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Bovine Trypanasomosis and Tsetse Fly Survey in Bure District, Western Ethiopia

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Astract: The study was carried out from October 2011 to May 2012 to estimate the prevalence of bovine trypanosomiasis, investigate the effect of parasitemia on packed cell volume (PCV) and determine tsetse fly density by using Giemsa stained thin blood smears examination and deployment of monopyramidal traps in selected peasant associations of Bure district, Western Ethiopia. The overall prevalence of bovine trypanosomosis in the study area was 6.1% (34/560). *Trypanosoma conglence* was identified to be the major cause of the disease accounting for 61.77% of the total infections observed followed by *T.vivax 35.3%* and mixed infection of *T. congolense* and *T. vivax* (2.9%). From the expected risk factors, origin, sex and body condition of the animal were not found to influence the prevalence of the disease ($P>0.05$) but, a significant association was obtained between the mean PCV and infection rate ($P<0.05$). The mean PCV was lower (16.5%) in parasitemic animal as compared to aparasatemic animals (26.1%). Furthermore, entomological survey using monopyramidal traps that were deployed near to the rivers and grazing field of the selected peasant associations indicated the presence of the two *Glossina* species, namely *Glossina pallidipes* and *Glossina morsitans*. Higher catches of *Glossina pallidipes* were registered. The high prevalence of trypanosomosis and the high catch of tsetse fly in all cases showed the seriousness of the problem. The present study clearly indicated that, trypanosomosis is one of the most important constraints for livestock production in the study area. The existence of *T.congolense* and *T.vivax* as most important Trypanosome species in terms of economic loss in livestock calls for further epidemiological investigation and application of appropriate trypanosomosis and tsetse control technologies in the study area.

Key words: Bovine Trypanosomosis · Prevalence · Buffy Coat · Tsetse Flies · Western Ethiopia

INTRODUCTION

Livestock are the main stay of the vast majority of African people. They contribute a large proportion of the continents GDP and constitute a major source of foreign currency earning for a number of countries. Livestock production, indeed, contributes to improve food security and poverty alleviation in developing world. Tsetse transmitted trypanosomosis continues to be the major constraints of livestock production in Sub-Saharan Africa, jeopardizing the lives of 55 million people. The risk of infection in humans as well as in domestic animals has been known to greatly affect social, economical and agricultural development of communities within tsetse infested areas which roughly constitutes more than a third (10 million km^2) of African between 14°N and 29°S of the continent [1, 2]

Ethiopia's economy is largely dependent on crop and livestock production. Besides its direct contribution in terms of GDP and foreign exchange, livestock provides virtually all the draught power for cultivation and transportation of agricultural crops and people in rural of the country [3].

Statistical estimates show that 88% of the human population and 70% of livestock exist in the highland of Ethiopia that constitutes only 36.3 % of the country's land area. Most of which is heavily degraded and unable to provide sufficient food for the people living on it.

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On the other hand, low lands of the country that accounts about 63.7% of the total land, supports only 12% of humans and 33% of livestock population. Substantial proportion of the fertile agricultural low lands is rendered inaccessible by the threat of tsetse and trypanosomosis. The North West region is affected by tsetse and non-tsetse transmitted trypanosomosis [4, 5]. According to Langridge [6] the tsetse flies in Ethiopia are confined to the southern and western regions between longitude 33° and 38° E and latitude 5° and 12° N infesting areas which together amount to 97,855 km^2.

Increasingly tsetse and trypanosomosis control Schemes become concentrated in selected areas of high priority; the areas where control is technically feasible and where economic returns are considerable [7]. In attempt to solve this bottleneck problem of the country, it necessitates further up dated research on description of host, agent and environment relationship in Bure.

The present research was initiated to generate base line data that may assist in decision making to apply appropriate tsetse and trypanosomosis control.

Therefore, the present study aims at the following objectives

- To determine the prevalence of bovine trypanosomosis in selected areas of Bure district.
- To identify vector species and their apparent density.
- To assess the risk factors associated with the disease.

