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# Study on Bovine Trypanosomiasis and Associated Risk Factors in Benatsemay District, Southern Ethiopia

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**ABSTRACT:** Trypanosomosis is an endemic livestock disease in Ethiopia that hinders livestock production and productivity, especially in fertile agricultural western and southwestern areas. A cross-sectional questionnaire-based and parasitological studies were conducted from October 2020 to July 2021 in the Benatsemay district, southern Ethiopia to assess the knowledge of livestock owners about trypanosomosis, its prevalence, and host-related risk factors associated with bovine trypanosomosis in the area. According to the questionnaire survey, trypanosomosis was the main bottleneck to cattle in two of the selected study Sites in the Benatsemay district. The parasitological survey revealed that 11.46% (44/384) of the cattle were infected with trypanosomosis. Moreover, *Trypanosoma congolense* (9.11%) is the leading trypanosome species in the area, followed by *T. vivax* (31.8%). The adult age group (16.15%), poor-conditioned cattle (22.22%), and black-skinned cattle (34.24%) were significantly associated ( $P < .05$ ) with trypanosomosis infection in the study area. Furthermore, the mean packed cell volume (PCV) of parasitaemic cattle (22.75%) was significantly ( $P < .05$ ) lower than that of aparasitaemic cattle (29.23%). Therefore, the present study revealed that the prevalence of bovine trypanosomosis in the study area and participatory vector control and the rational use of trypanocidal drugs should be implemented to control trypanosomosis in the area.

**KEYWORDS:** Cattle, risk factors, *Trypanosoma congolense*, *Trypanosoma vivax*

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## Introduction

In Ethiopia, trypanosomosis is one of the major impediments to livestock development, and agricultural production contributes negatively to the overall development of agriculture, in general, and food self-reliance efforts of the nation in particular.<sup>1</sup> While tsetse-borne trypanosomosis excludes some 180 000 to 200 000 km of agriculturally suitable land to the west and southwest of the country, 14 million heads of cattle, an equivalent number of small ruminants, nearly 7 million equines, and 1.8 million camels are at risk of contracting trypanosomosis at any 1 time.<sup>2</sup>

Trypanosomosis is a complex disease caused by unicellular flagellate protozoan parasites (trypanosomes) found in the blood and other tissues of vertebrates, including cattle and humans.<sup>3–5</sup> It is mainly transmitted by *Glossina* species which are responsible for cyclical transmission,<sup>6,7</sup> whereas other biting flies such as *Tabanus*, *Hematopota*, and *Stomoxys* in domestic and wild animals were responsible for mechanical transmission of trypanosomosis.<sup>7,8</sup> In Ethiopia, *Trypanosoma congolense*, *T. vivax*, and *T. brucei* in cattle (Nagana), sheep, and goats, *T. evansi* in camels (surra), and *T. equiperdium* in horses (dourine) are the most important trypanosome species.<sup>9–12</sup>

Trypanosomosis is a progressive and not always fatal disease, and its main features are anemia, tissue damage, and immunosuppression.<sup>13,14</sup> Furthermore, fever and loss of appetite occur intermittently during parasitaemic peaks, the latter becoming marked in the terminal stages of the disease. Typically, the disease is chronic, extends over several months, and usually terminates fatally if untreated. The effect of trypanosomiasis is not

only its direct losses resulting from mortality, morbidity, infertility of infected animals, and costs of controlling the diseases but also its indirect losses, which include exclusion of livestock and animal based crop production from huge fertile tsetse infested areas.<sup>8,15</sup>

In pastoral and agro-pastoral (PAP) regions, animal trypanosomosis is a serious constraint to cow production, posing a threat to household food security and livelihoods. The disease costs the cattle sector a lot of money since it causes mortality, milk and weight loss, and significant control and treatment costs.<sup>16,17</sup> Bovine trypanosomosis is highly abundant and widespread throughout Ethiopia's most agricultural and fertile areas, particularly in the lowlands and major river basins of the Abay, Omo, Akabo, Didessa, Ghibe, and Baro.<sup>18,19</sup> The disease has been documented in several locations of the country, with prevalence rates ranging from 1.38% to 17.15% whereas only a few published studies have been found in Afar and Tigray.<sup>20</sup>

Currently, 5 *Glossina* species, namely *Glossina pallidipes*, *Glossina morsitans submorsitans*, *Glossina fuscipes*, *Glossina tachinoides*, and *Glossina longipennis*, infest around 220 000 km<sup>2</sup> of the country's above-mentioned regions. *Trypanosoma congolense*, *Trypanosoma vivax*, and *Trypanosoma brucei* are the most regularly reported and important *Trypanosoma* species infecting cattle in the country.<sup>21,22</sup> Wet mount, buffy coat technique (BCT), and the polymerase chain reaction (PCR) technique are some of the diagnostic procedures available to test trypanosomosis. The PCR test is more sensitive than classical parasitological methods. The use of PCR results in the proper amplification of products from the disease agent of



