

Original Article

Prevalence of bovine trypanosomosis and vector density in a dry season in Gamo-Gofa and Dawuro Zones, Southern Ethiopia

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ARTICLE INFO

Keywords:

Cattle
Dawuro
Ethiopia
Gamo-Gofa
Vector
Prevalence
Trypanosomosis

ABSTRACT

Trypanosomosis remains one of the biggest constraints of livestock productivity in sub-Saharan Africa. It is of particular concern in Ethiopia where crop production is largely dependent on animal traction power. This study was conducted between November 2015 and March 2016 to estimate the prevalence of bovine trypanosomosis and vector density in Gamo-Gofa and Dawuro Zones located in Southern Ethiopia. For the entomological survey, a total of 305 NGU traps were deployed for three consecutive days at different positions in the two study areas. For parasitological study, blood samples were collected from 2402 cattle and examined for the presence of trypanosomes by the buffy coat technique (BCT). Blood samples that were positive in the BCT were further tested with Giemsa-stained thin smears for identification of *Trypanosoma* species. In the entomological survey, a total of 4113 flies were captured of which 1605 (39%) were tsetse flies while 2508 (61%) were other biting flies of the genus *Stomoxys* and *Tabanus*. *Glossina pallidipes* was the only tsetse fly identified in the study areas. The overall mean apparent density of *G. pallidipes* was 1.8 flies per trap per day (FTD). The overall prevalence of trypanosomosis in the study areas was 5.1% (95% CI: 4.2–6.0); however, it was significantly ($p < 0.001$) higher in Dawuro Zone (10%) than Gamo-Gofa (1.9%). Prevalence was also noted to be significantly ($p < 0.001$) higher in cattle with black coat colour and those with poor body condition. Trypanosomosis was caused mainly by *Trypanosoma congolense* (61.8%) and to a lesser extent by *T. vivax* (28.5%). Mixed infection by the two spp. was seen in 9.8% of the total positive animals. Evaluation of the mean packed cell volume (PCV) of the study animals with infection status revealed a significantly ($p < 0.001$) lower mean PCV in parasitaemic animals ($21.5 \pm 0.46\text{SE}$) than aparasitaemic ones ($24.3 \pm 0.11\text{SE}$). Generally, the prevalence noted in the current study is moderate. However the observation of such level of infection in a dry season suggests that the disease is still an important constraint of cattle production in the study areas. Thus, we recommend that the existing parasite and vector control interventions need to be strengthened with special emphasis to Dawuro Zone where the prevalence was significantly higher. As the current sampling was done only once and in a dry season only, further longitudinal studies including all the seasons of the year need to be considered in the future.

1. Introduction

Animal trypanosomosis is a very important disease that affects the health and productivity of livestock in sub-Saharan Africa (Swallow, 2000; Sutcliffe et al., 2014; Alsan, 2015; Giordani et al., 2016). It is particularly important in Ethiopia where agriculture makes the backbone of the economy and crop production is largely dependent on

animal traction power. Cattle trypanosomosis is caused principally by three species of trypanosome (*Trypanosoma congolense*, *T. vivax* and *T. brucei*) which are transmitted biologically by several species of tsetse flies (*Glossina*) and also mechanically by *Tabanus* and *Stomoxys* spp (Steverding, 2008; OIE, 2013). Clinically, the disease is characterized by intermittent fever, anemia, rapid loss of body condition, emaciation, collapse, and death in untreated animal (Radostits et al., 2007; Taylor

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et al., 2007).

In Ethiopia, animal trypanosomosis is widely distributed across the tsetse infested belts found in the western and southern regions of the country. In these regions, about 220,000 Km² of fertile land is infested by *Glossina* species (Cecchi et al., 2008). Cattle production plays a key role in the livelihood of southern and western regions of Ethiopia; however, their production potential is not fully utilized due to endemic diseases like trypanosomosis (Abebe, 2005). Trypanosomiasis is threatening the agricultural production and cattle breeding more severely than any other livestock disease (Moti et al., 2015). Five species of *Glossina* have been implicated in the transmission of Trypanosoma species in Ethiopia including, *G. pallidipes*, *G. m. submorsitans*, *G. fuscipes*, *G. tachinoides* and *G. longipennis* (Abebe, 2005; Miruk et al., 2008; Fikru et al., 2012; Tafese et al., 2012). Nonetheless, the distribution and density of these vectors vary from area to area depending on the existing ecological conditions and vector control interventions.

