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Study on Bovine Trypanosomosis and Tse Tse Fly Challenge in Darimu District of Birbir Valley, South Western Ethiopia

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Abstract

This study was undertaken on bovine trypanosomiasis and its vectors at Birbir valley located in Darmu district, Illubababor zone. The parasitological examination was conducted using Buffy coat technique while vector survey was conducted using odour baited Monopyramidal trap. The objective of the study was to determine the prevalence of trypanosomiasis in cattle, to determine fly density and to identify associated risk factors. From 392 Blood samples were collected, 45(11.5%) were found to be positive by Buffy coat technique and trypanosome species identified by their motility were *T.congolense* 40 (88.9%) and T.vivax 5(11.1 %) and *Trypanosoma congolense* was the dominant species. A total of 52 Monopyramidal traps were deployed and 1836 (73%) tsetse flies and 676(26.9%) biting flies were caught. From flies captured, 971(52.9%), 540(29.4%) and 325(17.7%) *Glossina morsitans sub morsitans* of savannah flies, *Glossina fuscipes fuscipes* of reverine and *Glossina pallidipes* of savannah species were identified respectively. The overall apparent densities fly / trap / day (FTD) were 17.7 and 6.5 for tsetse and biting flies respectively. There was no statically significant difference ($P > 0.05$) in the prevalence of trypanosome infection between sex group while statistically significant difference was observed between age group($x^2=41.0, p=0.000, p<0.05$). The mean PCV of the parasitemic and aparasitemic animals were 21.3% and 24.3% respectively .The difference between the mean PCV value of the parasitemic and apparasitemic animals were statistically significant($P<0.05$). Designing and implementation of trypanosomosis control should be targeted to the major cyclically transmitting tsetse flies.

Keywords: Darimu; Cattle; Trypanosomiasis; Prevalence; Vector

Abbreviations: FAO: Food and Agricultural Organization; OIE: Office International des epizooties; ID: Identification; M.A.S.L: Meters Above Sea Level; MOA: Ministry of Agriculture; NTTICC: National tsetse and Trypanosomiasis Investigation and Control Center; PA: Peasant Association; PCV: Packed Cell Volume; GPS:

Geographical Positioning System; VSG: Variants Surface Glycoprotein; RBC: Red Blood Cell; RPM: Revolution Per Minute; MPCV: Mean Packed Cell Volume; F/T/D: Fly per Trap per Day.

Introduction

Trypanosomosis (which affects various livestock species as well as human) has been diagnosed in 40 countries of Africa; Comprising an area of 11 million km^2 or roughly one third of the continent [1] where as the primary vectors of the disease (tsetse fly) infest over 10 million km^2 of the most suitable land spread across 37 countries [2,3].

Trypanosomosis is the main constraint to the livestock production on the continent of Africa and prevents full utilization of land and impose significant negative impact in food production and Economic growth in many parts of the world, particularly in sub- Saharan Africa [1]. In addition it has greatly hampered people and animals settlement in a considerable part of the world [4]. About 30% of cattle population estimated at 160 million and comparable numbers of small ruminants are at risk from Trypanosomiasis in Africa [1]. Bovine trypanosomisis is an important protozoan diseases caused by the genus Trypanosome transmitted through bites by different species Glossina (cyclically) and a number of biting flies (Mechanically) such as *Tabanus, Haematopota and stomoxys* species [5]. In Ethiopia Trypanosomiasis which is locally called as "Gandi" is a serious constraint to the livestock production in tsetse infested area of south and south western part of the country [6].

The disease also found outside of the tsetse belt areas transmitted mechanically by biting flies. This type of transmission has caused the spread of *Trypanosome vivax* and *Trypanosoma evansi* outside tsetse area [7]. Economical important animal Trypanosome species in Ethiopia are, *T. Vivax, T. congolese T. brucie, T. evansi* and *T. equiperdium* [6]. Accordingly there are 5 species of *Glossina* in Ethiopia, namely, *G. pallidepes* and *G. morsitance sub morsitance* from *morsitance* group, *G. fuscipes* and *G. tachinodes* from *palpalis* group and *G. longpennes* from *fusca* group. All play their role in transmission of African animal Trypanosomosis [7].

Prevalence of the disease depend on rate of exposure, availability of infected animals, the insect reservoir and seasons [8] and a number of epidemiological studies have been conducted on cattle trypanosomosis [7,9].

