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Full Length Research Paper

Bovine Trypanosomosis and Tsetse Fly Densities during the wet humid period in Loka Abaya District of Sidama National Regional State, Southern Ethiopia

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The study was carried from October-December 2018 in the wet humid periods using a Cross-sectional study design to determine the prevalence of bovine trypanosomosis and the major species of trypanosomes and to identify the associated risk factors for the disease in selected localities of Loka abaya district. Blood sample collected from 384 randomly selected cattle to measure Packed Cell Volume (PCV). The buffy coat techniques (BCT) was employed for the detection of trypanosome parasite. NGU-2G traps were deployed for the collection of tsetse flies. From 384 blood samples 8.33% tested positive for trypanosomes. 24(75%) tested for *Trypanosoma congolense*, followed by *Trypanosoma vivax* 8(25%). Animals with poor body condition score showed statistically significant difference (P<0.049). The infection rates between anemic and nonanemic animals was significantly different (P<0.003). 1548 tsetse flies and other biting flies were trapped with 963(62.21%) tsetseflies, 271(17.51%) *Tabanus,* and 48(3.10%) were *Stomoxys.* The overall apparent density of tsetse and biting flies was 14.3 and 8.84 F/T/D, respectively. In conclusion the results of the present study confirmed that trypanosome parasites and tsetse flies were prevalent and still a challenge to cattle production demanding integrated community based strategies to eradicate or minimize their effects on livestock production.

Key words: Buffy coat techniques (BCT), Bovine, Loka Abaya District, packed cell volume (PCV), prevalence, risk factors, tsetse flies, Ethiopia.

INTRODUCTION

Trypanosomosis is the disease that affects a wide range of mammalian species including human (Taylor et al., 2016; Constable et al., 2017). It is a disease of poverty as it hampers livestock production in the continent. Trypanosomosis is mainly transmitted cyclically by tsetse flies (*Glossina* species) or mechanically by biting flies (Constable et al., 2017). The three main groups of tsetse flies for transmission of trypanosomosis as described by Tesfaye and Ibrahim (2017), are the open land of savanna inhabitants (*Glossina morsitans*), river banks and adjecent area inhabtants (*Glossina palpalis*) and those inhabiting dense forest areas (*Glossina fusca*).

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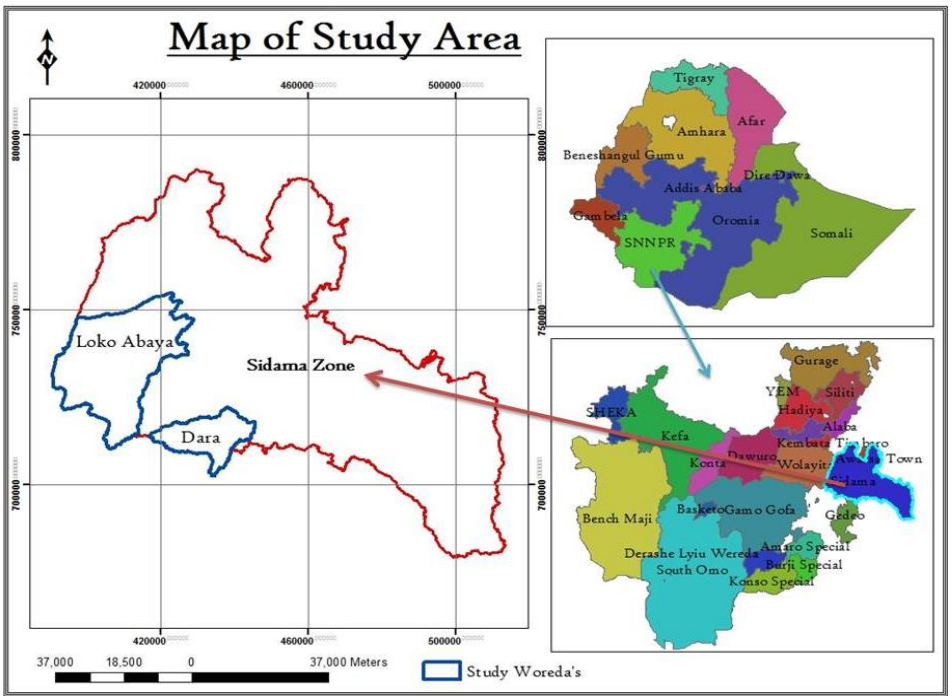