Despite various attempts over the past few decades to control animal trypanosomosis across the tsetse fly belts of Ethiopia, the disease persists as major animal health problem causing considerable morbidity and mortality in livestock. Perhaps, this is because the control interventions made so far were not well organized and sustainable. Also, the magnitude of the intervention varies from place to place. According to a more recent study, the prevalence of trypanosomosis in cattle in southern Ethiopia is 27.5% (Zeryhun and Abraham, 2012). Although there are several studies of bovine trypanosomosis in Ethiopia, most of the studies were restricted to small study areas and considered a small sample size which generated only limited information. Therefore, this study was aimed to estimate the prevalence of cattle trypanosomosis and apparent density of vectors, and identify the potential risk factors in a wider geographical area considering a larger sample size.

2. Materials and methods

2.1. Study area

The study was conducted in two purposively selected administrative zones, Gamo-Gofa and Dawuro located in Southern Nations, Nationalities and People's Regional State (SNNPRS) in Ethiopia. Gamo-Gofa zone is one of the areas where tsetse and trypanosomosis control had been carried out by the Southern Rift Valley Tsetse Eradication Project (STEP) while Dawuro had not been subject to control. A total of six districts (three from each zone) were selected randomly. The districts were Arbaminch Zuria, Mirab Abaya, Kamba, Gena Bosa, Tocha and Mareka. The first three districts were selected from Gamo-Gofa Zone whereas the other three were from Dawuro. Geographically, the districts are located between 5°72' – 7°20'N latitude and 36°93'–37°60' E longitudes (Fig. 1). The altitude of the districts ranges from 1003–1327 m above sea level.

2.2. Study population and production system

The study population constituted local zebu cattle (*Bos indicus*) kept under traditional husbandry practices. The predominant farming system in the study area is a mixed production system where by livestock and crop production practiced side by side. Cattle population in the area is largely dependent on grazing on naturally persistent communal pastures, but during dry period the main feed sources are crop residues. Cattle are kept mainly for milk production for household consumption, traction power, income generation, and manure. At night the animals were kept in houses shared with people or in some places in poorly constructed separate barns. According to CSA (2012) data, the total cattle population of Gamo-Gofa Zone is 1,308,815 whereas that of Dawuro Zone is 333,887.

2.3. Study design and sample size

A cross-sectional study was conducted in a dry season between November 2015 and March 2016. The sample size required for the parasitological study was determined by using the formula for simple random sampling given here under with 95% level of confidence (Thrusfield, 2005).

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

where, n is required sample size, P_{exp} is expected prevalence, and d is desired absolute precision. The sample size was calculated based on expected prevalence of 30% (Ayele et al., 2012) and absolute precision considered was 2%. Accordingly as per the predetermined parameters, the sample size computed was 2402 cattle. This was proportionally allocated to the two zones taking in to account the total cattle population. Thus, a sample size of 953 and 1449 were considered for Dawuro and Gamo-Gofa Zones, respectively. The sample size allocated for each Zone was further sub divided into the three districts again based on their total population size. At each district, the study animals were selected by systematic random sampling technique from the cattle herds grazing in communal pasture lands. Cattle herds found in a district were the smallest sampling unit in this study. As it was an extensive production system, a herd was defined as those cattle grazing on the same communal pasture/field.

2.4. Entomological survey

For the entomological survey, a total of 305 NGUtraps were deployed in 19villages as described by FAO (1992). Acetone and cow urine were used as a bait to attract flies. The traps were deployed at an interval of about 200–250 m apart and remained at one site for 72 h. All trap sites were geo-referenced using hand held global positioning system (GPS) units. Tsetse and other biting flies trapped were collected and counted. The apparent density was determined based on mean catches of flies in traps deployed and expressed as the number of fly catch/trap/day (FTD). Tsetse flies were identified to the species level while others were identified only to the genus level according to their characteristic morphological features (FAO, 1992).

