic methods such as PCR can also be used to detect trypanosoma DNA in blood or other tissues (Desquesnes and Dávila, 2002).

2.9.1. Parasitological Diagnosis

Trypanosoma detection can be conducted on a variety of samples including blood, lymph nodes, cerebrospinal fluid, genital secretions and organ smears. The choice of sample depends on the stage of the disease, the species of trypanosoma and the diagnostic method used. Depending on the test, the sample can be processed as a fresh preparation or on fixed or frozen biological material whereas, for epidemiological surveys, this method does not provide a large enough volume for blood banking purposes. Use of anti-coagulant-containing tubes is recommended although a capillary tube can be prepared using a dry tube immediately after the sample has been collected (Wells, 1984).

a) Wet blood film

This method is called the direct microscopic method. It involves placing a droplet of blood on a microscope slide, covering it with a cover-slip and examining it under a microscope at ×400 total magnification. Trypanosomas can be recognized by their movement among the red blood cells and depending on their size and movements, a presumptive diagnosis of the trypanosoma species can be made. However, final confirmation of the species must be made by examining stained preparations such as GSBS.The wet blood film technique is simple, inexpensive and provides immediate results. However, its diagnostic sensitivity is generally low and depends on the examiner's experience and the level of parasitemia. The test may be positive when the parasitemia is above 104 parasites/ml. Sensitivity can be improved by lysing the red blood cells before examination using a haemolytic agent. Due to its low sensitivity, this technique is generally used to follow-up experimental infections rather than to detect infections in field samples (Desquesnes and Davila, 2002).

b) 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. It is stained for 30 minutes with 4% diluted Giemsa stain in phosphate buffered saline, with pH 7.2. Staining time and stain dilution may vary with stain and individual technique.

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 ×400 to ×1000 total magnification.The method is simple and relatively inexpensive, but results are delayed because of the staining process; however commercial kits are available for quick staining. Trypanosomas are recognized by their general morphology, but may be damaged during the staining process. This may make it difficult to identify the subgenus. The test is positive when the parasitaemia is above 104–105 /ml. Thin blood smears are generally preferred to thick ones, because of the lower specificity of the latter (Uilenberg, 1998; Desquesnes *et al*., 2017).

c) Thin blood smear

These are made as in the case of blood smears to detect on the blood parasites like trypanosomas. 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 trypanosomas (Murray *et al*., 1977).

d) Parasite concentration techniques (Microhematocrit Centrifugation Technique (mHCT))

The microhaematocrit centrifugation technique, or Woo method (1970), is widely used for the diagnosis of animal trypanosomosis. It is based on the separation of the different components of the blood sample according to their size, shape and density. Fresh ear vein blood (about 70 µl) is collected into heparinised capillary tubes and one end is sealed by using cristalseal. The sealed capillary tube is then placed in one of the numbered grooves in a microhaematocrit centrifuge with the sealed end facing outwards and against the rubber band around the capillary centrifuge rotor and centrifuged for 5 min in 12, 000 rpm. After centrifugation, the haematocrit value is read using a microhaematocrit capillary tube reader and is expressed as % (PCV). A Neubauer cell can be used or alternatively a capillary tube holder consisting of a slide on which 2 pieces of glass (25 x 10 x 1.2 mm) are glued 1.5 mm apart, to form a groove in which the capillary tubes are placed for examination and the plasma/white blood cell interface (buffy coat) is examined by slowly rotating the tube. Trypanosoma movement can first be detected using the X40 objective at a distance that provides the appropriate depth of field through the capillary tube.

The microhaematocrit centrifugation technique is more sensitive than direct examination techniques. In the case of infections with *T. vivax*, the sensitivity of the Woo method is close to 100% when parasitaemia is above 700 trypanosomas/ml of blood. However, sensitivity falls to 50% when parasitaemia is between 60 and 300 trypanosomas/ml of blood. Compared with the microhaematocrit centrifugation technique, the buffy coat technique has the added advantage that preparations can be fixed and stained for more accurate identification of species and for retention as a permanent record. It also has advantage that the PCV and hence the level of anaemia, can be determined at the individual animal and/or herd level (Uilenberg, 1998).

e) Dark-ground or phase-contrast buffy coat technique

The most steps of the buffy coat technique, or Murray method, are identical to steps of the mHCT techniques as described above, but in this method, the tube is cut with a diamond tipped pencil 1 mm below the buffy coat to include the top layer of RBCs. The buffy coat and the uppermost layer of RBCs are extruded onto a microscope slide and covered with a cover-slip (22 x 22 mm). And the microscopic examination is performed with the light condenser in the dark ground position and using a X40 or X100 phase-contrast objective lens. Approximately 200 fields of the preparation are examined for the presence of motile trypanosomas, at a X400 magnification using a dark-field or phase-contrast microscope. Like the mHCT the buffy coat technique is more sensitive than direct examination techniques. The sensitivity of the buffy coat method can be improved by using the double-centrifugation technique (Murray *et al*., 1977).

2.9.2. Molecular Methods

The principle of molecular methods is to detect DNA sequences that are specific for trypanosoma subgenus, species, subspecies, type or strain. There are a number of PCR based techniques that have been developed for identification of trypanosoma species (Thumbi *et al*., 2008). These molecular techniques rapidly detect and identify trypanosoma species in both mammalian hosts and the tsetse vector with high sensitivity and specificity (Cox *et al*., 2005; Mwandiringana *et al*., 2012), even in cases of low parasitaemia; however, none have been validated for routine diagnostic purposes (Deborggraeve and Buscher, 2010).

a) The Polymerase Chain Reaction (PCR)

It is based on an enzyme DNA polymerase, which amplifies (multiplies, copies) sequences of DNA bases, unit sufficient material is produced to be detected. It does so by polymerization ("sticking together") of nucleic acids (Uilenberg, 1998). Parasite DNA is denatured (separated by heat into the two single strands). Two primers are used, which are short sequences of nucleotides (one for single strands), each constructed so as to be complementary to a specific site on one of the two single parasite DNA strands. The primers attach to the sites for which they are complementary and DNA polymerase then starts to reproduce the rest of each complementary sequence which follows from that primer. This occurs in opposite direction until the entire sequence of double-stranded DNA between the primers has been doubled (as a complementary strand is produced from each primer) (Delespaux *et al*., 2003).

The polymerase can of course only do its work when nucleic acids are added to the test material. The cycle is then repeated, the two double stranded DNA sequences are chain denatured, the primers attach again, and the polymerase amplifies. In the end PCR product is submitted to electrophoresis and bands are detected by special staining (IAEA, 2007).

