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A Cross-sectional Study of Bovine Trypanosomosis in Sayo District, Oromia Regional State, Western Ethiopia

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Abstract: A cross sectional study of bovine trypanosomosis was conducted in Sayo district of Kellem Wollega zone, Western Oromia Region, during early dry (December, 2016) and early rainy (June, 2017) seasons employing parasitological blood examination and survey of vectors. A total of 860 representative blood samples were collected from randomly selected cattle and examined using standard methods of parasitological examination techniques. Accordingly, the overall prevalence of bovine trypanosomosis was found to be 11.16%. The species of trypanosomes encountered include *Trypanosoma congolense* (52.10%) followed by *T. vivax* (32.30%), *T. brucei* (10.40%) and mixed infection of *T. congolense* and *T. vivax* (5.20%). The seasonal prevalence of bovine trypanosomosis was 15.11% and 7.20% in early dry and early rainy seasons, respectively. There was a statistically significant difference (P <0.05) in the prevalence of bovine trypanosomosis between low land (13.8%) and mid land altitude (8.5%). Similarly, there was also a statistically significant difference (P <0.05) in the prevalence of bovine trypanosomosis between poor body conditioned cattle compared to both medium and good categories. On the contrary, there were no statistically significant differences (P>0.05) in the prevalence of bovine trypanosomosis between different sex categories. The mean PCV value of trypanosome infected animals was (22.94% ± 0.15%) compared to non infected groups (26.47 ± 0.35%) with statistically significant difference (P<0.05). As part of survey of vectors of bovine trypanosomosis a total of 1575 flies were caught during the study period. The findings encountered revealed that 807 (51.24%), 667 (43.35%) and 101(6.41%) were *Stomoxys, Glossina* and *Tabanus* respectively. The apparent density of *Glossina*, *Stomoxys* and *Tabanus* were 4.16, 5.04 and 0.63 f/t/d, respectively. The present study disclosed that despite vector control implementation practices in the study area, bovine trypanosomosis still remain to be a core problem. Therefore, the current control strategies being implemented in the study area should be further assessed and consideration of integrated trypanosomosis and vector control approaches should be instituted.

Keywords: Bovine, Kellem Wollega, Tsetse, Prevalence, Trypanosomosis, Western Ethiopia

1. Introduction

African animal trypanosomosis is a vector-borne disease, which is transmitted biologically by different tsetse flies [1, 2] and mechanically by a number of biting flies of genus diptera [3]. African Animal trypanosomosis (AAT) is a major constraint to livestock and mixed crop-livestock production in tropical Africa [4]. The distribution of the disease is parallels the distribution of tsetse flies and comprises of an area approximately 10 million Km^2 [5] in 37 sub-Saharan countries [6]. Most species of domestic animals are to some degree susceptible to trypanosomosis, but it is of major importance in cattle [7, 8]. Trypanosomosis can exert serious losses in pigs, camels, equines, goats and sheep as well. In cattle, the disease is caused by *T. vivax*, *T. congolense* and *T. b. brucei* [9, 10].

Animal trypanosomosis has a significant negative impact on economic growth in many parts of the world [11]; particularly in sub-Saharan Africa [12]. About 50 million of cattle and other livestock species are at risk of the disease and farmers lose 3 million cattle every year because of trypanosomosis [13]. In Africa the overall economic loss due to trypanosomosis was estimated at 4.5 billion USD [14]. Thus, animal trypanosomosis constitutes a major threat to food security and livestock production in several parts of sub-Saharan Africa [15], retarding agricultural development in large areas of the continent in terms of mortality, abortion, reduced fertility, milk and meat production, and ability to work as traction animals [16].

