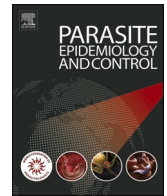




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Epidemiological investigation of bovine trypanosomosis and distribution of vectors in Jimma zone, Ethiopia

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ABSTRACT

Trypanosomosis is highly reliant on the distribution of vectors responsible for transmission. A cross-sectional study was conducted to determine the prevalence and associated risk factors of bovine trypanosomosis as well as the distribution of vectors in the Jimma zone, Ethiopia. Blood samples from a total of 2088 cattle were collected and tested using a buffy coat and Giemsa techniques. An overall 13.36% prevalence of trypanosomosis was recorded in study areas. The highest proportion of the infections was caused by *T. vivax* (44.80%) followed by *T. congolense* (36.92%) and mixed infection (18.28%) of both species. The study also revealed that trypanosomosis was associated with anemia as the mean PCV was significantly lower among trypanosome-infected animals (20.34 ± 4.39) than non-infected ones (27.98 ± 3.68). Moreover, anemia was more pronounced with *T. congolense* infection (19.54 ± 3.22) than *T. vivax* (21.07 ± 3.96) and mixed infection of both species (20.16 ± 2.71). This study identified age, body condition, and agro-ecology as risk factors for the occurrence of trypanosomosis in cattle. Vector survey was conducted by deploying 377 mono-pyramidal traps in selected districts. Accordingly, *Glossina* species and other biting flies (*Stomoxys* and *Tabanus*) were identified with an apparent density of 5.27 and 1.74 fly/trap/day, respectively. Moreover, a higher 4.49 fly/trap/day of *G. tachinoides* than *G. morsitans submorsitans* (0.79 fly/trap/day) was noted in study areas. The present study indicated that trypanosomosis is the major cattle production constraint in the areas. Hence, applicable management techniques of the disease and its vector should be implemented and further investigation involving molecular technique should be conducted in different seasons.

1. Introduction

Bovine trypanosomosis impedes cattle production, particularly in Sub-Sahara African countries. The disease occurs in large areas and constitutes a significant threat to the survival and productivity of cattle in these countries (Oluwafemi et al., 2007; Seyoum et al., 2013; Duguma et al., 2015). Trypanosomosis is caused by a unicellular protozoan parasite that belongs to the genus *Trypanosoma* (Holmes et al., 2004). The epidemiology of trypanosomosis in tsetse-infested areas of Africa is determined by the *Trypanosoma* parasite, tsetse fly, reservoir host, and livestock. Climate and vegetation are non-biological factors that influenced the epidemiology of the disease and those factors could directly affect birth, death, or migration rates of the vector as well as their density (Lord et al., 2018; Meharenet et al., 2020). Tsetse-transmitted trypanosomosis is much dependent on the distribution and capacity of *Glossina* species

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responsible for transmission. Among these species, the savannah and riverine species are the most important, since they inhabit areas suitable for grazing and watering lands of cattle (Cecchi et al., 2008).

Trypanosomosis is the major problem to cattle production and other agricultural development in Ethiopia. It is also an important blood parasite disease in humans (Denbarga et al., 2012; Seyoum et al., 2013). About 180,000–220,000 km² of potential land of Ethiopia was infested with tsetse flies and 14 million cattle are at risk of trypanosomosis (Abebe, 2005; Duguma et al., 2015). *Trypanosoma congolense*, *T. vivax*, and *T. brucei* are the most pathogenic *Trypanosoma* species within the country that transmitted by tsetse flies. Several studies indicated that five *Glossina* species have existed in Ethiopia, however, only four of them (*G. morsitans submorsitans*, *G. pallidipes*, *G. tachinoides*, and *G. fuscipes fuscipes*) are widespread and economically important (Abebe, 2005; Meharenet et al., 2020). Moreover, trypanosomosis is also transmitted mechanically by biting flies like *Stomoxys* and *Tabanus* (Cheremet et al., 2004). The distribution of *Trypanosoma* species is mainly limited tsetse belt area of the country like the west, southwest, and southern parts of Ethiopia. Nevertheless, *T. vivax* can cause disease outside of the belt area (Getachew, 2005; Girma et al., 2014).

Trypanosomosis affected directly the meat and milk productivity of cattle, increase abortion, and mortality rate (Leta et al., 2016). About 200 million US dollars were lost from the national economy due to the direct and indirect impact of trypanosomosis on agricultural and livestock production (Seyoum et al., 2013). Trypanosomosis decreased the work efficiency of oxen and hinder the introduction of drought cattle in tsetse-infested areas for crop farming (Siyum et al., 2014). The magnitude of the problem requires a multidisciplinary approach for effectively promoting sustainable agriculture and rural development strategies (Tulu, 2019).