2.9.3. Serological Test

The aim of serological tests is to detect specific antibodies developed by the host against the infection or inversely, to demonstrate the occurrence of circulating parasitic antigens in the blood by the use of characterized specific antibodies. The detection of antibodies indicates that there has been infection, but as antibodies persist for some time (weeks, sometimes months) after all trypanosomas have disappeared from the organism (either by drug treatment or self-cure) a positive result is no proof of active infection. On the other hand, circulating trypanosomal antigens are eliminated quickly after the disappearance of the trypanosomas and their presence therefore shows almost always that live trypanosomas are present in the animal (Uilenberg, 1998).

a) Trypanosoma Antigen Detection Assays

Several ELISA antigen detection methods based on immune sera or monoclonal antibodies, have been described for trypanosomosis. However, field evaluations of these tests have given variable and generally unsatisfactory results (Desquesnes, 1996).

i. The indirect Enzyme-Linked Immunosorbent Assay (ELISA)

The binding of anti-trypanosomal antibodies to the antigen is shown by a conjugate of antibovine (if the test serum is bovine) immunoglobulins labelled with an enzyme, which can be visualized by adding an appropriate chromogenic substrate (i.e. the interaction between enzyme and substrate will create a colour). Usually solubilized antigens obtained from disrupted trypanosomas are used (instead of smears containing whole trypanosomas) and the soluble antigens are coated in the wells of microtrays ("stuck" on the surface of the well, as it were). Each microtray contains usually 96 wells (Uilenberg, 1998).

ii. Antigen-Detecting Tests (Ag-ELISA)

These tests have been developed for the detection of circulating trypanosomal antigens. The surface antigens of trypanosomas are variable; only one or two of the many different variants are present in the blood at any one time and unless one would possess mixtures of antibodies to all the possible variants, their detection is not reliable. Therefore, the tests that have been developed are based on so-called monoclonal antibodies against invariable (internal) antigens. The wells of a microplate are coated with a monoclonal antibody, which is specific for an invariable (non-surface) antigen of a trypanosoma species.

Serum of the animal to be tested is allowed to react in one of the wells and if there is the corresponding circulating antigen, its presence in the well after washing off the test serum can be shown by finishing off the test with a conjugate of the same monoclonal antibody marked with a suitable enzyme and the presence of the enzyme is shown by use of the suitable chromogenic substrate, as in the normal ELISA. In particular, Ag-ELISA tests based on monoclonal antibodies presented a lack of specificity between trypanosoma species and there were also cross-reactions with other, non-identified parasitic agents, resulting in high rates of false-positives in non-endemic areas (Desquesnes, 1996; Uilenberg, 1998).

b) Detection of IgM Antibodies

The CATT (Card Agglutination Test for Trypanosomas)/*T. evansi* is an agglutination test that can detect recent infections with *T. evansi*. It consists of mixing fixed parasites and serum (or blood) containing immunoglobulins M (IgM). Although the test was developed with *T. evansi*, it can be crossed with other trypanosoma species and thus also detect IgM antibodies directed against *T. vivax* and to a lesser extent, those directed against *T. congolense*. As IgM antibodies are consumed during IgM parasite immune reactions, their availability in the blood fluctuates, which means that the test can give false-negative reactions (when the IgM antibodies have been consumed in eliminating a large population of parasites) (Desquesness *et al*., 2017).

2.9.4. Animal Inoculation

The inoculation of blood into rodents, usually mice or rats, is particularly useful in revealing subpatent infections. The laboratory animals are injected intraperitoneally with 0.1–0.5 ml (depending on the size of the rodent) of freshly collected blood on anticoagulant. Artificial immunosuppression of recipient animals by irradiation or drug treatment (e.g. cyclophosphamide 200 mg/Kg) (Endoxan®) will greatly increase the chances of isolating the parasite. A drop of blood is collected from the tip of the rodent's tail three times a week for 2 weeks to 2 months. The fresh blood is placed on a slide, covered with a cover-slip and examined directly under the microscope (preferably by phase-contrast with a magnification of X400) (Desquesnes *et al*., 2017).

2.10. Treatment and Control

2.10.1. Treatment

In trypanosoma endemic areas trypanocidal drugs are the most widely used methods of trypanosomosis control, ensuring maximum effects at relatively little cost (Clausen *et al*., 2010).

This approach of controlling the parasite in the host relies on limited and extensively used trypanocidal drugs with little or no regular monitoring (Sow *et al*., 2012).

Diminazene aceturate (Berenil®, Veriben®, Ganaseg®, etc.). is used widely against *T. vivax* and *T. congolense* as a curative and sanative drug at 3.5-7mg/kg BW. It is well-tolerated by ruminants and it is one of the two recommended drugs for bovine trypanosomosis. It is not well-tolerated by horses. Homidium bromide (ethidium) and homidium chloride (novidium): are also widely used against *T. congolense* and *T. vivax* as curative and sanative drugs at 1 mg/kg BW (Constable *et al*., 2017).

Isometamidium (Samorin or Trypamidium): is the other preferred drug against *T. vivax* and *T. congolense* in ruminants. It is used as a curative and prophylactic drug at 0.25–0.5 mg/kg (1.25–2.5 ml/50 kg), 1 .0 mg/kg (2.5 ml/50 kg) BW (Desquesnes, 2004). At much higher doses (12. 5-35 mg/kg BW), it can be used prophylactically against *T. simiae* in pigs but not without the risk of death from acute cardiovascular collapse (Constable *et al*., 2017).

2.10.2. Control

The control of trypanosomosis in enzootic countries involves control of tsetse fly population, prophylactic treatment, good husbandry of animals at risk and use of trypanotolerant animals (Constable *et al*., 2017).

a) Control of Fly

A wide variety of tsetse control techniques have been developed and have undergone trial. These control techniques include: ecological methods, use of insecticides, use of traps and targets, use of insecticide treated cattle, use of sterile insect technique or reducing the risk of exposure through changes in livestock management (Allsopp and Hursey, 2004; Bouyer *et al*., 2014; Shaw *et al*., 2015; Holt *et al*., 2016).

Use of Sterile Insect Technique (SIT)

The Sterile Insect Technique (SIT) is a biologically-based method for managing insect pests of agricultural and medical/veterinary importance. The SIT involves mass production of the target insect in specialized production centers, sterilization of the males by gamma irradiation and systematic release of the sterile males over the target area in a ratio of about 50 sterile males to every wild male (Vreysen *et al*., 2000; Okhoya, 2003).

The principle of SIT is that the released sterile males compete with the wild male population for mating opportunities with wild females, resulting in a reduction in the number of offspring produced. Over time, this can lead to a decrease in the overall population of the target insect.The SIT has been successfully used to control populations of various insect pests, including the tsetse fly, which is a vector for several diseases, including African trypanosomosis. The SIT has the advantage of being environmentally friendly and highly specific to the target pest, as it does not affect other non-target species (Vreysen *et al*., 2000; Okhoya, 2003).

