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Prevalence of Bovine Trypanosomosis and Apparent Density of Its Vectors in Shabe-Sombo District, Jimma Zone, Ethiopia

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Abstract: Animal trypanosomosis is one of the major disease problems affecting livestock development and agricultural productivity in Ethiopia as well as sub-Saharan African countries. A cross sectional study was conducted from November 2017 to March 2018 in four peasant associations (PAs) of Shabe-Sombo district, Oromiya regional state to determine the prevalence of bovine trypanosomosis and apparent density of tsetse flies. A total of 376 blood samples were collected from randomly selected animals. Packed Cell Volume (PCV) was determined and samples were examined for the presence of trypanosomes using the buffy coat technique and confirmed by Giemsa staining for positive samples. Thirty two (8.51%) from the tested samples were positive for trypanosomes. The dominant identified trypanosome species was *Trypanosoma congolense* (50%) and *T. vivax* (31.25%), *T. brucei* (6.25%) and mixed infections (*T. congolense* with *T. vivax*) (12.5%). The difference between prevalence of trypanosomes among study sites was statistically insignificant ($p>0.05$). The mean PCV value of parasitemic animals (20.64%) was significantly lower ($p<0.05$) than that of aparasitemic animals (27.46%). A total of 648 tsetse flies and 86 biting flies were caught by deploying 67 different types of traps. Of these tsetse flies, 60.2% was *Glosssina pallidipes* and the remaining were *G. fuscipes* (24.1%) and *G. morsitans-submorsitans* (15.7%). The overall apparent density of tsetse flies was 4.84 flies per trap per day (F/T/D). In conclusion, this study revealed that trypanosomes and their vectors are prevalent and have been posing a huge threat to cattle production in the area. Therefore, proper intervention strategies should be put in place and implemented to minimize the burden of the disease.

Key words: Bovine · Buffycoat · Prevalence · Shabe-Sombo · Trypanosomosis · Vectors

INTRODUCTION

Livestock is backbone of the socio economic system of most of the rural communities of Africa [1]. Ethiopia is known for its large and diverse livestock resource endowments. Livestock is primarily kept on small holdings where it provide drought power for crop production, manure for soil fertility and fuels, serves as a sources of grazing family diet and sources of cash income (from livestock and livestock products). Despite large livestock population, Ethiopia fails to optimally utilize this resource due to different constraints facing the livestock subsector. Shortage of nutrition, reproductive insufficiency, management constraints and animal disease are the major constraints [2]. One of the diseases hampering the livestock subsector is trypanosomosis [3].

Animal trypanosomiasis is a devastating disease caused by protozoan parasite of the different species of genus *Trypanosoma* that inhabits the blood and other tissues of vertebrates, including livestock, wildlife and human [4-7]. With exception of *T. equiperdum*, which is a venereal disease, all have arthropod vectors in which transmission is either cyclical or noncyclical. In cyclical transmission the tsetse fly is necessary, an intermediate in which the trypanosomes multiply, undergoing a serious of morphological transformations before it forms infective stage for next mammalian host are produced [8].

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Ethiopia is believed to have the largest livestock population in Africa, which is currently estimated to be 54 million cattle, 25.5 million sheep and 24.1 million goats [9]. However, the livestock industry of the country is suffering from debilitating diseases such as trypanosomosis with approximately 15% of all arable land or 220,000 km^2 area infested with tsetse flies [3]. Morbidity and mortality losses from ruminant livestock alone are estimated to be USD 200 million [10]. A number of studies have been undertaken in different parts of the country to determine the magnitude of this economically important disease [11-14]. So far one study shows a 14.23% prevalence of bovine trypanosomosis in Shebe-sombo district [15].

However, due to climatic change, ecological disturbances and human interventions the distribution of trypanosomes is dynamic. As a result, updating the prevalence (even in already studied areas) and status of the disease have paramount importance for understanding the epidemiology of the disease, to facilitate the choice of suitable control methods and to help in planning for development programs in the area. Therefore, the objectives of this study were to determine the prevalence of bovine trypanosomosis and its vectors density in Shabe-Sombo district.

