



STUDY ON BOVINE TRYPANOSOMOSIS AND ASSOCIATED RISK FACTORS IN ASSOSA DISTRICT

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ABSTRACT: Across sectional study on prevalence and associated risk factors of Bovine Trypanosomosis was conducted in Assosa district, from March to June, 2022 to determine the prevalence of Trypanosomosis in Bovine and the prevailing species of trypanosomes, associated risk factors and its vector density. Blood samples were collected from (n=384) randomly sampled cattle and examined using parasitological (buffy coat technique) and hematological (measurement of packed cell volume) procedures. An overall, 10.15% (39/384) prevalence was recorded. The infection was caused by *T. congolense* 32/48 (66.66%), *T. vivax* 9/48 (18.75%), *T. brucei* 3/48(6.25%) and mixed infection was found to be 4/48 (8.33 %). The infection rate was found statistically significant (P<0.000) among trypanosome species. Mean packed cell volume (PCV) value of infected animals was lower (19.06%) than non-infected animals (26.01%) and the variation was statistically significant (P<0.000). Non- significant difference was recorded within study sites, sex and age categories of animals (P>0.05), where as significant association was observed in body conditions. *G. morsitans submorsitans* were the tsetse fly caught and its mean apparent density measured as fly per trap density was 2.99. In addition, other mechanical vectors such as Stomoxys, tabanids and haematopota with f/t/d of 1.77, 0.26 and 0.25 were recorded respectively. In conclusion, the result of the current study showed the economical importance of trypanosomosis in the study area signaling for strategic control efforts.

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Key words: Blood, Packed cell volume, risk factor, Trypanosomosis, and Tsetse fly

1. Introduction

Trypanosomiasis is a devastating disease of livestock caused by protozoal parasites of the genus trypanosoma that inhabits blood and other tissues of vertebrates including animals, wildlife and human (Adam *et al.*, 2003; Gupta *et al.*, 2009; Bal *et al.*, 2014). It is a vector borne disease that is transmitted biologically by tsetse flies and mechanically by other biting flies (FAO, 2002; OIE, 2009). It is a major constraint contributing to direct and indirect economic losses to crop and livestock production (Abebe, 2005) and has a significant negative impact on economic growth in many parts of the world (Taylor *et al.*, 2007; Sharma *et al.*, 2013), particularly in sub-Saharan Africa (Cecchi *et al.*, 2008).

Tsetse transmitted animal trypanosomiasis is one of the major constraints to socio-economic development in Africa. Tsetse flies infest approximately 10 million km² of the continent affecting 38 countries. It is considered that 7 million km² of this area would otherwise be suitable for livestock and or mixed agricultural development. About 30% of the 147 million cattle in countries affected by tsetse are exposed to the disease. The situation with regard to sheep, goats, pigs,

horses, donkeys and camels is probably similar but is less well documented. The annual losses in livestock production due to trypanosomiasis are estimated at \$5 billion. Data available at present indicate that the overall situation is deteriorating. Since the 1950's the areas of savanna tsetse infestation have continued to increase. As a result there is increasing pressure on tsetse-free pasturages (Getachew A., 2005).

Livestock is backbone of the socio economic system of most of the rural communities of Africa (Elnasri H., 2005). Ethiopia is known for its large and diverse livestock resource endowments. Livestock is primarily kept on small holdings where it provide drought power for crop production, manure for soil fertility and fuels, serves as a sources of family diet and sources of cash income (from livestock and livestock products). Despite large livestock population, Ethiopia fails to optimally utilize this resource due to different constrains facing the livestock subsector Shortage of nutrition, reproductive insufficiency, management constraints and animal disease are the major constraints (Bekele. J *et al.*, 2010). One of the diseases hampering the livestock subsector is trypanosomosis (Getachew A., 2005).

In Ethiopia, tsetse transmitted animal trypanosomosis is a serious constraint to livestock production and agricultural development, exorcising farmers and livestock keepers out of areas having very high potential for growth, and forcing them to live on a highly degraded highlands of the country (Abebe, 2005). The problem caused by tsetse and trypanosomosis is not only limited to inflicting diseases but also leading to significant negative impacts such as losses due to mortality and morbidity in domestic animals, cost of livestock treatment and tsetse control, and getting rid of draught animals from their infestation areas (Juyal *et al.*, 2005).

Trypanosomosis is a complex disease caused by unicellular parasites found in the blood and other tissues of vertebrates including livestock, wild life and people. 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 equiperdium* in horses (Abebe G, 2005). The Diagnosis of trypanosome infection is based on clinical signs; but the clinical signs of the African Animal trypanosomosis are indicative but are not sufficiently pathognomonic. Therefore, standard methods have been developed and applied practically to diagnose the disease in animals. The methods include: direct microscopic examination of blood, either by the wet film method; but it is insensitive (Getachew, 2005). Stained thin and thick smear techniques permit detailed morphological studies and identification of different *Trypanosoma* species by light microscopy. Sensitivity can be improved through parasitological buffy coat techniques of concentration of the parasites by centrifugation and blood inoculating into susceptible laboratory animals (Getachew, 2005).