The impact of tsetse and Trypnsosomiasis on socio – economic sphere is therefore complex and enormous in animals lost, excluding traction power, manure value, nutritional value of animal products (milk and meat), lose of employment opportunity that could have been created with production expansion and high annual cost for the treatment of trypanosomosis. Although few studies were with limited scope conducted in the Western Ethiopia, no study was conducted in Illubabor zone, Darimu district. So there is a lack of base line data of current tsetse flies density and prevalence of bovine trypanosomiasis. Studies on the epidemiology of Trypnsosomiasis are crucial to design and implement evidence based interventions.

Therefore, the major objectives of this study were:

- To determine the prevalence of bovine Trypanosomiasis
- To identify the associated risk factors
- To determine apparent density of flies (F/T/D) and their distribution

Materials and Methods

Study Area

The Study site is located in Oromia regional state, Illubabor zone, Darimu District and lies at 035°15 to 035°32 E longitudes and 08°30 to 08°44 latitude N of equator. The area of the district is 1387.97sq.km and the altitude ranges from 1200 to 1800 m.a.s.l. with the topography of mountains, platue, marshy area, steep slope and with Varity of vegetation. The agro climatic situation is mid high land 53.5% and low land 46.6%. Metrology station recorded two season in the area, long rainy (June – September) with mean annual rain fall of 1456 mm and November-march long dry season, and with mean lower temperature 18°C and the highest average 25°C. Animal population of the district is cattle 96,484, sheep 34,432, goats 20,556 and equine 5,483. The study was conducted in 4 peasant association namely, Ilala, Bena 2, Abuna Gali and Dade Botor. There are rivers which flow throughout the year to Baro Akobe river system, namely, Birbir, Golol, Asas and Gaba River with small stream and seasonal rivers. . The type of vegetation coverage is savannah grassland, forest, and the tall reverine tree spp. Wild game such as buffalo, bush pig, buffalos, crocodile and hippotatamus are most common. Most people in the area engaged in crop and livestock production. All cattle in the study area are local and kept under traditional management system, and used for

drought power, milk, meat production and as means of income generation.

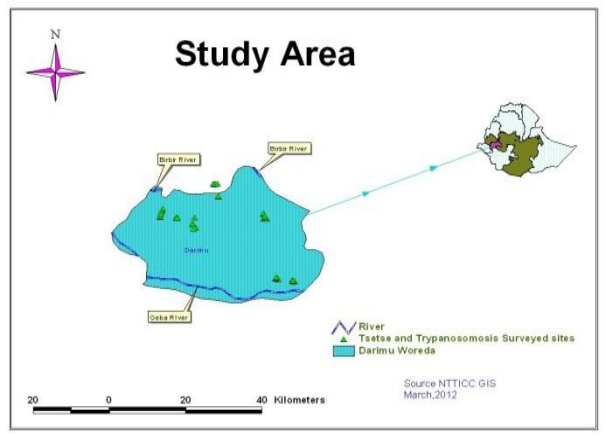

Figure 1: Geographical location of Darimu districts.

Case Report

Tsetse transmitted Trypanosomiasis occurs only in sub- Saharan Africa where they are present as sleeping sickness in humans and as Nagana in domestic livestock. They are severing limits to health and socio Economic development over about half of the sub continents. With the single exception of Trypanosome equiperdum of equine which is transmitted through coitus, all others Trypanosome have vectors in which their transmission is either cyclical or acyclical. In Darimu district Nagana is commonly occurring in virtually all places where tsetse and domestic animals co- exist. The clinical signs are not pathognomonic and hence clinical examination is of little help in pinpointing their diagnosis and characterized mainly by intermittent fever, progressive anemia, and loss of body condition of susceptible hosts, weakness and drop in production. Finally if untreated lead to heavy mortalities. To control the disease several strategies have been applied by using the limited range of trypanocidal drugs to treat or protect livestock, insecticide treated cattle (spot-on) and insecticide impregnated target to control tsetse flies. There is no vaccine against the disease due to the ability of its antigenic variation of variant surface glycoproteins (VSGS) and no new drugs have been marketed for the treatment or prevention of bovine trypanosomosis in the past 50 years [10].

Sample Size Determination

The Sample size was determined according to Thrusfield M [11] and random sampling method was used for the population with 95% confidence level, 5% desired absolute precision and 50 % expected prevalence.

$$N = \frac{1.96^2 \, p^{exp}\left(1 - p^{exp}\right)}{d^2}$$

Where
n= required sample size
1.96= the value of Z at 95% confidence interval
P^{exp}= expected prevalence of trypanosomosis (50%)
d=desired absolute precision level at 95% confidence interval

Study Animals

The study animals were local cattle that are managed under traditional communal grazing (extensive System). In each site animals were randomly selected from all age and sex group. A total of 392 cattle were examined and all appropriate information's like sex, age, peasant association were recorded.