Mating between sterile and fertile insects can cause sterility in their offspring, as female tsetse flies typically mate only once in their lifetime. This means that the sterile males released through the SIT can effectively outcompete wild males for mating opportunities with wild females, leading to a reduction in the overall population of the target insect. The SIT is generally considered to be most effective when used as part of an AW-IPM approach to controlling tsetse fly populations. The AW-IPM approach involves the coordinated use of multiple control methods, including the SIT, to achieve sustainable removal of an entire tsetse fly population within a defined geographic area (Vreysen *et al*., 2000; Feldmann, 2004; Dyck *et al*., 2005).

b) Prophylactic Treatment

Attempts at trypanosomosis control have also been directed to prophylactic dosing with chemicals such as suramin, prothridium and isometamidium (Samorin). Prophylaxis is used along with other methods in areas where there is a heavy tsetse challenge. The prophylactic effect is supplemented by the development of antibodies, and the total period of protection may be as long as 5 months. However, it is customary to give four or five treatments per year. The productivity response to this pattern of treatment is good if general husbandry is also adequate (Constable *et al*., 2017).

c) Use of Trypano-tolerant Animals

Trypanotolerance is the characteristic of an animal that enables it to remain productive under tsetse and trypanosomosis challenge (Courtin *et al*., 2008). The most well-known trypanotolerant breeds are West African short-horned cattle (Muturu, Baoulé and N‟dama), (Naessens, 2006). In East

Africa, the Maasai Zebu and OrmaBoran breeds show reduced susceptibility (Uilenberg, 1998). The four Ethiopian cattle breeds Abigar, Gurage, Horro and Sheko are related to trypanotolerance (Desta *et al*., 2011). A major factor enabling these animals to cope with trypanosoma infections is a better capacity to limit both anaemia and parasitaemia (Naessens, 2006).

2.11. Status of Bovine Trypanosomosis in Ethiopia

Eighty-eight percent (88%) of human population and 70% livestock population exist in the highland area of Ethiopia. From the total land coverage of the country, 36.3% of it is highland which is heavily degraded and unable to provide enough food for the community living there. In contrast, low-lands of the country that accounts for 63.7% of the total land coverage, supports about 12% of humans and 33% of livestock population. Tsetse and Trypanosomosis threat have rendered these fertile lands inaccessible (Dagnachew *et al*., 2020).

Varying degrees of tsetse risk occur in all areas of Ethiopia except the central highlands and the northeast and southeast arid areas of the country. The highest risk areas were localized in the western zones associated with the river valleys of Blue Nile, Didessa, Baro, Akobo, Gojeb, Ghibe and Omo. The risk area extended from the southern part of the Rift valley, around the southwestern corner of the country and along the western lowlands and escarpments to the Blue Nile. The risk area further extends north from the Blue Nile to Tekeze River along the Sudan border (Abebe *et al*., 2004).

In the east the risk area spreads to the north along with the eastern escarpment and Awash River through the northern part of the Rift Valley of Ethiopia. Foci of risk areas were observed along the Wabi-Shebele and Genale Rivers flowing towards Somalia. Varying degrees of tsetse risk areas were found in five of the nine regional states namely, Amhara, Benshangul, Gambella, Oromiya, Southern Ethiopia. According to the risk model, the eastern lowlands (Afar and Ogaden) and the central highlands of Ethiopia were free from tsetse-transmitted trypanosomosis (Abebe *et al*., 2004).

Five species of Glossina (*G. morsitans sub-morsitans*, *G. pallidipes*, *G. tachinoides*, *G. fuscipes fuscipes* and *G. longipennis*) have been recorded from Ethiopia, but only four are widespread and cause significant economic importance. These are *G. morsitans submorsitans* and *G. tachinoides*, which have a west to east distribution across Africa south of the Sahara desert and *G. pallidipes* and *G. fuscipes*, which often occur together in East Africa, although the former extends far to the south whereas the latter has an essentially central African distribution (NTTICC, 2000).

Table 2: Tsetse species and their distribution reported in Ethiopia

Tseste species	Location	Reference
G. tachnoides	Pawi (Metekel Zone)	Mekuria and Gadisa, 2011
G. pallidipes, *G. m. submorsitans* and *G. f. fuscipes*	West Shewa (south west oromia)	Ayele *et al*., 2012
G. tachinoides	Diga and Sasiga districts (East Wollega zone)	Tafese *et al*., 2012
G. pallidipes	Ameya District (South West Shewa)	Firaol *et al*., 2014
G. m. submorsitans, G. pallidipes, G. tachinoides and *G. fuscipes*	south-western Ethiopia	Duguma *et al*., 2015
G. tachinoides	Bullen district of Metekele zone, Benshangul Gumuz	Aki *et al*., 2016
G. m. submorsitans and G. tachinoides	Gimbi district (West Wollega)	Geremew *et al*., 2016
G. pallidipes and *G. fuscipes*	escarpment of Omo River, Loma district, southern Ethiopia	Eyasu *et al*., 2021
G. m. submorsitans and *G. f. fuscipes*	Gidami district (western oromia)	Degneh, 2021
G. pallidipes, *G. m. morsitans*, *G. tachinoides* and *G. f. fuscipes*	Sayo district (west oromia)	Degneh, 2021
G. pallidipes, *G. m. morsitans*, *G. tachinoides* and *G. fuscipes*	Western Ethiopia (Kellem wollega)	Tsegaye *et al*., 2021
G. pallidipes	Rift Valleys of Gamo Zone, Southern Ethiopia	Syoum *et al*., 2022

Trypanosoma species affecting livestock in Ethiopia are; *T. congolense, T. vivax* and *T. brucei* in cattle, sheep and goats. *T. evansi* in camels and *T. equiperdium* in horses (Abebe, 2005).

Table 3: Some of Trypanosoma species and their distribution reported in Ethiopia

Prevalent Trypanosoma species	Location	Prevalence	Reference
T. vivax, T. congolense, and Mixed infection	Kindo Koisha district, Wolaita Zone	15%	Kidanemariam *et al*., 2002

T. vivax, T. congolense and T. brucei	Guraferda and Sheko districts, Bench Maji Zone	4.4%	Abebayehu and Biniam, 2010
T.congolense T. vivax and Mixed infection	West Shewa, South Western Oromia	25.7%	Ayele *et al*., 2012
T. brucei, *T. congolense* and *T. vivax*	Genji District, Western Ethiopia	9.89%	Bogale *et al*., 2012
T. congolense and *T. vivax*	Arba Minch Zuria, southern Ethiopia	17.7%	Teka *et al*., 2012
T. congolense, T. vivax and Mixed infection	KindoKoish District, Southern Ethiopia	6.3%	Adale and Yasine, 2013
T. congolense, *T. brucei* and Mixed infection	Ameya District, South West Shewa	1.4%	Firaol *et al*., 2014
T. congolense	Ghibe valley, Southwestern Ethiopia	5.83%	Moti, 2014
T. vivax, T. congolense, T. brucei and Mixed infection	south-western Ethiopia	9.61%	Duguma *et al*., 2015
T. congolense, T. vivax and Mixed infection	Bullen District of Metekele zone	5.6%	Aki *et al*., 2016
T. congolense and *T. vivax*	Mareka District, Southern Ethiopia	8.3%	Eshetu *et al*., 2016
T. congolense and *T. vivax*	Chilga District, Northwest Ethiopia	6.2%	Seyoum and Abera, 2016
T. vivax, *T. congolense*, *and T. brucei*	Gena-Bossa, Dawuro Zone	15.38%	Adisu and Wale, 2017
T. congolense and *T. vivax*	Quara District, North Western Ethiopia	6.77%	Alemu and Alemneh, 2017
T. vivax, *T. congolense*, *T. brucei*, and Mixed infection	Dara District, Sidama Zone	14.8%	Migbaru *et al.*, 2017
T. congolense and *T. vivax*	EnemorenaEner District, Guraghe Zone	5.2%	Tamirat and Tsegaye, 2018
T. congolense and *T. vivax*	Boloso Bombe District, Southern Ethiopia	5.4%	Loha, 2019
T. congolense	West Gojjam, Northern Ethiopia	1.9%	Girmaw, 2020
T. brucei, *T. congolense* and Mixed infection	JimmaArjo District, East Wollega Zone	4.39%	Abdeta, 2021
T. congolense	Halu District of Illubabur Zone	5.32%	Bekele and Beshir, 2021