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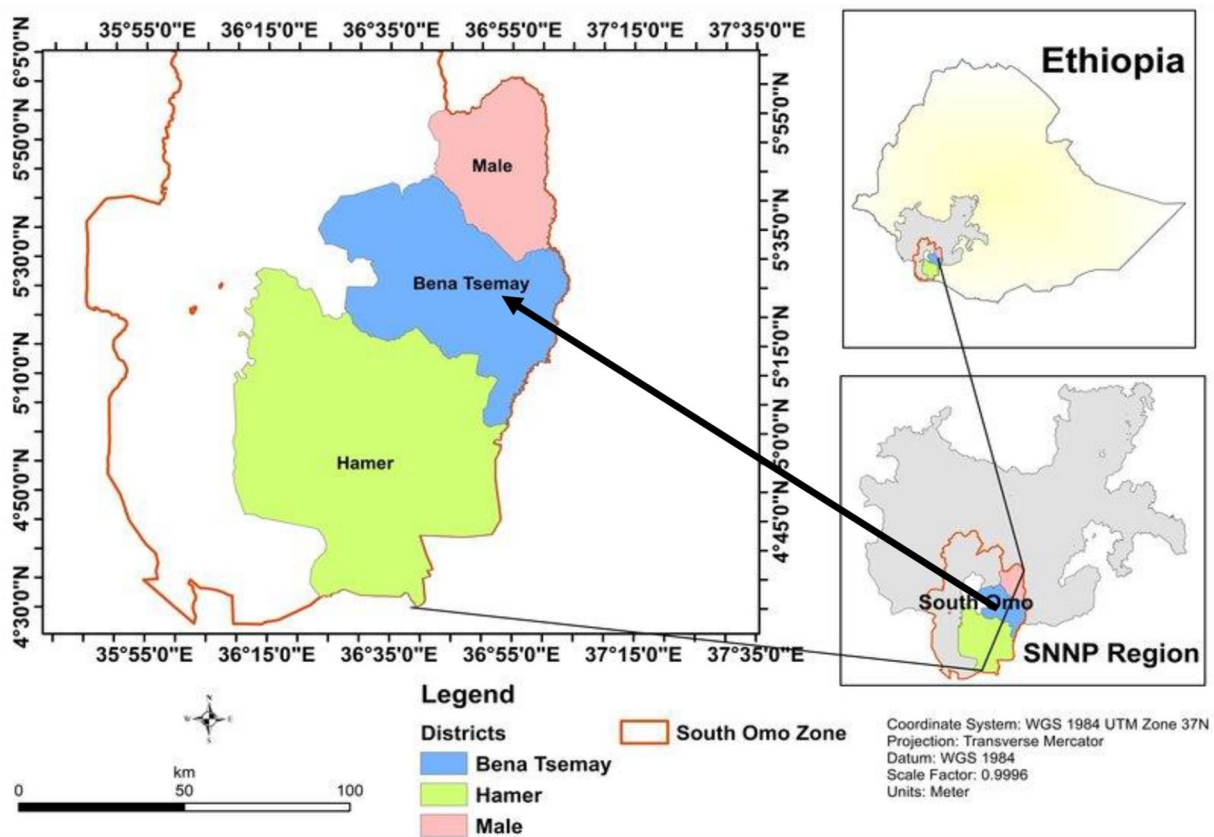


Figure 1. Map of Benatsemay district.

interest, which can inform treatment options that can be carried out as soon as possible in the field, allowing for better control programs.<sup>23</sup>

Despite intensive research on trypanosomosis, there is no effective vaccine against it, and unlikely to appear shortly because of the ability of trypanosomiasis to readily change its glycoprotein surface coat through a process called antigenic variation.<sup>6</sup> This striking nature of continuously escaping the immune system of the host results in exhaustion and suppression of the immune system to fight the disease. Due to this scenario, trypanosomosis is currently controlled by using a trypanotolerant host, vector control, and trypanocidal drugs or a combination of the above 3 methods. However, in poor rural communities, which are mostly affected by the disease, control mainly relies on the use of trypanocidal drugs. The main drugs used by livestock keepers are Isometamidium chloride (ISM), diminazine aceturate (DA), Homidium salts, and ethidium bromide.<sup>24,25</sup>

Despite the presence of a huge livestock population in the Benatsemay district, the production and productivity, as well as economic yield from the livestock sector, are very low due to different ailments affecting the livestock population, among which trypanosomosis is the primary ailment. Moreover, there are several factors that the disease is epidemic in the district due to conducive conditions such as suitable vector multiplication sites, the closeness of grazing areas to wildlife conservation

areas, and irrational use of drugs or drug abuse. However, there are scarce data on the parasitological prevalence of bovine trypanosomosis in the area. Therefore, this study aimed to identify the prevalence and host-related risk factors for bovine trypanosomes in the Benatsemay district.

