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# Prevalence and Associated Risk Factors of Bovine Trypanosomiosis in Ofa Wereda of Wolaita Zone, SNNPR, Ethiopia

**Wondimu Tessema, Kasech Dana**

School of Veterinary Medicine, Wolaita Sodo University, Wolaita Sodo, Ethiopia

**Email address:**
wonduhopful@gmail.com (W. Tessema)

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**Abstract:** Trypanosomiosis is an important parasitic protozoan disease of livestock in the area and causes severe disease which results in loss of livestock and agricultural productivity with serious socio-economic consequences. A cross-sectional study was conducted from June 2021 to November 2021 in selected kebeles in Ofa Wereda of Wolaita Zone with the objectives of determining the prevalence of trypanosomiosis and associated risk factors. Blood samples were collected from 280 selected cattle of the study villages and evaluated through standard parasitological methods. The overall prevalence was 15.00% (42/280). Trypanosoma congolense was the predominant species in the area (10.35%). Among species of trypanosomiosis, *T. conglence* and *T. vivax* were identified in 29 (10.35%) and 10 (3.57%) in examined samples respectively. Mixed infection by two species was noted in 3 (0.11%) of the samples. Meanwhile from considered epidemiological factors body condition and PCV-value were showed statistically significance difference ($P < 0.05$) with the overall prevalence of trypanosoma infections in cattle. On the other hand, trypanosoma infection among age, agro-ecology and sex showed no statistically significant difference ($P > 0.05$). In conclusion, bovine trypanosomosis is economically important disease that affects the health as well as productivity of cattle in Ofa Wereda. Hence, appropriate disease prevention and control methods should be undertaken to improve livestock production and agricultural development in the area.

**Keywords:** Associated Risk Factor, Bovine, Prevalence, Trypanosomiosis, PCV, Ofa Wereda

## 1. Introduction

### *1.1. Background*

Ethiopia economy is largely dependent on agriculture that is influenced by livestock, which provides more than 90% drought energy required for crop production. The cause of food deficiency in the country could be numerous and it is difficult to separate. However, some livestock and human disease have obvious influences on the country are self reliance in food production [36].

Trypanosomosis is a parasitic disease caused by unicellular protozoan parasites of the genus trypanosoma and family trpanosomatidae. They multiply in blood stream, lymphatic vessels and tissue, including cardiac muscle and the central nervous system [90]. Trypanosomosis is transmitted by tsetse flies (Glossina spp) are believed to be the most important infectious disease holding back development of livestock production in Africa [54].

African animal trypanosomosis (AAT), also called Nagana is the most important disease constraint to livestock and mixed crop-livestock farming in tropical Africa [23]. The distribution of the disease is parallels the distribution of tsetse flies and comprises of an area approximately 10 million $Km^2$ [78] in 37 subSaharan African countries [83]. Nagana puts 55 million cattle at risk and leads to the death of three million animals every year, inflicting a direct annual loss of USD 1.0-1.2 billion in cattle production [21, 17].

Although, most species of domestic animals are to some degree susceptible to AAT, but it is most important in cattle and small ruminants, as they are the most frequently reared animals in sub-Saharan Africa [53, 91, 75]. Many wild animal species in Africa also host one or more trypanosome species and can serve as reservoirs for both human and

domestic animals [9]. However, certain breeds of African cattle have been shown to exhibit a level of tolerance to trypanosome infection [72].

Trypanosomosis is a haemoprotozoan disease of animals and humans caused by several species of parasites of the genus Trypanosoma [18]. The most common pathogenic trypanosome species affecting cattle in Africa are T. congolense, T. vivax and to a lesser extent, T. b. brucei [87, 78]. In areas where more than one trypanosome species is present, mixed infections in domestic animals are often encountered [93, 70].

Trypanosomosis is a vector-borne disease known to be transmitted cyclically by tsetse flies [58, 103] and mechanically by a number of biting flies of genus diptera such as Tabanus, Hematopota, Chrysops and Stomoxys [32, 57]. The main tsetse-transmitted trypanosomes include T. congolense, T. vivax, T. b. brucei and T. simiae [73]. Mechanical transmission of T. congolense has been shown under experimental conditions and can therefore not be excluded from contributing to its spread in Africa [32]. In addition, T. equiperdum is transmitted sexually [51, 74]. Moreover latrogenic transmission could also occur when using the same needle or surgical instrument on more than one animal, at sufficiently short intervals, that the blood on the needle or instrument does not dry [31].