Study Design

A cross sectional type of study was conducted from November 2014 to May 2015 to determine the prevalence of bovine Trypanosomiasis and apparent density of flies (F/T/D) and their distribution.

Sample Collection and Examination

Vector Survey: Entomological data was collected once during dry season period of January 2015. The flies were caught with Mono pyramidal trap baited with acetone, octenol and cow urine [12]. The traps were deployed where animals are staying for longer period of time for watering and grazing. 52 traps were deployed at an interval of 250 meter. The bottoms of each pole of the trap were smeared with grease in order to prevent the ants climbing up the pole towards the collecting cage that could damage the tsetse flies. The traps were kept in position for 48 hrs. The different fly catches in each trap were counted, sexed, identified based on their species and morphological characteristics and other biting flies. Tsetse fly apparent density mean catches in the trap deployed was expressed as the number of tsetse catch /trap / day [13].

Figure 2: Monopryamidal trap used during tsetse survey in Darimu district.

Entomological examination: All caught tsetse and biting flies were sorted by sex and species and counted immediately at collection site using hand lens. A stainless steel entomological pin was pushed through dorsal side of the thorax and preserved by pinning and keeping the flies in the fly box. The flies should be placed more than half way up the pin. A data table was added underneath and the point of the pin pushed well in to the cork lining of the storage box. The box was made up of wood, so that the insect may not be damaged for a long period of time [14].

Blood samples: Blood samples which were randomly collected in heparinized capillary tubes were transported after sealed with crystal sealant at one end, and by keeping with in capillary tube holder without exposing to direct sunlight and then processed immediately at the study site using Buffy coat method. From positive blood samples, thin smears were prepared, air dried and fixed with methanol alcohol and store in slide box and taken to the laboratory for morphological identification.

Parasitological examinations: The blood sample collected from marginal ear vein randomly using heparinized capillary tubes were filled up to ¾ of its length and sealed with crystal sealant. The sample was centrifuged at 12, 000 RPM for five minutes then the trypanosomes concentrate between RBC and plasma. To assess the anemic status the packed cell volume (PCV) was determined for each sample using haematocrit reader [15]. Low PCV is an indication of anemia that almost always accompanies trypanosome infection [10]. The capillary tubes were broken just 1 mm below the Buffy coat and the fluids were extruded on a slide and examined under a microscope of 40x power of magnification for the presence of motile trypanosomes. For the purpose of morphological identification a thin blood smear was prepared from the Buffy coat for those samples that were positive on examination. The thin blood smears were air dried, fixed with methanol for 3 minutes and stained by Giemsa solution (1:10) dilution for 30 minutes, then the smear was dried and examined under 100x oil immersion objective for morphological identification of trypanosome species .

Data Analysis

The collected data were entered in to Excel and analyzed by SPSS version 20 Software. For the tsetse and biting flies' survey result data were analyzed by fly per trap per day (F/T/D). The prevalence of the trypanosomosis in different variable (sex and age) was compared by chi square (x^2) test while mean PCV value in parasitemic and aparasitemic animals were compared by t test.

Results

Entomological Survey

The fly survey results shows that three species of *Glossina* and other biting flies were present. The F/T/D/ result of tsetse flies indicated that 42.67, 18.46, 3.35 and 2.04, were recorded in Abuna Gali, Dade Botor, Ilala and Bena2 respectively. The surveyed site infested in their order of decreasing with *G.m.sub morsitans, G. fuscipes* and *G. Pallidipes* respectively. During survey 52 mono pyramidal traps were deployed and a total of 2512 flies were caught, of which 1836 (73%) tsetse flies, Stomoxys 669 (26.6%), Tabanus 6(0.23%) and Haematopota 1 (0.03%) were recorded respectively. From total tsetse flies caught 1697 (92%) were female and 139 (7.6 %) were male.

G. morsitanse sub morsitanse 971 (52.9%), was the dominant Tse species followed by *G.fuscipes fuscipes* 54 (29.4%) and *G.pallidipes* 325 (17.7%).