## Materials and Methods

### *Description of study area*

The study was carried out in 3 selected sites (Chali, Gurmamero, and Geisma) in the Benatsemay district. It is located approximately 702 km southwest of Addis Ababa and 42 km southeast of the zonal town, Jinka, and it is one of the 10 districts in the South Omo Zone, with latitude ranges between 5°00'31"N and 5°41'47"N and longitudes range between 36°12'13"E and 37°03'50"E. Benatsemay district is located in the tropics between low to high altitudes. The temperature of the district swings between 15.4°C and 43°C, and rainfall, which falls from February to May and July to October, is low, ranging from 250 to 1200 mm. The altitude ranges from 500 to 2696 m above sea level. The Woito River separates it from the Malle districts and Oromia Region. The western part of this district is included in Mago National Park (Figure 1).

According to the census of the Central Statistics Agency of Ethiopia in 2017,<sup>26</sup> the total cattle population of the district was estimated to be approximately 525 941.

### Study animal and sampling methods

The local breed of zebu cattle managed under extensive pastoral and agro-pastoral management systems were included in this study. The age of the study animals was determined based on the dentition as described by Pace and Wakeman<sup>27</sup> and classified into 3 groups: <1 year (young), 1 to 3 years (adult), and >3 years (old). The body condition score of cattle was determined according to Nicholson and Butterworth's<sup>28</sup> method and classified as poor, medium, and good. Six kebeles/sites from the Benatsemay district of the south Omo zone in southern Ethiopia were purposively selected from 34 kebeles (PAs) for sampling based on accessibility and tsetse infestation within each district sample frame gathered from district agriculture offices. Farmers/pastoralists were notified to bring their cattle for evaluation by local agricultural or animal health extension agents and community leaders. Then, among those registered on each sampling day, a systematic random selection of households that had brought their cattle for examination at defined sites was made. Animals were also chosen at random from among the cattle brought to the examination site.

### Study design and sample size determination

A cross-sectional study design was employed from October 2020 to July 2021 to determine the prevalence of trypanosomiasis in the study animals and to extract information on the most prominent disease of cattle by interviewing household owners. The numbers of animals required for this study were determined by using the formula given by Thrusfield<sup>29</sup> with 50% expected prevalence and 5% accepted error at a 95% confidence interval as follows.

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

where; N=required sample size, P<sub>exp</sub>=expected prevalence, d=desired absolute precision

Accordingly, a total of 384 cattle of different age groups, sexes, and body condition groups, were randomly selected from selected sites for parasitological investigation. For the questionnaire survey, a total of 30 households were selected based on the number of cattle populations, available number of households, and accessibility to the road.

### Sample collection

The cattle were examined by visualization of the vital signs such as the mucus membrane respiratory condition, heartbeat, pulse rate, body temperature, lymph nodes, animal behavior (standing position and posture), and presence were examined before taking the sample. After proper restraining, blood samples were collected from the marginal ear vein with heparinized microhematocrit tubes by puncturing the ear vein using a

lancet after disinfecting with 70% alcohol and had been sealed with wax at one end for hematological analysis. For parasitological examination, blood samples were collected from the ear vein of 384 cattle.

### Laboratory analysis

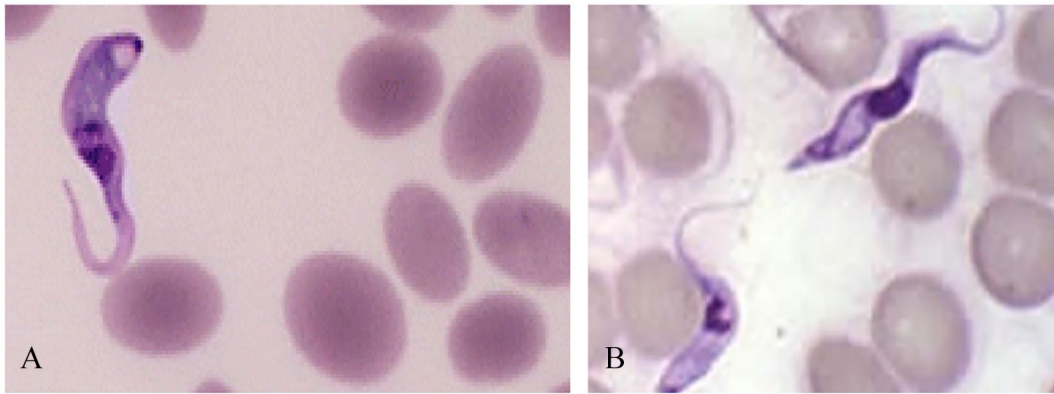
The buffy coat method was used to study the prevalence rate of trypanosome infection. Blood was collected into capillary tubes by capillary attraction until the tube was filled  $\frac{3}{4}$  ways. Each capillary tube was sealed at one end using a sealer and centrifuged at 12000 rpm for 5 minutes. After centrifugation, the PCV level of individual cattle was determined by reading centrifuged capillary tubes on a hematocrit reader to classify animals into anemic and non-anemic. All parasitological diagnostic tests and procedures were conducted as described by Paris et al.<sup>30</sup> and OIE.<sup>31</sup> The parasitological investigation using the buffy coat method in this study was similar in terms of the principle of the hematocrit centrifugation technique; however,<sup>30</sup> also compared the dark ground/phase contrast buffy coat method with the hematocrit centrifugation technique and the former was proved to be more sensitive than latter one.