MATERIALS AND METHODS

Study Area: The study was conducted in Shabe-Sombodistrict which is located in Jimma zone of southwestern Oromiya regional states and it is found about 50kms from Jimma town and 406 kms from Addis Ababa. Geographically, Shabe-Sombo town falls between 7° 50'00''N latitudes and 36° 51' 67''E longitudes. The total land area covers 1691km^2 with altitudes of 1400 to 2010 meter above sea level. The annual mean temperature ranges from 17°C to 23°C and receives annual rain fall greater than 1400mm. The livestock populations of the district were estimated to be 121,367 cattle, 40,592 sheep, 27,985 goats, 3,932 horses, 6,439 mules 7,548 donkeys and the livestock are free grazing. However, about 159,647 estimated numbers of animals live in the tsetse belt areas [16].

Study Population: The study population constituted of zebu cattle of various sexes, age groups and body conditions managed under small holder mixed crop-livestock farming system. The study was conducted on 376 randomly selected local breed cattle, selected from 4 peasant associations (PAs). Of these animals, 75 from Machi, 70 from Angacha, 118 from Kishe and 113 are from Gasera.

Study Design: A cross-sectional study was employed from November 2017 to March 2018 to determine the prevalence of bovine trypanosomosis and apparent density of its vectors in the study area. The PAs were selected purposively based on the information obtained from the district's office of livestock and fishery. The animals were selected randomly and restrained by farmers for sampling. Blood samples were collected by puncturing ear vein using lancet and collected in to heparinized microhaematocrit tubes and Buffy coat technique was used for diagnosis. A total of 376 animals were examined in this study. During sampling, sex, age and body condition of the animals were recorded. Body condition score for each cattle was estimated based on the technique given by [17] by visual and palpation of the body as good, medium and poor and with the amount of fat covering the rump, loin and degree of depression in the tail head area. The age of the animals were also estimated by their dentition as described by [18] and based on owners' information.

Sampling Method

Sample Size Determination: The sample size was determined based on the expected prevalence of 14.23% as previously reported by [15] and absolute desired precision of 5% at 95% confidence level as described by [19].

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

where, **n**= Sample size, **P**= the expected prevalence, **d**= the desired absolute precision

Accordingly, 188 animals were required to be sampled during study season. To increase precision, the obtained minimum sample size (188) was inflated by two folds, to account for the effect of randomness and representative population in the study. Therefore, a sample size of 376 was considered for this study. The limits of the associated interval indicate the specified bounds within which the estimate will lie with the defined level of confidence.

Sampling Strategy: A random sampling technique was employed to sample individual animal caught at communal grazing points of each village. A total of 376 samples were collected during the study period from all settlements. The species of animals sampled are only bovine and the sample size for each settlement varies with the availability of cattle.

Study Method

Survey of Trypanosomosis: Blood samples were collected aseptically in to heparinized microhaematocrit tubes by puncturing margin of ear vein with the tip of lancet. Then one end of the capillary tubes were sealed with sealant (Hawksley Ltd, Lancing, UK) and spun at 12,000 revolutions per minute (rpm) for five minutes to concentrate trypanosomes as Buffy coat and to separate blood cells. Then packed cell volume (PCV) were read on haematocrit reader and recorded. The capillary tubes were then broken just 1mm below Buffy coat and expressed on microscopic slide, mixed and covered with a 22x22mm cover slip. Then it was examined under x40 objective of microscope using dark ground Buffy coat technique to detect the presence of motile trypanosomes. For positive samples, Geimsa stain of thin blood smears were made, fixed with methanol for 5 minutes and examined under oil immersion using x100 objective to identify the species of trypanosomes [20, 21].

Entomological Survey: A total of 67 baited mono-pyramidal, mono-conical and bi-conical traps were deployed along suitable tsetse habitats such as bank of river and savannah lands to assess the apparent densities, distributions and species of tsetse flies involving in transmission of trypanosomes. All traps were baited with acetone, octenol and cow urine in three separate bottles and deployed at an interval of 200-250 meters [22]. To prevent caught of flies from insects like ants the underneath of each trap pole is smeared with grease. After 48 hours of trap deployment time the cages were collected and captured flies were identified according to morphological characteristics [23, 24].

Data Analysis: Raw data were entered into a Microsoft Excel spreadsheet. Data analysis was made using Statistical Package for Social Science (SPSS), version 20 software and descriptive statistics were used to summarize the data. The prevalence was calculated for all data as the number of infected individuals divided by the number of individuals examined and multiplied by 100. The association between the prevalence of trypanosome infection and different variables like age, sex and body conditions were assessed by odd ratio. The mean PCV values between trypanosome positive and negative animals were also assessed. The test result was considered as significant, when the calculated p-value was less than 0.05 at 95% confidence interval.