PA	No. Traps deployed	Flies/trap/day					
		Tsetse spp.			Biting flies		
		G.m.subm	*G.p*	*G.f*	Tabanus	stomoxys	Haem
Benna 2	14	0	0	2.04	0	4.82	0
Dade Botor	14	2.29	0.86	15.32	0	11.11	0
Abuna Gali	14	32.39	8.36	1.93	0.14	5.39	0.04
Ilala	10	0	3.35	0	0.1	3.6	0
Total	52	9.34	3.13	5.19	0.06	6.43	0.01

Table 1: Overall apparent density of flies in different PA's of Darimu districts.
G.m.sub m= G. morsitanse sub morsitanse G.p= G.pallidipes G.f= G.fuscipes fuscipes

Parasitological Examination Results

From the total of 392 cattle examined 45(11.5%) were found to be positive for trypanosome. The identified trypanosome species were *T. congolense* 40(88.9%), *T. vivax* 5(11.1%) which are entirely cyclical in their mode of transmission and *T. vivax* that can be transmitted mechanically too.

No	PA's	No of animals examined	No of animals infected	Non infected	Trypanosome spp. Identified			Prevalence Rate %
					T.c	T.v	Mixed	
1	Bena 2	96	13	83	13	0	0	13.54
2	Dade botor	80	10	70	7	3	0	12.5
3	Abuna gali	120	16	104	14	2	0	13.3
4	Ilala	96	6	90	6	0	0	6.25
	Total	392	45	347	40	5	0	11.5

Table 2: Overall prevalence of different species of trypanosome in four PA's of Darimu districts.
T.C= Trypanosome congolese, T.V = Trypanosome vivax, Mixed= *Trypanosome Congolese + Trypanosome vivax*

Associated Risk Factors

Sex: Out of 392 examined animals higher prevalence rate was recorded in male 14.6% and 8.5% in female. But the difference was not statistically significant (x^2= 0.10, P >0.05).

Sex	No. Examined	Non Infected	Infected	Trypanosome Spp. identified		Prevalence rate %
				T.c	T.v	
Male	192	164	28	24	4	14.6
Female	200	183	17	16	1	8.5
Total	392	347	45	40	5	11.5

Table 1: Prevalence of Trypanosomes infection by sex.
T.c=Trypanosome congolense,T.v=Trypanosome vivax

Age: Lower prevalence of trypanosome infection was detected in age group ≤2 years (1.3%) and high prevalence was recorded in age group >2 year (14%).There was statically significant difference between age groups (p <0.05).

Age(Year)	No. of examined animals	Non infected Animals	Infected Animals	TrypanosomaSpp. identified		Prevalence Rate %
				T.c	T.v	
≤2	78	77	1	1	0	1.3
>2	314	270	44	39	5	14
Total	392	347	45	40	5	11.5

Table 2: Prevalence of Trypanosomiasis by Age.

Hematological findings: Out of 392 examined animals parasitemic cattle were 11.5% and that of aparasitemic animals were 88.5% and the overall MPCV were 21.3 and 24.5 respectively. 35.2% animals had anemia (MPCV <24) without having trypanosome infection, 3.8 % animals had normal PCV and infected by trypanosome, 7.7% were infected in which low PCV was observed and 53.3 % of all examined animals were not anemic.

	Examined	Mean	Standard deviation	95.00% Confidence Interval	
				Lower	upper
Aparasitemic	347	24.314	4.302	23.86	24.768
Parasitemic	45	21.533	4.803	20.09	22.976

Table 3: Comparison of mean PCV's of Parasitemic and a parasitemic animal.
PCV= packed cell volume
Those parasiteamic animals have statistically lower mean PCV than aparasitemic animals ($p<0.05$).

Discussion

During entomological survey, a total of 2512 flies were caught, of which 1836 (73%) tsetse flies, 669 (26.6%) Stomoxys, 6(0.23%) Tabanus and 1 (0.03%) Haematopota were recorded. A previous study in Estern Wolega Zone showed out of 1151 flies were caught, 822 (71.42%) belong to *Glossina* species, 198 (17.20%) were *Stomoxys* and 131 (11.38%) were *Tabanus* [16].

The overall apparent density of tsetse flies of flies was 16.6 F/T/D. Earlier studies in the western part of the country, reported the apparent density of Glossina species ranging from 0.3 to 24.4 F/T/D [17,18]. Such wide variations may be attributed to the differences in season and density of vegetation cover, types of traps deployed and type and volume of odour attractants utilized during the studies.

