Prevalence on Bovine Trypanomosis and Vector Density in Four Selected settlements of Dangur Area, North Western Ethiopia

Lijalem Nigussie [2], Geremew Haile[1*], Abriham Kebede[1], Negesse Mekonnen [3]and Walkite Furgassa[1]

[1] Wollega University, School of Veterinary Medicine, Nekemt, Ethiopia [2] Jigjiga University, College of Veterinary Medicine, Jigjiga, Ethiopia, [3]National Animal Health Diagnosis, Sebeta, Ethiopia
Email: geremewlov@gmail.com

Abstract: A cross-sectional study was conducted from December 2015 to April 2016 in four selected settlements of Dangur area northwestern Ethiopia with the aim to determine the prevalence of bovine trypanosomosis and to assess apparent densities of vectors. Baited mono pyramidal traps were used for the vector survey while Buffy coat technique was used for the determination of prevalence of trypanosomosis. One species of the genus *Glossina (Glossina tachynoides)* and three genera of biting flies (*Stomoxys, Tabanus and heamatopota*) were caught and identified. The overall apparent density of tsetse flies and other biting flies caught were 1073 (9.75) flies per trap per day) and 852(7.74) respectively. A higher number of female *Glossina* species 659(61.4%) was caught than male 414(38.6%). Out of a total 439 cattle examined, 43 (9.8%) with 95% CI: [8.26% – 12.38%] were found infected with trypanosomes. The species of trypanosomes encountered in the current study were Trypanosoma *congolense* and Trypanosoma *vivax* which accounted for 72.09% and 28.91% of the overall infection, respectively. The prevalence in all selected peasant associations (PAs) were 10.35%, 8.50%, 13.89% and 5.89% for Demtsatse, Azarti kitili, Alkasha and Burji respectively. In this study, there was no statistically significant difference (P>0.05) between PAs, body condition, age and sex. Statistically significant difference (P< 0.05) was observed with the mean PCV values between parasitaemic (21.9%) and aparasitaemic animals (29.3%) and species of trypanosoma. According to this study, the prevalence of bovine trypanosomosis and the presence of its vector are evident that can pose impact to the performance of livestock in the area. Therefore, possible control options should be implemented to reduce the disease associated economic loss.

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1 Introduction

In developing African countries, livestock production remains crucial and represents a major asset among resource-poor smallholder farmers by providing milk, meat, skin, manure and traction. However, the economic benefits of livestock populations remain marginal due to prevailing livestock diseases which are among the principal hindrance to livestock performance and cause of high economic losses of the resource of poor farmers [1]. About 30% of the total cattle population in the African continent and about 60 million pastoralists in 37 Sub- Sahara African (SSA) countries are exposed to African animal Trypanosomosis (AAT) and Human African Trypanosomosis (HAT) or human sleeping sickness, respectively [2].

Trypanosomosis is the main haemoparasites disease in domestic animals that causes a significant negative impact in food production and economic growth in many parts of the world, particularly in Sub-Saharan Africa and is caused by the protozoan parasite Trypanosome [3]. The most important Trypanosoma species affecting livestock are Trypanosoma *congolense,* Trypanosoma *vivax,* Trypanosoma *brucei,* in cattle, sheep and goat, Trypanosoma *evansi* in camel andTrypanosoma *equiperdum* in horse [4].

In Ethiopia, trypanosomosis is one of the major impediments to livestock development and agricultural production due to its high prevalence in the most arable and fertile land of South West, West and North West part of the country following the greater river basins of Abay, Omo, Ghibe, Baro, Akobo and Didessa [4, 5]. Currently, about14 million heads of cattle, an equivalent number of small ruminants, nearly 7 million equines and 1.8 million camels are at risk of contracting trypanosomosis at any one time [6].