Benishangul Gumuz is one of the five regions of Ethiopia infested with more than one species of tsetse flies (Keno, 2005). Five species of Glossina (*G. morsitans submorsitans*, *G. Pallidipes*, *G. tachnoides*, *G. f. fuscipes* and *G. longipennis*) have been registered in the region (Keno, 2005; ARAHDL, 2016/17). The tsetse flies (vectors), *G. fusca*; the bush fly, *G. morsitans*, which inhibit principally savannah area and *G. palpalis*; a riverine species, effectively prevent the rearing of the cattle over the large area of the region (NTTICC, 2004). And nearly 31,000 km² or 62% of the Benishangul Gumuz region's total land area is believed to be infested with tsetse fly (NTTICC, 2004).

In addition to this, the major livestock diseases such as pasteurellosis, black leg and parasitism due to both internal and external parasites are identified in the region, despite this fact, very scant information is available about the disease epidemiology and its vector with baseline data in the Assosa district. The aims of the

present study was, therefore, to assess the epidemiology of trypanosomosis and its vector density in six kebeles of Assosa district.

Therefore, the objectives of the study were

- To determine the prevalence and assess associated risk factor of bovine trypanosomosis
- To determine apparent density of tsetse and other biting flies.

2. Materials and Methods

2.1 Study area

The study was conducted from March to June, 2022 in Assosa district. It was conducted in six kebeles here after called sites namely: Assosa town, Amba-8, Megel 29, Megel 32, Amba5 and Komoshiga 25. The district has 74 kebeles covering an area of 2317 kilometer square with human population of 47666. Assosa is located between 8030'' and 40°27" N and 34°21" and 39°1" E. It has an altitude range of 1000-1570 meter above sea level. Its annual temperature ranges between 16^oc- 34^oc and its annual rainfall range is 900-1300 mm and it extends from May to October with peak rainy periods from June to August (NMSA, 2007). The livelihood of the society largely depends on mixed livestock and crop production having livestock population of 21,216 Cattle, 24,560 Goat, 4,073 Sheep, 4265 Equines, 4,372 donkeys, 66 horses, 50,315 Poultry & 26336 beehives. Extensive livestock husbandry, feeding and outdoor housing system was found, Back yard chicken Management system is practiced, where local breeds are allowed to scavenge, what nature provides them. In the woreda, animal movement was due to agriculture and trade purpose. Animal in the area was used for meat, milk, ploughing, traction power, and income generation. Socio economy of the people in the area was mainly depending on mixed farming (CSA, 2015 and Assosa woreda agriculture office, 2021).

2.2 Study Design and Study Animals

Cross- sectional study design was used. A local zebu cattle, which are usually kept under an extensive husbandry system grazing the communally owned pasture land throughout the year were randomly sampled. They grazed together during the day time and returned to their individual owner's farmstead each evening. The body condition of each of the study cattle was scored as good, medium and poor as designed by Nicholson M, and Butterworth M (1986). Concurrently, their age was categorized in years (< 2, 2-7, >7) based on De-Lahunta A, and Habel RE (1986).

2.3 Sampling Techniques and Sample Size Determination

This study was conducted in six peasant associations including Assosa town, Amba5, Amba8, Megelle 29,

Megele 32 and komoshiga 25. The study sites were selected purposively as convenient, particularly based on accessibility, agro ecology, and presence of cattle. The animals were sampled randomly involving both sexes, all age groups, and all types of body conditions. The desired sampling size was calculated according to the formula given by (Thrusfield, 2005). The sample size was determined based on the expected prevalence of 50%; confidence level of 95%, and 5% desired absolute precision.

So, it was calculated by using the formula
$$n = \frac{1.96^2 * 50\% (1 - 50\%)}{(5\%)^2} = \frac{3.84 * 0.5 (1 - 0.5)}{(0.05)^2} = 384$$

As result a total of 384 cattle were calculated, and these cattle were sampled at their communal grazing area using simple random sampling.

2.4 Study methods

2.4.1 Packed cell volume (PCV) determination

Blood samples were obtained by puncturing the marginal ear vein with a lancet and collected directly into a pair of heparinized capillary tubes. The tubes were then sealed at one end with crystal seal. PCV was measured in a micro-hematocrit centrifuge (Hermmlle Labortechnik, type Z, Germany). The capillary tubes were placed in micro hematocrits centrifuge with sealed end outermost. Then, the tube was loaded 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 5 minutes. After centrifugation, the capillary tubes were placed in a hematocrit reader. The length of the packed red blood cells column is expressed as a percentage of the total volume of blood. Animals with PCV less than 24% were considered to be anemic (OIE, 2008).