After centrifugation, the capillary tubes were placed in a hematocrit reader to determine packed cell volume (PCV) expressed as a percentage of the total volume of blood, taking PCV values  $\geq 24\%$  to 46% as normal for zebu cattle.<sup>32,33</sup> During wet smearing, a 40 $\times$  microscope objective lens was used to identify motile trypanosomes, and the different types of species were confirmed through Giemsa staining at 100 $\times$  magnification on the basis of their morphology.<sup>30,34</sup> Species identification of the parasite was conducted by observing the motility of the parasite, which was described by OIE,<sup>31</sup> and Paris et al.,<sup>30</sup> as *T. vivax* (extremely active, traverses the whole field very quickly, pausing occasionally with a long free flagellum, an inconspicuous undulating membrane, a rounded posterior end, and a large terminal kinetoplast) and *T. congolense* (sluggish, adheres to red blood cells by its anterior extremity).

### Questionnaire survey

A pretested semi-structured questionnaire was employed to extract information on the most prominent disease of cattle by interviewing household owners (pastoralists) of the study district. The survey included questions about the socioeconomic aspect, frequent animal diseases in the study areas, animal groups, breed, age group, and sex most infected. More information was gathered from participants in order to analyze their perceptions of trypanosomiasis patterns, seasons, and transmission methods.

A total of 30 household owners were purposively selected from the 3 study sites based on their willingness of the household owners to participate, accessibility of the site, presence of tsetse infestation, and availability of households at the time of



**Figure 2.** (A) *Trypanosoma vivax* with a long free flagellum, a modest undulating membrane, a rounded posterior end, and an anterior end kinetoplast and (B) *Trypanosoma congolense* with a subterminal/medium kinetoplast, and no flagellum.

the survey since the households were nomadic. Before dissemination of the questionnaire to respective participants, the questionnaire was first translated into the Bena/Tsemay language. Secondary Information from the study district was also recorded and analyzed (Supplemental File). Informed consent was obtained through both written and verbal consent forms before interviewing participants and their involvement in the study was voluntary. Additionally, the owners' consent has been obtained for publishing the data and this was approved by the Research Ethics and Review Committee of Wolaita Sodo University.

#### Data management and statistical analysis

Data collected were entered into a Microsoft Excel spreadsheet and then analyzed using STATA version 13. The prevalence of trypanosomosis was calculated as the number of cattle infected with trypanosomes divided by the total number of sampled animals, which was expressed as the percentage. Data generated from the questionnaire survey were expressed by descriptive statistics such as means, frequencies, and percentages for different parameters. Logistic regression analyses were conducted using trypanosome infection as outcome variables against each of the explanatory variables of the hypothesized risk factors (sex, age, body condition score, coat color, and study sites).

The explanatory variables with a  $P$ -value  $\leq$  of .25 in univariable analyses were selected for multiple logistic regression analyses. The final multiple logistic regression models were manually built using a forward stepwise selection approach. A variable was considered as a confounder if it changed the coefficient of the significant variables by more than 25%. Multicollinearity of the predictors in the models was also assessed using Kruskal gamma statistics, and those variables whose gamma value ranged between  $-0.6$  and  $+0.6$  were considered in a multivariable logistic regression model. The odds ratio (OR) and its 95% confidence interval (CI) of the variables associated with the outcome variables were calculated from the

final multivariate logistic regression models. Levels of significant differences were considered at a  $P$ -value less than .05.

## Results

### Parasitological prevalence and trypanosome species identified

From a total of 384 cattle examined by using the buffy coat method, 11.46% (44/384) were infected with trypanosomosis. Moreover, the Giemsa stain of the blood smears revealed that *Trypanosoma vivax* and *Trypanosoma congolense* was identified using a 100x magnification lens (Figure 2).

### Analysis of trypanosomosis with risk factors

A higher prevalence of bovine trypanosomosis was recorded in Gurmamero sites (18.33%), followed by Geisma (9.2%) and Chali (6.93%). The disease prevalence was statistically significant ( $P < .05$ ) among the study sites. *Trypanosoma congolense* (9.11%) and *T. vivax* (2.34%) were the 2 species identified in the study district. There was no report of mixed infection in the area (Table 1).