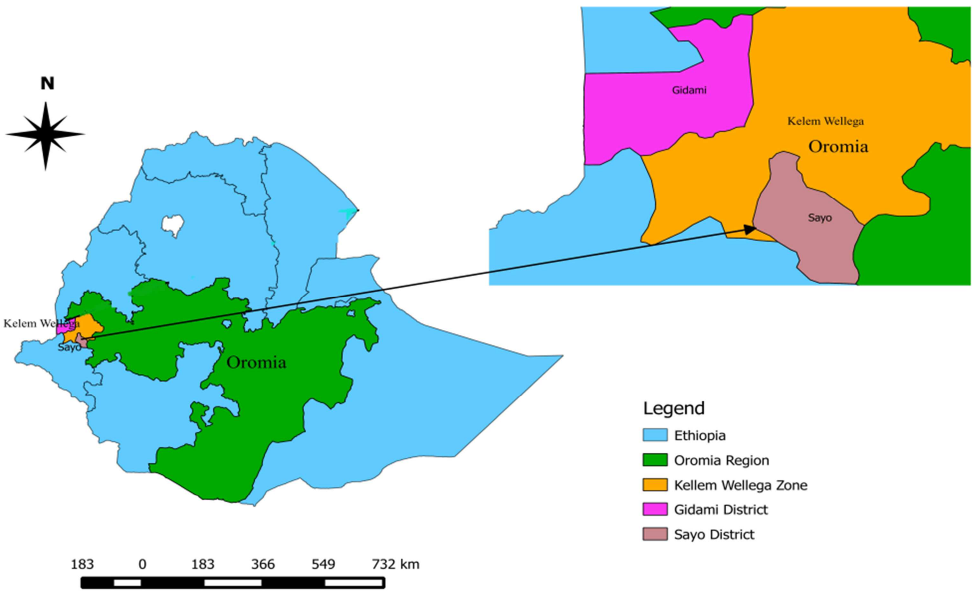
In Ethiopia trypanosomosis is widespread in domestic livestock in the Western, South and South western lowland regions and the associated river systems (Abay/Dedessa, Ghibe/Omo and Baro/Akobo and rift valley) [17], limiting livestock productivity and agricultural development in the country [18]. Currently, about 220,000 Km^2 areas of fertile land are infested with five species of tsetse flies namely *Glossina pallidipes*, *G.morsitans morsitans*, *G. fuscipes*, *G. tachinoides* and *G. longipennis* [18]. 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. equiperdium* in horses (17]. Annual losses to the national economy are estimated to exceed $200 million because of mortality and morbidity of livestock, denied access to land resources and the costs of controlling this disease [19].

The distribution of trypanosomes is dynamic due to climatic change, ecological disturbances, and human interventions. Previous studies conducted so far in Sayo District [20] did not considered the different altitudes and seasonal dynamics of the disease prevalence as well as the flies' density. In addition, as part of prevention and control of the disease, regular surveillance of the vector and parasite need to be considered in tsetse infested areas of the country. Therefore, the present study was initiated and designed to identify the species of trypanosomes affecting cattle and the vector and their dynamics in early dry and early rainy seasons of the year in different altitudes of the study area.

2. Materials and Methods

2.1. Study Area Description

This study was conducted in six selected villages of Sayo district. Sayo district is located in Oromia regional state, Western Ethiopia at about 636 Km's to the West of the capital city, Addis Ababa. The total surface area of the district is estimated 127,800 hectares. The district has an altitude ranging from 1100 to 2000 meters above sea level. Based on altitude the district is subdivided in to three climatic zones: highland 29%, midland 46% and lowland 25%. The area has two distinct seasons: the dry season extending from December to May and the rainy season extending from June to October. The annual rainfall and temperature ranges of the area are 1200-2000 mm and 15-25^{O}C, respectively [21]. The livelihood of the society largely depends on mixed livestock and crop production. The main crops cultivated in the area are maize, teff, sorghum, pepper, and barley. Coffee is the main source of income for the society. The livestock populations of the district are 59,593 cattle, 53,377 sheep, 6225 Goats, 11, 3149, equines [22].



Source: [21]

Figure 1. *Map of study area.*

2.2. Study Design

A cross-sectional study design was employed to determine the prevalence of bovine trypanosomosis and apparent density of vectors during early dry (December 2016) and early rainy season (June 2017). In the present study, six peasant associations were purposively selected for the sake of convenience and accessibility, three from lowland (<1500 meters above sea level) and the rest three from midland (>1500 meters above sea level). A simple random sampling method was used to select the study animals from animal various gathering points located within the peasant associations.