2.5. Parasitological study

For parasitological examination, blood samples were collected from marginal ear vein of 2403 animals using heparinized capillary tubes which were sealed at one end with wax. The capillary tubes were filled to ¾th of their length and transferred to a haematocrit centrifuge and spun at 12,000 rpm for 5 min. The packed cell volume (PCV) was measured using a haematocrit reader for determination of the level of anemia. The capillary tubes were then cut using a diamond tipped pen 1 mm below the buffy coat to include the upper most layers of the red blood cells and 3 mm above to include the plasma. The content was expressed on to a clean microscopic slide, mixed well and covered with a 22 × 22 mm cover slip. This wet smear was examined by a microscope using 40 x objective lens for the presence of motile trypanosomes (Woo, 1987). Confirmation of trypanosome species was done by examination of Giemsa stained thin smears at 100 x magnification (Luckins, 1992; Murray, 1977). During sampling data about the age, sex, coat colour and body condition score (BCS) of each animal was recorded on specially designed format. The BCS was recorded as “good”, “medium” or “poor” based on the appearance of ribs and dorsal spines according to the description given for zebu cattle by Nicholson and Butterworth (1986).

2.6. Data analysis

Data collected from both parasitological and entomological studies

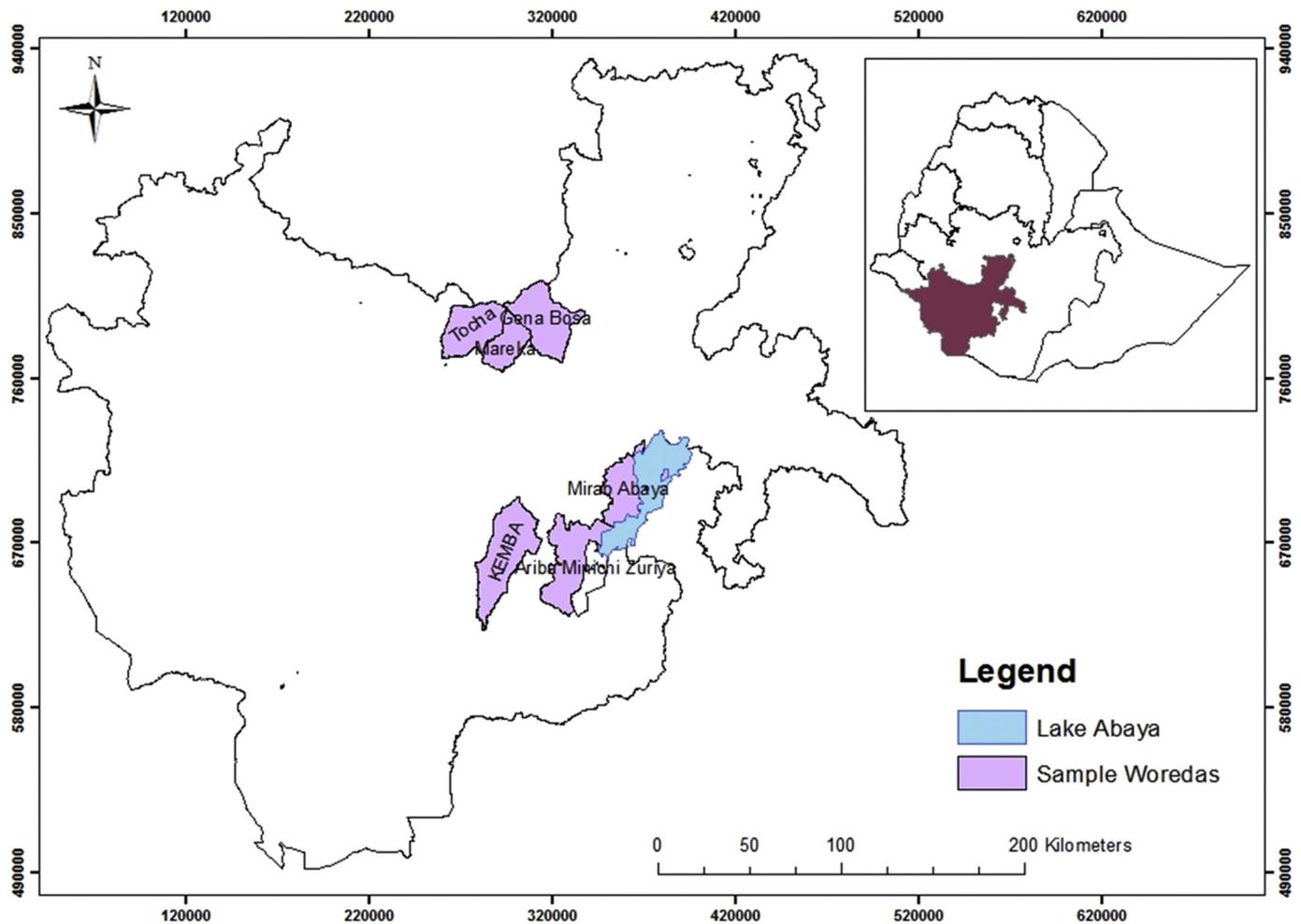


Fig. 1. Map of tsetse and trypanosomosis study districts in the southern Ethiopia.

were coded and entered in to a Microsoft Excel Spreadsheet. All statistical analyses were performed using Stata statistical software version 14.2 (Stata Corp, 4905 Lakeway Drive, College Station, Texas, 77845, USA) using survey command. Prevalence was estimated as the proportion of trypanosome positive animals over examined. The difference in the prevalence of trypanosomosis (dependent variable) between the study areas, sex, age and cattle of different coat colors (independent variables) was analyzed by using univariable and multivariable logistic regression analysis. The association of trypanosomosis with anemia (as determined by low mean PCV %) was evaluated by Student's *t*-test. PCV value cut off of 24% was used to determine the presence of anemia in the study animals (Latimer, 2011). The apparent tsetse density (AD) was expressed as the number of flies per traps per day (FTD). Level of precision was held at 95% and $p < 0.05$ set for significance.