Although there was a slightly higher prevalence of trypanosome infection in female animals than in male animals, the difference was statistically insignificant ( $P > .05$ ). However, the prevalence of bovine trypanosomosis was statistically significant ( $P < .05$ ) among age groups, that is, it was significantly higher and more prevalent in older animals (age  $>3$  years) than in young age groups (Table 1).

The body condition score and body color of the study animals were significantly associated ( $P < .05$ ) with trypanosome infection. Accordingly, poor body conditioned animals were more infected than medium and good body conditioned animals. Similarly, trypanosome infection was higher in black-coated animals than in red and mixed-coated animals (Table 1).

According to multivariate logistic regression analysis, age, hair coat color, body condition, and study sites were found significantly ( $P < .05$ ) associated with trypanosomosis (Table 2).

**Table 1.** Association of bovine trypanosomosis prevalence with potential risk factors.

FACTORS	CATEGORY	NO. OF EXAMINED	POSITIVE	PREVALENCE (%)	CHI-SQUARE	95% CI	P-VALUE
Sex	Male	183	17	9.28	1.01	0.36-1.30	.24
	Female	201	27	13.43			
Age	<1 year	35	1	2.85	12.46	1.29-3.27	.002
	1-3 years	120	6	5.00			
	>3 years	229	37	16.15			
Body condition score	Poor	108	24	22.22	17.16	1.41-3.61	.003
	Medium	178	13	9.20			
	Good	98	7	7.14			
Hair coat color	Red	121	6	4.95	46.39	0.15-0.42	.0001
	Black	73	25	34.24			
	Mixed	190	12	6.84			
Study sites	Chali	101	7	6.93	8.45	1.17-2.85	.015
	Gurmamero	120	22	18.33			
	Geisma	163	15	9.20			

**Table 2.** Multivariate logistic regression analysis of risk factors for trypanosomosis.

RISK FACTORS	MULTIVARIATE LOGISTIC REGRESSION ANALYSIS	
	AOR (95% CI)	P-VALUE
Sex		
Female	Ref	Ref
Male	0.55 (0.23-1.33)	.19
Age		
<1 year	0.76 (.077-7.64)	.82
1-3 years	Ref	Ref
>3 years	4.63 (1.72-12.50)	.002
Body condition score		
Good	Ref	Ref
Medium	0.66 (0.13-3.33)	.62
Poor	7.33 (1.76-30.55)	.006
Hair coat color		
Black	1.44 (0.77-2.68)	.252
Red	0.63 (0.30-1.30)	.212
Mixed	Ref	Ref
Study sites		
Geisma	1.69 (0.38-7.51)	.48
Gurmamero	3.30 (1.09-9.94)	.03
Chali	Ref	Ref

### *Effect of trypanosomosis on packed cell volume (PCV)*

The investigation of PCV results in the cattle studied for trypanosomosis infection revealed that the packed cell volume (PCV) of parasitaemic animals ( $22.75 \pm 2.78$ , 95% CI: 21.90-23.59) was significantly lower ( $P < .05$ ) than that of non-parasitaemic animals ( $29.24 \pm 3.94$ , 95% CI: 28.82-29.66). There was a statistically significant difference in the mean PCV value between the infected and noninfected animals ( $t = 10.58$ ,  $P = .0001$ ) (Table 3).

During the study period, a total of 44 (11.46%) animals were between the PCV range of 16 and 24, which was anemic, while the rest of the 340 (88.54%) animals were between the PCV range of 24 and 39, which were non-anemic (Figure 3).