Trypanosomosis transmission in livestock is either cyclically (*T. congolense, T. brucei*) by tsetse flies or non-cyclically (T. *evansi,* T. *vivax*) by haematophagus flies (like *tabanids* and *stomoxys*) The exception is infection with *T. equiperdum*, which is transmitted by sexual contact [7; 8]. Most tsetse (*Glossina*) transmissions are cyclical, which is the primary vector of African trypanosomosis and infest

the physical landscape covering approximately 11.6 million km^2 of Africa representing 37% of the land area of the continent and affecting 37 countries in Africa [9]. At present, 23 different species and eight sub species of the genus *Glossina* are recognized belonging to three groups on the basis of their preference for habitat: the riverine (*palpalis*) group, the forest (*fusca*) group and the savannah (*morsitans*) group [10].

The course of trypanosomosis in cattle is variable depending on the factors associated with the host and the parasite. Generally, *Nagana* in cattle and other domestic animals is characterized by the intermittent presence of parasites in the blood, intermittent fever, wasting, lymphadenopathy, lacrimation and abortion in pregnant animals. Post-mortem examination of an animal that died of AAT reveals generalized carcass emaciation, enlarged lymph nodes, and enlarged liver and petechial haemorrhages of the serosal membranes, especially in the peritoneal cavity [11].

There are a number of drugs available for the treatment of AAT [12]. Early diagnosis of the disease is important for effective treatment. The choice of drug, dosage and route of administration vary depending on the animal species affected, local preference and presence or absence of trypanosome drug resistance. The appearance of resistant strains of trypanosomes has been associated with the extensive and prolonged usage of the few anti-trypanosomal drugs like Diminazene aceturate, Homidium, Isometamidium, Quinapyramine and Suramin that available on the market [13].

The control of trypanosomosis is facilitated through understanding of the disease epidemiology as well as knowing of its vector (tsetse flies) distribution in the infected area [14]. Tsetse flies in Ethiopia, distributed in south western, western and North western regions between longitude 33° and 38°E and latitude 5° and 12°N. These tsetse infested areas lie in the low lands and also in river valleys of Baro, Akobo, Didessa, Abay, Ghibe and Omo. The areas were dominated by 5 species of tsetse fly (*Glossina*) namely Glossina *morsitans sub morsitans,* Glossina *pallidipes,* Glossina *fuscipes,* Glossin*a tachinoides,* Glossina *longipennis* among these species the first four are wide spread and more economic importance while *G. longipennis* is of minor economic importance [4].

Dangur woreda has a high livestock population, which plays a substantial role in the livelihood of the farmers, for the agricultural community in both the market and the households' level. Unfortunately, animal productivity is very low in the area; there are many reasons for this, among which is the major obstacle of animal trypanosomosis. The disease commonly occurs in areas associated with tsetse flies, where the disease is rapidly transmitted by *Glossina* species as an intermediate host [15].

The main objectives of this paper were;

- To estimate the prevalence of bovine trypanosomosis in selected settlements in Dangur Area.
- To indentify and determine the dominant trypanosome species in the study area.
- To estimate the apparent density of trypanosoma vectors in the study area.

2 Materials And Methods

2.1 Study Area

The study was conducted in Beninshangul Gumuz regional state, Metekele zone, in selected settlement which is found in Dangur area that lies at longitude of 30°E and latitude 11°north of equator. The area was covering an area of 8387km^2 with altitudinal range of 1200-3131 meters above sea level. The study was conducted in four selected settlements hereafter called sites namely: Demtsatse, Azarti kitili, Alkasha and Burji. The average annual rainfall is 1250mm and its average temperature is 28°C [16].

The livelihood of the society largely depends on mixed livestock and crop production having livestock population of 38300 cattle31597 goats, 15056 sheep, 7102 equines and 68088 poultry 31597 goats, 15056 sheep, 7102 equines and 68088 poultry. The major livestock diseases of the area are Trypanosomosis, PPR, sheep and goat pox, Lumpy skin disease and Newcastle disease [17].