Figure 1. Map of Study District. Source: Sidama Adminstration (2014).

Moreover from epidemiology perspective it was reported as three factors influence the disease, namely the distribution of the vectors, the virulence of the parasite and response of the host (Urquhart et al., 1996; cited in Lejebo et al., 2019). Trypanosomes transmitted by *Glossina* species are endemic in sub-Saharan Africa known as the ″tsetse belt″ that occur between 20°N and 30°S. *Glossina* species infested 10 million Km^2 of Africa involving 38 countries (Constable et al., 2017). Tsetse flies are vectors of blood parasite of the genus Trypanosoma,which causes trypanosomiasis to both human beings and animals. According to Getachew (2005); cited in Lejebo (2019) the most important species of trypanosomes affecting cattle, sheep and goats in Ethiopia are *Trypanosome congolense, Trypanosoma vivax* and *Trypanosoma brucei*. According to Lemu et al. (2019), animal Trypanosmosis in Ethiopia is wide spread in the Western, South and Southwestern lowland regions associated with the major river basins of Abay, Omo, Ghibe and Baro. These tsetse infested valleys and lowlands are the most fertile and suitable for agricultural development and human settlement.

In Ethiopia, the prevalence of cattle trypanosomosis, only with concentration diagnostic technique, range from 2.1% (Yigzaw et al., 2017) to 41.6% (Wogayehu et al., 2017). According to Mulatu et al., 2016), tsetse infested areas amounts to about 200,000 km^2 of land in the five adminstative regions of Ethiopia. In the Southern region alone, about 49.000 km^2 area is estimated to be infested by tsetse flies affecting 4 to 5 million heads of cattle. While in the rift valley region of the southern Ethiopia, 25,000 km^2 is supposed to be infested by tsetse flies putting about 2.6 million heads of cattle at risk (STEP, 2013). Eventhough, trypanosomosis is economically important livestock disease, in the study localities there was no study conducted in the district. Therefore, the present study was carried out to;

(i) investigate the status of (prevalence) of bovine trypanosomosis in selected localities of Loka abaya district.
(ii) identify vector species and their distribution with their apparent densities
(iii) determine the potential risk factors of trypanosomosis.
The outcome of this study will be used for the strategic monitoring and suppression of the vectors to integrate all efforts to undertake area wide activities to reduce the infection of cattle and promote the livelihood of the farmers and other community members.

METHODOLOGY

Study settings

The study was conducted in the wet-humid period from October-December, 2018 in three selected localities of Loka Abaya district, Sidama National Regional State, Southern Ethiopia. The district is bordered by Bilatie river basin and Wolaita zone in the west, Gedeo zone in the south, and Oromia Regional State in the east and north. The area is characterized with mixed, crop-livestock production, farming system (Sidama Administration, 2014) (Figure 1). Loka Abaya has an altitude of 1001-1500 m above sea level in which large part is hot arid and some part as semi-arid agro-ecology. The

mean annual maximum and minimum mean annual temperature of the area was 22 and 26°C respectively, and average annual rainfall ranges 900-1400 mm with bimodal rain pattern, major short rainy seasons (September- December) and February to April respectively.