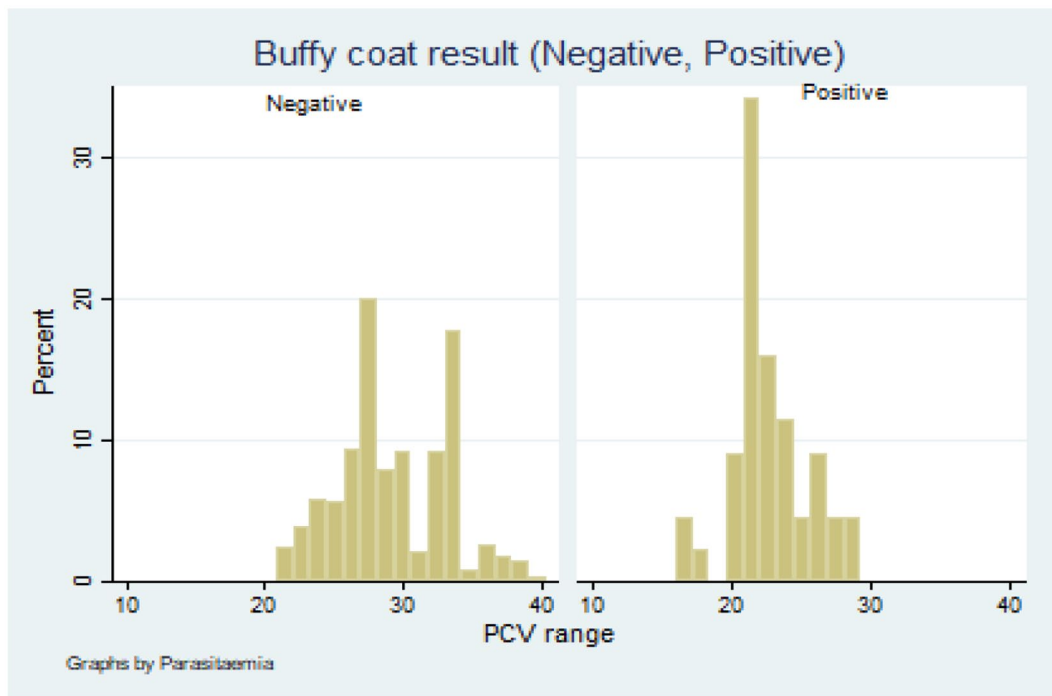
### *Questionnaire survey results*

According to the survey, trypanosomosis was the most commonly mentioned disease by the respondents in Chali (40%) and Gurmamero (40%) sites whereas 30% of the respondents in Geisma trypanosomosis was their main challenge next to pneumonia (40%) (Table 4).

Respondents of the study area also revealed that trypanosomosis dominantly occurs in large ruminants (90%) compared with small ruminants and equines. The disease occurrence is equal in all breeds of large ruminants present in the area. Moreover, an interviewee perceived that trypanosomosis was more serious in the old age groups (53.33%) than in the young age groups, but it was equally dangerous in both sex categories (Table 5).

**Table 3.** Analysis of the association of trypanosome infections with mean PCV (%) of cattle.

PARASITAEMIC STATUS	NO. OF EXAMINED	MEAN PCV $\pm$ SD	95% CI	T-VALUE	P-VALUE
Parasitemia	44	22.75 $\pm$ 2.78	21.90-23.59	10.59	.0001
Non-parasitemia	340	29.24 $\pm$ 3.94	28.82-29.66		

**Figure 3.** PCV distribution among the infected and non-infected cattle.**Table 4.** Ranks of common animal diseases in the study areas.

S. NO.	STUDY SITES	LIST OF COMMON DISEASES	FREQUENCY	PERCENTAGE (%)
1	Chali	Trypanosomosis	4	40
		Blackleg	2	20
		Ectoparasite	1	10
		FMD	3	30
2	Gurmamero	Trypanosomosis	4	40
		Blackleg	1	10
		Ectoparasite	2	20
		Pneumonia	3	30
3	Geisma	Trypanosomosis	3	30
		Blackleg	2	20
		Ectoparasite	1	10
		Pneumonia	4	40

According to respondents, trypanosomosis frequently occurs during the wet season (50%) compared with the dry season, and its trend is increasing over time. About 77% of the respondents

replied that trypanosomosis was transmitted by the bite of flies. All the respondents disclosed that they relied on trypanocidal drugs for the control of trypanosomosis (Table 6).

**Table 5.** Interviewee perception of the animals most affected by trypanosomiasis.

INTERVIEW DESCRIPTION	RESPONSES	FREQUENCY	PERCENTAGE (%)
Animal groups most infected	Large ruminants	27	90.00
	Small ruminants	2	6.66
	Equines	1	3.33
Breeds most infected	Local	8	26.66
	Exotic	3	10.00
	Equal in both breeds	19	63.33
Age groups most infected	Old	16	53.33
	Young	14	46.66
Sex groups most infected	Male	1	3.33
	Female	7	23.33
	Equal in both sex groups	22	73.33

**Table 6.** Interviewee perception of the season of trypanosomosis infection, trends of trypanosomosis, and method of transmission.

INTERVIEW DESCRIPTION	RESPONSES	FREQUENCY	PERCENTAGE (%)
Season of frequent trypanosomosis occurrence	Dry	4	13.33
	Wet/rainy	15	50.00
	Equal in both season	11	36.66
Trends of trypanosomosis	Increasing	16	53.33
	Decreasing	11	36.67
	I don't know	3	10.00
Methods of transmission	Flies	23	76.67
	Ticks	2	6.67
	I don't know	5	16.67

## Discussion

According to this study, trypanosomosis was the major bottleneck for the cattle of Benatsemay district, and it was ranked as the number one enemy for livestock production in the area. Moreover, this survey also revealed that large ruminants are more susceptible to trypanosomosis than small ruminants. This might be due to the vector (*Glossina*) preference to feed on large ruminants rather than small ruminants. The multivariate analysis revealed that age, body condition score, and body color were among the determinants of trypanosomosis in the area. A higher occurrence of trypanosomosis in older animals than in young animals was perceived by the respondents as weakness and infection by other diseases of the former compared to the latter. The wet season was known as “fly swarming” in the area; therefore, the respondents associate the frequent occurrence of trypanosomosis during this season with overrunning fly vector, which extensively feeds on grazing animals.