Ethiopia is believed to have the largest livestock population in Africa. An estimate indicates that the country is a home for about: 59.50 million cattle, 30.70 million sheep, 30.02 million goats, 11.01 million equines, 1.21 million camels and 56.53 million chickens [24]. However, Ethiopia is one of the countries suffering from trypanosomosis with approximately 220,000 Km² of arable land is infested with five species of tsetse flies: namely Glossina pallidipes, G. m. submorsitans, G. fuscipes, G. tachinoides and G. longipennis [77]. According to [60], the prevalence of bovine trypanosomosis in Ethiopia range from 1.38 to 17.15%. The most important trypanosome species affecting livestock in Ethiopia are T. congolense, T. vivax and T. brucei in cattle, sheep and goats, T. evansi in camels and T. equiperdum in horses [45, 51]. In Ethiopia, the direct loss (mortality) due to trypanosomosis is estimated to amount 1.5 to 2 billion Birr per year [38].

### *1.2. Statement of Problem*

In Wolaita zone as a genearal and Ofa Wereda particular, 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. To overcome the problem and to assess the epidemiology of trypanosomiosis, many researches are conducted previously in Wolaita zone and souroundings by many researchers. Some of them are [4, 94, 49, 10] and they found the causative factors of trypanosomiosis. So to know the current status of bovine trypanosomiosis in the area, the current study needed to conduct and to address epidemiology of trypanosomiosis.

### *1.3. Objectives*

#### *1.3.1. General Objectives*

A general objective of this study was to determine the overall prevalence of trypanosomiosis and its associated risk factors in cattle in study area.

#### *1.3.2. Specific Objectives*

1) To estimate the prevalence of trypanosomiosis in study area.
2) To investigate associated risk factors that predisposes the occurrences of trypanosomiosis.
3) To identify species of trypanosoma from infected animals.

### *1.4. Research Questions*

Trypanosomiosis raises several questions due to its etiological causes and associated risk factors. In this study trypanosomiosis has the following questions that should be solved accordingly after the relevant research conducted.

1) What is the overall prevalence of trypanosomiosis in the cattle in the study area?
2) What are the major risk factors associated with the disease in the study area?
3) What are the major species of trypanosomiosis that causes infection in cattle in the study area?

### *1.5. Significance of Study/Benefit and Beneficiary/*

Bovine trypanosomiosis exerts a great negative impact on the socio economic aspect especially for those of farmers' and private organizations whose income rely on ruminant production. So at the end, this research will identify the cause of the trypanosomiosis infection and associated risk factors. Subsequently the study will benefit farmers and small ruminant private organization by obtaining information pertinent to trypanosomiosis infection. The finding might also help to the researchers and as a baseline data for further researches activities. The study will generate data for policy makers, governmental and non-governmental organization to undertake and develop different prevention and control strategies.

### *1.6. Scope of Study/Delimitation/*

The current study will play a great role in estimating the prevalence of bovine trypanosomiosis, identification of species and its associated risk factors in the study area. Generally, this research needs a wider agro-ecology, a seasonal variation, a large study population, species and breeds diversification to identify exact root cause and associated factors to the societies and the government as a whole.

But some of this aim will not achieve due to many reason. Some of them are financial shortage, a non-suitable agro-ecology for transportation and short study period.

# 2. Literature Review

### *2.1. Trypanosomiosis*

Bovine Trypanosomosis (Nagana) is a disease complex caused by several species of unicellular protozoan parasites of the phylum Sarcomastigophora, order Kinetoplastida, family Trypanosomatidae and genus *Trypanosoma*. 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 [33].

### *2.2. Ethiology*

Trypanosomes are flagellated protozoan parasites that live in the blood and other body fluids of vertebrate hosts. 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*., 2004). In Ethiopia there are three important species of trypanosomes are recorded in cattle. These are *T. congolense*, *T. vivax* and *T. brucei*. *T. vivax* and *T. congolense* are the main pathogens of cattle. Trypanosomosis outside "tsetse belt" is caused by mechanically biting flies; the main etiological agent of mechanically transmitted trypanosomosis is *T. vivax* [33].

### *2.3. Morphology*

The different trypanosome species differ in morphological characteristics as described by [65]. Trypanosoma congolense is smaller in size, usually without free-flagellum, but has marginally located medium sized kinetoplast [84]. It is divided into four subtypes, with different distributions and pathogenicity: savannah type, forest type, Tsavo type, and Kilifi type [63]. Trypanosoma congolense savannah type is the most pathogenic of the four and is capable of causing severe anaemia and even death of infected cattle [12]. Other *T. congolense* types cause mild disease that in certain instances does self-cure.