RESULTS

Parasitological Results: A total of 376 animals were sampled including 75, 70, 118 and 113 from Machi, Angacha, Kishe and Gasara Peasant Associations, respectively. Out of these, 32 animals were tested positive for trypanosomes and the overall prevalence of trypanosomosis was 8.51% in the study area. It was determined to be 10.67%, 8.57%, 11.02% and 4.42% in Machi, Angacha, Kishe and Gasera peasant associations respectively (Table 1). Higher infection rate occurred in female than male animals. The infection rate in male and female cattle was 7.03% and 10.16% respectively, But no significance difference was observed in prevalence between sex groups ($P>0.05$) (Table 2).

Prevalence of bovine trypanosomosis among age group was studied. The highest prevalence was observed in adult age groups (8.97%) and young cattle (6.67%). Both age groups were infected with trypanosoma species, but the obtained result indicates that there was no statistically significant difference in prevalence of bovine trypanosomosis among age groups of animals ($p>0.05$) (Table 3). Based on their body condition score, studied animals were categorized into poor, medium and good body conditions. A higher prevalence (19.75%) was seen in animals with poor body condition than those with medium (5.88%) and good (4.62%) body condition for the concerned blood parasite.

Hematological Result: The mean PCV of individual animals was measured and recorded before buffy coat examination to assess degree of anemia. As a result, the mean PCV of parasitaemic animals were 21.53±4.348 and aparasitaemic animals were 25.89±3.801 with statistically significant variation ($p<0.05$) (Table 4).

Distribution of the Species of Trypanosomes: Prevalence of trypanosomosis was varies within the species. The majority of trypanosome species identified was *Trypanosoma congolense* (50%) and *T. vivax* (31.25%) and the remaining 6.25% and 12.5% were, *T. brucei* and mixed infections (*T. congolense* with *T. vivax*), respectively (Table 1).

Table 1: Species of trypanosomes and their prevalence at four PAs of study area

				Trypanosoma species			
Pas	Number of examined	Number of positive	Prevalence (%)	*T. congo*	*T. vivax*	*T. brucei*	*Mixed*
Machi	75	8	10.67	4	2	1	1
Angacha	70	6	8.57	2	3	1	0
Kishe	118	13	11.02	7	4	0	2
Gasara	113	5	4.42	3	1	0	1
Total	376	32	8.51	16	10	2	4

Note: PAs=Peasant Associations, T. congo= T. congolense, Mixed (T. congolense and T. vivax)

Table 2: Species of tsetse flies and biting flies caught in 4 peasant associations of the study area

				Glossina Species Caught										
				G. p		*G.m.sub*		*G. f*				Biting flies		
Peasant Associations	Altitudes (masl)	Nºs of traps	Nºs of days traps deployed	M	F	M	F	M	F	Total	FTD	Tabanids	Stomoxys	Total
Machi	1326-1545	17	2	44	76	24	58	23	37	262	7.71	17	13	30
Angacha	1320-1341	16	2	32	50	-	-	9	14	105	3.28	14	6	20
Kishe	1286-1322	18	2	48	80	08	12	11	38	197	5.47	16	7	23
Gasera	1360-1383	16	2	18	42	-	-	08	16	84	2.63	9	4	13
Total		67		142	248	32	70	51	105	648	4.84	56	30	86

NOTE: M= male, F= female, FTD= Fly/Trap/Day, G.p= *Glosssina pallidipes*, G.m.s= *Glossina morsitans sub-morsitans*, G.f= *Glossina fuscipes*

Table 3. Prevalence rate of bovine trypanosomosis on the basis of body condition, age, sex and PAs of the animal at study area

							95% C.I	
Risk factors	Category	Nºs examined	Nºs positive	Prevalence (%)	P-value	OR	Lower	Upper
BC	Poor	81	16	19.75	0.002	0.197	0.069	0.564
	Medium	187	11	5.88	0.648	0.777	0.263	2.298
	Good	108	5	4.62				
Age	Young	75	5	6.67	0.524	1.380	0.513	3.712
	Adult	301	27	8.97				
Sex	Male	199	14	7.03	0.279	0.668	0.322	1.387
	Female	177	18	10.16				
Pas	Machi	75	8	10.67	0.109	0.388	0.122	1.235
	Angacha	70	6	8.57	0.259	0.494	0.145	1.683
	Kishe	118	13	11.02	0.070	0.374	0.129	1.086
	Gasera	113	5	4.42				
Total		376	32	8.51				