The surveyed site infested with *G. morsitanse sub morsitanse* 971 (52.9%) followed by *G.fuscipes fuscipes* 54 (29.4%) and *G.pallidipes* 325 (17.7%). However a study conducted in North Western Ethiopia [19] revealed a relative abundance of *G. tachinoides* (71.8%) than *G. m. submorsitans* (28.2%). This may be due to ability of *G. tachinoides* to adapt to unsuitable habitats and riverine flies appear to be largely unaffected by human population density and can even adapt to human-made environments.

The current finding showed an overall prevalence of Bovine trypanosomosis 11.5%. Earlier studies indicated the prevalence of bovine trypanosomosis ranging from 8.6 to 9% and from 8.5 % to 11.3% in southwestern and north western parts of the country respectively [19,20,16]. However, our finding was higher than previous reports from districts in southern part of Ethiopia that showed the diseases prevalence ranging from 4.2% to 4.4% [17,21]. These variations could be attributed to seasonal differences during sampling periods and methods employed for the studies. Besides, in Southern Ethiopia tsetse control has been carried out by the southern tsetse and trypanosomosis control project for many years which significantly reduced the prevalence.

The present study revealed that *T. congolense* 40(88.9%) and *T. vivax* 5(11.1%) were the identified trypanosome species which are entirely cyclical in their mode of transmission and *T. vivax* that can be transmitted mechanically too. The present finding was in line with previous report in Northern Ethiopia [19] that showed the majority (95.5%) of the infections is caused by *T. congolense* and the remaining 5% is caused by *T. vivax.* The predominance of *T. congolense* in tsetse infested areas of Ethiopia has been reported by many authors. The present finding is also supported by earlier works done in which 82.4% *T. congolense* and 5.9% *T. vivax* infections in Arbaminch, southern Ethiopia has been reported [17].

The predominance of *T. congolense* infection in cattle may be due to the high number of serodems of *T. congolense* as compared to *T. vivax* and the development of better immune response to *T. vivax* by the infected animal [19].

A higher prevalence rate was recorded in male 14.6% and 8.5% in female. But the difference was not statistically significant (x^2= 0.10, P >0.05) showing that both male and female cattle were equally susceptible to trypanosomosis infection. This is in agreement with the findings of previous studies in Ethiopia [22].

Lower prevalence of trypanosome infection was detected in age group ≤2 years (1.3%) and high prevalence was recorded in age group >2 year (14%).There was statically significant difference between age groups (p <0.05) [23]. Also reported that highest prevalence was observed in adult animals. This could be due to the fact that young and suckling graze at homes until they were weaned off. While adult animals travel long distance for grazing and draught to tsetse challenge areas.

Out of 392 examined animals parasitemic cattle were 11.5% and that of aparasitemic animals were 88.5%. The mean PCV value of parasitemic animals was 21.3 which are significantly lower than that of aparasitemic 24.5. This finding agreed with the report of Endalu M, et al. [19] in North Western part of Ethiopia. 35.2% animals had anemia (MPCV <24) without having trypanosome infection. 7.7% were infected in which low PCV was observed and 3.8 % animals had normal PCV and infected by trypanosome. Likewise, similar findings were observed in Bullen district [24]. This might be due to the fact that buffy coat method is effective at the early stages of the infection where a large numbers of parasites are circulating in the peripheral circulation. At the latter phase when the disease progresses into chronic state, however, the trypanosome count sharply drops in the circulation making too difficult to detect with the routine buffy coat procedures.

Conclusion & Recommendations

The present study indicated that bovine trypanosmosis prevails in the different peasant associations of the district. *Trypanosoma congolense and Trypanosoma vivax* were the dominant trypanosome species in the study area. The occurrence of trypanosomosis was associated with high challenges of different species of tsetse flies, namely, *G.m.sub morsitance, G.pallidepes* and G.*fuscipes* and other biting flies in the district. Trypanosomosis is an important disease that limits livestock rearing and agricultural activity in the study area. Therefore a progressive and integrated control of tsetse and Trypanosomosis is quite necessary. To minimize the effect of trypanosomosis and tsetse challenge, sustainable control with the participation of the community is very important. Based on the above conclusion, the following recommendations are forwarded.

- Designing and implementation of trypanosomosis control should be targeted to the major cyclical vectors of the savannah and riverine tsetse flies by using integrated tsetse control method such as insecticide treated cattle (Spot- on), odour baited insecticide impregnated target and trypanocidal drugs to treat clinically sick animals.
- Strict supervision on the usage of trypanocidal drugs (dosage) should be put in place to avoid trypanocidal drug resistant.

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