Trypanosoma vivax is a monomorphic parasite with distinct free flagellum and larger and terminal kinetoplast. It shows variable levels of virulence and distinct pathogenicity in West African isolates, causing an acute disease in cattle often accompanied by weight loss, reduced milk yield, abortions and mortality, whereas the East African isolates largely cause chronic infection [42]. In East Africa, there are two types of *T. vivax* isolates: the haemorrhagic T. vivax that causes an acute haemorrhagic syndrome and the mild strain [13, 62]. Cattle infected with the haemorrhagic *T. vivax* produce auto-antibodies to red blood cells, a phenomenon that is not observed in the nonhaemorrhagic *T. vivax* [13].

Parasites in Trypanosoma brucei group show pleomorphism 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* [65].

### *2.4. Life Cycle*

The life cycle of trypanosome in tsetse involves cyclical development for varying length of time, depending on species and ambient temperatures. Most tsetse-transmission 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 hypo pharynx 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 one week with *T. vivax* or extend to a few weeks for *T. brucei* species [35].

### *2.5. Epidemiology*

The epidemiology of trypanosomosis is highly dependent on the parasite, vector and host factors. Trypanosome species occur in a variety of genotypes with different strains, virulence, immunogenicity and response to chemotherapeutic agent. Since the parasite infects a wide range of animals including wild animals which constitute the reservoirs of the disease, the epidemiology of trypanosomosis is extremely complex. The degree of risk to which domestic animals are exposed to the trypanosomosis depends on the species and density of tsetse present, infection rate in tsetse, species and strain of trypanosomes, source of infection (wild or domestic animals) and feeding preference of the flies [102].

### *2.6. Pathogenesis*

Pathogenesis of trypanosomosis in most species is a progressive, but not always fatal disease and the main features are anemia, tissue damage and immunosuppression. Metacyclic trypanosomes are inoculated intradermally as the fly feeds. They multiply at this site provoking a local skin reaction (Chancre), which is most pronounced in a fully susceptible host and may be slight or absent with some strains or species of trypanosomes. Within the chancre, metacyclic parasites change to trypomastigote form, enter the bloodstream directly or through the lymphatics and initiate

characteristic intermittent parasitemias. Their behavior thereafter depends largely on the species of trypanosome transmitted and the host [55]. *T. vivax* usually multiplies rapidly in the blood of cattle 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. Both species exert their effect mainly by causing severe anemia and mild to moderate organ damage. The anemia has a complex pathogenesis involving mainly increased erythrophagocytosis, some hemolysis and dyshemopoiesis. Very acute infection with *T. vivax* in cattle causes parasitemias and disseminated intravascular coagulation (DIC) with hemorrhages. *T. brucei* and rarely *T. vivax* have the capability of escaping from capillaries into interstitial tissues and serous cavities where they continue to multiply. The cerebrospinal fluid is often invaded by *T. brucei* alone or mixed with other species or as a relapse after an apparently successful treatment. Animals chronically infected with any pathogenic trypanosome may develop concurrent and even fatal bacterial, viral and other protozoan infections as a result of immunosuppressant [81].

### *2.7. Clinical Signs*

There are no pathgnomonic signs that would help in pinpointing a diagnosis. The general clinical sign are there but variations determined by the level of tsetse challenge, the species and strain of the trypanosome, the breed and management of the host. Acute episodes last for a few days to a few weeks from which the animal dies or lapses in to a sub-acute to chronic stage, or the illness may be chronic from the beginning. Chronic cases may run a steady course, may be interrupted by periodic incidents of severe illness, or undergo spontaneous recovery. 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, anorexia 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, 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 [16].

### *2.8. 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. Besides clinical diagnosis, parasitological, serological and molecular methods with varying degrees of sensitivity and specificity are available for the diagnosis of trypanosomosis [25].

#### *2.8.1. Clinical Diagnosis*

Severity of disease varies with species and age of the animal infected and the species of trypanosome involved. *T. Congoles* and *T. vivax* are highly pathogenic for cattle and *T. brucei* infections are generally regarded as being low pathogenicity. The primary clinical signs are intermittent fever, anaemia and weight loss. Cattle usually have a chronic course with high mortality, especially if there is poor nutrition or other stress factors [82].

Clinical diagnosis is found to have a good sensitivity (78%) but a low specificity (27%) when compared to parasitological tests. Under field conditions, in the absence of simple and portable diagnostic tools or access to laboratory facilities, veterinarians could rely on clinical sings and direct parasitological diagnosis to screen and treat cases of bovine trypanosomosis presented by farmers [90].

#### *2.8.2. Parasitological Diagnosis*

These are made by placing a drop of blood on a microscope slide and covering with a cover slip. The blood is examined microscopically using an x40 objective lens. Approximately 50-100 fields are examined. Trypanosomes can be recognized by their movement among the RBC. The method is simple, inexpensive and gives immediate results. Depending on the trypanosome size and movements, a presumptive diagnosis can be made of the trypanosome species [104].

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 [76].