2.3. Study Animals and Sampling Strategy

The study animals consisted of all zebu cattle above one year of age and both sexes. The study animals were kept under an extensive husbandry system. The sample size was determined using the formula given by [23] with an expected prevalence of 16.9 % [20], 95% confidence interval and 5% desired absolute precision. Accordingly, sample size of 215 was obtained. Thus, 215 animals each were sampled from midland and lowland in two different seasons of the year. Overall, a total of 860 cattle were sampled in both seasons and altitudes. Potential host related risk factors associated to the disease such as age, sex and body condition score of the studied animals were recorded during sampling. Based on the appearance of ribs and dorsal spines for zebu cattle as per the description given by [24], animals were grouped into poor, medium, and good body condition categories. The age of study animals was estimated by means of their dentition as described by [25] and conventionally categorized as young (≤3 years) and adult (>3 years).

2.4. Examination Techniques and Protocol

2.4.1. Parasitological Examination

For parasitological examination, blood sample collection was performed by piercing the marginal ear vein of cattle with a sterile lancet in to heparinized capillary tube. Following, centrifugation of capillary tubes at 12,000 rpm for 5 minutes was undertaken in a microhaematocrit centrifuge. Then, the PCV for each sample was determined using the hematocrit reader [26]. Animals with PCV ≤ 24% were considered to be anemic [27]. Then the Buffy coat zones were examined to reveal trypanosomes as per recommendation by [28] under 40 x magnifications as a wet preparation for motile trypanosomes under the light microscope [29]. Thin blood smears were made and stained with Giemsa staining for the purpose of species identification by light microscope under oil immersion [30]. The trypanosome species were distinguished using their size, position of the kinetoplast, degree of the development of undulating membranes and presence or absence of free flagellum [31].

2.4.2. Vector Survey

Vector survey was conducted in four villages of Sayo district in early dry and early rainy seasons only in lowland areas. A total of 80 monoconical traps (40 in early dry and 40 in early rainy) were deployed at approximate intervals of 100 to 200 meters for 48 hours at watering and grazing points and baited with acetone, octenol (1-3-octane) and 3 days old cow urine filled in separate bottles [32]. The underneath of each trap pole was smeared with grease in order to prevent the ants climbing up on the pole towards the collecting cage that could damage the tsetse flies. After 48 hours of deployment, tsetse flies in the cages were counted and identified based on their habitat and morphology to the genus and species level [33]. Biting flies were identified to genus level based on their morphological characteristics such as size, color, wing venation structure, and proboscis [34]. Sexing was done for tsetse fly by observing hypopygium on the posterior end of the ventral aspect of male flies [35]. The Apparent density of the tsetse fly was calculated as the number of tsetse catch/trap/day [36].

2.5. Data Management and Analysis

The collected data during the study period were entered into Microsoft Excel 2007 program and was decoded. Descriptive statistics was employed in analyzing the data. Prevalence was expressed as percentage of number of animal found positive versus the total number of sampled cattle. The association between the prevalence of bovine trypanosomosis and host related risk factors were assessed by logistic regression, whereas the student's *t*-test was used to assess the difference in mean PCV between trypanosome positive and negative animals. All statistical analyses were conducted using SPSS version 20.0 software. The test result was considered significant when the calculated p-value was less than 0.05. The apparent density of fly population was calculated by dividing the number of flies caught by the number of traps deployed and the number of days of deployment and expressed as fly/trap/day (FTD).

3. Results

3.1. Parasitological Findings

Out of the total 860 cattle randomly selected and examined, 96 were found to be positive for trypanosomosis with an overall prevalence of 11.16%. The prevalence of bovine trypanosomosis was 15.11% and 7.20% in early dry and early rainy seasons, respectively. There was a statistically significant difference (P<0.05) in the prevalence of trypanosomosis between the two seasons. Cattle were invariably infected with different species of trypanosomes. *Trypanosoma congolense* (52.10%) was the dominant trypanosome species followed by *T. vivax* (32.30%), *T. brucei* (10.40%) and mixed infection from *T. vivax* and *T. congolense* (5.20%). In the present study, trypanosome prevalence was (13.76%) in lowland and (8.5%) in midland areas, with a statistically significant difference (P <0.05) (Table-1). The highest prevalence of trypanosomosis was recorded in poor body condition score (22.40%) followed by medium body condition (9.70%) and the lowest in good body conditioned animals (3.54%), with statistically significant difference (P<0.05). The prevalence of trypanosomosis was higher in males (13.22%) as compared to female animals (9.23%), showing no statistically significant difference (*P* >0.05). Concerning age of cattle, the prevalence of trypanosomosis was statistically significantly different (P <0.05) in the adult age group 12.89% than in younger animals (7.22%) (Table-1).