Table 4: The mean PCV values of parasitaemic and apparasitaemic animals

				95%CI		
PCV	Nºs examined	Mean	Standard deviation	Lower	Upper	P-value
Aparasitemic	344	25.89	3.801	25.49	26.29	0.001
Parasitemic	32	21.53	4.348	19.96	23.10	

Entomological Survey Results: A total of 648 tsetse flies and 86 biting flies (Tabanids and Stomoxys) were caught by deploying 67 traps mono-pyramidal, mono-conical and bi-conical traps. Tsetse flies caught during the study period were *Glossina pallidipes*, *G. m. submorsitans* and *G. fuscipes* (Table 2). The overall apparent density of tsetse fly and biting fly was 4.84 Flies/Trap/Day (FTD) and 0.64 F/T/D respectively. Female accounts 65.28% of total captured tsetse fly. Therefore, the overall apparent density of tsetse fly was found to be higher than that of biting fly during the study period.

DISCUSSION

In Ethiopia, animal trypanosomosis is one of the most important diseases limiting livestock productivity and agricultural development due to its high prevalence in the most arable and fertile land of Southwestern

and Northwestern part of the country following the greater river basins of Abay, Omo, Ghibe and Baro [25]. The study result showed that the disease, bovine trypamosomosis still remains great threat to the study area by impeding livestock production and hampering agricultural activities.

The overall prevalence of bovine trypanosomosis in present study area revealed that 8.51%. This finding was in line with previous result obtained on bovine trypanosomosis from different parts of Ethiopia by [26] (8.5%), [27] (8.3%), [28] (8.6%) and [29] (8.2%), from Dangur district of North Western, Mareka district of Southern part, Hawagelan district and East Gojam, respectively.

This present finding was decreased when compared with the reports of [15] who reported a prevalence of 14.23% in the study area. Similarly this result was slightly lower than the findings of [30-33] who reported an overall prevalence rate of 14.1% from Gidami, 12.4% from Abeshige, 13.14% from Seyo and 13.44% from Gawo Dale district, respectively.

The possible suggestions in the reduction of bovine trypanosomosis could be due to the presence of strategic and frequent tsetse fly control carried out by National Tsetse and Trypanosomosis Investigation and Control Center (NTTICC). The center applies variety of tsetse fly control methods, such as 1% deltametrin pour-on and insecticide impregnated targets which significantly reduces the prevalence of disease in the study area. Another possible reason for the low prevalence of the disease might be due to the differences in agro-ecology, sampling season, vector infection rate and animal susceptibility, therapeutic and prophylactic treatments with trypanocidal drugs, which obviously mask the epidemiological situation of the disease. Despite different trypanosomosis control methods has been practiced in the study area, the finding was still higher than the finding of [34-36] who reported 1.3%, 4.4% and 4.86 % from different parts of Ethiopia, respectively.

Among the different species of trypanosomes detected in study period, *T. congolense* (50%) was the most prevalent trypanosome species followed by *T. vivax* (31.25%). Mixed infection of *T. vivax* with *T. congolense* was also prevalent (12.5%) and the lowest prevalence was observed on *T. brucei* (6.25%). According to [5], *T. congolense* and *T. vivax* are the most prevalent trypanosomes that infect cattle in tsetse infested and tsetse free areas of the Ethiopia, respectively. The high proportion of *T. congolense* in tsetse infested areas of Ethiopia has been reported by many authors. Hence, it is in close agreement with the previous report of [10] (58%), [18] (61.9%), [36], (64%) [37] from southwestern parts of Ethiopia and [38] (61.77%) from western Ethiopia and it is far below the report of [28] (73%) and [39] (84.21%) from Hawagelan and Yayo district, respectively.

During the study period, the prevalence of bovine trypanosomosis was assessed between sexes of animals and among 32 trypanosome positive animals 10.16% of them were females and 7.03% were male animal. The trypanosome infection in female animals was slightly higher than in the male animals and this show that both female and male cattle were equally susceptible to trypanosomosis infection. This result is similar with previous results of [34, 37, 40] who obtained no significant difference ($p>0.05$) in susceptibility between the two sexes. Therefore, they have equal chance of coming in contact with flies and eventually harbor a disease.