Thick Blood Smear Technique is simple and relatively inexpensive, but results are delayed because of the staining process. Trypanosomes are easily recognized by their general morphology, but may be damaged during the staining process. This may make it difficult to identify the species (Taylor, 1998). Usually, both a thin and thick smear is made from the same sample. Thick smears contain more blood than thin smears and, hence, have a higher diagnostic sensitivity. Thin smears on the other hand allow trypanosome species identification [48].

### *2.9. Treatment*

Trypanosomosis can be treated with trypanocidal drug for therapeutic and prophylactic purpose. A therapeutic drug includes diminazene aceturate, homidium bromide and homidium chloride. Prophylactic drugs for cattle include homidium (bromide, chloride) and isomethamidium [5].

### *2.10. Control and Prevention*

The control of trypanosomosis in enzootic countries involves control of tsetse fly population, prophylactic treatment and good husbandry of animals at risk and use of trypanotolerant animals. Control of tsetse has been successfully attempted, but reinvasion is frequent if the land is not properly utilized. More recent methods involved the use of insecticides applied strategically in the form of ground and aerial spraying over large expanses of land [46].

As tsetse is sensitive to insecticides and no resistance has developed, considerable successes were achieved in some countries. However, spraying insecticides is costly and harmful to the environment. Other effective methods involve targets impregnated with insecticides and traps that attract and catch tsetse. These are simple and cheap and can be constructed and maintained by local communities. Furthermore, they do not pollute the environment and are suitable for both small and large scale farming [15].

Another method is the sterile insect technique. Since the female tsetse only mates once in a life time, this technique is theoretically able to eradicate a targeted tsetse species in areas where other methods have been used to reduce its density but it is expensive. Attempts at trypanosomosis control have also been directed to prophylactic dosing with chemicals such as homidium (bromide, chloride) and isomethamidium. 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 [37]. The productivity response to this pattern of treatment is good if general husbandry is also adequate. The downside of this approach is that it has reportedly led to drug resistance in many countries. In the absence of a vaccine, control methods must combine reduced exposure to the vectors (large scale tsetse trapping and pour-on applications) with strategic treatment of exposed animals (chemotherapy Chemoprophylaxis) along with use of trypanotolerant animals when feasible [81].

### *2.11. Trypanosomosis in Ethiopia*

In Ethiopia, trypanosomosis is widespread in domestic livestock in ve heart failure or intercurrent bacterial infections that frequently take advantage of the weakened immune system [19].

## 3. Materials and Methods

### *3.1. Description of Study Area*

The study was conducted in Ofa Wereda of Wolaita zone, SNNPRS, Ethiopia. Ofa Wereda is located at a distance of 358 km south of Addis Ababa between the coordinates of 6° 3' 0"-6° 48' 0"N Latitude and 37° 26' 0" - 37° 37' 0" E Longitude and (Figure 1). The altitude of Ofa Wereda is 1500 to 2900 m.a.s.l and the average temperature varies between 17.6°C to 22.5°C and average annual rainfall of 900mm. The Weredas has 24% Kola, 60% Weinedega and 16% Dega and the main crops grows in the Wereda are Teef, maize, H/bean, sweet potato, cassava, check pea, rish potato, wheat sorqhum, pea bean, barely and enset. Ofa Wereda has a livestock population of 135,864 cattle, 73,391 sheep, 8,488 goats, 182,241 poultry, 16,128 donkeys, 1,543 hoarse and 1,103 mules respectively [105].