T. congolense, *T. vivax*, *T. brucei* and Mixed infection	KellemWollega, Western Oromia	12.62%	Degneh, 2021
T. vivax and *T. congolense*	Sodo Zuriya District, southern Ethiopia	5%	Hundesa *et al*., 2021
T. brucei, *T. congolense* and Mixed infection	Kellemwollega, Western Ethiopia	8.7%	Tsegaye *et al*., 2021
T. congolense and *T. vivax*	Gamo zone, Southern Ethiopia	10.17%	Seyoum *et al*., 2022
T. congolense, *T. vivax*, *T. brucei* and Mixed infection	Sayo District, Oromia Regional State, Western Ethiopia	11.16%	Efrem *et al*., 2023

3. MATERIALS AND METHODS

3.1. Study Area

The study was conducted in the selected Kebeles of the Western Sidama, Southern Ethiopia from December 2022 to October 2023 in both dry (January to March 2023) and wet (June to August 2023) seasons of the study period. The study Kebeles were located in the formerly Loko Abaya District which is currently splited to Darara and Loko Abaya Districts. The study sites were selected purposively based on their location in the tsetse and bovine trypanosomosis belt in the area as well as the complaints of the community about the disease. From the two Districts three kebeles namely; Chirecha from Loko Abaya and Fokabadelecha and Tulito from Darara were selected for the study (Figure 1).

The area is characterized by a mid-altitude tropical climate, with an average elevation of around 1,500 meters above sea level. It is located along the Bilate river tsetse belt and has a mean annual minimum and maximum temperature of the area is 22 and 26°C respectively and average annual rainfall ranges from 900-1400 mm with bimodal rainfall pattern. The area is known for its fertile soils, which support a variety of agricultural activities, including coffee and maize cultivation as well as livestock rearing. It is bordered by Bilate river basin and Wolaita zone in the West, Gedio Zone in the South and Oromia Regional State in the East as well as North. The livestock population of the area was 100,493 cattles, 61,526 Goats, 42,117 sheep, 3186 Equines and 166,860 poultry (DWNRB, 2023).

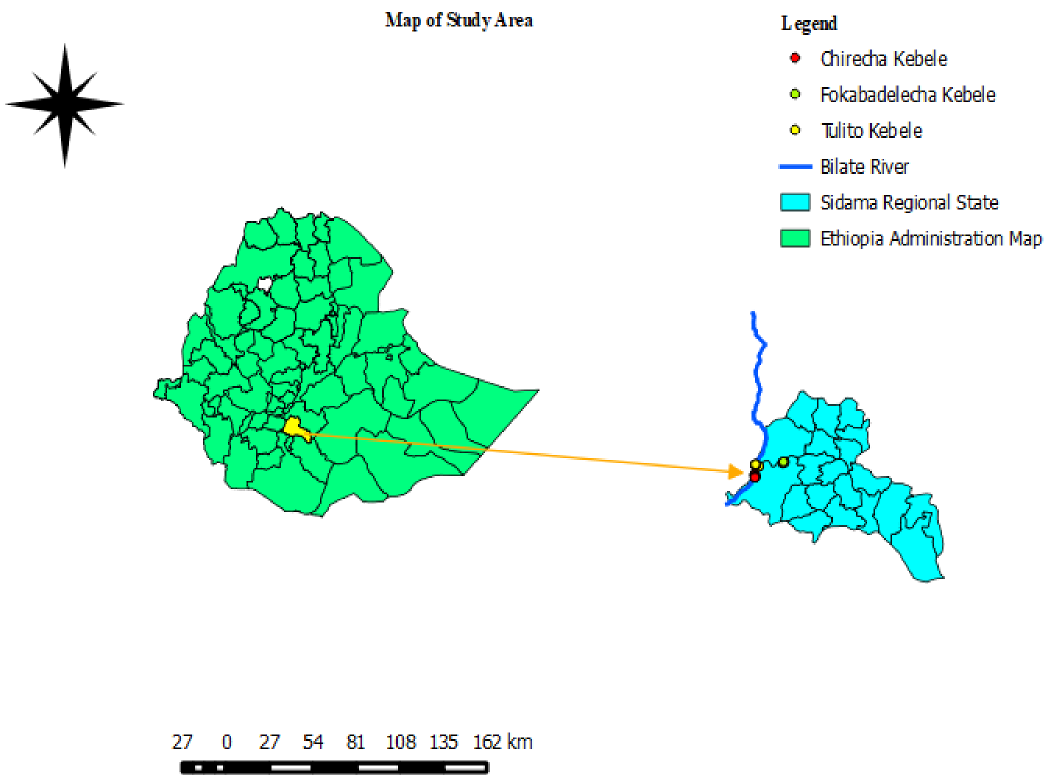


Figure 1: Map of study area

3.2. Study Population

Local (zebu) cattle breed which were kept by extensive management system was incorporated in the study. They herded together during the day time and returned to their individual owner's homestead at each night. The study animals were selected by using systematic random sampling method by taking breed, age, sex, coat colour and body condition into account. The age of study animals was estimated by means of their dentition as described by Torell *et al*. (1998) and categorized as young (≤3 years) and adult (>3 years). The body condition of animals grouped in to poor, medium and good based on criteria described by Nicholson and Butterworth (1986).

3.3. Study Design and Sample Size Determination

A repeated cross-sectional study was conducted to estimate the seasonal prevalence of bovine trypanosomosis and the apparent density of tsetse fly in the study area. The sample size required for the study was calculated by considering the prevalence of 8.3% reported by Abebe *et al*. (2021) from Loko Abaya, 95% CI and 5% desired absolute precision. For sample size computation the following formula described by Thrusfield (2018) was used.

$$n = \frac{(1.96^2 * P_{exp} * (1 - P_{exp}))}{d^2}$$

where n is the required sample size, P_{exp} is the expected prevalence, and d is the desired absolute precision.

So, the calculated sample size was 117. But, to increase the Precision of the study, the sample size was increased by more than 2x and the resulting sample size was about 240 in each season of the study period (i.e. an overall of 480 samples).

3.4. Blood Sampling and Examination

Blood samples were collected by puncturing the superficial ear vein of animal by using sterile blood lancet into heparinized capillary tubes and one end was sealed with Cristaseal. Then the capillary tubes were transferred to a hematocrit centrifuge and centrifuged immediately for 5 minute at 12,000 rpm. Packed cell volume (PCV) was measured by using a hematocrit reader for the determination of the level of anemia. Animals with PCV $<25\%$ were designated as anemic (Douglas and Wardrop, 2010).