The principle of prevention and control of trypanosomosis depends on reducing the contact between cattle and vectors. The control methods of trypanosomosis mainly include control of tsetse fly numbers, use of a trypanocidal drug, and use of cattle breed that tolerate the disease (Achenef and Bekele, 2013; Bouyer et al., 2014). To effectively control trypanosomosis, it is important to know the epidemiology of the disease and its vector distribution in the areas (Ebhodaghe et al., 2018). Therefore, this study was conducted to determine the prevalence and associated risk factors of bovine trypanosomosis, distribution, and density of vectors in the study areas.

2. Materials and methods

2.1. Study areas and design

The cross-sectional study was conducted in selected districts of Jimma zone, namely, Limu Seka, Goma, Gumay, and Dedo from October 2018 to October 2019. Jimma zone is located in the southwestern part of Ethiopia about 345 km far from Addis Ababa, the capital city of the country. It covers a total area of 19,305.5km². The zone is situated at an altitude range of 1000 to 3360 m above sea level. The average temperature varied from 25 to 30 °C maximum and 7–12 °C minimum. It received the highest annual rainfall up to

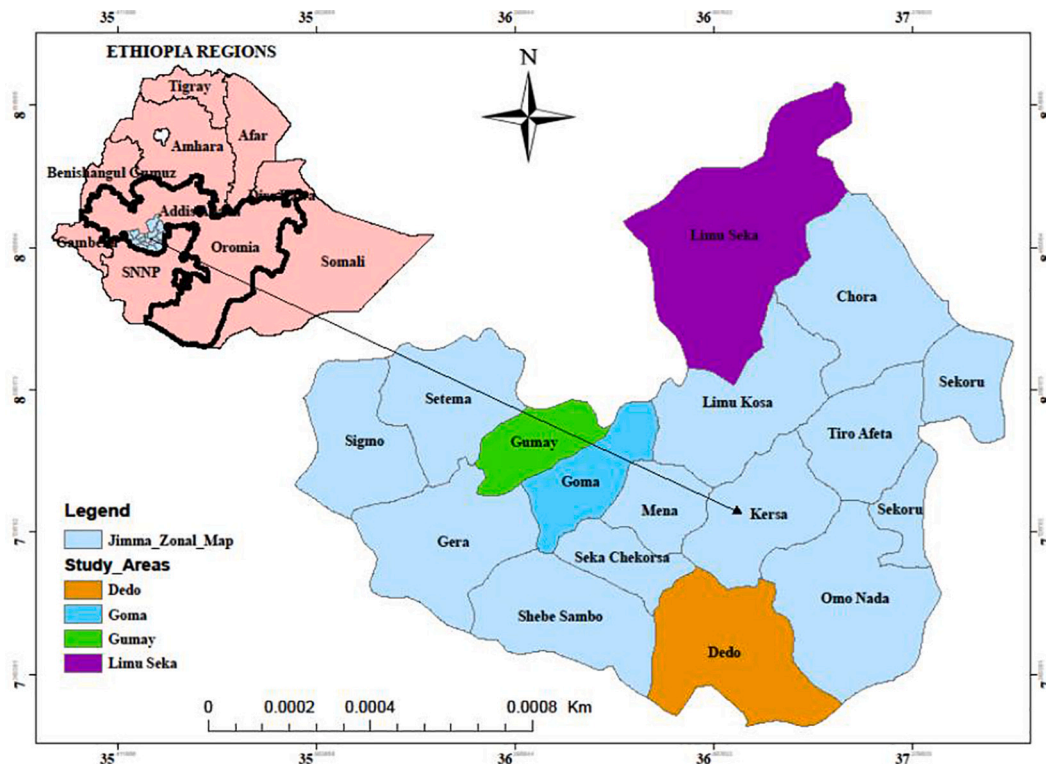


Fig. 1. Map showing study districts in Jimma zone.

1200–2800 mm per year. The rivers located in the zone are Didessa, Ghibe, and their tributaries which created valleys suitable for insect reproduction. Savanna grassland, forest, riverine, and bush lands are the major vegetation in the areas (Fig. 1). According to Libeau et al. (1995) the agro-ecology of Ethiopia was classified as lowland (500–1500 m above sea level) and midland (1500–2300 m above sea level). Several wild animals such as African buffalos (*Syncerus caffer*), bush pig (*Potamochoerus larvatus*), warthog (*Phacochoerus africanus*), bush buck (*Tragelaphus scriptus*), kudu (*Tragelaphus imberbis*), hippopotamus (*Hippopotamus amphibius*), crocodile (*Crocodylus porosus*), hyena (*Crocuta crocuta*), and snakes (*Serpentes*) are claimed to serve as sources of food for the vector of *Trypanosoma* parasite (Tola et al., 2016). Jimma zone has an estimated 2,324,110 head of cattle, 760,330 head of sheep, and 439,562 head of goats (CSA, 2017). An extensive system is the major management system with communal herding in the zone. Similarly, local cattle breeds are the dominant breed in the areas.