The overall prevalence (11.45%) of the current study was lower than the previous study reported by Chanie et al.,<sup>35</sup> and Leta et al.,<sup>20</sup> who reported bovine trypanosomosis prevalences of 13.8% and 13.3% in the Borena and Benishangul Gumuz regions, respectively. Also, Terefa<sup>36</sup> and Bekele et al.<sup>37</sup> reported a prevalence of 15.1% and 21% in tsetse-infested Western and Southern Rift valley areas, respectively. Similarly, a higher prevalence of 17.2% was reported in tsetse-infested Metekel areas by Afewerk et al.<sup>38</sup>

However, a higher bovine trypanosomosis prevalence was reported by Ayele et al.,<sup>39</sup> and Tegegn et al.,<sup>40</sup> in the Deramallo of Gamo zone and Gngangatom district of the South Omo zone, respectively. On the other hand, lower prevalence reports from Sodo Zuriya District (5%), the Kaffa zone (6.9%), Didessa district (5.47%), and Dale-Wembera district (2.86%) were disclosed by Hundessa et al.,<sup>41</sup> Alemayehu et al.,<sup>42</sup> Bekele and Nasir,<sup>43</sup> and Biyazen et al.,<sup>19</sup> respectively.



In comparison to other tsetse-infested locations, this study demonstrated a relatively lower prevalence of trypanosomiasis in the study district. This variation is due to a variety of reasons, including the decreased prevalence of trypanosomiasis in this study location, the laboratory technique used, and the study period. The investigation was conducted during the dry season and early wet season, which may have influenced the vector population and, also the prevalence of trypanosomes infection.<sup>44</sup> In addition, the expansion of veterinary services to peasant associations, deforestation for crop cultivation (settlement), frequent use of trypanocidal/insecticidal drugs and animal husbandry practices, and the presence of a trypanosomiasis and fly control program in the area may have all contributed to the lower prevalence.

Furthermore, the significant difference in trypanosomiasis prevalence among the study sites might be associated with the difference in location of each study site and their proximity to suitable *Glossina* habitats in the district. Gurmamera site is located close to Mago National Park, which is the origin of the *Glossina* infestation of the South Omo zone. Due to the proximity of this site to a wildlife conservation area, most animals from the Site graze in and around the park, especially during the dry season, which easily predisposes the animals to an infected starving tsetse vector. Geisma Site, on the other hand, is close to the Woito River, which is also suspected to be a suitable habitat for tsetse flies so that the animals in the area can easily become infected. In line with the current study, Van den Bossche study also revealed that the prevalence of trypanosomiasis and its impact on livestock productivity vary by location and are heavily influenced by the level of interaction between tsetse, domestic, and game animals.<sup>45</sup>

According to this study, male and female animals were equally infected. This might be due to the grazing of all sex groups together on grazing land. In agreement with our results, previously Nigatu,<sup>46</sup> and Tilahun,<sup>47</sup> reported equal infection of bovine trypanosomiasis in males and females from Abbay Basin areas and Southwest Oromia region, respectively. However, the report of Tegegn et al,<sup>40</sup> showed significantly higher infection in females (29.3%) than males (18.5%) from the Gngatom district of the South Omo zone.

The age of the study animals was significantly associated with trypanosome prevalence, that is, higher infection in older animals than young. Previous research findings of Alemayehu et al,<sup>42</sup> Gona et al,<sup>48</sup> and Tegegn et al,<sup>40</sup> were consistent with our current result, which reported a significant difference in the prevalence of the disease among different age groups, that is, there was lower disease prevalence in young age groups than in older animals. However, Nigatu,<sup>46</sup> Ayele et al,<sup>39</sup> and Tilahun,<sup>47</sup> disagree with the current finding, who stated an insignificant infection rate of trypanosomiasis among different age groups. This might be due to the effect of maternal immunity in young age groups as well as the preference of tsetse flies to feed on old animals than young animals. Younger calves are often kept in

the farmstead and do not venture far for grazing and watering, that reducing the risk of tsetse fly exposure. However, in the field, at grazing and watering spots, the older age groups were more exposed to the tsetse risk. While traveling great distances to the working area, draught oxen are also at risk of being bitten by the tsetse fly.