Agricultural crops include, haricot bean (*Phaseolus vulgaris*), Maize (*Zea mays*), Teff (*Eragrostistef*), sweet potato (*Ipomeabatatas*). According to the reports of Berou of Finace and Economic Development (BoFED, 2012) the livestock population of Loko Abaya was estimated to be 886, 242 bovine, 117,274 ovine, 99,817 caprine, 41160 equines and 442,428 poultry, 1,792,333 cattle, 456,740 sheep, and 324, 032 goats.

Most of the livestock were kept under transhumance way of livestock management by moving between different grazing lands, and due to the mobility cattle herds exposed to tsetse fly biting, vector of trypanosomosis, on the river banks (Gidabo, Bilatie) and near to Lake Abaya. These were areas where grazing land and water is abundant. The district consists of different types of vegetation including dry land savanna in dry tropical and warm temperate regions. Near to the study village there exists a park known as Loko Abaya National Park with an area of 500 km^2. In the study areas and its surrounding there are various wildlife resources, like, Lion, Antelope, Bush pig, Bush bug, Hippopotamus and Crocodiles. Moreover, there were three rivers namely: Bilatie, Gedabo and Derba that drain into Lake Abaya.

Study design

Study population and type

The study employed zebu cattle of with differences in sex, age, existing body conditions scores found on the same communal grazing land.Cattle population in three localities of Loka Abaya district (that is, Bura Bukito, Tulto Chericha and Abaya Zuria) were purposively selected based on accessibility, farmers cooperation and large cattle population. The design of study used was cross-sectional study type.

Sample size and sampling method

Study cattle were randomly and purposefully selected based on the factors, age, sex and body conditions. Sample size was estimated using the improved formula adapted from Thrusfield (2018). The samples were registerd by the livestock owners name and code or local name of the animal. Young and adult cattle were sampled following the procedures of (Bitew et al., 2011). Above one year old, both sexes of cattle with different body conformation were sampled randomly from a village of each study locality for examination of blood parasite (trypanosome). The body condition of sampled cattle was also estimated based on the parameters for zebu cattle (Nicholson and Butterworth, 1986), with numbers from 1 to 3 (good, medium and poor) respectively. Moreover, determination of parasitological samples from study localities was determined based on the preliminary assessment of the number of cases of bovine *trypanosomosis* and fly density reported by Dilla tsetse eradication project office. The study sites selected were tsetse and trypanosomosis control sites with continuous monitoring activities. Using the formula (Thrusfield, 2018), and considering an expected prevalence of 50 and 95% confidence level and 5% desired precision, the calculated sample size was 384.

$N=1.96^2 x P_{exp} (1-P_{exp})/d^2$

Where N=# of sample size
P_{exp}=expected prevalence
d^2=desired absolute precision

Study methodology and procedures

A total of 384 cattle between 1 and 10 year of age with differences in body conditions and color were sampled during wet-humid (October-Decmber, 2018) periods. Hematological, parasitological and entomological studies were conducted following the procedures below.

Parasitolohical and hematological servey

i) Measuring of packed cell volume (PCV): Blood samples were collected by puncturing marginal ear vein with a sterile lancet using a pair of heparinized capillary tubes. The tubes were placed in micro-hematocrit centrifuge fter symmetrically loading the tubes and closing the centrifuge lid,the specimen were allowed to centrifuge at 12,000 revolutions per minute (rpm) for 5 min. Then, using hematocrit capillary reader tubes were read, recorded and expressed as a percentage of packed red cells to the total volume of whole blood for each cattle to identify the general health condition of the animal. Animals with PCV < 25% were considered to be anemic (Murray et al., 1983).

ii) Buffy coat technique (BCT): After the centrifugation of blood sample, the contents of the capillary tube was cut using a diamond tipped pen 1 mm below the buffy coat to include the upper most layers of red blood cells.The contents of the capillary tube was expressed on to glass slide and covered with a 22 × 22 mm coverslip. The slide was examined under x40 objective and x10eye piece to identify trypanosome parasites by their movement (Murray et al., 1983; MOARD, 2007).