2.2. Study animals

The study population includes cattle of different ages, body condition, and sex kept under an extensive management system in selected districts of the Jimma zone. The body condition score (BCS) of zebu cattle was recorded by grouping as poor (score 1&2), medium (score 3), and good (score 4&5) based on the appearance of ribs and vertebral spinous processes (Nicholson and Butterworth, 1986). The hair color coat was categorized as white, red, black, and mixed color based on observation during sample collection. Classification of management system was done based on the criteria adopted by Richard (1993). Consequently, extensive management system for those cattle that were kept outdoor during the daytime and allowed to graze on communal or private owner pasture land. The age of study cattle was estimated using their dentition as described by Pasquini et al. (2003) and categorized as young (1 to 3 years) and adult (>3 years). Study sample units were local cattle breeds with one year age and above.

2.3. Sampling methods and sample size determination

A multistage sampling method was conducted with the zone as the highest and individual animals as the lowest sampling stage and district, peasant association (PA), village, and herd in between the two stages. Jimma zone was selected purposively due to a large number of cattle and the zone was thought to be highly infected with trypanosomosis. From a total of ten districts in the Jimma zone, four districts were selected by the lottery method, namely, Limu Seka, Goma, Gumay, and Dedo. Similarly, twenty-four PAs were selected from Limu Seka, Goma, Gumay, and Dedo districts using a simple random sampling method. A total of 48 villages were selected from these PAs based on the number of the villages in PAs. A total of 142 herds were selected from forty-eight villages using the lottery method. Herd known as *Ulle* in the local language (Afan Oromo) was a clustering of cattle that share common grazing areas and watering points. Likewise, *Abba Ulle* was an important contact person in the village that facilitates cooperation among cattle owners (Robi and Gelalcha, 2020). The sampling frames of the individual cattle were obtained from *Abba Ulle* in each respective village. A simple random sampling method was conducted to sample individual cattle from each herd. The sample sizes selected from each herd varied depending on the number of animals in each herd. The sample size was determined according to the formula given by Thrusfield (2005) with an expected prevalence of 13.02% (Ebhodaghe et al., 2018) at 95% confidence interval and 5% desired absolute precision for four districts. Hence, a total of 174 animals were required to be selected from each district (a total of 696 cattle). However, to increase precision, the sample size was increased by three-fold. Thus, a total of 2088 cattle were included in the study. Presumptive risk factors for bovine trypanosomosis were recorded during sampling.

2.4. Parasitological study

Blood samples were collected from the marginal ear vein by piercing the vein with a sterile lancet. Then, blood was drawn by a heparinized capillary tube. The collected blood samples were centrifuge at 12,000 rpm for 5 min immediately in a hematocrit centrifuge. The tubes were taken from the hematocrit centrifuge and placed on the microhematocrit reader to determine the PCV for each sample. Packed cell volume (PCV) below 24% was considered as anemia (Van den Bossche et al., 2000; OIE, 2008). The contents of the capillary tube (about 1 mm above and below the buffy coat) were examined using the buffy coat technique to reveal the *Trypanosoma* parasite under $\times 40$ magnification using a light microscope (Murray et al., 1977). A thin blood smear was prepared from *Trypanosoma* positive samples and stained with *Giemsa* for *Trypanosoma* species identification under oil immersion $\times 100$ objective lens. The *Trypanosoma* species were distinguished using their size, position of the kinetoplast, presence of undulating membranes, and length of the free flagella according to Picozzi et al. (2002) and OIE (2008).

2.5. Entomological study

A total of 377 mono-pyramidal traps were deployed in the selected district of the Jimma zone. The mono-pyramidal trap was used due to its ability to collect and conserves the tsetse flies, which were used to determine and monitor the entomological parameters such as apparent density. This type of trap has proven to be highly effective in controlling the tsetse flies population. The mono-pyramidal trap is also acceptable for large-scale *Glossina* flies management. Furthermore, this type of trap is simple to construct and transport (Simarro et al., 1990; Gouteux and Lancien, 1986). Three tsetse fly attractants (acetone, octenol (1-oct-3-nel), and three days old cow urine) were used (Brightwell et al., 1997; Kassa, 2005). These attractants were placed on the ground about 30 cm upwind of the trap in separate bottles. The traps were positioned for two consecutive days with a mean interval between traps of 250 m in most likely areas for finding tsetse based on the presence of gallery forests and the location of rivers and streams after clearing up to 2-3 m radius of the

trap site to enhance the visibility of the traps and to prevent from the possible fire damage (Gouteux and Lancien, 1986). The trap deployment sites were selected to represent all vegetation type could be associated with the tsetse fly, reproduction, behavior, feeding, and others. After 48 h of deployment, the catchments of each trap were sorted by fly species and then counted, identified, and analyzed. The sex of flies was identified by observing the posterior end of the ventral aspect of the abdominal by hand lens and stereomicroscope. The male flies were identified by having enlarged hypopygium in the posterior ventral part of the abdomen which is lacking in female flies. The Brunhes et al. (1998) key was used to determine the species in the reference laboratory. The coordination and altitude of each trap were recorded using GPS. The apparent density (arithmetic mean catches per trap per day) of flies was calculated according to the formula given by Leak (1999).