3. Results

3.1. Entomological result

A total of 305 NGU traps were deployed in both of the study zones and about 4113 flies were captured in 72 h. From these, 1605 (39%) were tsetse flies of *Glossina* spp. while 2508 (61%) were other biting flies (*Stomoxys* and *Tabanus*). The overall apparent density of tsetse flies was 1.8F/T/D and of that of biting flies was 2.7F/T/D. Only one species of *Glossina*, *Glossina pallidipes*, was identified in both study areas (Table 1).

3.2. Prevalence of trypanosomosis and risk factors

From a total 2402 cattle examined by using the buffy coat technique, 123 (5.1%) animals were found infected by trypanosomes. Prevalence varied from 1.6% (Mirab Abaya) to 11.2% (Mareka) between the districts included in the study (Table 2).

Trypanosomosis was found to be significantly ($p < 0.05$) associated with study zone, coat colour and BCS while sex and age of the animals showed no significant effect ($p > 0.05$). Accordingly, prevalence was noted to be significantly higher in Dawuro than Gamo Gofa Zone, in black skinned animals than in others and in poorly conditioned than well-conditioned animals (Table 3).

Those risk factors with P -values < 0.25 in linear logistic were subjected to multivariable logistic regression analysis and the result shown in Table 4. The result of multivariable logistic regression analysis showed that only zone, body condition and colour were significant variation in the prevalence of trypanosomosis.

3.3. Species of trypanosomes identified

Two species of trypanosomes, *Trypanosoma congolense* and *Trypanosoma vivax*, were identified in both Gamo-Gofa and Dawuro zones. In both of the study areas, *T. congolense* was the predominant species which accounted for 61.8% of the overall infection. *T. vivax* was identified in 28.5% of the parasitaemic animals while mixed infection by the two species was observed in 9.8% (Table 5).

Table 1
Relative abundance of *Glossina pallidipes* and other biting flies in Gamo-Gofa and Dawuro Zones.