2.2 Study Animals

The study was conducted on 439 randomly selected local breed cattle, selected from four peasant associations (PAs). The management system for all cattle was extensive in which animals kept under free grazing. Of these animals, 116 were from Demtsatse, 106 from Azarti kitili, 115 from Alkasha, and 102 from Burjii. Vary number from different association were based on total population of the Pas. Examination and evaluation of body condition were accomplished during sample collection. They were classified as poor, medium and good by observing the body condition of the animals in the field according to the method described by Nicholson and Butterworth [18]. The ages of animals were also estimated by the dentition method [19] and from owner information.

2.3 Study Design and Sampling Method

Cross-sectional study was conducted to determine the species bovine trypanosomosis, prevalence of trypanomosis and the density of tsetse and biting flies' population. Study animals were selected with a simple random sampling.

2.4 Sample Size Determination

A sample size was determined by the expected prevalence of bovine trypanosomosis in the district which was 11.27% [20] and the minimum sample size for this cross-sectional study was calculated using the formula by Thrusfield [21] with 95%confidence level and 5% absolute precision.

$$n = \frac{1.96^2 \, Pexp \, (1 - Pexp)}{d^2}$$

Where: n = required sample size

P exp= expected prevalence

d = desired absolute precision

Thus, 151 cattle were needed for the study. However, to increase the precision, the sample was increased to 439.

2.5 Study Methodology and Procedures

2.5.1 Hematological Survey

First, cattle to be sampled were restrained properly and areas around ear vein prepared aseptically for sampling. The tip of lancet was used to prick ear vein of cattle. Blood samples were collected directly into heparinized microhematocrit capillary tube up to ¾ of the capillary tube. The capillary tubes holding the sample were sealed and placed in the rack. The rack holding the tube was then placed in the ice box and taken to the laboratory for examination. After reaching the laboratory, the capillary tubes were taken out from the box and placed in a micro hematocrit centrifuge with sealed end outer most. Load the tube symmetrically to ensure good balance. After screwing the rotary cover and closing the centrifuge lid, the specimens were allowed to centrifuge at 12, 000 rpm for five minutes. Tubes were then taken out and placed in hematocrit reader and expressed the reading as a percentage of packed red cells to the total volume of whole blood.

2.5.2. Parasitological Survey

After determination of the PCV, the previously centrifuged capillary tube containing the blood was then cut using a diamond tipped pen 1mm below the Buffy coat to include the upper most layers of the red blood cells and 3mm above to include the plasma, then the contents of capillary tube poured onto a clean microscopic slide, covered with a 22x22 mm cover slip and examined under X 40 objective and X 10 eye piece for the presence of motile Trypanosomes. A sample was considered positive for trypanosomosis when Trypanosome was detected with the Buffy Coat Technique (BCT) [22; 23].

Trypanosomes positive Buffy coat samples were analyzed and Trypanosome species were identified based on their morphological structure from Giemsa-stained thin films. A small drop of blood from a micro hematocrit capillary tube was applied to a clean slide and spread by using another clean slide at angle of 45 degree, air dried and fixed for 2 minutes in methyl alcohol then, immersed in Giemsa- stain (1:10 solution) for 50 minutes. Drain and wash of excess stain using distilled water and allowed to dry by standing up right on the slide rack and examined under the microscope with oil immersion X100 objective lens [6].

2.5.2 Entomological Survey

The apparent densities of tsetse and biting flies were determined based on the mean fly catches in mono pyramidal traps baited with acetone, octenol and cow urine which were deployed at an interval of about 100-200 meters along livestock, watering points in selected settlements of the District [24]. In this study, 55 mono pyramidal traps were deployed early in the morning and maintained in position for two consecutive days at four different peasant associations (PAs) within eight days. The cages from these traps were emptied. Caught tsetse flies and biting flies were counted, identified and sexed for the tsetse fly, other biting flies according to their morphological characteristics such as size, color, wing venation structure and proboscis at the genus level [25; 26].