The capillary tubes were then cut using a diamond pen at about 1 mm below the buffy coat to include the uppermost layers of the red blood cells and plasma. The contents of the capillary tube were pressed onto a microscopic slide, mixed and covered with a 22 × 22 mm cover slip. Finally, it was examined under ×40 and/or ×10 objectives for the presence of motile trypanosomes (Woo, 1970). The trypanosoma species were identified based on their movement pattern during the buffy coat examination as described by Murray *et al*. (1977). The confirmation of trypanosoma species was done by morphological characteristics after staining the thin blood smear with Giemsa and examination with oil immersion microscopy with ×100 power of magnification (Luckins, 1992).

3.5. Entomological Study

The entomological study was conducted in the dry season (January to March 2023) and the wet season (June to August 2023). A total of 40 standard NGU traps were deployed around the watering and grazing areas to trap tsetse and other biting flies, with 20 traps deployed in each season. The traps were deployed at regular intervals of 200 meters for a period of 48 hours at the watering and grazing points of animals. To prevent ants from climbing up the supporting pole towards the collecting cage and damaging the tsetse flies, the lower portions of the supporting pole were greased and the Acetone was used as attractant (Appendix ii). The location of each trap was recorded using a GPS and a device known as "Thermohygrometer" was utilized to measure the temperature and humidity (Appendex ii). After 48 hours of deployment, the tsetse flies in the cages were collected, counted and identified based on their morphology to the genus level. The species of captured flies were determined according to procedures and keys provided in the guidelines for the collection of entomological baseline data for tsetse area-wide integrated pest management programs (Pollock, 1982). Biting flies were also identified to the genus level based on their morphological characteristics such as size, color, wing venation structure and proboscis (Wall and Shearer, 1997; Marquardt *et al*., 2000). Sex identification was done for tsetse flies by observing the hypopygium on the posterior end of the ventral aspect of male flies.The apparent density of the tsetse fly was calculated as the number of tsetse caught per trap per day (Pollock, 1982).

3.6. Data Analysis

The collected data were entered into a Microsoft Excel spreadsheet, coded edited and then summarized by descriptive statistics. The prevalence of trypanosomosis was calculated by dividing the number of infected cattle by the total number of sampled animals and then multiplying by 100. For the statistical analysis STATA software version 14 was used. The association between the risk factors and trypanosoma infection was analyzed using univariable logistic regression. Risk factors with p-values less than 0.25 were checked for collinearity and the non-collinear factors further subjected to multivariable logistic regression analysis and finally the model was validated by assessing Goodness of fit and its predictive ability. The mean PCV in parasitaemic and aparasitaemic animals was compared using a t-test. The apparent tsetse density (AD) was expressed as the number of flies per trap per day (F/T/D).

4. RESULTS

4.1. Parasitological Findings

During this study, a total of 480 cattles were examined in both dry and wet seasons and 30 cattles found positive for bovine trypanosomosis, giving an overall prevalence of 6.3% (95% CI: 4.4-8.8; OR: 1.54). The prevalence of bovine trypanosomosis was relatively higher in the wet season compared to the dry season. Though, statistically there is no difference ($p > 0.05$) in prevalence between the two seasons (Table 4).

Table 4: Seasonal Prevalence of Bovine Trypanosomosis in the western sidama, Southern Ethiopia

Season	No of animals examined	No of Positive animals	Prevalence (%)(95% CI)	OR	p-value
Dry	240	12	5.0(2.9-8.6)		
Wet	240	18	7.5(4.8-11.6)	1.5	0.26
Total	480	30	6.3(4.4- 8.8)		

Two species of Trypanosoma encountered in the study area namely, *T.congolense* and *T.vivax* (Figure 2).

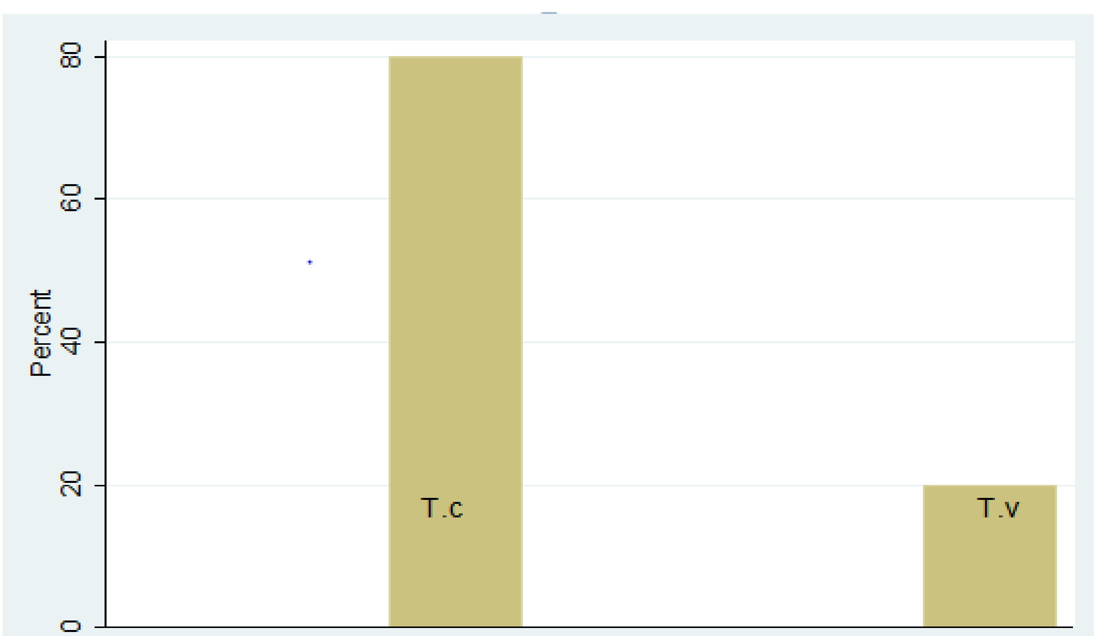


Figure 2: Prevalence of Trypanosoma species in the study area

The univariable logistic regression analysis was conducted to assess the risk factors associated with the prevalence of trypanosoma infection. The analysis output showed that the prevalence of trypanosomosis was significantly higher ($p < 0.05$) in Chirecha (12.0%) compared to Tulto (2.8%) and Fokabadelecha (2.1%). Nevertheless, there was no significant difference in the prevalence of bovine trypanosomosis among animals with different body condition scores (poor, medium and good) ($p > 0.05$), prominent proportion of animals with poor body conditions (7.1%) were infected than medium (6.8%) and good (4.7%). The prevalence of bovine trypanosomosis was higher in adult animals (6.5%) than in young animals (5.5%), but the difference was not statistically significant ($p > 0.05$). There was also no significant difference in the prevalence of trypanosoma infection between males (8.8%) and females (5.7%) ($p > 0.05$).

Interestingly, there was a statistically significant difference in the prevalence of the disease among animals with different coat colours ($p < 0.05$). Black-coated animals had the highest prevalence (19.4%), followed by red (6.2%), mixed (4.6%) and white (3.4%) coated (Table 5). After checking colinearity, those variables with $p < 0.25$ in the univariable logistic regression analysis (kebele and coat colour) were subjected to multivariable logistic regression analysis.

