

Epidemiological Status and Vector Identification of Bovine Trypanosomiasis in Didesa District of Oromia Regional State, Ethiopia

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Abstract: A cross sectional study was conducted in Didesa district of Oromia Regional State from November 2013 to June 2014 to determine the prevalence of trypanosomiasis and tsetse density. Simple random sampling was used to select 556 cattle from the purposively selected four PAs for collection of blood sample. Buffy coat technique was used to determine prevalence of bovine trypanosomiasis in the study area and trap was deployed for collection of tsetse flies. Blood sample was examined and it was found that 27 (4.86%) were parasitic positive. The prevalence was insignificant ($P>0.05$) in sex group, age, body condition score and between peasant association. But PCV between parasitemic and aparasitaemic is significant ($p<0.05$). In this study the most common trypanosome species identified were *T. congolense* (17/27, 62.96%) followed by *T. vivax* (9/27, 33.33% and mixed *T. vivax* and *T. congolense* (1/27, 3.70%). The proportional prevalence of *T. congolense* is significantly higher ($P=0.000$) than the other trypanosome species. The mean PCV values recorded were 21.52% in parasitaemic and 28.49% in aparasitaemic animals with a statistical significant difference ($P<0.05$). About 40 traps were deployed for 48 hours (2 days) for collection of tsetse fly. A total of 557 flies were collected from a study area, of which the higher density was for tsetse fly 382 (4.90 flies per trap per day) followed by 137 *Stomoxys*, 32 *Tabanus* and 6 *haematopta*. Generally, this study showed that trypanosomiasis is still present and becomes a constraint for livestock production of the study area. So control and prevention mechanisms must be continued to reduce prevalence of the disease and tsetse flies population.

Keywords: Trypanosomes, Tsetse Flies, Bovine, Didesa and Pcv

1. Introduction

Ethiopia has enormous livestock resource with a total contribution of 15% to gross domestic product (GDP) and 33% to agricultural output. Current estimates of livestock population show that there are 41.5 million heads of cattle, 41 million sheep and goats, 5.8 million equines, 1 million camels, and over 52 million poultry (DACA, 2006). Despite the importance of livestock to the larger sector of the population and to the economy at large, the sub-sector has remained untapped. The little benefit from the enormous livestock resource of the country is attributable to a multitude of problems. This comprises of diseases, age-old traditional management system, inferior genetic make-up coupled with under nutrition and complicated by malnutrition and absence of well-developed market infrastructure (MoARD,

1997). While tsetse- borne trypanosomiasis is excluding some 180,000 to 200,000 km² of agriculturally suitable land in the west and south west of the country, 14 million heads of cattle, an equivalent number of small ruminants, nearly 7 million equines and 1.8 million camels are at risk of contracting trypanosomiasis at any one time (MoARD, 2004).

Trypanosomiasis is a complex disease caused by unicellular parasites (trypanosomes) found in the blood and other tissues of vertebrates including cattle and man (Tesfaye, 2002; Uilenberg, 1998). It is flagellated protozoa, which are transmitted by a number of different arthropod vectors but mainly by biting flies (Urquhart et al., 1996). The most important trypanosome species affecting livestock in Ethiopia are *Trypanosoma congolense*, *Trypanosoma vivax*

and *Trypanosoma brucei*, in cattle, sheep and goats, *Trypanosoma evansi* in camels and *Trypanosoma equiperdum* in horses (Getachew, 2005).

Trypanosomosis is a major constraint contributing to the direct and indirect economic losses to crop and livestock production (Abebe, 2005). Trypanosomosis in Africa costs livestock producers and consumers an estimated US \$ 1 billion each year (Kristajonson et al., 1999). It is a severe problem to agricultural production in widespread areas of the tsetse infested regions (Slingenbergh, 1992) that accounts over 10 million square kilometers of the tropical Africa (Budd, 1999). In Ethiopia, Trypanosomosis is widespread in domestic livestock in the Western, South and South-western lowland regions and the associated river systems (i.e. Abay, Ghibe Omo and Baro/Akobo) (Abebe and Jobre, 1996; Abebe, 2005; Langridge,). Currently about 220,000 Km² areas of the above mentioned regions are infested with five species of tsetse flies namely *Glossinapallidipes*, *G. morsitans*, *G. fuscipes*, *G. tachinoides* and *G. longipennis* (NTTIC, 2004).