2.6 Data Analysis

The data recorded was entered into Microsoft excel data base system and statical analysis was done. SPSS statical software was used to analyse the data. The association between trypanosomosis infection rate and study variables (such as age, sex and PCV) was determined by Pearson's chi- square (X2) test. A statically significant association between variables exists when $p<0.05$ and at 95% confidence level (CI). Finally, the density of fly population 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 (F/T/D) [27; 28]. (Lelisa *et al*., 2014; Geremew et al 2015).

Results

2.7 Entomological Results

One species of *Glossina (G. tachynoides*)and three genera of biting flies (*Stomoxys, Tabanus and heamatopota)*were encountered during the entomological survey. The relative abundance of *Glossina* species and other biting flies is shown in Table 1. The overall *Glossina* species caught per 48 hours in Demtsatse, Azarti kitili, Alkasha and burjii were 465 (41.71%), 37 (3.56%), 122 (11.70%), and 449(43.03%), respectively. The male and female proportions of Glossina between the peasants association are shown in Table 2.

Table 1: Tsetse and other biting flies caught in study areas

Area	No of Trap	Glossina species			Other biting flies		
		Gt	F/T/D		Stom.	Tab.	Heam. F/T/D
Demtsatse	13	465	17.88		131	128	57
zarti kitili	10	37	1.85		91	81	59
Alkasha	12	122	5.08		16	17	4
Burji	20	449	11.23		88	111	69
Total	55	1073	9.75		337	326	189 7.74

Gt= Glossina tachynoides Tab. = Tabanus Stom. =*StomoxysHeam. =Heamatopot*a
F/T/D =Fly per trap per day

Table 2: Proportion of male and female Glossina species on the study area

Pas	**Total number of Glossina**	**Male**	**Female**
		Number (%)	**Number (%)**
Demtsatse	465	172(37)	293(63)
Azarti kitili	37	9(24)	28(76)
Alkasha	122	78(64)	44(36)
Burjii	449	155(34.5)	294 (65.5)
Total	**1073**	**414(38.6)**	**659 (61.4)**

2.8 Parasitological Results

Out of 439 cattle examined, 43 (9.8%) with 95% CI: (2.36% – 6.01%) were found to be infected with trypanosome species. Of the 43 cattle positive for trypanosomes, 31 (72.1%), 12 (27.9%) cases were caused by *T. congolense and T. vivax* respectively which was statistically significant ($p<0.05$) (Table 5). The association between infection and study variables (PAs, body conditions, age and sex) of cattle infected in the areas is shown in Table 3.

Table 3: Prevalence of trypanosomosis based on PAs, body condition, age and sex

Origin of animals (PAs)	Number Examined	Positive	Prevalence (%)	X^2	P-value
Demtsatse	116	12	10.35		
Azarti kitili	106	9	8.50	4.22	0.24
Alkasha	115	16	13.91		
Burjii	102	6	5.89		
Total	**439**	**43**	**9.8**		
Body condition					
Poor	235	28	11.92	2.6	0.27
Medium	113	8	7.08		
Good	91	7	7.70		
Total	**439**	**43**	**9.8**		
(Age category) Young	100	14	14	4.12	0.173
Adult	125	17	13.6		
Old	214	12	5.6		
Total	**439**	**43**	**9.8**		

High infection rate was recorded in Alkasha, followed by Demtsatse, Azarti kitili and Burjii. The infection was also high in cattle with poor body condition and age between 1- 2(young) years and slightly higher in males than females. No significant variation was seen in the infection status and between the variables above ($p > 0.05$). The relationship between infection rate and PCV is shown in table 4 bellow. The mean PCV of parasitic and aparastemic cattle was 21.9% and 29.3%, respectively and is statistically significant ($p<0.05$).