2.4.2 Buffy coat technique

Heparinized microhaematocrit capillary tubes, containing blood samples were centrifuged for 5 minutes at 12,000 rpm. After the centrifugation, trypanosomes were usually found in or just above the buffy coat layer. The capillary tube was cut using a diamond tipped pen 1 mm below the Buffy coat to include the upper most layers of the red blood cells and 3 mm above to include the plasma. The content of the capillary tube was pour onto a glass slide, and covered with cover slip. The slide was examined under x40 objective and x10 eye piece for movement of parasite (Paris, 1982). Trypanosome species were identified according to their morphological descriptions as well as movement in wet film preparations (OIE, 2008).

2.4.3 Entomological survey

A total of 56 odour baited traps of monoconical, biconical and mono pyramidal were deployed at 200-250 m intervals to assess the density and species of tsetse

flies during the study. Each and every trap was odour baited with acetone and cow urine. The underneath of each trap pole was smeared with grease in order to prevent ants climbing up the pole towards the collecting cage that could damage the tsetse flies. The trap deployment time was 48 hours. After capturing the flies in the collecting cage, they were then sorted by sex and species and recorded. 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 (Fischer and Say, 1989). Sexing was done for tsetse fly just by observing the posterior end of the ventral aspect of abdomen by hand lens, accordingly, male flies were easily identified by enlarged hypophageum. The apparent density of tsetse flies was determined based on the daily mean number of flies captured in odour-baited traps and recorded as fly per trap per day (F/T/D) (Leak *et al.*, 2009).

2.5 Data management and Analysis

All the collected raw data and the results of parasitological and hematological examination data were entered into a Microsoft excel spread sheets program and then was transferred to Intercool STATA version 11.0 for analysis. The prevalence of trypanosome infection was calculated as the number of positive animals as examined by buffy coat method divided by the total number of animals examined at the particular time. Data collected on PCV values were analyzed by ANOVA one to compare the mean PCV values of infected animals against that of uninfected animals. Pearson's chi-square (χ^2) was used to evaluate the association of different variables with the prevalence of trypanosome infection. In all of the statistical analysis, a confidence level of 95% is used and P-value of less than 0.05 (at 5% level of significance) was considered as statistically significant.

4. RESULT

4.1 Parasitological result

Out of the total animals examined (n=384), 39/384(10.15%) were found to be infected with trypanosomes (Table-5). The prevalence in terms of trypanosome species was 8.33% for *T. congolense*, 1.56 % for *T. vivax*, 0.52% for *T. brucei* and 0.78 % was found to be mixed infection. The proportion of trypanosome species was 32/43(74.41%) for *T. congolense*, 6/ 43(13.95%) for *T. vivax*, 2/43(4.65%) for *T. brucei* and 3/43 (6.97%) for mixed infection and the infection rate was found to be statistically significant (P<0.000) among trypanosome species (Table 4).



Table 1. Species based prevalence of bovine trypanosomosis in Assosa district

Trypanosomes	No. positive	Positive (%)	X ² (p-value)
<i>T. congolense</i>	32	74.41	308.81(P<0.000)
<i>T. vivax</i>	6	13.95	
<i>T. brucei</i>	2	4.65	
Mixed(<i>T.congolense</i> & <i>T.vivax</i>)	3	6.97	
Total	43	100	

X²= chi square

4.2 Hematological result

The mean PCV value for all examined animals was 23.02. However, the mean PCV value for non-infected and infected animals was 26.01 and 19.06 respectively. The mean PCV values of cattle were significantly ($\square < 0.000$) influenced by trypanosome infection as 19.06 % and 26.01 % PCV values in trypanosome positive and negative animals were registered, respectively (Table 7).

4.3 Trypanosomosis associated Risk factors

The highest prevalence (11.80%) of trypanosomosis was recorded in animals >7 years old whilst the lowest prevalence (8.63 %) was recorded in animals 2-7 years of old and the association was not found statistically significant among the age groups (Table 6). Slightly, higher prevalence was registered in female animals (11.16 %) than in male animals (8.98 %), which was not found to be statistically significant ($p > 0.05$) (Table 6). Trypanosomosis was recorded across the study sites with the highest and lowest prevalence of (18.29%) and (4.54 %) in mati and matha respectively and prevalence of trypanosomosis was not significant across the study sites (Table 5). The highest prevalence of trypanosomosis (16.82%) was found in animals with poor body condition while the lowest (6.89 %) was recorded in animals with medium body conditions respectively, and the difference was significant ($p < 0.000$). The effect of age, sex, sites and body condition on prevalence of trypanosomosis is summarized in Table 5 and Table 6.