2.6. Data analysis

Data recorded during the study were stored in Microsoft Excel® window 2010 and analyzed by STATA version 11.0 for Windows (Stata Corp. College Station, TX, USA). The prevalence of the parasite was computed by dividing the number of positive samples by total samples. For each prevalence, binomial exact 95% confidence interval (CI) was calculated using EpiTool. The mean PCV values of infected cattle against that of non-infected cattle were compared using a *t*-test. Moreover, the mean PCV among three *Trypanosoma* species were compared using one-way ANOVA. The correlation between the altitude (altitude of the site where traps were deployed) and density of *Glossina* species was analyzed using Pearson correlation. The correlation coefficient was categorized as weak (0–0.30), moderate (0.30–0.70), and strong (0.70–1.00). Screening of different potential risk factors associated with *Trypanosoma* infection was done using the univariable random effects logistic regression analysis. The herd was used as a random effect to interpret the potential clustering of cattle in the herds and for the difference in herd sizes. The variables with $p \leq 0.25$ in univariable logistic regression, after checking for multicollinearity using collinear matrix index and interaction effect using cross-product terms were taken forward for multivariable modeling. The backward stepwise procedure was used for a further selection of the variables. Potential risk factors of *Trypanosoma* infection were identified by multivariable random effects logistic regression analysis. The strength of risk factors associated with bovine trypanosomosis was assessed using the adjusted odds ratio (AOR). A covariate was considered confounder and included in the model if its inclusion altered the odd ratio of the estimated risk by >20% (Dohoo et al., 2009). The model fitness was observed using the Hosmer-Lemeshow test. The model validation was also evaluated using the ROC curve. In all cases, differences between parameters were tested for significance at probability levels of 0.05 and a 95% confidence interval (CI). The apparent density of flies was calculated by dividing the total number of tsetse flies captured ($\sum F$) by-product number of functional traps used to catch them (T) and the number of days for which the traps were operational (D): $FTD = \sum F/T \times D$.

3. Results

3.1. Parasitological study results

From a total of 2088 examined cattle, 279 were positive for the *Trypanosoma* parasite using the parasitological technique. An overall 13.36% of trypanosomosis prevalence was recorded in the study areas. The highest (15.49%) and lowest (11.70%) prevalence of the parasite was recorded in Limu Seka and Gumay districts, respectively. *Trypanosoma vivax* was the dominant species with a proportion of 44.80% followed by *T. congolense* (36.92%) and mixed infection of *T. vivax* and *T. congolense* (18.28%) as presented in Table 1.

This study indicated that trypanosomosis was associated with anemia as the mean of PCV of infected cattle ($20.34\% \pm 4.39$ SD) was significantly lower than non-infected ones ($27.98\% \pm 3.68$ SD). The anemia was highly associated with *T. congolense* ($19.54\% \pm 3.22$ SD) compared to *T. vivax* ($21.07\% \pm 3.96$ SD) and mixed of both species ($20.16\% \pm 2.71$ SD) as stated in Table 2.

In univariable analysis of the hypothesized risk factors of *Trypanosoma* infection, there was a statistically significant difference ($p = 0.020$) between the young and adult cattle. Adult cattle had higher odds (OR = 2.4) of *Trypanosoma* parasite infection compared to younger cattle. Moreover, a significant difference ($p = 0.032$) was observed among the body condition of cattle. The odds of trypanosomosis were 2.5 times higher among cattle with poor body condition score than those in good body condition. However, study district, agro-ecology, sex, and skin coat color were not statistically significant (Table 3).

The multivariable logistic analysis showed that age, body condition score, and agro-ecology were associated with the occurrence of bovine trypanosomosis (Table 4). An insignificant difference was observed among variables regarding their interactions and

Table 1
Prevalence and distribution of *Trypanosoma* species in study areas.