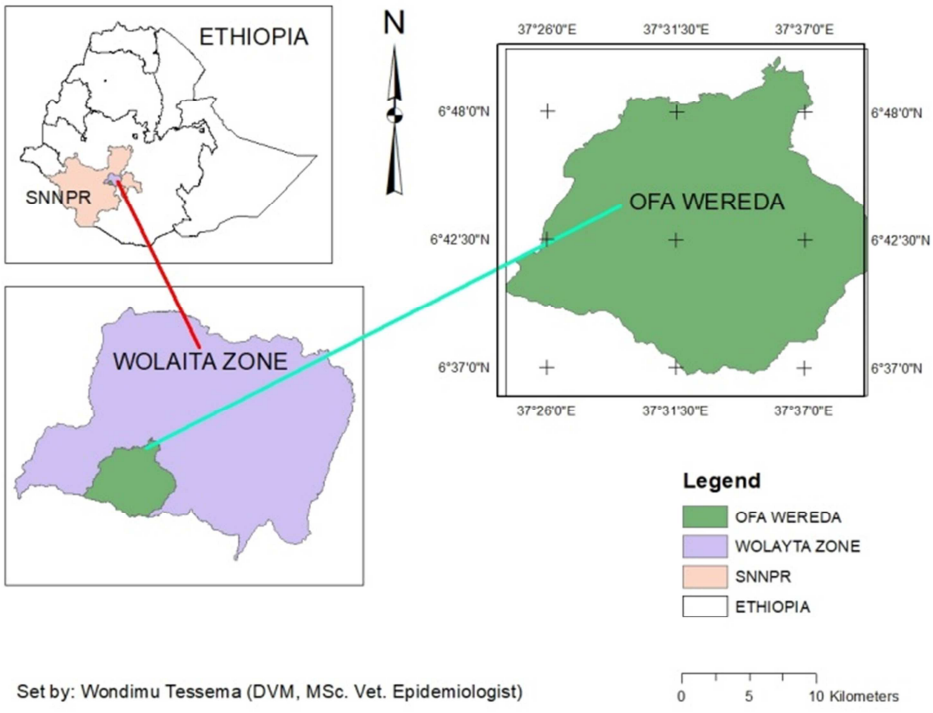


***Figure 1.*** *Study Area.*

### *3.2. Study Population*

The study animals were local breed of cattle which selected from study area that managed in extensive management system and consisting of different age, sex and body condition and will be selected from 6 purposely selected rural kebeles. During sampling, age, sex, species, agro-ecology and body conditions of the animals will be recorded. According to [43, 92] the body condition scoring of animal classified as poor, medium and good and the age of animals will estimated through dentition and categorized as young (< or = to one years) and adult (> than one years).

### *3.3. Study Design*

Cross-sectional study design was carried out from June 2021 to November 2021 to estimate the prevalence and associated risk factors of bovine trypanosomiosis.

### *3.4. Sampling Technique*

Sampling techniques was used in this study are purposive sampling and simple random sampling technique. In purposive sampling technique, Wereda and study kebeles namely Okoto, Tida, Busha, Galako, Galda and Mancha are selected based on transport access and animal population numbers. In simple random sampling technique animals will sample from selected kebeles.

### *3.5. Sample Size Determination*

For estimation of the disease prevalence, the sample size was determine by assuming the expected average prevalence to be 10.09%, the statistical confidence level 95%, while the desired precision taken is 5%. According to [100] formula the sample size calculated as the following.

$$n = \frac{Z^2 Pexp.(1-Pexp)}{d^2}$$

Where: n = required sample size; Pexp = expected prevalence; d = desired absolute precision (5%). Since there was published data previously in Wolaita zone in different Weredas by [4, 94, 49, 10] who reported 6.1%, 13.4%, 6.67% and 14.2% of prevalence of trypanosomiosis respectively. So, the expected average prevalence for this study was (10.09%). According to the above formula, the calculated sample size was 140. To increase precision and also to be representative, sample size was inflated 2 times. Therefore, the total sample size required for this study was 280. Due to difference in cattle population in six selected kebeles, the sample size allocated proportionally based on number of cattle population.

### *3.6. Parasitological Data Collection*

#### *3.6.1. Packed Cell Volume (PVC) Determination*

After well straining of animal in the field, blood will collected from an ear vein into heparineized micro-haematocrit capillary tubes. Each capillary tube will filled to its last third and sealed with crystal seal at one end and centrifuge immediately in a micro heamatocrite centrifuge for five minute at 1500rpm. After centrifugation, the capillary tubes are placed in a haematocrit reader. The length of the packed red blood cells column is expressed as a percentage of the total volume of blood. Then the packed cell volume (PCV) will determined. Animal with PCV less than or equal to 24% are considered to be anemic [81].

#### *3.6.2. Buffy Coat Technique*

In Buffy Coat technique, blood will collected from an ear vein using heparinized micro-haematocrit capillary tube and the tube will sealed. A heparinized capillary tube containing blood will centrifuged for 5 minutes at 12, 000 rpm. After centrifugation, tryponosomes are usually found in or just above the buffy coat layer. The capillary tube will cut using a diamond tipped pen 1mm below the buffy coat to include the upper most layers of the red blood cells and 3mm above to include the plasma. The content of the capillary tube will expressed onto a slide and covered with cover slip. The slide will examined under x40 objective and x10 eye piece for movement of parasite. Trypanosome species are identified according to their morphological descriptions as well as movement in wet film preparations provided by OIE [80].

#### *3.6.3. Thin Blood Smear*

A small drop of blood from a micro haematocrit capillary tube will applied to a clean slide and spread by using another clean slide at an angle of 45°. The smear was dried by moving it in the air and fixed for 2 minutes in methyl alcohol. The thin smear will flood with Giemsa stain (1:10 solution) for 30 minutes. Excess stain will drain and washed by using distilled water. Then it will allow drying and examined under the microscope (x100) oil immersion objective lens [80].