Table 1. *Prevalence of bovine trypanosomes on the basis of altitude and season.*

Season	No. examined animals	No. infected animals	Prevalence (%)	X^2 test	P- Value
Altitude					
Low land	436	60	13.8	6.02	0.01
Mid land	424	36	8.5		
Season					
Early dry	430	65	15.11	13.55	<0.001
Early rainy	430	31	7.20		
Body condition					
Poor	250	56	22.40	50.71	<0.001
Medium	299	29	9.70		
Good	311	11	3.54		
Sex					
Male	416	55	13.22	3.44	0.06
Female	444	41	9.23		
Age					
1-3 years	263	19	7.22	5.92	0.01
>3 years	597	77	12.89		
Over all	860	96	11.16		

T.c = T. congolense, T.v = T. vivax, T. b. = T. brucei Mixed (T. congolense and T. vivax)

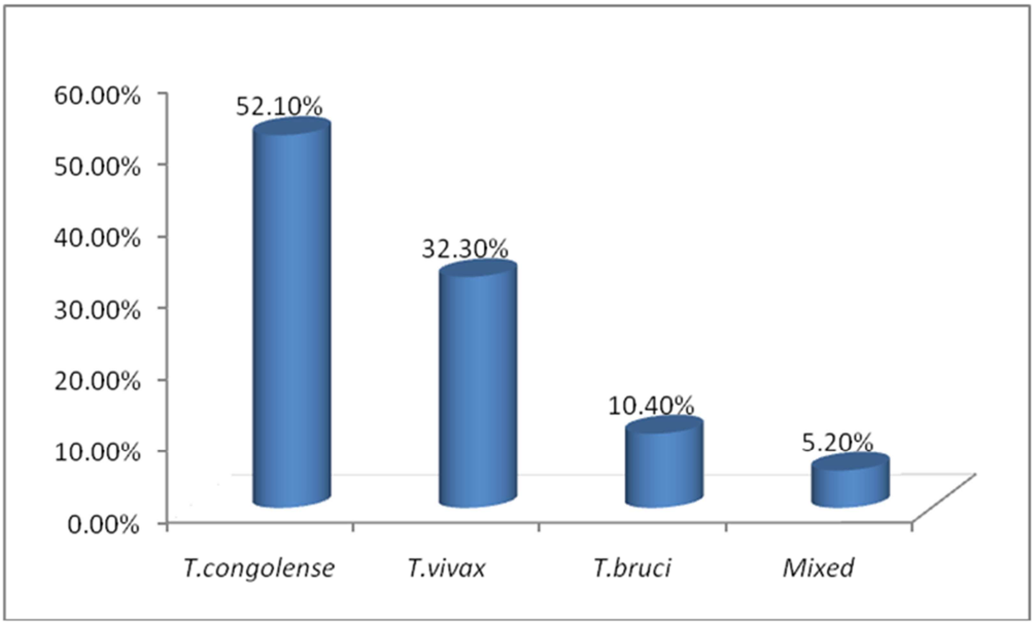


Figure 2. *Proportion of trypanosome species identified during the study period.*

3.2. Haematological Findings

From the total of 860 examined animals, 219 (25.46%) were anemic having PCV ≤24%. The anemic distribution was higher in parasitaemic cattle 58.33% than aparasitaemic ones (21.33%). The overall mean PCV values were statistically significantly different (P<0.05) between parasitaemic 22.94% (95% CI: 22.25-23.63) and aparasitaemic animals 26.47 (95% CI: 22.25-23.63) (Table-2).

Table 2. *Mean overall PCV in parsitemic and aparsitemic cattle.*

Status of animals	No. examined	Percentage of anemic animals	Mean PCV (%)	Std. Error	P-value	95% Confidence Interval
Aparsitemic	764	21.33	26.47	0.15	<0.001	26.17-26.77
Parsitemic	96	58.33	22.94	0.35		22.25-23.63

3.3. Entomological Findings

A total of 1575 flies were caught during the study period. Of these, 667 (42.35%) belong to *Glossina* species. Similarly, 807 (51.24%) and 101 (6.41%) were *Stomoxys* and *Tabanus*, respectively. The overall apparent fly density was 9.84 f/t/d. The relative abundance of *Glossina* and other biting flies is shown in Table 3. Out of total of 667 *Glossina* species captured, 301 (45.13%) flies were males and the rest 366 (54.87%) flies belong to females. The apparent density of *Glossina*, *Stomoxys* and *Tabanus* were 4.16, 5.04 and 0.63 f/t/d, respectively.