Zone	No of traps deployed	Days of deployment	<i>G. pallidipes</i> caught	F/T/D	Other biting flies caught	F/T/D
Gamo-Gofa	165	3	814	1.6	2423	4.9
Dawuro	140	3	791	1.9	85	0.2
Overall	305	3	1605	1.8	2508	2.7

FTD: flies per trap per day.

Table 2
Prevalence of trypanosomosis in cattle in Gamo-Gofa and Dawuro Zones, South Ethiopia.

Zone	District	No animals examined	No animals positive	Prevalence (%)	95% CI
Gamo-Gofa	Arbaminch	759	13	1.7	0.8–2.6
	Mirab-Abaya	306	5	1.6	0.2–3.1
	Kamba	384	10	2.6	1.0–4.2
Dawuro	Gena Bosa	196	13	6.6	3.1–10.1
	Mareka	561	63	11.2	8.6–13.8
	Tocha	196	19	9.7	5.5–13.8
Overall		2402	123	5.1	4.2–6.0

CI: confidence interval.

3.4. Hematological finding

The overall mean PCV value of all cattle was 24.1 (\pm 0.11SE). The mean PCV of parasitaemic animals (21.5 \pm 0.46SE) was significantly ($p < 0.001$) lower than that of aparasitaemic ones (24.3 \pm 0.11SE). Moreover, the study showed that 67.5% of the infected and 57.7% of uninfected cattle were found anemic based on a PCV cut off value of 24% (Latimer, 2011) (Table 6).

4. Discussion

In the present entomological survey, a total of 1605 tsetse flies and 2508 other biting flies were trapped by deploying 305 NGU traps for 72 h. *Glossina pallidipes* was the only species of *Glossina*/tsetse flies caught in all districts surveyed. This observation is consistent with previous studies, which reported *G. pallidipes* as the single or predominant tsetse fly in south-west areas and Omo-Ghibe tsetse belt of Ethiopia (Asha et al., 2008; Daya and Abebe, 2008; Zeleke, 2011; Ayele

et al., 2012; Teka et al., 2012; Girma et al., 2014; Sheferaw et al., 2016; Abebe et al., 2017). Generally the apparent density of tsetse flies (1.8 FTD) documented in the current study areas is lower than the figures reported by most of the studies conducted in the same geographical region (Zeleke, 2011; Ayele et al., 2012; Abebe et al., 2017) although it is comparable to that recorded by Sheferaw et al. (2016). Of the two study zones, the mean apparent density of *G. pallidipes* was relatively lower in Gamo Gofa zone. In the current study, other biting flies like *Tabanids* and *Stomoxys* were detected in a higher density than *G. pallidipes* and this signifies the possibility of mechanical transmission of trypanosomosis caused by *T. vivax* in the study areas even in the absence of *Glossina* species.

The overall prevalence of trypanosomosis in the current study areas was 5.1% and varied widely (1.6–11.2%) between the districts. Both linear and multivariable logistic regression analysis revealed that there was significantly ($p < 0.001$) higher prevalence in Dawuro zone (10.0%) than Gamo Gofa (1.9%). It was noted that the odds of trypanosomosis in cattle in Dawuro zone was 5.6 times higher than cattle in Gamo Gofa zone. The difference in trypanosomosis prevalence between the two zones matches with the entomological findings. The significant variation in prevalence between the two study Zones may be attributed to the interventions for tsetse and trypanosomosis control, which have been carried out over the past 20 years by STEP program in the Gamo Gofa zone but not in Dawuro zone.

Two species of *Trypanosoma*, namely *T. congolense* and *T. vivax*, were noted to cause bovine trypanosomosis in the present study areas albeit the majority of infection (61.8%) was caused by *T. congolense*. The predominance of *T. congolense* in the current study is in a general agreement with various reports from south western part of the country (Asha et al., 2008; Daya and Abebe, 2008; Miruk et al., 2008; Tadesse and Tsegaye, 2010; Ayele et al., 2012; Teka et al., 2012; Berhe et al., 2015; Sheferaw et al., 2016; Abebe et al., 2017; Bezabih and Bisho, 2017; Tadele and Ayichew, 2017). Perhaps the preponderance of *T.*

Table 3
Univariable logistic regression analysis for potential risk factors of trypanosomosis in cattle.