Recent findings indicate that the potential area of tsetse infestation is estimated to be ranging from 135,000- 220,000 square kilometres based on maximum dispersals up to 2000 meters above sea level (Slingenbergh, 1992). Economically the tsetse-transmitted trypanosomes (*Trypanosoma congolense*, *T. vivax*, and *T. brucei*) are most important in cattle with 14 million heads at risk in Ethiopia (Abebe, 2005; Upadhayaya, 2005). Past activities of tsetse and trypanosomosis control measures were initiated from early 1960s by French veterinary Assistance Mission followed by British Veterinary Assistance Mission up to 1976. The National Tsetse and Trypanosomosis Investigation and Control Centre (NTTICC) was established in 1971 to run activities on tsetse and trypanosomosis control (Lemecha, 1994). Since then different tsetse control projects were underway by NTTICC and thus meaningful achievements were recorded as some areas were freed of tsetse. These tsetse control activities against, mainly, *Glossina morsitans submorsitans* were undertaken in an area of over 4500km² of Didessa Valley as part of the Eastern Africa Regional Program.

However, only limited works were done on the prevalence of diseases, species of causative agents and vectors involved in Dideasa district as this information is important in preventing disease and reinvasion of the freed sites. This necessitates a continued follow-up and evaluation of the current status of tsetse infestation and occurrence of trypanosomosis in such controlled sites and their surrounding villages. Therefore, the present study was aimed at determining the status of the diseases and vectors with the following specific objectives:

Specific objectives:

- To investigate the status of bovine trypanosomosis and species identification
- To identify the tsetse species and density in the study area

2. Materials and Methods

2.1. Study Area Description

Didessa woreda is found in Illu Ababor zone of Oromiya region 430 km from Addis Ababa to western Ethiopia. The total area coverage of the district is 72,848 hector of which 10,970.75 hector is covered by forest, 10,466 hector is grazing land and 9,920.88 hector is cultivating land. The mean annual rainfall is 900-1000mm and the mean daily temperature is 12-18⁰c. The animals that found in the woreda are 107,526 bovine, 26,380 ovine, 9355 caprine, 19804 equines and 54750 poultry. The main crops cultivated in the area are maize, teff, sorghum, and barley (Didesa animal agency, 2012).

2.2. Study Design and Sampling Methods

A cross-sectional study design was used to determine current prevalence of bovine trypanosomosis in the study area from November 2013 to June 2014. The study district and peasant association was purposively selected. Study animals were selected with a simple random sampling. Desired sampling size was calculated according to the formula given by (Thrusfield, 2005). But to improve the degree of precision a total of 556 samples were taken for the present study.

2.3. Study Animals and Blood Sampling

Animals used in this study were local zebu cattle (*Bos indicus*), which are usually kept under an extensive husbandry system. Animals were allowed to graze freely during the day and housed in poorly constructed barns at night. Blood sample was taken from the randomly selected cattle. During sampling PAs, age, sex and body condition score of animals were recorded. The age was categorized in to three (0-2 years, 3-5 years and greater than five years). Body condition score was grouped in to poor and good conditioned animals based on the appearance of ribs and dorsal spines applied for Zebu cattle (Nicholson and Butterworth, 1986).

2.4. Parasitological Study

A total of 556 cattle were examined for blood sample collection. Blood sample was collected by puncturing of the marginal ear vein of each animal with a lancet and drawn directly into heparinized capillary tube and centrifuged with capillary hematocrit centrifuge (Woo, 1970). PCV measurement of trypanosomes was done by the dark ground Buffy coat technique (Murray et al., 1984). Positive samples were further processed for thin blood smear for confirmation of trypanosome species using their morphological characteristics (Paris et al., 1982) with Giemsa staining techniques.