All parasitological data are collected and conducted in Ofa Wereda Veterinary clinic and materials that required for this research are used from the Ofa Wereda Veterinary clinic and remaining materials like centrifuge, hematocrite capillary tube and hematocrite reader are temporarly taken from Sodo Regional Veterinary Laboratory.

### *3.7. Data Management and Analysis*

The well organized data and result of parasitological examination will be entered and managed in MS Excel work sheet and analyzed by using STATA version 14.2. The prevalence will summarized by using descriptive statistics and association of bovine trypanosomiosis prevalence with different potential risk factors such as age, sex, species, agro-ecology and body condition score will analyzed by logistic regression. A p value of 5% is used as cut off for statistical significance at 95% confidence interval.

## 4. Result and Discussion

### *4.1. Overall Prevalence*

In the present study, total of 280 cattle which belongs to

different age groups, sexes and agro-ecology that were managed under extensive management system were examined for the presence or absence of trypanosome in their blood by using the buff coat method. Out of 280 examined cattle, 42 were positive for trypanosomosis and the overall prevalence was 15.00% (42/280) (Table 1).

***Table 1.*** *Prevalence of trypanosomiosis and associated risk factors.*

| Risk factors | No of examined | No positive | Prevalence | |
|---|---|---|---|---|
| Age | Young | 59 | 9 | 12.25% |
| | Adult | 221 | 33 | 14.93% |
| Agro-ecology | Highland | 50 | 8 | 16.00% |
| | Midland | 95 | 10 | 10.53% |
| | Lowland | 135 | 24 | 17.78% |
| Body condition | Good | 187 | 21 | 11.23% |
| | Medium | 33 | 4 | 12.12% |
| | Poor | 60 | 17 | 28.33% |
| Sex | Female | 77 | 7 | 9.09% |
| | Male | 203 | 35 | 17.24% |
| PCV | <24 | 149 | 29 | 19.46% |
| | ≥24 | 131 | 13 | 9.92% |
| | Total | 280 | 42 | 15.00% |

Overall prevalence in the present study was in agreement with the results of [39, 29, 44] which found an overall prevalence of 14.2% in Humbo distrct, 14.1% in Gidami distrct, 15.57% in Eastern Wollega, and 12.28% in Dale Wabera distrct respectively. On the other hand, this finding was significantly lower than the reports of [106, 11, 2, 7] who reported 27.5%, 26.3%, 21.33%, and 21.0% prevalence of bovine trypanosomosis respectively at Wozeka, Nyangatom woreda, Konta special distrct, and Omo river basin of South Western Ethiopia. The reason for this difference may be associated with the decrease in the degree of tsetse infestation due to control measure taken. Additionally, this may be due to the continuous control effort being under taken which gradually decreasing the prevalence of the disease. Widespread use of trypanocidal drugs in the area might have also contributed to the low prevalence. In addition season of the study might have contributed to the finding of low prevalence. It is also possible that the continuous human settlement might have led to destruction of bush lands and forest with resultant destruction of the normal ecology of tsetse flies and there by decrease the vector density and hence infection level.

In the current study, regarding with age, the prevalence of trypanosome was slightly higher in adult cattle (14.93%) than young cattle (12.25%). Previous reports by [95, 88, 99] also revealed higher prevalence in older than young animals. It could possibly be attributed to the restriction of grazing in the young animals to a nearby homestead [6]. In addition, young animals are protected to some extent by maternal antibodies [41]. According to the [101] tsetse flies are attracted significantly more by odor of older animals. Other reports showed that T. congolense is a chronic disease increasing with age of animals and its infection is usually higher in adult animals than in young animals [66].

In the case of agro-ecology, Prevalence of trypanosomiosis infection was higher in lowland (17.78%) than highland (16.00%) and midland (10.53%). The result obtained here is in agreement with that of [64, 67] Higher prevalence in lowland areas might be attributed to the fact that animals in lowland areas are more challenged by vectors than animals in mid-altitudes. It is assumed that among many factors that contribute to the distribution of tsetse flies and tsetse transmitted trypanosomosis: altitude with temperature is one of the major biotic factors.

In the cases of body condition, the prevalence of trypanosomiosis infection was higher (28.33%) in animals of poor body condition than those of medium (12.12%) and good (11.23%). This finding agrees with previous reports [69, 39] 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.

Regarding with sex, the current finding showed that higher prevalence of trypanosomiosis infection was observed in male (17.24%) than female (9.09%). This result was in line with the reports by [62, 27, 97]. This might be due to the fact that both sexes have virtually similar exposure to biting flies in grazing areas. The slightly higher prevalence in males as compared to females may be attributed to stress factors related to work, where male animals are used for drought purpose [106].