Districts	Numbers of examined	Prevalence (95%; CI)	<i>Trypanosoma</i> species		
			<i>T. vivax</i> (%)	<i>T. congolense</i> (%)	Mixed (%)
Limu Seka	523	15.49(12.39–18.59)	41(14.70)	27(9.68)	13(4.66)
Goma	512	12.30(9.46–15.15)	24(8.60)	26(9.32)	13(4.66)
Gumay	530	11.70(8.96–14.43)	27(9.68)	21(7.53)	14(5.02)
Dedo	523	13.96(10.99–16.93)	33(11.83)	29(10.39)	11(3.94)
Overall	2088	13.36(11.90–14.82)	125(44.80)	103(36.92)	51(18.28)

CI: Confidence interval.

Table 2
Association between mean PCV and bovine trypanosomosis in study areas.

Infection status	Observation	Mean PCV \pm SD	CI; 95%	P-value
Non-infected	1809	27.98% \pm 3.68	27.81–28.15	
Infected	279	20.34% \pm 4.39	19.82–20.86	0.0001
With <i>T. vivax</i>	125	21.07% \pm 3.96	20.19–21.95	
With <i>T. congolense</i>	103	19.54% \pm 3.22	18.72–20.37	0.001
With mixed of both species	51	20.16% \pm 2.71	20.40–21.92	

PCV: Packed Cell Volume; SD: Standard Deviation; CI: Confidence Interval.

Table 3
Univariable logistic regression of putative risk factors of bovine trypanosomosis in study areas.

Variables	Category	Total animals examined	Total animals positive (%)	Crude OR(95%; CI)	P-value
District					0.267
	Limu Seka	523	81(15.49)		
	Goma	512	63(12.30)	1.3(0.92–1.86)	0.140
	Gumay	530	62(11.70)	1.4(0.97–1.97)	0.074
	Dedo	523	73(13.96)	1.1(0.80–1.59)	0.485
Agro-ecology					
	Midland	593	70(11.80)		
	Lowland	1495	209(13.98)	0.8(0.62–1.10)	0.188
Sex					
	Female	794	101(12.72)		
	Male	1294	178(13.76)	0.9(0.70–1.19)	0.500
Age					
	Young	920	112(12.17)		
	Adult	1168	167 (14.30)	2.4(2.05–2.74)	0.020
Body condition score					0.032
	Good	423	44(10.40)		
	Medium	885	109(12.32)	1.5(1.06–2.04)	0.022
	Poor	780	126(14.87)	2.5(2.06–4.04)	0.016
Skin coat color					0.220
	White	271	28(10.33)		
	Red	903	121(13.40)	1.3(0.89–1.87)	0.185
	Mixed	360	50(13.89)	1.2(0.80–1.91)	0.345
	Black	554	80(14.44)	1.6(1.03–2.35)	0.037

OR: Odds ratio; CI: Confidence interval.

multicollinearity. A Hosmer-Lemeshow test ($\chi^2 = 3.02, p = 0.56$) showed that the model was fit the data. Further, the area under the ROC curve (0.57) indicated that the model had a good predictive ability.

3.2. Entomological study results

From a total of 377 mono-pyramidal shape deployed traps, 5290 tsetse flies and other biting flies were caught during the study period. *Glossina* species accounted for 75.14% ($n = 3975$) of the total fly catch while other biting flies mainly genus *Stomoxys* and *Tabanus* contributed to 24.86% ($n = 1315$) as described in Fig. 2. Two *Glossina* species namely *G. tachinoides* and *G. morsitans submorsitans* were identified in the selected district of the Jimma zone. A higher proportion of *G. tachinoides* 85.11% than *G. morsitans submorsitans* 14.89% were identified in study areas. The apparent fly densities of *Glossina* species and other hematophagous insects

Table 4
Final multivariable logistic regression model for potential risk factors of bovine trypanosomosis in study areas.

Factors	Total animal examined	Total animal positive (%)	Adjusted OR(95%; CI)	P-value
Age				
Young	920	112(12.17)		
Adult	1168	167 (14.30)	2.4(2.06–2.75)	0.017
Body condition score				0.029
Good	423	44(10.40)		
Medium	885	109(12.32)	1.5(1.06–2.04)	0.023
Poor	780	126(14.87)	2.5(2.09–3.08)	0.013
Agro-ecology				
Midland	593	70(11.80)		
Lowland	1495	209(13.98)	2.6(2.11–3.29)	0.011

OR: Odds ratio; CI: Confidence interval.