Table 3. *Flies caught during the study period.*

Season	No. trap deployed	*G.m.m.*	*G.pal.*	*G.fusc.*	*G.tach.*	*Stom.*	*Tab.*	Total	FTD
Early dry	40	164	155	127	7	441	79	973	12.16
Male		76	70	51	3			200	2.50
Female		88	85	76	4			253	3.16
Early rainy	40	47	40	65	62	366	22	602	7.53
Male		13	11	22	17			63	0.78
Female		34	29	43	45			151	1.89
Total	80	211	195	192	69	807	101	1575	9.84

G.m.m: G. *Morsitans Submorsitans*; G.pal: *Glossina Pallidipes*; G. tach: *G. Tachnoides*
FTD: Fly/trap/day

4. Discussion

The current study indicated that trypanosomosis is the most prevalent disease of cattle causing considerable direct and indirect economic losses in the study area. The overall prevalence of bovine trypanosomosis was 11.16%. This is much lower than previous reports: 16.9% in Sayo district, Kellem Wollega Zone, Western Ethiopia [20], 17.5% in upper Didessa of tsetse infested regions [37], 20.40% in Wolyta and Dawero zones of southern Ethiopia [38] and 29% in Gawo Dale District of western Ethiopia [18]. The relatively low prevalence of trypanosomosis in this report is related to the vector control intervention practiced in the study area since 2014 by National Tsetse and Trypanosomosis Investigation and Control Center (NTTICC), which includes deploying of impregnated targets and application of delthamethrine pour-on.

The prevalence of bovine trypanosomosis was 15.11% and 7.20% during early dry and early rainy seasons, respectively, with statistically significant variations (P<0.05). During early rainy season it is obvious that the population of flies increases. Due to this farmers inject their animals with trypanocidal drugs and also use insecticide spray in this season better than any other time to minimize the effect of the disease. This result is inconsistent with the report of [39, 40], which indicated higher rate of *T. congolense* infection in dry season than in wet season. They suggested that presence of few ponds in the dry season might have forced the animals to come close together and also created a favorable ground for the tsetse flies.

The prevalence of bovine trypanosomosis in lowland areas was 13.76% as compared to the midland areas 8.5%. Higher prevalence in lowland areas might be attributed to the fact that animals in lowland areas are more challenged by vectors than higher altitudes. It is assumed that there are many factors that contribute to the distribution of tsetse flies and tsetse transmitted trypanosomosis: Among these, altitude with temperature is one of the major biotic factors that limit the distribution of flies. This finding is in agreement with many other studies [41, 42].

In this study three species of trypanosomes identified were *T. congolense* (52.10%), *T. vivax* (32.30%), *and T. brucei* (10.40%) and mixed infection with *T. congolense* and *T. vivax* (5.20%). These species were widespread in most parts of Western and South Western Ethiopia [43, 44]. The high proportion of *T. congolense* (52.10%) in the present study is in agreement with previous reports of [45] at Pawe, North west Ethiopia (60.9%), [46] in selected sites of southern region (63.4%) and [47] in West Gojam (54.3%). The predominance of *T. congolense* infection in cattle suggests that the major cyclical vectors or *Glossina* species are more efficient transmitters of *T. congolense* than *T. vivax* in East Africa and also due to the high number of serodems of *T. congolense* as compared to *T. vivax.* [48], indicated that *T. vivax* was highly susceptible to treatment while the problems of drug resistance were higher in *T. congolense*.

This study also showed that there is strong association between body condition score of cattle and trypanosome infection. The occurrence of infection was 22.40%, 9.70% and 3.54% in cattle with poor, medium and good body conditions, respectively. This finding was in agreement with the study of [47] and [49] who found the highest prevalence of trypanosomosis in animals with poor body condition. The majority of the infected animals manifested clinically poor body condition, as trypanosomasis is characterized by progressive weight loss [50]. In contrast, out of 250 poor body conditioned animals 194(77.6%) were not infected by trypanosomes. This could be due to malnourishment, internal parasites and other body loss causing diseases [51, 52].