In the current study, prevalence of trypanosomiosis was high in those animals that have PCV-value <24 (19.46%) than PCV-value ≥ 24 (9.92%). The low PCV value is an indicator of the anaemia which might be the infection of trypanosomiosis. Animal trypanosomoses is known to cause significant anaemia in infected animals [20, 26, 89, 30]. Anaemia appears with progressing parasitaemia and there is lysis of large numbers of red blood cells resulting in a drop in PCV%, haemoglobin and RBC counts [85] which may result from massive erythrophagocytosis by an expanded and active mononuclear phagocytic system of the host.

### *4.2. Identified Trypanosomosis Species*

The major species of trypanososme were morphologically identified by using thin blood smear in the study area were trypanososma congelence, trypanosome vivax and also mixed infection (trypanosome vivax and trypanosome bruei) were also observed (Table 2).

***Table 2.*** *Prevalence of identified species of trypanosomosis.*

| Species of trypanosomiosis | No. of examined | No. of infected | Prevalence |
|---|---|---|---|
| *T. congelence* | 280 | 29 | 10.35% |
| *T. vivax* | 280 | 10 | 3.57% |
| *T. vivax* and *T. brucei* | 280 | 3 | 0.11% |
| Total | 280 | 42 | 15.00% |

T. c = trypanosome congelence, T. v =trypanosome vivax, T. b= trypanosome brucei.

With regard to species of parasites, it was observed that *trypanosome congelence* was the predominant species in the

area followed that *trypanosome vivax*. This finding suported by [26, 40, 14, 34]. In several studies conducted in sub-Saharan Africa, *T. congolense* has been found to be the most prevalent trypanosome species in cattle [8, 71, 86]. The high ratio of *T. congolense* may be ascribed to the more efficient transmission of this species by tsetse flies than *T. vivax* in tsetse infested areas [66,]. [59] Indicated that *T. vivax* was highly susceptible to treatment while the problems of drug resistance were higher in *T. congolense*.

### *4.3. Univariable Logistic Regression Analysis of Associated Risk Factors*

Univariable logistic regression analysis was used to determine single predictors having crude significance levels at<0.25 that were a prior interest for further multivariable logistic regression analysis by using backward step wise selection method (Table 3) below.

***Table 3.*** *Summary of Univariable logistic regression analysis of associated risk factors.*

| Risk factors | Crude OR | P-value | 95% CI |
|---|---|---|---|
| Age | | | |
| Young | Ref | | |
| Adult | 0.9751773 | 0.951 | 0.4380388 - 2.170974 |
| Agro-ecology | | | |
| Highland | Ref | | |
| Midland | 0.6176474 | 0.345 | 0.2271079 - 1.679767 |
| Lowland | 1.135135 | 0.777 | 0.4730085 -2.72412 |
| Body condition | | | |
| Good | Ref | | |
| Medium | 1.090312 | 0.882 | 0.3488101 - 3.408101 |
| Poor | 3.125138 | 0.002 | 1.518036 -6.433635 |
| Sex | | | |
| Female | Ref | | |
| Male | 2.083333 | 0.094 | 0.8832929 - 4.913744 |
| PCV | | | |
| <24 | Ref | | |
| ≥24 | 0.4558738 | 0.028 | 0.2259724 - 0.9196739 |

Ref = reference category.

### *4.4. Multivariable Logistic Regression Analysis of Associated Risk Factors*

During multivariable logistic regression analysis, backward stepwise regression analysis was used to identify those predictors that predispose the occurrence of trypanosomiosis infection in the cattle this study (Table 4). During backward step wise regression analysis, in the first step those predictors were identified having crude significance level at<0.25 in univariable logistic regression analysis. Then in the second step multiple predictor models were fit by using step one. Finally in the third step multivariable model was fit that containing predictors with adjusted significance levels at<0.10 from step two.

In the current study, the analysis of infection of trypanosomiosis with body conditions of cattle showed that there was significant difference among poor, medium and good body conditioned cattle ($P$<0.05). It was observed that the odd of infection of trypanosomiosis in those cattle have poor body conditioned was 2.914 times higher than that of good body conditioned cattle where as those cattle with medium body conditioned were 8.26% more likely infected by trypanosomiosis than good body conditioned cattle. This finding was similar with [3, 4, 28, 47, 49, 50] Abiy (2002). The possible explanation of this result is the disease itself results in progressive emaciation of the infected animals; nevertheless, non-infected animals under good body condition have well developed immune status that can respond to any foreign protein better than those of non-infected cattle with poor body condition score which can be immune compromised due to other diseases or malnutrition and concurrent infections depress the immune responsiveness in the same cases [22].