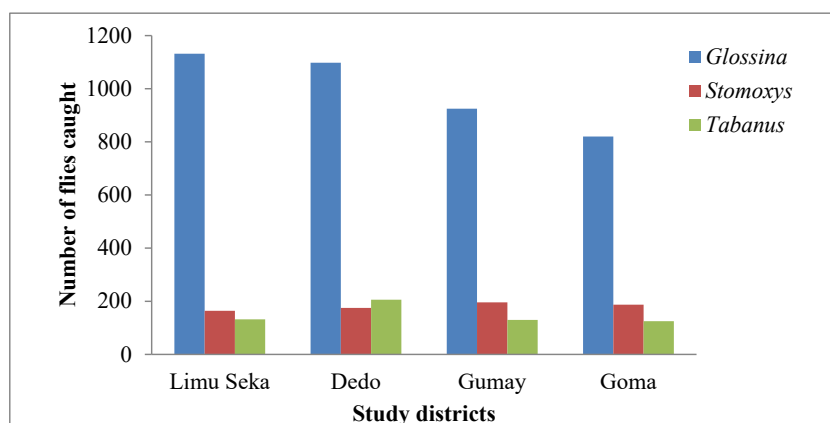


Fig. 2. Number of flies caught in each district.

were 5.27 and 1.74 fly/trap/day, respectively. Similarly, the apparent fly densities of *G. tachinoides* and *G. morsitans submorsitans* were 4.49 and 0.79 fly/trap/day, respectively. Out of the total *G. tachinoides* trapped, 66.83% were female and 33.17% were male for *G. tachinoides*. As well, 63.18% were female and 37.46% were male for *G. morsitans submorsitans* (Table 5). A strong negative correlation was observed between altitude (altitude of sites where traps were deployed) and density of *Glossina* species ($r = -0.748$, $p = 0.001$).

4. Discussion

This study helps to implement an appropriate method for the control and prevention of the disease and its vectors in the Jimma zone, Ethiopia. The current study revealed a prevalence of 13.36% bovine trypanosomosis caused by *T. vivax* and *T. congolense* and verified that two species of *Glossina* (*G. tachinoides* and *G. m. submorsitans*) and other biting flies service as potential vectors for *Trypanosoma* infection. In addition, the occurrence of bovine trypanosomosis is influenced by age, body condition of cattle as well as agro-ecology.

The prevalence (13.36%) of bovine trypanosomosis recorded in the present study is in close agreement with the finding of Degneh et al. (2017) and Feyissa et al. (2011), who reported a prevalence of 14.08% in Gidami and 14.20% in Humbo districts, respectively. This might be due to similar tsetse flies controlling methods that had been taken in the districts, the treatment strategies and the same diagnosis technique used. Another possible explanation for the similarity of prevalence might be because of similar vegetation-covered in the districts like savanna grassland, forest, riverine, and bush land. However, the prevalence recorded in the current study is lower than the value reported by Cherenet et al. (2006) 25.70% in northwest and Mulaw et al. (2011) 28.10% in western parts of the country. We found higher trypanosomosis prevalence than those reported by Tadesse and Tsegaye (2010), Duguma et al. (2015), and Meharennet et al. (2020). A significantly higher prevalence of trypanosomosis was recorded in lowland (13.98%) than in midland (11.80%). The cattle from lowland had three times higher odds of trypanosomosis compared to those in midland. This finding is in agreement with the observation of Sinshaw et al. (2006) in three highland districts bordering Lake Tana, Mekuria and Gadisa (2011) in Metekel and Awi zone, and Degneh et al. (2017) in the Gidami district. This could be agro-ecology directly influenced ecological parameters like vegetation, temperature, and humidity that affected vectors' survival, reproduction rate, and intensity (Cherenet et al., 2004; Van den Bossche et al., 2010). The highest (15.49%) prevalence of trypanosomosis was recorded in the Limu Seka district. However, no significant difference ($p = 0.267$) was observed among the study districts. Even though this result differs from some earlier studies (Tadesse and Tsegaye, 2010; Siyum et al., 2014; Meharennet et al., 2020), it is similar with those of Zemedkun et al. (2016) and Gebisa et al. (2020). Lack of significant variation in the prevalence of trypanosomosis among the districts sampled might be due to similar tsetse flies controlling methods that had been taken by Bedele NTTICC (National Tsetse and Trypanosomosis Investigation and Control

Table 5

Distribution of *Glossina* species in study areas.

Districts	Fly species								Total no. of fly	FTD	No traps	Alt
	<i>G. tachinoides</i>				<i>G. morsitans submorsitans</i>							
	Female	Male	Total	FTD	Female	Male	Total	FTD				
Limu Seka	768	364	1132	5.90	0	0	0	0	1132	5.90	96	1353
Gumay	533	287	820	4.61	0	0	0	0	820	4.61	89	1459
Goma	629	296	925	4.72	0	0	0	0	925	4.72	98	1393
Dedo	331	175	506	2.69	374	218	592	3.15	1098	5.84	94	1154
Total	2261	1122	3383	4.49	374	218	592	0.79	3975	5.27	377	

FTD: fly/trap/day, Alt: average altitude of the site where traps were deployed.