iii) Entomological study (tsetse survey): The tsetse flies were surveyed with the deployment of geo-referenced NUG-2G traps. The sites of traps deployment was on the four vegetation sites as described by Vreysen (2000), which includes Woody grassland (WGL) grassland with scattered trees, Bush land (BUL) habitats dominated by trees and shrubs, Reverie Forest (RF) trees along banks of rivers and streams and Cultivated land (CUL) habitat dominated by cultivated crops. 12 traps for each habitat were deployed following the standard procedures for *G. pallidipes* species. The traps were baited with decomposed cow urine using plastic bottles and acetone with dispensing vials after clearing the vegetation to increase visibility. Traps were deployed and stayed for 72 h within the altitude range of 1184 to 1350 masl. Each trap was set with approximate intervals of 100 m in shade and visible manner. After 72 h, trapped flies were collected from cages and counted, identified, sexed, and analyzed along with biting flies (IAEA, 2006). The apparent densities (AD) of identified tsetse flies and other mechanical vectors were calculated as fly catch/trap/day in relation to study sites and vegetarian types. *Glossina* spp. were characterised using identification keys including arista, shape of superior and inferior claspers and abdominal markings (FAO, 2008). Tsetse flies can be destinquished from other biting flies by two easily observed morphological features which includes the presemce of forward pointing proboscis on their heads and completely folded wings over the abdomen when at rest. *Stomoxys* spp. were identified using wing patterns, thoracic and abdominal markings, limb shape and colour, frons, mouth-parts and genitalia.*Tabanus* species were identified using several keys from different authors Oldroyd (1975), where the major taxonomic markers incude body shape, colour, sizes of distinct parts including head, thorax, abdomen, limbs, mouth-parts and wings.

Data analysis

Data collected from parasitological and entomological surveys were

Table 1. Trypanosomosis prevalence and identified *Trypanosoma* species in the study localities of Loka Abaya district.

Study localities	Nº examined	Nº positive (%)	*Identified Trypanosoma* species (%)	
			T. congolense	*T. vivax*
BuraBukito	128	10 (7.8)	9 (7.0)	1 (0.8)
Abaya Zuria	128	15 (11.7)	12 (9.4)	3 (2.3)
TultoChericha	128	7 (5.5)	3 (2.3)	4 (3.1)
Total	384	32 (8.3)	24 (6.3)	8 (2.1)

T. c :Trypanosoma congolense, T. v: Trypanosoma vivax.

Table 2. Results showing Univariable logistic regression analysis for the prevalence of Trypanosomosis and associated potential factors.

Potential factors	Levels of potential factors	Nº examined	Nº positive (%)	CI	χ^2	P-value
Sex	Male	92	5 (5.4)	2.3-12.5	Ref	
	Female	292	27 (9.2)	6.4-13.2	1.45	0.228
BCS	Good	61	2 (3.3)	0.8-12.4		0.039
	Medium	118	2 (1.7)	0.4-6.6	Ref	
	Poor	205	28 (13.7)	9.6-19.1	18.94	0.003
Age	≤3 years	63	7 (11.1)	5.3-21.7	0.88	0.341
	4-6 years	141	10 (7.1)	3.8-12.7	Ref	
	≥6 years	180	15 (8.3)	5.1-13.4		0.510
Localities	BuraBukito	128	10 (7.8)	4.2-14.0	3.32	0.295
	Abaya Zuria	128	15 (11.7)	7.2-18.6		0.081
	TultoChericha	128	7 (5.5)	2.6-11.1	Ref	
Color	Gray/White	170	11 (6.5)	3.6-11.3	Ref	
	Red/Bright brown	142	13 (9.2)	5.4-15.2	1.62	0.378
	Black-White	72	8 (11.1)	5.6-20.8		0.225
Total		384	32 (8.3)			