Regarding with PCV- value, the occurrence of trypanosomiosis infection were shown a significance difference ($P$<0.05) in those cattle that have low PCV- value (PCV<24). It was observed that the odds of infection of trypanosomiosis was 50.3% less likely in those cattle that have PCV-value ≥24 compare with those cattle PCV-value <24. The current finding is in agreement with many different research reports. The PCV value of parasitemic animals is found to be significantly lower than that of aparasitemic animals and this result is similar to the results obtained by [29, 52, 68, 107]. This could be attributed to the fact that trypanosomosis predisposes infected animals to other concurrent infection due to immune suppression [81] Which in turn could have caused a lower mean PCV in infected animals compared to non-infected ones.

***Table 4.*** *Multivariable logistic regression analysis of associated risk factors.*

| Risk factors | Adjusted OR | P-value | 95% CI |
|---|---|---|---|
| Body condition | | | |
| Good | Ref | | |
| Medium | 1.08216 | 0.893 | 0.3438175 - 3.406078 |
| Poor | 2.914854 | 0.004 | 1.404668 - 6.048669 |
| Sex | | | |
| Female | Ref | | |
| Male | 1.96895 | 0.134 | 0.8120897 - 4.773815 |
| PCV | | | |
| <24 | Ref | | |
| ≥24 | 0.4974729 | 0.055 | .2436248 - 1.015822 |

Ref= reference category.

## 5. Conclusion and Recommendations

The results of current study revealed that trypanosomosis is one of the constraints to animal production at the study area. Bovine trypanosomosis is one of the major impediments to livestock development and agricultural production in study area contributing negatively to the overall development in general and to food security in particular. The most prevalent species in the study area was T. congolense followed by T. vivax and and also mixed infection of T. vavix and T. brucei was also observed. Cattle with poor body conditioned were highly infected by trypanosomiosis than medium and good body conditioned cattle. The PCV values <24 was indicated that cattle those were anaemic and infected by trypanosomiosis. Based on the above conclusion, the following

recommendations are forwarded:

1) Proper and strict follow up trypanocidal drug utilization.
2) Awareness creation should be given to the farmers about risks of drug resistance.
3) Attempt should be made to expand government and private veterinary services to serve the community properly.
4) Suitable community-based tsetse and trypanosomosis control program should be designed and implemented.
5) Further studies on the epidemiological aspects and development of drug resistance in pathogenic trypanosomes are required.

## List of Abbreviation

| | |
|---|---|
| AAT | African Animal Trypanosomiosis |
| BCS | Body condition score |
| CSA | Central Statistical Agency |
| DIC | Disseminated Intravascular Coagulation |
| FAO | Food and Agricultural Organization |
| NTTICC | National Tsetse- Trypanosomosis Investigation and Control |
| RBCs | Red Blood Cells |
| SNNPR | South Nation Nationalities and People Region |
| USD | United Stated Dollar |

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## Appendix

***Appendix 1. Methods of Detection of Bovine Trypanosomiosis***

Thin blood smear

Procedure:-

Take a drop of blood on a grease free slide.

Spread the blood on the slide using a cover slip or a clean slide at an angle of 45°C.

Dry it quickly and fix with methyl alcohol for 2 minutes.

Stain with giemsa diluted 1:10 in neutral phosphate buffer for 30 minutes.

Wash with phosphate buffer at ph 6.8-7.2.

Allow it to dry by standing upright on the rack.

Examine under the microscope (x100).

Buff coat technique.

Procedure:-

Fill heparinzed or citrate tube with blood from the animal to be examine.

Centrifuge the sample using haematocrit centrifuge.

Transfer the capillary tube onto a slide.

Use a small adhesive tape to attach the tube onto a slide.

Examine the buff coat in the capillary tube under the microscope. (the buff coat is the grayish narrow space found between the plasma and the red blood cells in the capillary tube). The motile organism such as the trypanosomes is seen flickering at this junction.

Cut the capillary tube at the junction between the buff coat and the red blood cells.

Blow the capillary tube containing the plasma, the buff coat and some red blood cells on a clean slide.

Make a smear of this content and stain with giemsa to identify the organism.

***Appendix 2. Materials Used***

Grease free slide

Lancet

Cotton wool
A pair of scissor or sharp blade
Slide box
Microscope
Haematocrit reader
Haematocrit capillary tube
Generator
Centrifuge
Rack

***Appendix 3. Chemicals and Regents Used***

Alcohol
Methanol
Immersion oil
Xylene

***Appendix 1. Sample Collection Format***

| Ser. No. | Owner's name | Zone | Wereda | Kebele | Species | Breed | Age | Sex | Body condition | Agro-ecology | Remark |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | | | | | | | | | | |
| 2 | | | | | | | | | | | |
| 3 | | | | | | | | | | | |
| 4 | | | | | | | | | | | |
| 5 | | | | | | | | | | | |

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