Center), having the same flies' belt and the same treatment strategies. This may be contributed by the differences in agro-ecology, vector density, the practice of trypanocidal drug used, and fly control operation that affected the occurrence of the parasite (Maje-kodunmi et al., 2013; Geiger et al., 2015). It is noted that the parasitological method could be a relatively less sensitive diagnostic method as results of it fails to detect 66% of infected cattle (Marcotty et al., 2008). The molecular diagnostic techniques which enable precise identification of the parasite at the species level and serological diagnostic method are more sensitive (Murray et al., 1977).

In the current study *T. vivax* was the most prevalent *Trypanosoma* species responsible for bovine trypanosomosis. This finding is in agreement with the fact that *T. vivax* was the major pathogenic *Trypanosoma* species in western Ethiopia (Cherenet et al., 2004; Abebe, 2005; Mulaw et al., 2011) and other African countries (Leak, 1999; McDermott et al., 2003). The highest prevalence of *T. vivax* recorded in this study is also similar with previous findings in Ethiopia (Sinshaw et al., 2006; Gebisa et al., 2020) and elsewhere (Nimpaye et al., 2011; Swai and Kaaya, 2012). The dominance of *T. vivax* may be due to its ability to be transmitted both cyclically (tsetse flies) and mechanically (biting flies). Another possible explanation for the high proportion of *T. vivax* compared to the other *Trypanosoma* species could be that *T. vivax* has a shorter lifecycle within the tsetse fly proboscis and a fast multiplication of parasitaemia in their host that could lead to high detection in cattle (Jones and Dávila, 2001; Osorio et al., 2008). The mixed infection of *T. congolense* and *T. vivax* which is documented in this study is in agreement with the results of Dagnachew et al. (2005), Mekuria and Gadisa (2011), and Denbarga et al. (2012). The mixed infection often occurred in areas where more than one *Trypanosoma* species is present (Moti et al., 2015; Giordani et al., 2016).

Anemia is a well-known clinical feature of *Trypanosoma* infection in cattle. It is usually determined by measured the PCV of individual cattle (Marcotty et al., 2008). The present result revealed that trypanosomosis strongly causes anemia – PCV less than 24% (Van den Bossche and Rowlands, 2001). However, there were also non-infected cattle with the PCV value of less than 24%. This may be due to delayed recovery from anemia after treatment with trypanocidal drugs. Moreover, a low PCV is not only caused by trypanosomosis, but also by poor nutrition, helminthosis, and tick-borne disease (Moti et al., 2013). Furthermore, anemia was more severe in cattle infected with *T. congolense* than *T. vivax* and mixed infected of both species. Our finding is also agreed with the previous studies on bovine trypanosomosis (Mungube et al., 2012; Meharenet et al., 2020), in which anemia was more severe in *T. congolense* infected cattle compared to *T. vivax*. This may be due to the development of *T. congolense* mainly limited to the intravascular blood of host animals and result in hemolysis of red blood cells (Van den Bossche and Rowlands, 2001).

This study also revealed that body condition was significantly associated with the occurrence of trypanosomosis. The odds of trypanosomosis were 2.5 times higher among cattle with poor body condition scores than those with good body condition. The current study finding is in line with previous reports from Ethiopia (Degneh et al., 2017; Gebisa et al., 2020; Meharenet et al., 2020). This could be explained as cattle having poor body conditions were less resistant to parasite or trypanosomosis was mainly characterized by progressive weight loss (Radostits et al., 2007). Age was another factor that has shown significantly associated with the occurrence of trypanosomosis in cattle. Adult cattle were about two times more likely to be exposed to the *Trypanosoma* parasite compared to younger cattle. The association between the prevalence of trypanosomosis and the age of cattle in the present result was in agreement with the previous findings (Tadesse and Tsegaye, 2010; Ngongolo et al., 2019). This might be tsetse flies are attracted more by the odor of large animals. Moreover, Adult cattle were traveled a long distance for grazing which tends to increase the chance to contact a vector of the disease.

A slightly higher prevalence of trypanosomosis was observed in female than male cattle. However, an insignificant difference ($p = 0.500$) was observed between the sex of cattle and the prevalence of trypanosomosis. Similar findings were reported by Zemedkun et al. (2016) in Wolaita zone and Gebisa et al. (2020) in Buno Bedele zone. This attributed to cattle have an equal chance of exposure to the *Trypanosoma* parasite and its vectors in the districts. Similarly, a slightly higher prevalence of trypanosomosis was observed in cattle having black skin color following by mixed skin color. This related to tsetse flies are more attracted to large black colors like hides of cattle and are the reason why tsetse traps or targets are made out of black-colored material (Leak, 1999). However, no significant difference ($p = 0.220$) was observed among the different skin colors of cattle. This finding is supported by Teka et al. (2012) and Tulu et al. (2018), who reported that the occurrence of bovine trypanosomosis was not influenced by skin coat color of cattle.