Table 4: Association between PCV and infection rate

PCV category	Number of animals examined	Positive	Prevalence	Mean PCV	X^2	P-value
<24	193	31	16.06	21.9	15.42	0.00
24-37	242	12	4.96	29.3		
>37	4	0	0	38.25		
Total	**439**	**43**	**9.8**	**25.8**		

Table 5: Trypanosoma species and infection rate

Number of animal examined	Total positive	Infection rate			X^2	P-value
		T.congolences	T. vivax			
439	43	31 (72.09%)	12(27.91%)		439	0.000

3 Discusion

In this study, the entomological findings revealed that one species of *Glossina* (*G. tachynoides*) out of five reported in Ethiopia [4]. and other biting flies (*Stomoxys, Tabanus and Heamatopota* species) was detected in the selected settlement of Dangur area. This species of *Glossina* has also been reported in the Western, SouthWestern and North western parts of the country [29; 30]. The overall apparent density of *Glossina* species and other biting flies were 9.75 and 7.74 flies per trap per day respectively. This finding was much higher than the report of [31], who reported the density of vector in and around Arbaminch were 0.194 and 0.069 fly per trap per day for tsetse and biting flies, respectively and report of [30]. who reported the density of vector in Mandura district 0.06 fly per trap per day for tsetse fly.

The apparent densities of *Glossina* species were 17.88, 1.85, 5.08 and 11.23 flies per trap per day in Demtsatse, Azarti kitili, Alkasha and Burji respectively. A higher number of female *Glossina* species 659(61.6%) was caught than male 414(38.4%), which is in line with various reports from the country [27; 29; 30; 32]. This could be attributed to the longer lifespan of female compared to male *Glossina*.

The present study revealed that the overall prevalence of bovine Trypanosomosis in selected settlements of Dangur area was 9.8%, which is higher than the reports in different areas likes; 4.2% in Bedele district of South-West Ethiopia [33]. (Moti *et al*., 2013), 6.86% in Diga District of Eastern Wollega [34]. (Efrem *et al*., 2013), 6.1% in Bure district Western Ethiopia [35]. (Mezene *et al*., 2014) 5.43% in Mandura district North west Ethiopia [30]. 5.3% in Haro Tatessa settlement South west Ethiopia [36]. The relatively high prevalence of trypanosomosis in this area might be related to tsetse distribution and high fly–animal contact.

The prevalence is agreement with the previous study in Dangur district 11.27% [20]. However, the prevalence was relatively lower than that of 25% in Gawo Dale District of western Ethiopia [5]. and 17.07% and 12.35% in Abbay Basin area of North West Ethiopia [37]. In rainy and dry season respectively. This might be attributed to more tsetse density and biting fly in the District than the Dangur area. The study was carried out in the late rainy and dry seasons of the year and therefore there was no significant difference in the prevalence of cattle trypanosomosis amongst the four settlement areas. This reflects the presence of continued *Glossina* challenge to cattle as the animals are driven to water sources, where they congregate.

Two species of trypanosomes were identified in this study: *T. congolense* and *T. vivax*. The proportion of trypanosomes species in this study were 72.09% for *T.congolense* and 28.91% for T. *vivax*. There was no mixed infection detected in the current study. The domination of *T.* congolense over *T. vivax* is may be due to a highly presence of a biological vector (Glossina), whereas *T. vivax* is more readily transmitted mechanically by biting flies than tsetse flies [38]. The predominance of *T. congolense* infection in cattlemay be also due to the high number of serodems of *T. congolense* as compared to *T. vivax* and thedevelopment of better immune response to *T. vivax* by the infected animal [39]. According to [4], *T. congolense and* T. vivax are the most prevalent trypanosomes that infected cattle in the tsetse-infested and tsetse-free areas of Ethiopia, respectively.