Age wise comparison was also conducted at study area to assess prevalence rate of the bovine trypanosomosis by classifying animals in to young and adult age groups. The prevalence was found to be 6.67% in the young age group and 8.97% in the adult animals (Table 3). However, statistically there is no significant difference in infection rate among the different age groups ($p>0.05$). Body condition of the cattle was another factor that has shown strong association with trypanosomosis prevalence. A higher prevalence (19.75%) was seen in animals with poor body condition than those with medium (5.88%) and good (4.62%) body condition. However, no significant difference ($P>0.05$) was observed among the body conditions of cattle considered and infection with trypanosomes. This result was in close agreement with [37].

In the present study, the prevalence of the disease in the study peasant associations were 10.67%, 8.57%, 11.02% and 4.42% in Machi, Angacha, Kishe and Gasera, respectively and the difference was statistically non-significant ($p>0.05$). This might be due to that some sites of the study area have a common border sharing the same agro-ecology which is favorable for tsetse distribution. On other hand, uncontrolled animal movement between PAs and presence of different types of wild animals (which are important for the disease to act as a host) can facilitate distribution of the disease.

PCV of the animals could be an indicator of the health status of population under study. Even though other diseases such as helminthosis, thick borne disease and nutritional imbalances contribute to the low PCV values, trypanosomosis could also significantly contribute to

anaemia (low PCV). Of 376 examined animals, 106 (28.2%) of them had a PCV below normal range for bovine (24-46) [41] (Van den Bossche and Rowlands, 2001). Among 32 positive animals, 26 (81.25%) of them were anaemic (PCV<24). The mean PCV value of aparastaemic (25.89%) was higher than that of parasitaemic animals (21.53%) and the finding was agrees with the reports of [35, 39] who reported the mean PCV of parasitaemic animals to be lower than in a parasitaemic animals. As anaemia is the classical sign of the disease pathogenicity [42], the low PCV in parasitaemic animals could have contributed in reducing the mean PCV of the cattle.

In this study, the entomological findings revealed three species of tsetse flies (*Glossinapallidipes, G. m. submorsitans and G. fuscipes*) out of the six tsetse fly species reported in Ethiopia [3, 43]. Additionally, two genera of biting flies (Tabanus and Stomoxys) were identified during study period. These three species of tsetse fly along with biting flies were also reported by [44] and [45] in different part of Oromiya region, Ethiopia. The overall apparent density of *Glossina* species was found to be 4.84 flies/trap/day (FTD). This finding was in line with the report of [46, 47] who found an overall apparent density of 4.08 f/t/d and 4.5 f/t/d, respectively. The apparent fly density Tabanids and Stomoxys were found to be 0.42% f/t/d and 0.45% f/t/d, respectively.

The present entomological finding was far below the report of [48, 49] who reported 14.97 f/t/d and 11.9 f/t/d for tsetse at Arba Minch and Hewa Gelan, parts of Ethiopia, respectively. A higher number of female *Glossina* species 423 (65.28%) was caught than male 225 (34.72%), which is in line with various reports from the country [37, 38]. This could be attributed to the longer lifespan of female compared to male *Glossina* [50].

CONCLUSIONS AND RECOMMENDATIONS

The present study indicated that bovine trypanosmosis was found in different peasant associations of the Shabe-Sombo district. The overall prevalence of bovine trypanosomosis in present study area revealed 8.51%. *Trypanosoma congolense* and *T. vivax* were the dominant trypanosome species identified in the study area. Even though, the prevalence was low when compared with the previous study, currently the disease remains to be a serious threat to livestock production and agricultural activities in the settlement areas of the district and it have been associated with the presence of biological (Tsetse flies) and mechanical vectors. Based on the above conclusion, the following recommendations are forwarded: Designing and implementation of vector and trypanosomosis control should be continuously implemented in the area and neighboring woredas to reduce the burden of the disease and vectors, The vector population and the prevalence of the disease should be monitored regularly, Strict supervision on the usage of trypanocidal drugs (dosage) should be put in place to avoid trypanocidal drug resistant, Organize community-based intervention for the control and prevention of the disease and Use of the trypano-tolerant breeds of animals in the highly prevalent area should also be recommended.

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