2.5. Entomological Survey

The apparent densities of tsetse and biting flies were

determined based on the mean fly catches in traps baited with acetone, octenol and cow's urine. A total of 40 traps with a monoconical shape were placed approximately 100m apart and left in position for two consecutive days (48 hrs). The flies caught per trap were identified, counted and apparent fly density per trap per day (f/t/d) was recorded. The tsetse flies were identified to species level (Marquardt *et al.* 2000).

2.6. Data Analysis

Collected data was fed to Microsoft Excel spread sheet and process of coding, handling and validating was done on this sheet. Coded was transferred to SPSS version 20.0 for analysis. Descriptive statistics, student t-test and chi-square tests were used to express results and analysis of variables. The trypanosomiasis with variables; peasants association, age, sex and body condition score was compared by chi-square test. The mean PCV of infected and non-infected animals were compared with student t-test.

3. Results

3.1. Parasitological Findings

The overall prevalence of trypanosomiasis was 4.86% in Didesa district in the study period as shown in Table-1. The prevalence of trypanosome species in cattle within peasant association in the study areas were 5.43%, 4.87%, 5.4% and 3.93% in Busi, Doyo, Dingo and Hunqe respectively. There was no statistical significant difference between PAs ($P>0.05$). The prevalence of trypanosomes infection differed between age categories 0-2 years, 3-5 years and greater than 5 years but not significant ($P>0.05$). Higher prevalence observed 6.1% in age group <2 years compared to the 3-5 years age category (4.1). The prevalence of trypanosome infection was slightly higher in male 5.6% than female 4.32% animals but there was no statistically significant difference ($p>0.05$). The prevalence trypanosomes in body condition score shows no significant association but high prevalence found in poor (7.94%) followed by medium (4.32) and lowest in good (2.53%) as indicated in Table-2.

Table 1. Prevalence of Trypanosomiasis in Study area of PAs.

PA's	No. of animal examined	No. positive	Prevalence	X ²	p- value
Busi	92	5	5.43		
Dingo	164	8	4.84	0.44	0.93
Doyo	148	8	5.4		
Hunqe	152	6	3.94		
Total	556	27	4.86		

Table 2. Prevalence of Trypanosomiasis between Sex, age group and body condition score.

Factors	No. of animals examined	No. of positive	Prevalence (%)	X ²	p- value
Sex					
Female	324	14	4.32	0.49	0.488
Male	232	13	5.60		
Age					
1-2	82	5	6.1	0.495	0.71
3-5	171	7	4.1		
>5	303	15	4.9		
BCS					
Poor	126	10	7.94	3.77	0.15
Medium	351	15	4.32		
Good	79	2	2.53		

3.2. Hematological Findings

The proportion of trypanosome infection with species level indicated that 17(62.96%) cattle were found to be infected by *T. congolense*, 9(33.33%) cattle were found to be infected by *T. vivax* and 1 (3.70) cattle were found to be infected by mixed (TC & TV) as shown in Table-4. PCV of individual animals was measured for the assessment of degree of anemia. A mean PCV of 21.52% and 28.75 % were found for infected animals and non infected animals respectively as indicated in Table-3. The difference was statistically significant ($P = 0.000$).

Table 3. Mean PCV in Parasitemic and aparasitemic cattle.

Condition	Mean PCV%	95% CI	Std error	p- value
Infected	21.52	21.04-22.00	0.24	0.000
Non infected	28.75	28.50-29.03	0.14	

Table 4. Ratio of trypanosome species infection.