coded and entered in a Microsoft Excel sheet.The data for this study consists of nominal, ordinal, and scale data(continuous and categorical). All statistical analyses were done using SPSS (Statistical Packages for Social Sciences), version 20. Data was analyzed using Statistical Package for social Sciences (SPSS) version 20.0. Cross tabulations, Chi-square test, and Logistic regression were applied. The total prevalence rate was calculated by dividing the number of positives by the total number of animals examined and multipled by 100. The association between the trypanosome infection and associated risk factors where assessed by logistic regression.Chi-square test was employed to determine prevalence with sex, age and body condition scores ,where as two sample T-tests was used to compare the Mean PCV values between trypanosome positive and negative animals.In all analyses, the confidence interval level was 95% and p value $P<0.05$ was considered as significance. PCV value was grouped as anemic if it was less than 25% and normal if it was greater than 25% (Murray et al.,1983).The density of tsetse fly population was calculated by dividing the number of flies trapped by the number of traps deployed and number of days of deployment expressed as Fly/Trap/Day (Leak et al., 1987; Leak, 1999).

RESULTS AND DISCUSSION

Trypanosomosis prevalence and identified Trypanosoma species

384 cattle were examined to determine the presence of trypamosomosis by buffy coat technique.Trypanosomosis were detected in 32 cattle with an overall prevalence of 8.3%. Two species of trypanosomes were identified with *T. congolense* 24 as (6.3%) compared with *T. vivax* 8, as (2.1%), (Table 1). The prevalence of trypanosomosis within the three localities were 15 (11.7%), at Abaya zuria, 10(7.8%), at Bura bukito and 7(5.5%) at Tultochericha (Table 2).

PCV findings

The mean packed cell volume (PCV) value for all studied

Table 3. Mean PCV of apparently healthy and cattleinfected with *Trypanosoma* species.

Status	No examined	Mean% PCV	Std.Er	95% CI
Non-anemic	352	23.8	0.23	23.3-24.2
anemic	32	20.7	0.42	19.8-21.5
Overall	384	23.5	0.21	23.1-23.9

Table 4. Aparent density of tsetse flies and other biting flies in the study localities.

Localities	No of traps deployed	Days of deployment	*G. pallidipes (Tsetseflies)*	F/T/D	Stomoxys and Tabanids	F/T/D
Bura-Bukito	12	3	515	14.3	155	4.3
Tulto-Chericha	12	3	242	6.7	54	1.5
Abaya-Zuria	12	3	206	5.7	110	3.1
Overall	36	3	963	8.9	319	3.0

FTD: Flies per Trap per Day (Leak et al., 1987).

cattle was 23.5%. The mean PCV for parasitaemic and aparasitaemic cattle were 20.7% and 23.8, respectively (Table 3). So, the mean PCV was lower in cattle infected by trypanosome.

Entomological results

A total of 36 NGU traps were deployed at an interval of 100 m, and overall 1282 flies were caught in 72 h. From these, 963 (75.1%) 319 (24.9%) were *Glossina* species and other biting flies respectively. *Glossina pallidipes*, was the only Glossina species caught in the area. Other biting flies including Tabanids and Stomxys were also trapped. The Apparent Density (AD), calculated was 8.9 F/T/D and 3.0 F/T/D for tsetse flies and all otherthe biting flies respectively (Table 4).

DISCUSSION

In this study the overall prevalence of bovine trypanosomosis was 8.3%, and the prevalence's in the study localities, (Bura-Bukito, Tulto-Chericha and Abaya-Zuria) slightly varied and ranged from 7.0 to 11.7%. Most recent studies in this regard show that the estimates of bovine trypanosomosis falls within the range of 7.2 and 23% (Lelisa et al., 2014; Duguma et al., 2015; Leta et al., 2016; Abebe et al., 2017; Eshetu et al., 2017). The higher prevalence of the disease in Abaya zuria locality compared with other localities may be attributable due to the habitas (grassland and bush coverages) suitable for the vector (tsetse flies). Strong associations between the body conditions of cattle and trypanosome infection was also observed.The occurance of infection was 3.3, 1.7 and 13.7% in cattle with good, medium and poor body conditions respectively. The majority of the infected cattle had poor body condition because of the effect of trypanosomosis. Cattle with poor body condition are unable to tolerate the infection; and hence lead to chronic infection that is the nature of trypanosoma infection. However, poor body condition might be due to other pathogens and nutritional defeciencies (Mulatu et al., 2016).