MATERIALS AND METHODS

Study Area: The study area is located in Oromia regional state, Illubabore zone and lies at 08° 11' 20" to 08° 20' 53" E longitude and 035° 01' 27" to 035° 15' 13" latitude north of equator. Altitude of the area ranges from 720 to 1800 m.a.s.l. According to the Bure district Agricultural and Rural Development Office, the climatic condition alternates with long summer rain fall (June-September), short rainy season (March-April) and winter dry seasons (December-February) with mean annual rain fall of 1700mm. The mean lower and higher average temperature are 15°C and 27°C respectively. The dominant vegetation types are *Acacia jacaranda, ficas sycomors* and *Cordia africana* trees. The wooded grassland (Savannah) is dominated with Pillistigma trees. It has long perennial grass with mountains and hills of various heights. Wild games like buffalo, bush pig, kudu, warthog, hippo and crocodiles are the most commonly found in the study area. Agriculture is the main stay of lively hood of people with a mixed farming system and livestock plays and integral role for agriculture. The main crop types cultivated include barley, sorghum, coffee, teff and bean.

Study Population: The study was conducted on 560 local breed cattle selected from five peasant associations in the districts and individual animals were selected by simple random proportion using lottery method Of these animals, 90 were originated from Dorani dibi, 133 were originated from Megersa, 155 originated from Kufi, whereas the remaining 96 and 89 were from Nabo-miriga and Addisalem respectively. The origin, age, sex and body condition of the animal were explanatory variables used to associate with prevalence rate.

Study Design: A cross-sectional study design was employed to determine the prevalence of trypanosomosis in the study animals. On the other hand for the entomological survey baited traps were deployed at 200-250 meters interval.

Sample Size Determination: The number of animals required for the study was determined using the formula given by Thrustfield [8] for simple random sampling.

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

Where,

N = required sample size
P_{exp} = expected prevalence
d = desired absolute precision

The sample size was determined using 95% level of confidence, 50% expected prevalence and 0.05 desired absolute precision. Therefore, a total of 384 cattle were needed for this study. However, samples were collected from 560 animals to increase precision.

Study Method and Sample Collection

Parasitological Survey

Buffy Coat Examination: Blood samples were collected in to heparinized microhematocrit capillary tubes after piercing the ear vein using lancet. Then one end of the capillary tube was sealed with sealant and centrifuged at 12,000 rpm for five minutes to separate the blood cells and concentrate trypanosomes in the buffy coat zone. Then, PCV (Packed Cell Volume) was determined using hematocrit reader and animals with PCV = 24 were

considered anemic [9]. The capillary tubes were then broken just below buffy coat and expressed the contents on microscope slide mixed and covered with a 22x22 mm cover slip. Then, it was observed under x40 objective of microscope using dark ground buffy coat technique to detect the presence of parasites and for positive samples Giemsa stain of thin blood films were made. This technique was reported as the most sensitive of the parasitological tests for the detection of *T. vivax* and *T. congolense* [10, 11].

Entomological Survey: During tsetse fly survey study 90 baited monopyramidal traps were deployed at the side of river and woody grass land. Out of total 90 traps used, 18 were deployed in Doranidibi, 12 were in Megersa, 12 were in Nabomiriga, 24 were in Kufi, 12 were in Edgetfana and 12 were in Addisalem. Every trap was odour baited with acetone, octenol and cow urine as odour attractants for tsetse [12]. The underneath of each trap pole was smeared with grease in order to prevent the ants climbing up the pole towards the collecting cage that could damage the tsetse flies. The trap deployment time was 48 hours and flies captured were then sorted by sex, species and sites. The species of tsetse was identified based on the characteristic morphology. Other biting flies were also separated according to their morphological characteristics such as size, color, proboscis and wing venation structures at the genus level [13, 14].

Data Management and Analysis: Data collected were stored in MS Excel spreadsheet to create a data base and transferred to the SPSS software programmed of the computer before analysis. The SPSS versions 20 Software of the computer programmed were used for the statistical analysis. The prevalence rate was calculated by dividing the proportion of cattle infected by the total number of animals examined multiplied by 100. The determinant factors were investigated using Pearson's chi-square. A statically significant association between variables was said to exist if the calculated $P<0.05$ at 95% confidence interval level. Finally, the fly density was calculated by dividing the number of flies caught by the number of traps deployed and number of days of deployment and expressed as fly/trap/day.