Table 5: Univariable logistic regression analysis for Bovine trypanosomosis with associated Risk Factors

Variable	Categories	No of animals examined	No of positives	Prevalence (%)(95% CI)	OR	P-Value
Kebele	Fokabadelecha	144	3	2.08(0.6-6.3)	-	Ref.
	Chirecha	192	23	12.0(8.1-17.4)	6.4	0.003
	Tulto	144	4	2.8(1.0-7.2)	1.3	0.703
Season	Dry	240	12	5.0(2.9-8.8)	-	Ref.
	Wet	240	18	7.5(4.8-11.6)	1.5	0.26
BCS	Good	149	7	4.7(2.2-9.5)	-	Ref.
	Medium	205	14	6.8(4.1-11.2)	1.5	0.405
	Poor	126	9	7.1(3.7-13.2)	1.6	0.39
Age	Young	110	6	5.5(2.4-11.7)	-	Ref.
	Adult	370	24	6.5(4.4-9.5)	1.2	0.69
Sex	Female	389	22	5.7(3.7-8.4)	-	Ref.
	Male	91	8	8.8(4.4-16.7)	1.6	0.27
Coat color	White	88	3	3.4(1.1-10.1)	-	Ref.
	Red	226	14	6.2(3.7-10.1)	1.87	0.334
	Mixed	130	6	4.6(2.1-10)	1.37	0.662
	Black	36	7	19.4(9.4-35.8)	6.84	0.008

Based on the results of the multivariable logistic regression analysis, it was found that only Kebele and coat colour were significant risk factors associated with the prevalence of trypanosoma infection ($p < 0.05$)(Table 6). The Hosmer-Lemeshow goodness-of-fit test was conducted to evaluate how well the multivariable logistic regression model fits the data. The result showed that the model fits the data well ($HL\chi^2 = 1.87$; $p = 0.9317$), indicating that the model is reliable in predicting the risk of trypanosoma infection. The Receiver Operating Characteristic (ROC) curve was also generated to evaluate the performance of the multivariable logistic regression model. The area under the curve (AUC) was found 0.7673, which suggests that the model has a fair to good discriminatory power in distinguishing between infected and non-infected animals.

Table 6: Multivariable logistic regression analysis for potential risk factors

Variable	Category	No of animals examined	Prevalence (%) (95% CI)	OR	P-value
Kebele	Fokabadelecha	144	2.1(0.6-6.3)	-	Ref.
	Chirecha	192	12.0(8.1-17.4)	6.56	0.003
	Tulto	144	2.8(1.0-7.2)	1.44	0.640
Coat colour	White	88	3.4(10.1-10.2)	-	Ref.
	Red	226	6.2(3.7-10.2)	2.24	0.220
	Mixed	130	4.6(2.1-10.0)	1.97	0.356
	Black	36	19.4(9.4-35.8)	7.32	0.007

4.2. Haematological Findings

The haematological study showed that a significant proportion of cattle 170(35.4%) were found anaemic, with a PCV value falled in 12-24% and of which, 25(14.7%) were infected with trypanosomosis. On the other hand, only 5(1.6%) of the non-anaemic animals were positive for trypanosomosis. The overall mean PCV value of the animals was 25.98% ±5.28 (95% CI: 25.50-26.45). The mean PCV value was significantly lower ($p < 0.05$) in the Trypanosoma infected animals (21.1±3.39, 95% CI: 19.80-22.33) than non-infected animals (26.30±5.23, 95% CI: 25.82-26.79). Moreover, the study found a significant difference ($p < 0.05$) in the mean PCV value between the wet (27.13±4.55) and dry seasons (24.83±5.72) (Table 7).

Table 7: t-test analysis of mean PCV(%) with associated risk factors

Variable	Category	Animals observed	Mean PCV(%)	Std.dev.	t-test	p-value
Infection	Aparasitemic	450	26.30	5.23	5.4	0.00
	Parasitemic	30	21.1	3.39		
Season	Dry	240	24.83	5.72	4.88	0.00
	Wet	240	27.13	4.55		
	Overall	480	25.98	5.28		

4.3. Entomological Findings

A total of 256 flies were caught by using 40 NGU traps in the dry and wet seasons. Among the total flies caught, 79(30.9%) were Glossina, 56(21.9%) were Tabanus and 121(47.2%) were Stomoxys. It was observed that, the *G. Pallidipes* was the only Glossina species found in the study area during the study period. Furthermore, the study found that higher numbers of flies were caught during the wet season (249) than dry season (7). The average temperature of the area during the dry season was measured 32.8°C (with a range of 31.0°C to 33.5°C), while during the wet season, it was 29.1°C (with a range of 25°C to 30.2°C). In addition, the average humidity in the dry season was recorded as 40.8% (with a range of 34% to 46%), while during the wet season, it measured 60.7% (with a range of 48% to 85%) as shown (Table 8).

Table 8: Seasonal Distribution of Vectors of Trypanosomosis in the area

Season	Average T (^{O}C)	Average H (%)	Tsetseflies			Biting flies			Overall
			M	F	Total	T	S	Total	
Dry	32.8	40.8	0	1	1	6	0	6	7
Wet	29.11	60.7	25	53	78	50	121	171	249
Total			25	54	79	56	121	177	256

NB. T=temperature, H=humidity, M=male, F=female, T=tabanus, S=Stomoxys

Out of 79 *G. Pallidipes* caught during the study period, 54(68.4%) were females and 24(31.6%) were male.

4.3.1. Apparent Density of Flies

This study revealed that the overall apparent density of flies was 3.2 flies per trap per day (F/T/D). The apparent density of *G. pallidipes* was 0.99F/T/D and that of biting flies was 2.2F/T/D (Tabanus 0.7F/T/D and Stomoxys 1.5F/T/D). The seasonal apparent density of biting flies (2.2F/T/D) was relatively higher than that of *G. pallidipes* (0.99F/T/D) in the study area. Moreover, the overall apparent fly density recorded during the wet season (6.23F/T/D) was higher than dry season (0.18F/T/D). Further analysis based on Kebeles, showed the Glossina density in Chirecha kebele was higher than Tulito during both study seasons. Specifically, during the wet season, *G. pallidipes* had an apparent density of 1.95F/T/D and biting fly 4.28F/T/D. In other hand, during the dry season, *G. pallidipes* had an apparent density of 0.025F/T/D and biting flies had an apparent density of 0.15F/T/D (Table 9).

Table 9: Seasonal apparent density of flies in the study area

Season	Kebele	No of traps deployed	Tsetsefly (*G.p*)				Bitingfly(T and S)		Overall
			M	F	Total	f/t/d	Count	f/t/d	f/t/d
Dry	Chirecha	10	0	1	1	0.05	4	0.2	0.25
	Tulito	10	0	0	0	0	2	0.1	0.1
	Total	20	0	1	1	0.025	6	0.15	0.18
Wet	Chirecha	10	16	31	47	2.35	89	4.45	6.8
	Tulito	10	9	22	31	1.55	82	4.1	5.65
	Total	20	25	53	78	1.95	171	4.28	6.23
Overall		40	25	54	79	0.99	177	2.2	3.2

NB. M=Male, F=Female, *G.p*= *Glossina pallidipes*, T=Tabanus, S=Stomoxy, f/t/d=fly/trap/day

5. DISCUSSION

The study investigated the seasonal distribution of Glossina species apparent density and the prevalence of bovine trypanosomosis in selected kebeles in the Darara and Loko Abaya districts of the Western Sidama, Southern Ethiopia. The overall prevalence of bovine trypanosomosis in the current study area was 6.3%, which is comparable with the prevalence reported from various parts of Ethiopia.