T. species	No. of positive	Percentage (%)	X ²	p- value
<i>T. congolense</i>	17	62.96		
<i>T. vivax</i>	9	33.33	54.00	0.00
TC & TV mixed	1	3.71		
Total	27	100.00		

3.3. Entomological Findings

A total of 577 flies were caught. Of these, 392 belong to *Glossina* species, 147 were *Stomoxys*, 32 were *Tabanus* and 6 were *heamotopota*. Furthermore, all *Glossina* species caught were *Glossina tachinoides*. The overall apparent fly density was 3.56 f/t/d as it is put in Table-5.

Table 5. Flies FTD between PAs.

PA's	No. of trap	Glossina species		Other biting flies				Over all total	Over all FTD
		Tach.	FTD	Tab	Stom	Heam	total		
Busi	10	88	4.4	00	13	1	14	0.7	
Dingo	10	20	1	1	46	4	51	2.56	577
Doyo	10	131	6.55	19	65	1	85	4.25	
Hunqe	10	153	7.65	12	23	00	35	1.66	

4. Discussion

The present study revealed that from a total of 556 randomly selected cattle's in the study area, 27 (4.86%) of animals were positive for trypanosomes. Similar findings of 4.43% from Arbaminch (Teka *et al.*, 2012) were reported. But this is lower than previous reports: 12.41% in Metekel and Awi zones of northwest Ethiopia (Solomon & Fitta, 2010) and 20.40% in Wolyta and Dawero zones of southern Ethiopia Miruk *et al.*, 2008. The relatively low prevalence of trypanosomosis in this study may be related to tsetse distribution and low fly-animal contact and parasite and vector control programmes practiced in the area by Bedele NTTICC annually. In Didesa woreda every farmer in tsetse belt area has trypanocidal drugs in his home that he injects his cattle by non professional individuals.

In another way low sensitivity of direct parasitological Buffy coat examination may contribute for low prevalence that chronic stage is characterized by low parasitemic which is difficult to confirm by parasitological diagnosis (Simukoko, 2011). In very low sensitivity of Buffy coat method 50% of infected animals remained undetected using parasitological diagnostic tools as compared to the molecular analysis animals (Simukoko, 2011). In this study the prevalence of bovine trypanosomosis between peasant associations was not significant; even though it is higher than others in busi (5.43). This may be the result of uncontrolled animal movements between PA's by buying and selling cattles between the PA's.

The animals examined were categorized in 3 age groups; 1-2 (young) age, 3-5 age, and >5 years old. The trypanosome infection prevalence was found to be 6.1% in the 1-2 age group, 4.1 in 3-5 age group and 4.9% in above 5 years old animals as indicated in table-2. 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 by Cheren *et al.* (2006) and Habtamu (2009) in the Jawi district of the Amhara region, Ethiopia. During the study period, the prevalence of bovine trypanosomosis was assessed between sexes of animals and there is no significant difference. Among 27 trypanosome positive animals; 14 (4.32%) of them were female animals and 13 (5.6.52%) of them were male animals. Even though the trypanosome infection in male animals is slightly higher than in the female animals; both sexes were equally susceptible to trypanosomosis infection. This result is similar with previous results of (Teka *et al.*, 2012) and Daya and Abebe (2008) who obtained no significant difference in susceptibility between the two sexes.

The occurrence of disease in three different body condition (poor, good and medium) animals shows the highest prevalence in poor body condition (7.94%) followed by in medium (4.32%) and good body condition (2.53%). This finding is consistent with the observations of Tadesse and Tsegaye (2010) and Bitew *et al.* (2011). In contrast, 21.93% aparasitaemic cattle were with poor body

condition and this indicates that other factors such as diseases, nutritional factors as well as management system may have contributed for the poor body condition of cattle (Smith, 2009). The absence of trypanosome infection in the poor body condition animals might be due to mal-nourishment, internal parasites and other body loss causing diseases (OIE, 2009).