Although there was no significant difference (P>0.05) in prevalence of trypanosomosis between sex, in this study, highest prevalence of trypanosomosis was recorded in male animals (10.42%) than female animals (9.31%). This agrees with the previous report by [34] in Lalo Kile District of Western Ethiopia. These could be possibly due to the more use of male animals for draught purposes, travel long distances to an area of tsetse challenge for grazing or plowing and stressed by draught power and as a result the risk of contracting trypanosomosis is high [40].

The animals examined were categorized in 3 age groups; 1-2(young) age, 3-5(adult) age, and >5 (old) age and there was no significant difference (P>0.05)

in prevalence among age group in this study. The trypanosome infection prevalence was found to be 14% in the young age group, 13.6 in adult age group and 5.6% in aold age group animals as indicated in. Even though prevalence in young animals was not with higher significant variation, this might be because of an equal chance of exposure to parasites. Similar findings were also reported in the Jawi district of the Amhara region, Northern Ethiopia [41; 42]. The prevalence of cattle with poor, medium and good body condition was 11.92%, 7.07% and 7.70%, respectively. This is not statistically significant due to that poor body condition is not only due to trypanosomosis and could therefore result from other pathogens and nutritional stress. The infection rate of cattle with good body condition indicated recent infection that will progress to chronic one if not treated.

The prevalence of this study trypanosomosis was higher in anemic animals than non-anemic (Table 6). This might indicate that trypanosome involve in reduction of PCV. The mean PCV values of infected and non-infected animals were also assessed and trypanosome infection and mean PCV obtained between them had statistically significant difference (P<0.05) and it was lower in parastemic animal than aparasitemic one. This was due to the lower PCV value that might be resulted from the debilitating nature of the disease. Poor nutrition and intercurent gastro-intestinal helminthes infection could also contribute to the general low PCV So that, in absence of these two factors anemia is a good indicator of trypanosomosis [27; 43].

The results of the current study indicated that one species of *Glossina* and other biting flies that serve as potential vectors for trypanosomes present in the settlements of Dangur area and hence can sustain the occurrences of trypanosomosis in cattle. *G.tachynoides* was the common species of *Glossina* identified, whilst *T. congolense* is frequently detected, followed by *T. vivax*. The relative abundance of *Glossina*species caught and the prevalence of cattle trypanosomosis investigated could indicate a serious problem in the area. Therefore, progressive control methods aimed at reducing the *Glossina* species burden would be necessary to minimize the impact of trypanosomosis. Success of control options depends on active community participation. Therefore, mobilizing the community and increasing their participation in control activity could play a key role in reducing the impact of the disease and increasing animal productivity.

4 Conclusion And Recommendation

In general, this study indicate that the prevalence of bovine trypanosomosis in selected settlements of Dangur area was high that can pose threat to livestock owners in the area, due to loss in production and productivity of cattle.In the findings of the study, trypanosomosis was found to be negatively affecting the PCV value and body condition score of affected animals. Also young aged animals were more likely to be infected with trypanosomosis. The most commonly encountered trypanosoma species in cattle of the area was T.*cogolense* followed by T. *vivax*. Entomological survey from this study indicated that, relatively there was a higher tsetse fly (*Glossina*) density than other biting flies which play the main crucial role in the prevalence of trypanosomosis particularly to the area.

Based on the above conclusions, the following recommendations are forwarded:

- Tsetse burden in the area should be reduced through continual use of traps and insecticide-impregnated targets or through application of available chemicals on the animal.
- Regular screening of bovine trypanosomosis and early treating of positive animals with trypanocides are necessary.
- Educating farmers, especially those nearest to the main tsetse challenge areas, is critical to reduce the chance of contact of animal with flies.
- Continuous community-based tsetse monitoring and more trypanosomosis surveillance programs should be instituted in tsetse infested areas of the district.

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***Corresponding author:**
Geremew Haile
Wollega University, School of Veterinary Medicine, P. O. Box 395, Nekemt, Ethiopia,
Email: geremewlov@gmail.com

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