Risk factor	No of cattle examined	Number positive	Prevalence (95% CI)	OR (95% CI)	P-value
Study Zone					
Gamo-Gofa	1449	28	1.93 (1.2–2.6)	Ref	–
Dawuro	953	95	10.0 (8.1–11.9)	5.6 (3.7–8.6)	< 0.001
Coat colour					
White	543	16	2.9 (1.5–4.4)	Ref	–
Black	367	46	12.5 (9.1–15.9)	4.7 (2.63–8.48)	< 0.001
Red	1328	48	3.6 (2.6–4.6)	1.2 (0.70–2.19)	0.471
Gray	164	13	7.9 (3.8–12.1)	2.8 (1.33–6.03)	0.007
Sex					
Male	1139	52	4.6 (3.5–5.9)	Ref	–
Female	1263	71	5.6 (4.5–7.0)	1.2 (0.9–1.8)	0.278
Age					
Young (\leq 3 years)	753	44	5.8 (4.4–7.8)	1.2 (0.8–1.8)	0.242
Adult (> 3 years)	1649	79	4.8 (3.9–5.9)	Ref	–
BCS					
Poor	767	62	8.1 (6.4–10.2)	10.4 (1.4–75.6)	0.021
Medium	1516	60	4.0 (3.1–5.1)	4.9 (0.7–35.4)	0.118
Good	119	1	0.8 (0.1–5.8)	Ref	–

CI: confidence interval, OR: odds ratio, Ref: reference category.

Table 4
Multivariable logistic regression analysis for potential risk factors of trypanosomosis in cattle.

Risk factor	No of cattle examined	Number positive	Prevalence (95% CI)	OR (95% CI)	P-value
Study Zone					
Gamo-Gofa	1449	28	1.93 (1.2–2.6)	Ref	–
Dawuro	953	95	10.0 (8.1–11.9)	6.1 (3.7–10.1)	0.000
Coat colour					
White	543	16	2.9 (1.5–4.4)	Ref	–
Black	367	46	12.5 (9.1–15.9)	1.9 (1.0–3.5)	0.044
Red	1328	48	3.6 (2.6–4.6)	0.9 (0.5–1.6)	0.678
Gray	164	13	7.9 (3.8–12.1)	3.8 (1.7–8.7)	0.001
Body condition score					
Poor	767	62	8.1 (6.4–10.2)	10.9 (1.5–80.5)	0.019
Medium	1516	60	4.0 (3.1–5.1)	4.8 (0.6–35.3)	0.127
Good	119	1	0.8 (0.1–5.8)	Ref	–
Constant					0.000

CI: confidence interval, OR: odds ratio, Ref: reference category.

Table 5
The proportion of *Trypanosoma* species encountered in parasitaemic animals in the study areas.

Study area (Zone)	Number of animals infected	Number and percentage of single infections		Number and percentage of mixed infections
		<i>T. congolense</i>	<i>T. vivax</i>	
Gamo-Gofa	28	18 (64.3%)	9 (32.1%)	1 (3.6%)
Dawuro	95	58 (61.1%)	26 (27.4%)	11 (11.6%)
Overall	123	76 (61.8%)	35 (28.5%)	12 (9.8%)

congolense in the present study areas might be due to exposure of cattle to *G. pallidipes*, which is the only tsetse fly caught in the study areas and thought to be more efficient transmitter of *T. congolense* than *T. vivax* in East Africa (Langridge, 1976).

Bovine trypanosomosis in the present study was found to be influenced by the colour of cattle. As shown by univariable and multivariable logistic regression analysis, trypanosomosis was significantly higher in cattle with black coat colour ($P < 0.001$) than in white skinned animals. The odd of trypanosomosis in cattle with black coat colour was 1.9 times higher than that of white skinned animals. This finding is in agreement with the observation of Abebe et al. (2017) in Omo-Ghibe tsetse belt, Sheferaw et al. (2016) in selected parts of the southern rift valley and Girma et al. (2014) around Arba-Minch town. The present finding also accords with the assumption that tsetse flies are more attracted to large dark colors like the hides of cow and is the reason why tsetse traps or targets are made out of dark-colored material (Seifert, 1996; Leak, 1999).