6.3% from Kindo Koisha District of Wolaita Zone (Adale and Yasine, 2013), 6.25% from Bako Tibe (West Shoa) and Gobu Seyo (West Wollega Zone)(Abera *et al*., 2014), 5.83% from Ghibe valley, Southwestern Ethiopia (Moti, 2014), 6.2% from Chilga District of Northwest Ethiopia (Syoum and Abera, 2016), 8.3% from Bedele District of Buno Bedele Zone (Degneh *et al*., 2021), and 4.98% from the escarpment of Omo River, Loma district, southern Ethiopia (Eyasu *et al*., 2021). The correspondence in the overall prevalence of bovine trypanosomosis with the current study may be due to similarity of agro-ecological conditions and management systems practiced in these areas.

In contrast, some reports from different parts of Ethiopia and neighboring countries reported higher prevalence of bovine trypanosomosis than the current study. Mulaw *et al*. (2011) reported a prevalence of 28.1% from Asosa district of Benishangul Gumuz region, western Ethiopia, Keffale *et al*. (2017) reported 14.8% from Dara District, Sidama Zone, Southern Ethiopia, Degneh (2019) reported 14.08% and 11.16 % from Gidami and Sayo Districts, Kellem Wollega Zone, Oromia Regional State, Ethiopia, respectively. Also Emiru *et al*. (2022) reported 11.16% from Sadi Chanka District, Western Oromia. Likewise, higher prevalence of trypanosomosis have been reported from other parts of Africa, including 40% and 29% in Suba and Teso in Kenya (Thumbi *et al*., 2010) and 25.5% in East Zambia (Mulenga *et al*., 2022).

The lower prevalence in the current study areas than some above listed areas might be due to low density of vectors, treatment of infected animals by using trypanocidal drugs by animal owners as well as the animal health experts and progressive control measures such as chemical application on the back of the animals, the use of insecticide-impregnated targets under taken by the Arbaminch Animal Health Research Center (AAHRC) and Arbaminch Animal Health Research Center Dilla branch.

However, the overall prevalence found in the current study was relatively higher than the prevalence reported from the various parts of the country, such as 2.86% from Dale Wabera District of Kellem Wollega Zone, Western Ethiopia (Biyazen *et al*., 2014), 1.3% from Arbaminch of Gamogofa Zone, Southern Ethiopia (Girma *et al*., 2014), 4.17% from Western Kenya (Kivali *et al*., 2020) and 2.0% in Lira District of Uganda (Katabazi *et al*., 2021). Generally, the variation in prevalence reported in this study and others might be due to various factors such as differences in the study area, investigator experience and the time of the study.

Two species of trypanosoma namely, *T. congolense* and *T. vivax* with higher disease occurrence due to *T. congolense* than *T. vivax* were identified in the study area. This finding of higher infection of cattle by *T.congolense* than *T.vivax* is consistent with reports from Loka Abaya (Abebe *et al.*, 2021), escarpment of Omo River, Loma district, southern Ethiopia (Eyasu *et al*., 2021), Benatsemay District, Southern Ethiopia (Fesseha *et al.*, 2022) and Rift Valley of Gamo Zone (Syoum *et al.*, 2022).

The studies suggested, the higher prevalence of *T. congolense* infection in cattle could be due to high serodemes (serological variation) of *T. congolense* compared to other Trypanosoma species as well as the development of improved immune responses against *T. vivax* by infected animals. In addition, the predominance of *T. congolense* infection in cattle of tsetse-infested areas may be due to the high tendency of the parasite to develop new strains capable of escaping the host immune response. The host has the potential to the development of better immune response against *T. vivax* compared to *T. congolense* infections (Leak, 1999).

Among the potential risk factors considered during the study, Kebele and coat colour of the animal were siginificantly influencing the prevalence of trypanosomosis ($p<0.05$). The prevalence of trypanosomosis was significantly higher in Chirecha kebele than in Fokabadelecha and Tulto; and significantly higher in black coloured cattles than other colours. The higher prevalence in Chirecha Kebele than other two Kebeles might be due to the availability of suitable agro-ecology that support the reproduction of the vectors (Glossina and other biting flies) (i.e. higher fly density). This is also ascertained by the current study finding of the entomological survey (i.e. showed a relatively higher fly apparent density in Chirecha Kebele than the other).

The current study finding of higher prevalence of trypanosoma infection among the black coated cattle was comparable to the reports from the escarpment of Omo River of Loma district, southern

Ethiopia (Eyasu *et al*., 2021) and the Rift Valley of Gamo Zone (Syoum *et al*., 2022), who also reported higher prevalence of trypanosomosis in the black coloured cattles. This may be explained, Glossina, the vector for trypanosomas, are attracted to dark colors and shiny surfaces. Black-coated cattles may therefore be more attractive to Glossina and more likely to be bitten, which increases their risk of contracting trypanosoma (Pollock, 1982).

In this study, comparatively elevated prevalence of bovine trypanosomosis was observed in the wet season compared to the dry season, although there was no statistically significant differences ($p>0.05$). Likewise, other studies which used the same parasitological technique for diagnosis, such as (Eyasu *et al*., 2021; Eticha *et al*., 2022), reported the highest prevalence of trypanosomosis during the wet season. However, the difference was significant in these studies. The largest disease occurrence in the wet season may be justified by during the wet season, there is an increase in vegetation growth, which provides more food and shelter for the Glossina and other bitingflies. The humid conditions also favor the survival and reproduction of the fly, which can lead to an increase in the population size. As the population of Glossina increases, the risk of transmission of the trypanosoma parasites they carry also increase (Delespaux, 2007; Shaw and Cecchi, 2014).

On the other hand, the insignificance in this study might be attributed to the shortage of feed and water during the dry season, which leads owners to travel their animals long distances in search of food and water to high Glossina infested areas. Consequently, the likelihood of being bitten by Glossina and other biting flies is higher during this time. As a result, the prevalence of the disease may not exhibit significant variation when compared to the wet season.

The overall mean PCV value of all studied cattles was 25.98% (±5.28). The mean PCV value was significantly higher ($t=4.88$, $p < 0.05$) in the wet season than the dry season, which is in line with reports of Ayele *et al*. (2012) and Eyasu *et al*. (2021). In this study, the mean PCV of trypanosoma infected cattles was significantly lower ($t=5.4$; $P<0.05$) than non-infected cattles. The lowered mean PCV of trypanosoma infected animals was also reported from various parts of Ethiopia (Degneh, 2019; Tsegaye *et al*., 2020; Abdeta, 2021; Efrem *et al*.,2023) and Africa, Zambia (Marcotty *et al*., 2008).

One of the common symptoms of animal trypanosomosis is anemia, which is characterized by a decrease in the number of red blood cells. This decrease in red blood cells can lead to a reduction in the PCV of the animal, which is a measure of the percentage of red blood cells in a volume of blood (Marcotty *et al*., 2008; Desquesnes *et al*., 2013; Gonzatti *et al*., 2014).