In this study, there was a significant difference between mean PCV values of infected and non-infected animals. This factor may be related to the debilitating nature of the disease (Radostits *et al.*, 2007). In the absence of other diseases causing anemia, a low PCV value of individual animals is a good indicator of trypanosome infection (Abebe, 2005; Marcotty *et al.*, 2008). During the study period, cattle with $PCV \leq 24\%$ were considered anemic (Van den Bossche *et al.*, 2001) which is said to be the principal sign of trypanosomosis in livestock (Gardiner, 1989). In the present study, the mean PCV value for the parasitemic cattle was 21.52% while the mean PCV value for the aparasitaemic cattle was 28.75%; which is similar with the report of (Rowlands *et al.*, 1993) in Ghibe valley at South Western Ethiopia, in which was stated that the average PCV of parasitological negative animals was significantly higher than the average PCV of parasitological positive animals.

From the total cattle populations sampled during study period, 19.42% of cattle populations have $PCV \leq 24\%$, but some of them react negatively for trypanosomosis infection and this may have occurred due to the inadequacy of detection method used or delayed recovery of anemic situations after recent treatment with trypanocidal drugs or may be due to the compound effect of poor nutrition and hematophagous helminthes infection such as haemonchosis and bunostomiasis (Afework, 1998). The present study also revealed that almost 2.23% of the cattle have a PCV value in the normal range ($PCV > 24\%$) but they react positively to trypanosomosis infection and this may have occurred due to recent infection with trypanosomosis. This result agree with the previous result of (Garoma, 2009) who conclude that cattle's having PCV value of normal range were shown to be infected with trypanosome parasite.

Morphological identification of the species of trypanosomes involved in the study area was *T. vivax* and *T. congolense*. In the present study the prevalence of *T. congolense* was (62.96%) higher than the prevalence of *T. vivax* (33.33%) and TC & TV mixed infection (3.70). The high proportion of *T. congolense* detected in this study agreed with the report of Abebe (2007) which is 58% due to *T. congolense*. Such a high ratio of *T. congolense* may be caused by the presence of a biological vector (Glossina), whereas *T. vivax* is more readily transmitted mechanically by biting flies than tsetse flies (Langridge, 1976) and *T. congolense* is mainly confirmed in the blood, while *T. vivax* and *T. brucei* also invade the tissues (Hoare, 1972;). Other studies by Rowlands *et al.* (1995), Leak *et al.* (1999) have indicated that *T. vivax* is highly susceptible to treatment while the problems of drug resistance are higher in *T.*

congolense.

The density of tsetse population in the study area, and the level of their contact with the host, can be determined by the level of infection (Radostits *et al.*, 2007). The average tsetse fly density in the area was 382 (4.91 F/T/D) and over all density of all flies are (3.56 F/T/D). This finding seems to be slightly lower than the previous report 11.2 F/T/D by SRVL (2006) in daremello district. Concerning tsetse fly species only *Glossina tachnoides* were observed and among mechanical transmitters of trypanosomiasis found in the study area were *Tabanus*, *stomoxys* and *haematopota*.

5. Conclusions and Recommendations

Our study results revealed that bovine trypanosomiasis and apparent tsetse density survey in four villages of Didesa Woreda indicated that an overall 4.86% prevalence of the disease and density of tsetse flies with an overall apparent density of 3.56flies/trap/day. In this study *T. vivax* (33.33%) and *T. congolense*(62.96%) are trypanosome species identified and on entomological survey, only one species of tsetse fly identified was *G. tachnoides*. Higher prevalence of trypanosomiasis infection was observed in animals with poor body condition and low Pcv animals. From the total risk factors only pcv found significant and others are in significant.

Based on the conclusion, the following recommendations are forwarded:

- Strategic control of bovine trypanosomiasis including vector control should be strengthened to improve livestock production and agricultural development in the area.
- Attempt should be made to expand government and private veterinary services to serve the community in the study areas.
- Further surveys and studies should be conducted and appropriate, feasible control of trypanosomiasis must be done.
- Educating the public in the tse tse belt or affected areas of trypanosome to participate in control strategies.

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