RESULTS

Parasitological Findings: The overall prevalence of bovine trypanosomosis in the study area was 6.1%. The prevalence of trypanosomosis in each peasant association was determined to be 1.1% in Dorani dibi and Megersa, 1.8% in Kufi, 0.9% in Nabomiriga and 1.3% in Addisalem [Table 1]. Among those five peasant associations, Kufi showed the highest prevalence rate (1.8%) and the lowest being in Nabomiriga (0.9%).

Table 1: The prevalence of bovine trypanosomosis, in different peasant associations.

	Number of animal		
Peasant Association	Examined	Positive	Prevalence
Doranidibi	90	6	6.67%
Megersa	133	6	5.26%
Kufi	155	10	6.45%
Nabomiriga	96	5	5.21%
Addisalem	86	7	6.98%
Total	560	34	6.1%

Table 2: Association between prevalence and risk factors

		Non Infected	Infected	Prevalence	X^2-value	P-value
BCS	Good	135	6	4.4%	4.929	0.678
	Medium	289	16	5.5%		
	Poor					
	136	12	8.8%			
Age	2-5	159	6	3.8%	2.313	0.678
	<2	389	27	6.9%		
	>5					
	12	1	8.3%			
Sex	Female					
	268	14	5.2%	1.347	0.510	
	Male	292	20	6.8%		
PCV	≥25	300	25	3%	14.315	0.001
	<25	226	9	11.1%		

A comparison of trypanosome infection between male and female showed overall prevalence of 6.8% and 5.2%, respectively. Although the prevalence of trypanosome in male was higher than female, there was no statistically significant difference (X^2=1.347; P>0.05) in trypanosome infection between male and female.

A higher prevalence rate of 8.8% was seen in animals with poor condition than that of medium body condition (5.5%). The lowest infection rate of 4.4% was obtained in animals with good body condition [Table 2].

Body Condition Score: As statistical test applied to evaluate the presence of association between disease and mean PCV values showed a highly significant association between the mean PCV value and occurrence of parasitaemia (P<0.05). Animals without parasites were observed with a higher mean PCV value 53.6% as compared to parasitaemic animals 1.6%.

Up on the study two species of trypanosome were detected: *T.congolense*, *T. vivax* and mixed infection (*T. congolense* and *T. vivax*). A total of 34 infected animals 21 (61.765%) were found to be infected with *T. congolense*. Therefore, *T. congolense* considered as the predominant species responsible for infection of cattle residing in the five PAs. The remaining, 12(35.3%), 1(2.94%) were found to be infected by *T.vivax* and mixed (*T. congolense* and *T. vivax*) respectively.

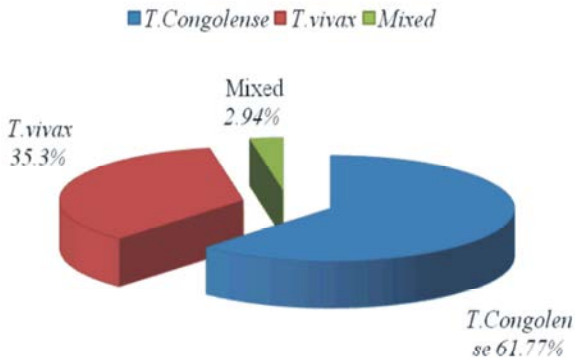

Fig 1: Trypanosome species detected in the selected study areas.

Entomological Finding: A total of 90 baited monopyramidal traps were deployed in five peasant associations of Bure District. Out of 90 traps, 18 were deployed in Dorani-dibi, 12 were in Megersa, 12 were in Nabo-miriga 24 were in Kufi,12 were in Edgetfana and 12 were in Addisalem. Four genera of flies: *Glossina, Tabanus stomoxys* and *chrysops* were caught. The total flies caught were 1883, of which 563 were *stomoxys,* 10 were *tabanus*, 2 were *chrysops* and 1308 were *Glossina* species. The mean catch of all flies was 10.46 flies/trap/day. The mean catch was found higher in Nabo Miriga (16.5 flies/trap/day).