Bloodsucking insects like *Stomoxys* and *Tabanus* other than *Glossina* species were abundant in the study areas. This result is supported by previous findings (Kone et al., 2011; Baldacchino et al., 2014), who reported that transmission of the *Trypanosoma* parasite also occurs through biting flies of the genus *Stomoxys* and *Tabanus*. This might be due to *Tabanus* and *Stomoxys* genus was a world-wide distribution even outside the tsetse belt areas of Africa (Nakayima et al., 2012). The apparent density (5.27 fly/trap/day) of *Glossina* species recorded in the present study agreed with Meharenet and Alemu (2020), who discussed the abundance of *Glossina* species were highly related to ecology, vegetation, and habitat factors. Moreover, it is also similar with several findings in different areas in Sokoru district (Meharenet, 2018), Limu Kosa district (Meharenet and Alemu, 2020), and in Kellem Wollega zone (Kassaye and Tsegaye, 2016). This could be attributed to similarities in ecology conditions characterized by forest, riverine, bushes and grasslands, and similar tsetse fly management activities that had been taken by Bedele NTTICC in the districts. However, we found a lower value for fly/trap/day than those reported by Megersa et al. (2019) and Ayele et al. (2012) 10.9 fly/trap/day in Boter-Tolay and 23 fly/trap/day in Darmallo districts, respectively. In other words, the variability in ecology, vegetation, and habitat factors in different areas are determinant in the flies' populations.

Two *Glossina* species were identified in study districts, namely; *G. tachinoides* and *G. morsitans submorsitans*. These species were also reported by other researchers (Dagnachew et al., 2005; Meharenet et al., 2020). Dedo district is populated by these two *Glossina* species whereas only *G. tachinoide* was identified in Limu Seka, Goma, and Gumay districts. Limu Seka, Goma, and Gumay districts are drained by large rivers such as Didessa, Ghibe, and their tributaries which explain the existence of riverine flies (*G. tachinoides*). The districts are also characterized by a spoiled savannah environment since *G. m. submorsitans* is sensitive to encroachment (Duguma et al., 2015). However, the natural vegetation in the Dedo district is characterized by riverine, bush-glass land, and open glass land which is suitable

for different *Glossina* species (Bezabih et al., 2016). Moreover, the ecological features of Dedo district are more diverse, providing niches for different coexisting species. The density of *Glossina* species was varied among districts because of differences in altitude (1154–1459 m above sea level). The low altitude was significantly and negatively associated with the density of *Glossina* species ($r = -0.748$, $p = 0.001$). Indeed, altitude directly influences ecological parameters such as vegetation cover and inducing particularly microclimatic zones of different elevations (Leak, 1999; Lord et al., 2018). In the present study higher females (66.29%) than male (33.71%) *Glossina* species were trapped. This is in close agreement with the finding of Leak (1999), who reported that females would be 70% to 80% in unbiased sampled. This may be due to several factors altered the favor of female flies like selective attractiveness of the trap, longer life expectancy of female compared to male, and a higher mortality rate of the male because of their higher susceptibility to insecticides (Rowlands et al., 1993; Leak, 1999).

The study did not include molecular techniques due to the limitation of facility and resources. Thus, further study including the molecular techniques should be conducted on trypanosomosis and its vectors. This study also did not allow the collection of blood samples from cattle during all the seasons to evaluate the seasonal effect on disease prevalence. It also important to conducted future studies with a longitudinal study design over more extended periods of time.

5. Conclusion

The finding of this study showed a high prevalence of trypanosomosis in cattle and this indicates that trypanosomosis is the major constraint to cattle production in the areas. *Trypanosoma vivax* and *T. congolense* were the two species responsible for trypanosomosis in study areas although the former is more dominant. This study also identified age, body condition, and agro-ecology as risk factors for the occurrence of trypanosomosis in cattle. Anemia was one of the characteristics of the *Trypanosoma* infection in cattle. *Glossina tachinoide* and *G. morsitans submorsitans* were two *Glossina* species prevalent in the areas with the dominance of *G. tachinoide*. Therefore, the appropriate management techniques of trypanosomosis and its vectors should be designed and implemented. Moreover, a further study involving more sensitive molecular techniques should be conducted on bovine trypanosomosis and its vector by considering different seasons.

Ethical statement

All procedures were carried out according to the experiment practice and standards approved by the animal welfare and research ethics committee at the National Institute for control and Eradication of tsetse fly and trypanosomosis, Bedele that is followed the international guidelines for animal welfare. Besides, a verbal agreement was got from cattle owners. Full cooperation and voluntary participation of all cattle owners were obtained by assuring them the confidentiality of their involvement.

Declaration of Competing Interest

The authors have not declared any conflict of interests.

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