Although anemia is a common symptom of animal trypanosomosis, not all animals infected with the parasite developed anemia in this study. This may possibly explained the severity of the anemia can vary depending on several factors, including the species and breed of the animals, the strain and virulence of the parasite, the duration and intensity of the infection, and the nutritional status of the animal (Morrison *et al*., 2008).

The entomological survey conducted in this study revealed the presence of only one Glossina species known as *G. pallidipes* as well as other biting flies such as Stomoxys and Tabanus. This finding is consistent with reports from Arbaminch areas, Gamogofa Zone, Southern Ethiopia (Girma *et al*., 2014) and Loka Abaya (Abebe *et al*., 2021) and in the Rift Valley of Gamo Zone (Syoum *et al*., 2022) who reported single species *G. pallidipes*.

The overall apparent density of *G. pallidipes* was 0.99F/T/D and 2.2F/T/D for biting flies (Tabanus 0.7F/T/D and Stomoxy 1.5F/T/D). Related, findings were reported from other parts of Ethiopia. 0.75F/T/D of *G. m. submorsitans*, *G. pallidipes*, *G. fuscipes fuscipes* and *G. tachnoides* from Gambela and Abobo districts (Kedir *et al*., 2016) and 0.48 F/T/D of *G. pallidipes* as well as *G. fuscipes* from Mareka District of Dawuro Zone, Southern Ethiopia (Eshetu and Barata, 2017). This relation in the apparent density of Glossina might be due to the similarity in the spatial factors which affects the distribution and occurrence of the fly such as climate, vegetation, rain fall and land utilization (Rogers *et al*., 1996).

However, the higher overall apparent density of Glossina were reported from Gidami district (3.06F/T/D) and Sayo district (4.16F/T/D) (Degneh, 2019), Dale Wabera district (9.1F/T/D) and Dale Sadi district (9.8F/T/D) (Tsegaye *et al*., 2021) and Rift Valley of Gamo Zone, Southern Ethiopia (12.85F/T/D) (Syoum *et al*., 2022). The study showed that the seasonal apparent density of biting flies was relatively higher than that of *G.pallidipes* in the area. Moreover, the apparent fly density recorded during the wet season was higher than that of the dry season. Similarly higher seasonal apparent density of flies in the wet season than dry season was reported from West Shewa, South West Oromia (Ayele *et al*., 2012).

The reasons for the lower density of *G. pallidipes* in the dry season over the wet season could be the rise in average temperatures and lower humidity in the dry season and this has the direct impact on the breeding patterns and survival rates of fly. First, the high temperatures themselves kill a flies, particularly teneral flies (i.e teneral flies unable to survive in high temperatures). Second, the high heat waves also coincided with the lower rainfall in that season results decline in the number of mammalian hosts, so that fly population decreased by dying of flies due to the drought (Hargrove, 2003).

In general, the differences in overall as well as seasonal apparent densities and distributions of Glossina in the current study area and other parts of Ethiopia as well as Africa could be differences in Habitat, Climatic condition, Host availability, Control measures undertaken and Human activities (Leak *et al*., 1999; Simarro *et al*., 2010 and Mihret *et al*., 2015).

A higher number of female *G. Pallidipes* were caught than male and this is in accordance with the reports from the Sayo District, Oromia Regional State, Western Ethiopia (Efrem *et al*., 2018) and Kellem Wollega Zone, Oromia Regional State, Ethiopia (Degneh, 2019). This could be attributed to the longer lifespan of female compared to male Glossina (Pollock, 1982).

6. CONCLUSION AND RECOMMENDATIONS

The study confirmed the widespread presence of bovine trypanosomosis and its vectors in the specified area. The overall prevalence of bovine trypanosoma infection was found 6.3%, with a slightly higher infection during the wet season (7.5%) compared to the dry season (5%), although the difference between the two seasons was not statistically significant. The study also identified a significantly higher prevalence of trypanosoma infection in Chirecha Kebele and in black-coated cattle. Two species of trypanosoma namely; *T. congolense* and *T. vivax* were identified in the study area. During the entomological study, a total of 256 flies (79 *G. pallidipes* and 177 biting flies such as Stomoxys and Tabanus) were caught by using NGU traps. A higher number of female flies (54) compared to male flies (25) of *G. pallidipes* were caught. The overall apparent density of *G. pallidipes* was 0.99F/T/D, while the apparent density of biting flies was 2.2 F/T/D. The wet season exhibited a higher apparent density of *G. pallidipes* (1.95 F/T/D) and other biting flies (4.28 F/T/D) compared to the dry season (0.025 F/T/D and 0.15 F/T/D), respectively.

Based on the conclusions drawn from the study, the following recommendation was forwarded:

- Control measures such as use of traps, targets, deltamithrin and trypanocidal drugs should be consolidated to reduce the prevalence of trypanosoma infection, particularly in high-risk areas such as Chirecha Kebele and black coated cattles.

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8. APPENDIX

Appendix i: Data collection format

Table 1: Format for parasitological study

Region_____________ Kebele_______________ Latitude______________

Zone_______________ Village_______________ Longitude____________

District_____________ Altitude______________ Date________________

S.NO	Owner name	Animal ID or name	Sex	Age	BCS	Colour	PCV (%)	Infection	Trypanosoma spps

Table 2: Format for entomological data collection

Region_____________ Kebele_______________

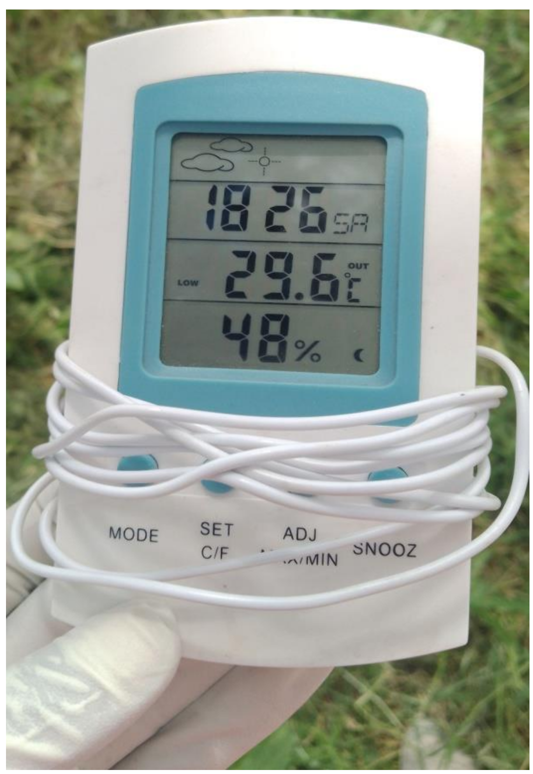
Zone_______________ Village_______________

District_____________

Trap type	Alt	Lat	Long	Deployed date	Deployed time	Collection date	Collection time	Tsetse spp	F	M	Tot	Biting fly	Tot

Appendix ii: Pictures taken during the study period

Picture 1: NGU trap deployed for entomological study



Picture 2: Thermohygrometer

Picture 3: Cattles brought for blood collection in Chirecha Kebele

Picture 4: Microscopic examination blood film