Table 3: Apparent density (F/T/D) of fly at different PAs of Bure district, Western Oromia

		Fly species caught-(F/T/D*)					
Pas	Altitude range (m)	*stomoxys*	*tabanus*	*chrysops*	*glossina*	*No of traps*	Mean catch
Dorani dibii	1423-1587	109	2	2	344	18	12.94
Megersa	1621-1693	59	0	0	40	12	4.13
N/miriga	1657-1747	109	1	0	286	12	16.5
Kufi	1460-1742	189	4	0	364	24	11.6
E/Fana	1594-1682	81	5	0	103	12	7.88
Addis Alem	1652-1678	53	6	0	171	12	9.58
Total		600	18	2	1308	90	

*F/T/D = Fly/trap/day

Table 4: The mean catch of Glossina speceis in six PAs of Bure District

		G.morsitans		*G.pallidipes*		
Pas	Altitude range	*Male*	*female*	*Male*	*Female*	F/T/D
Doranii dibii	1423-1587	14	11	147	172	9.56
Megersa	1621-1693	0	0	14	26	1.67
N/miriga	1657-1747	6	9	75	196	11.92
Kufi	1460-1742	3	6	55	300	7.58
E/Fana	1594-1682	0	0	24	79	4.29
Addis Alem	1652-1678	2	0	65	104	7.13
Mean catch		0.138	0.144	2.11	4.87	7.262

F/T/D= Fly per trap per day

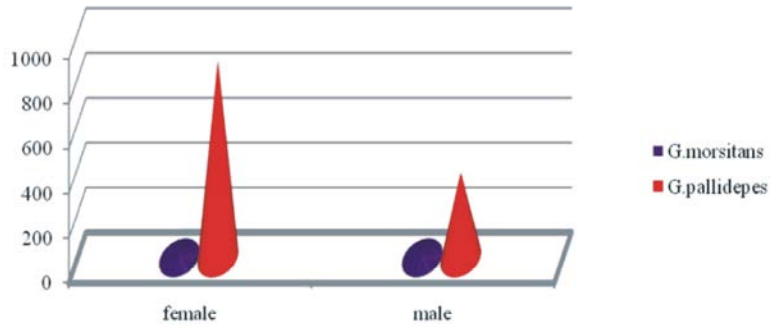

Fig 2: The number of female and male of Glossina species in six PAs.

During the survey periods two species of Glossina were caught namely*:* *G.morsitans* and *G.pallidipes*. A total of 1308 tsetse flies, were collected of which 903 (69.04%) flies were female and 405 (30.96%) were males. There was statistically significant difference between two sexes (P<0.05).

DISCUSSIONS

The overall prevalence of bovine trypanosomosis of the study area was 6.1% which disagree with the result of Mulaw *et al* 2011[12]. The prevalence of infection between sex categories was 6.8% for male and 5.2% for females, but there was no statistically significant difference between sex groups (P>0.05). The difference of prevalence of trypanosomosis among the body condition score was also not siginificant(P>0.05) being higher in poor body conditioned than the other group in consistence with Bacha *et al* 2013 [15].

In this study, age-wise analysis revealed that there was no significant difference in prevalence between age groups where a higher infection rate was recorded in older once(8.3%). This may be due to long time exposure to the vector and physiological stress associated with age and utilization. Less than 2 years old had the lower prevalence(6.9%) while in bovine between 2 to 5 years old had the lowest prevalence [3.8%]. These results agree with that of Dagnachew *et.al* [16] and Bogale *et al* 2012 [17].

Measuring of PCV values for each animal samples in the pre-intervention survey in all five sites were analyzed and marked difference was noticed. Accordingly, parasitaemic animals had generally lower mean PCV value than aparasitaemic ones. And in addition to this, about 73.53% of the parasitaemic animals had their PCV below 25%. This result in agreement with the work of Bekele [18] in which the mean PCV of parasitaemic animals became 94.87% in site one and 81.25% in site two in southern valley and Dagnachew *et al.* [16] in which 88% of all parasitaemic animals had mean PCV below 26% in Abay basin of north western Ethiopia, Bekele *et al.* [14] in which they reported the mean PCV of aparasitaemic and parasitemic animal 25.65% and 18.8 % respectively.