Poor body-conditioned animals were more infected than medium and good body-conditioned animals. Previously, Gona et al,<sup>48</sup> Tegegn et al,<sup>40</sup> and Ayele et al,<sup>39</sup> also reported similar findings of higher trypanosomiasis prevalence in poorly conditioned cattle than in medium and good body conditioned animals. Animals become emaciated (poor) due to other disease infections and poor nutritional status. This might be attributed to the decreased ability of emaciated (poor) animals to defend against trypanosome infection compared to medium- and good-body-conditioned animals. Significantly higher trypanosomiasis prevalence in black-colored animals might be associated with a color preference of *Glossina* vector to feed on black animals than other color types since the tsetse fly was more attracted by black color due to their shade-loving behavior during their flight from one area to another.<sup>49,50</sup>

The higher proportion of *T. congolense* over *T. vivax* in our current study area might be associated with effective transmission of *T. congolense* by a dominant cyclical vector in the area, *Glossina pallidipes*, than *T. vivax*. It might also be associated with increased trypanocidal drug resistance by *T. congolense*, which makes it dominant during parasitological surveys. The current study revealed that *T. brucei* was not identified after the buffy coat technique. Similarly, the study in Zambia revealed that *T. congolense* and *T. vivax* were the most prevalent pathogenic trypanosome species affecting cattle with *Trypanosoma brucei brucei* infecting cattle to a lesser extent.<sup>51</sup> Furthermore, *T. brucei* is clinically less apparent in cattle unless there are adverse climatic conditions such as droughts, the clinical presentation may not be as prominent, leading to farmers failing to seek veterinary assistance for sick animals.<sup>52</sup>

In the present study, the buffy coat technique was used to examine the presence of parasitemia. The parasitological prevalence of bovine trypanosomiasis observed using the buffy coat method in this study was similar to the prevalence observed during other studies<sup>51,52</sup> and this technique has low sensitivity<sup>53</sup> as compared to molecular diagnosis such as PCR diagnostic methods.<sup>54</sup> According to different studies, the PCR technique is a more sensitive parasitological technique that will provide better results through the proper amplification of products from the disease agent of interest.<sup>23</sup>

Packed cell volume (PCV) is one of the most fundamental quantitative measurements to estimate the anemic status of the tested animal. Since anemia is one of the striking pathological manifestations of trypanosomiasis that happens due to massive destruction of RBCs by the trypanosome. Also, by measuring PCV, our study animals were classified as anemic and nonanemic by setting the cut-off value of PCV measurement for

bovine species, which was  $<24$  (for anemic) and  $\geq 24$  (for non-anemic). Other studies found the same patterns in mean PCV values for trypanosome-infected and noninfected cattle. In the North Omo Zone, Muturi (1999) found mean PCV values of 16.7% and 28.0%; Alekaw (2004) found 21.60% and 25.40% in districts bordering Lake Tana in northwest Ethiopia, and Feyisa (2004) found 21.65% and 25.54% in southwest Ethiopia for parasitaemic and non-parasitic cattle, respectively. The significant decrease in mean PCV value of infected than non-infected cattle might be due to the erythrocyte destructive nature of trypanosomes in infected animals.<sup>55-59</sup>

The limitation of this study was that it doesn't use parasitological diagnostic methods like the PCR technique which will provide a better prevalence of trypanosomosis. Moreover, the study was only conducted in 3 epidemiological areas due to logistic and financial limitations.

## Conclusion

Trypanosomosis was ranked as the number 1 livestock ailment and was found to be the bottleneck for livestock production and productivity in the Benatsemay district of the South Omo zone. According to this study, trypanosomosis in the study district was caused by 2 prominent species of *Trypanosoma*, *T. congolense* and *T. vivax*, with an alarming prevalence level. According to livestock owners' insight, trends of this disease increased over time. The use of trypanocidal drugs was the dominant option for treating trypanosomosis in the area.

Thus, the present study revealed that the predominance of bovine trypanosomosis in the study area and factors such as age and body condition had a significant effect on the prevalence of trypanosomosis in cattle. The government and the public should then collaborate to control the vector as well as the disease in a sustainable way. The study should be in larger epidemiological areas using parasitological diagnostic methods such as PCR should be used to get a better prevalence of trypanosomosis in the study sites.

## Authors Contributions

HF, EE, MM, and TT collected data, design the research, analyzed, and interpreted the data, wrote and edit the original paper. All authors have approved the final submitted version of the manuscript.

## Availability of Data and Materials

All datasets used and analyzed during the study will be available upon reasonable request of the corresponding author.

## Ethics Approval and Consent to Participate

Informed consent was obtained from the participants through both written and verbal consent forms before collecting blood samples, and the survey protocol of the study was approved by the Research Ethics and Review Committee of Wolaita Sodo University. Participants' involvement in the

study was voluntary; participants who were unwilling to participate in the study and those who wished to quit their participation were informed to do so without any restriction.

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## Supplemental Material

Supplemental material for this article is available online.

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