The study also shows that the majority 75% of the infection was caused by *T. congolense* and 25% by *T. vivax*. The overall prevalence of *T*. *congolense* was significantly higher (P< 0.05) than *T*. *vivax*. The predominance of *T*. *congolense* in *Glossina* infested areas of Ethioipa and east Africa has been reported by many authors. Reports from various parts of the country, which is infested by *Glossina* support this opinion (Bekele and Nasir, 2011; Lelisa et al., 2014; Sheferaw et al., 2016; Degneh et al., 2017). The mean PCV of cattle with trypanosomosis (95% CI=19.8-21.5) was significantly higher than the apparently trypanosomosis free (95% CI= 23.3-24.2) cattle. This finding is in agreement with other many reports from various areas (Duguma et al., 2015; Eshetu et al., 2017). It is known and established fact that one of the major effect of infection with pathogenic trypanosomosis is anaemia (Constable et al., 2017).

The entomological survey results revealed that among the *Glossina* species the only tsetse fly species in the study area was *G*. *pallidipes*. Other biting flies include *Stomoxys* and*Tabanus* species. The overall apparent density of *G. pallidipes* was 8.9 Flies/Trap/Day compared with 3Flies/Trap/Day for other biting flies detected. It is well known that *G. pallidipes* is widespread and the only species that was reported from areas near to lake Abaya and around Arba Minch (Zekarias et al., 2014; Girma et al., 2014; Rodrigues et al., 2019). Apparent density of *Glossina* species ranging from 0.3 to 24.4 F/T/D was

reported from western part of Ethiopia. Such varations might have been due to differences in season and density of vegetation cover (Teka et al., 2012: cited in Mulatu et al., 2016). The mechanical vectors, *Stomoxys* and *Tabanus* species, widespread in Trypanosome infested areas of the country (Girma et al., 2014; Lemu et al., 2019).

CONCLUSION AND RECOMMENDATIONS

In conclusion,*T. congolense (*75%*),* was the predominat species of trypanosome followed by *T. vivax* (25%) in the study localities of Loka Abaya district. The overall prevalence of bovine trypanosomosis in Loka Abaya district was 8.3%. The overall apparent density of tsetse, Tabanus and Stomoxys were 8.9F/T/D, 2.5F/T/D and 0.4F/T/D, respectively. During entomological survey ,*G. pallidepes* was the only tsetse species identified in the study localities. The study also reveals infections with trypanosomosis negatively affects the body condition and PCV of animals. Significantly, the highest prevalence of trypanosome infection was observed in animals with poor body condition. It is therefore, possible to conclude that tsetse born trypanosomosis is still posing a threat to cattle production and management in the area. Based on the finding of this study the following recommendations are forwarded.

Further study to identify the prevalence of trypanosome parasites should be carried out. Control strategies that involve the use of chemicals (pour on), batied traps and targets and appropriate utilization of prophylactic and therapeutic majors should be adapted. Attempts should be made to extend and increase government and non-government veterinary services to give proper services to the challenged community by trypansomosis. Above all awareness creation of animal owners especially about proper drug use, and correct dose for treatment. Community participation in tsetse and trypanosomosis control will play major role in the reduction of the distribution and prevalence of tsetse fly and trypanosoma, respectively. Further surveys and studies should be carried out to strengthen feasible control methods of trypanosomosis and appropriate vector management strategies.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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