The mean PCV of animals from all sites did not show marked difference and the majority of animals had relatively medium PCV value, although there were animals with low PCV values in all cases. The appearance of parasitologically negative animals with PCV values of less than the threshold value (25%) may be due to the inadequacy of detection method used [11] or delayed recovery of anaemic situation after current treatment with trypanocidal drugs and occurrence of positive animals with PCV of greater than 25% might be thought of recent infection of animals. Trypanosome infection and mean PCV values obtained in this study in the parasitaemic animals was found to be highly associated. Similar results were reported by Bekele [18] at southern valley in southern Ethiopia that as PCV increased the proportion of samples detected parasiteamic correspondingly decreased. Hence, the mean PCV could be an indicator of the healthy status of cattle population under study.

It was generally accepted that the mean PCV value is affected by many factors other than trypanosomosis. However, these factors have been reported to likely affect both trypanosomosis positive and negative animals [9]. Other diseases considered to be affecting the PCV values in animals are helmenthiasis, tick borne diseases and nutritional imbalance. On the other hand most of the parasitaemic animals in the lowland areas were in good body condition despite having low PCV values. This could be attributed to the fact that animals in low altitude were at high plan of nutrition due to availability of sufficient pasture [16].

The proportion of *T.congolense* in all sites of this study was highest (61.77%). This result is consistent with the report by Abebe and Jobere [19] in which they

reported 58% of the total trypanososomes detected were *T.congolense.* The five PAs in the current study have shown insignificant difference in trypanosome prevalence (P>0.05).

The overall mean catch of tsetse flies was 7.3 flies/trap/day. This result was not in agreement with the previous report by Tilahun *et al.* [20] in which they reported the fly density between 16 to 22.4 flies/trap/24hrs in Tana Bales valley and by Regassa and Abebe [21], were they reported the mean catch of tsetse flies was 10.68 flies/trap/day in upper diddessa valley. This finding was higher than the result obtained during the study by Bekele [18], which he had caught 1.35 flies/trap/day in site one and 0.9 flies/trap/day in site two of his study area. This result is of course far from the work of study by Sinashaw [5] in which they had caught no tsetse fly.

The entomological data analysis of pre-intervention survey revealed that for Doranidibi and Nabomiriga PAs showed insignificance difference. This has been related to the similarity in the categorization of ecological situations in three sites; there is significance difference between the two PAs and the rest of the three PAs namely: Addisalem, Megersa and Edgetfana, which has been related to the difference in habitat classification particularly, riverine was available in Megersa and Edgetfana in addition to grasslands and bush lands. The populations of *G.pallidipes* were highest than *G.morsitans*; this could be related to varieties in the type of vegetation.

On the other hand, difference had occurred in the mean catch of tsetse flies with respect to classification of vegetation types in which grass land type had higher results. Such results of higher catch was reported by Hasan [22] where he indicated the highest total catch to grass land vegetation classification (47% in dry season and 72 % in wet season). Msangi [23] stated that *G.pallidipes* was wide spread being detected in all types of vegetation, the highest relative density being detected in bush land vegetation. According to Leak [24], vegetation is vital for providing a suitable condition.

Catching of female flies showed a greater degree of deviation from the expected 50:50 ratio with a significant relationship. Females accounted for 69.04% catch during this study. This result is similar to the report of Bekele [18] and Bancha [25] where this indicated about 63.2% and 60% catch of female respectively. Female *G.pallidipes* showed relative high density than others this result is found to be consistent with previous study by Bekele *et al.* [14] in which they reported 63.2% female *G.pallidipes*. Phelps and Lovemore [26] associated higher catches of female *G.pallidipes* to be attributable to their longer life span (Average of 8 weeks) than males living about 4 weeks, so that more catch of females could appear.

In conclusion the present study indicated that trypanosomosis is one of the most important constraints for livestock production in the area. Thus, trypanosomosis and tsetse control methods should be expanded to reach all infested areas besides participatory extension packages to create public awareness. Further epidemiological investigation is also a necessity to synchronize control efforts at national level.

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