During the study period, the prevalence of bovine trypanosomosis was 13.22% and 9.23% in male and female animals, respectively. Different works on the other hand reported higher prevalence in male than female cattle [9, 53] and the possible suggestion to this finding could be that male animals travel more long distances to tsetse abundant areas for draught purpose. On the other hand, [54] and [55] in separate studies showed that no statistically significant difference in the prevalence of bovine trypanosomosis by sex. [56, 57] and [58] were also reported similar results were they observed no significant difference in trypanosome infection between the two sexes.

The trypanosome prevalence was found to be 12.90% and 7.22% in older and younger animals, respectively. This is in agreement with the findings of [56]. [59] and [60] were also found significantly higher prevalence of trypanosome infection in older animals. This could be associated with the fact that older animals travel long distance for grazing and draught as well as harvesting crops to high tsetse challenge areas, while young animals are usually kept around the homestead. According to the [61] tsetse flies are attracted significantly more by odor of older animals. In addition, young animals are protected to some extent by maternal antibodies [62]. 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 [63].

Anemia is an important clinical sign and indicator of trypanosomosis [50]. In the present study, 58.33 % of the parasitemic cattle were anemic. The mean PCV of parasitemic animals was significantly lower than that of aparasitaemic animals. Such significant difference of PCV values in infected cattle had been reported in Ethiopia: [38, 47] and [64]. In the absence of other diseases causing anemia, a low PCV value of individual animals is a good indicator of trypanosome infection [65]. The aparasitaemic cattle with PCV ≤24% in this study could be due to the low sensitivity of buffy coat method in chronic cases of Trypanosomosis [31] or due to other anemia causing diseases [66], or delayed recovery of the anaemic situation after current treatment with trypanocidal drugs. However, 40 infected animals had a PCV value >24. This could be explained as new infections that had not progressed to chronic stage [66].

Results of entomological survey revealed the presence of four *Glossina* species (*G. m. submorsitans, G. pallidipes*, *G. tachinoides* and *G. fuscipes*) and two genera of biting flies (*Stomoxys* and *Tabanus*). These four species of *Glossina* have also been reported in the Western and South Western parts of the country [56, 67]. The apparent density of *Glossina*, *Stomoxys* and *Tabanus* were 4.16, 5.04 and 0.63 f/t/d, respectively. Our finding is lower than [58] and [68] who reported 14.97 f/t/d and 11.9 f/t/d, respectively. The relative low level of tsetse population in the present study may be due to the control intervention under taken in the area by National Tsetse and Trypanosomosis Investigation and Control Center (NTTICC) and expansion of farmlands leading to the destruction of tsetse habitat and elimination of their wild hosts [69]. A higher number of female tsetse species 366 (54.87%) were caught than male 301(45.13%), and this is in line with reports from the country [56] and [44]. This could be attributed to the longer lifespan of female compared to male *Glossina* [48].

5. Conclusions and Recommendations

The current study disclosed that bovine trypanosomosis in Sayo District with a prevalence of 11.33% remains to be a significant constraint to livestock productivity despite tsetse and trypanosomosis control efforts. Three species of trypanosomes, *T. congolense, T. brucei*, and *T. vivax* were responsible for disease of trypanosomosis in the study area. The occurrence of trypanosomosis was associated with high challenges of tsetse and biting flies. The present cross-sectional studies confirmed that, altitude, age and body condition score of animals are significant risk factors for trypanosomosis distribution in the area. This study also revealed the negative impact of trypanosomosis on the PCV value-of infected animals.

In nutshell, in Sayo district the ongoing tsetse and trypanosomosis control intervention decreased the prevalence of trypanosomosis and the tsetse sustained support and interventions are required from local government bodies in mobilizing and increasing participation of the farmers. Trypanocidal drug efficacy studies should be undertaken using the common drugs circulating in the area. Strengthening community awareness on trypanosomosis control methods considering the risk of drug resistance and improper drug usage is essential.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Ethical Considerations

This research was approved by the animal research ethics committee of the College of Veterinary Medicine and Agriculture of the Addis Ababa University.

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