Both in univariable and multivariable logistic regression analysis, body condition score was found to be one of the factors that significantly associated with trypanosomosis in cattle. It is well established fact that trypanosomosis is a wasting disease that results in a progressive loss of body condition (Radostits et al., 2007), although other factors such as parasitism and nutritional stress could play a role in the development of poor body condition (Steverding, 2008). Conversely, poor body condition can also be a risk factor for trypanosomosis as animals with poor condition have weaker immunity and thus are at

Table 6
Mean PCV of trypanosome infected and uninfected cattle in the study areas.

Infection status	No Animals	Animals proportion with PCV < 24%	Mean PCV%	SE	95% CI for mean	t	P
Infected	123	67.5	21.5	0.46	20.6–22.4		
Uninfected	2279	57.7	24.3	0.11	24.1–24.5	5.82	< 0.001
Overall	2402	58.2	24.1	0.11	23.9–24.3		

CI: confidence interval; PCV: Packed cell volume,

higher risk of infection by trypanosomes. Generally the present study finding is in agreement with the previous reports from Ethiopia (Ayele et al., 2012; Lelisa et al., 2014; Abebe et al., 2017).

It is known that anemia is one of the main clinical features of African animal trypanosomosis. It is usually determined by measurement of PCV of individual animals (Van den Bossche and Rowlands, 2001; Marcotty et al., 2008). Assessment of PCV in the present study resulted in an overall mean value of $24.1\% \pm 0.11$ (95% CI: 23.9–24.3) in the study animals regardless of the infection status. This figure is almost a cut off point for determination of anemia in cattle (Latimer, 2011). However, evaluation of PCV with trypanosomosis status revealed a significantly ($p < 0.001$) lower mean value in parasitaemic (21.5 ± 0.46) than aparasitaemic cattle (24.3 ± 0.11). This finding is in line with other bovine trypanosomosis studies in different parts of the country (Daya and Abebe, 2008; Tadesse and Tsegaye, 2010; Ayele et al., 2012; Tafese et al., 2012; Lelisa et al., 2014; Sheferaw et al., 2016; Abebe et al., 2017; Bezabih and Bisho, 2017). Although mean PCV was significantly lower in parasitaemic animals, a considerably higher (57.7%) proportion of aparasitaemic animals were noted to be anemic, that is their PCV was below 24%. The detection of anemia in large number of aparasitaemic animals could be due to other factors like malnutrition, helminthosis or tick-borne haemoparasites. This warrants the need for investigation of the causes of anemia in cattle in the study areas other than trypanosomosis.

5. Conclusion

The observation of 5.1% mean prevalence of trypanosomosis in a dry season in the current study suggests that the disease is still an important problem of cattle production in Gamo Goffa and Dawuro Zones. The study showed bovine trypanosomosis in the study areas was mainly caused by *T. congolense* and transmitted predominantly by the cyclical vector, *G. pallidipes*. However, the study further revealed that there is a potential for mechanical transmission even in the absence of tsetse by other haematophagous flies like *Tabanids* and *Stomoxys*. Thus, we recommend that the existing vector and parasite control interventions in the study areas need to be strengthened and the strategies should also

consider the mechanical vectors as well. During control, special emphasis should be given to Dawuro zone, which had not been subjected to control through STEP project and where the prevalence was noted to be significantly higher. Finally, as the current study was done in a dry season and sampling was done only once, it might not have reflected the actual status of trypanosomosis and vector density in the study areas. Thus, further longitudinal studies including all the seasons of the year need to be considered in the future.

Ethical statement

As the local ethics committee ruled there is no formal ethics approval was required to conduct this research.

Declaration of Competing Interest

The authors declare that they have no financial and personal conflict of interest with any people or organizations.

Acknowledgement

The authors highly acknowledge the great assistance provided by Southern Tsetse Eradication Project. We are also grateful to the owners of the animals included in this study for their cooperation. The authors are also thankful to Mestewat Simeon for her great help in preparing map of the study areas.

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