Table 2: Origin based Prevalence of bovine trypanosomosis in Assosa District

Sites(PA)	No. examined	No. positive	(%) positive	χ ² (p-value)
Assosa town	72	7	9.72	9.40(P>0.09)
Amba 5	64	4	6.25	
Amba8	44	2	4.54	
Megell 29	81	6	7.40	
Megelle 32	41	5	12.19	
Komoshiga 25	82	15	18.29	
Total	384	39	10.15	

Table 6: Prevalence of trypanosomosis infection with different potential risk factors

Risk factors	No. examined	No. positive	(%) positive	χ^2 (p-value)
Sex				0.49(P>0.05)
Male	178	16	8.98	
Female	206	23	11.16	
Total	384	39	10.15	
Age(years)				0.78(P>0.05)
< 2	101	10	9.90	
2 – 7	139	12	8.63	
> 7	144	17	11.80	
Total	384	39	10.15	
Body conditions				7.32(P<0.02)
Good	161	13	8.07	
Medium	116	8	6.89	
Poor	107	18	16.82	
Total	384	39	10.15	

Table 7: Mean PCV comparison of parasitaemic and aparasitaemic animals

Status	Frequency	Mean PCV (%)	SE	X ² (p-value)
Infected	179	19.06	02.9	25.18(p<0.000)
Non-infected	205	26.01	01.18	
Total	384	23.02	01.54	

PCV: packed cell volume; SE: standard Error

4.4 Entomological survey result

The present survey of tsetse flies depicted that *G. morsitans sub morsitans* is the only species of tsetse fly responsible for cyclical transmission of trypanosomosis in the study area. Tsetse fly survey was carried out in six kebele of the study district by deploying a total of 56 geo-referenced traps (16 mono-conical, 20 mono-pyramidal and 20 biconical traps) in the river border, open wood land (savanna grass land) and on grazing fields of cattle, the number of tsetse flies captured in each study site after 48 hour is 109, 79, 68, 92, 122, 142 for Assosa town, Amba 5, Amba 8 , Megelle 29, Megelle 32 and Komoshiga 25 respectively. A total of 612 tsetse and biting flies were caught from different sites during the study period. The mean apparent density of *G. m.sub morsitans* in the survey sites was investigated as 2.99 f/t/d while the mean apparent density of mechanical vectors such as stomoxys (1.77 f/t/d), tabanids (0.26 f/t/d) and haematopota (0.25 f/t/d) were recorded. The highest fly density was observed in komoshiga 25 peasant association (7.55 f/t/d) and the lowest was recorded in Amba8 (3.4 f/t/d) (Table 8).

Table 8: Flies caught in different areas of survey sites at Assosa district

Sites	Total flies caught	No. of traps	Tsetse flies caught					Biting flies		
			No.	species	M	F	*F/T/D	Stomoxys	Tabanid	Haematopota
Assosa town	109	10	52	GT	22	30	2.6	30	4	3
Amba 5	79	9	44		15	29	2.4	26	5	4
amba8	68	10	30		11	19	1.5	28	7	3
Megell 29	92	9	61		22	39	3.38	20	5	6
Megell 32	122	9	71		26	45	3.94	43	3	5
Komoshiga 25	142	9	71		28	49	4.27	52	6	7
Total	612	56	335		124	211	2.99	199(1.77)	30(0.26)	28(0.25)

F/T/D=fly per trap per day, GT= Glossina m.sub morsitans, M=male, F=female

5. DISCUSSION

10.15 % of Cattle Trypanosomosis prevalence were reported in the study area. This finding was agreed with the study conducted by (Aki A *et al.*, 2015) who reported 8.96% bovine trypanosomosis prevalence in Kameshi District, Benishangul Gumuz region, western Ethiopia. In contrast, 22.38 % bovine trypanosomosis prevalence was reported by Bayisa *et al.* (2015) in Asossa district, which was high as compared to the current study. Similarly, 26.30% cattle trypanosomosis prevalence was reported by Aki A *et al.* (2017) in Mandura district which were higher than the present findings.

This research showed that the infection was predominantly caused by *T. congolense* 32/43 (74.41%), *T. vivax* 6/43(13.95%), and *T. brucei* 2/43(4.65%) and mixed infection 3/43(6.97%). This result is in line with the reported proportions of *T. congolense* (75.5 %) followed by *T. vivax* (14.28%) from Metekel and Awi zones (Mekuria *et al.*, 2011). This result was also in consistent with prior reports of (Mulaw *et al.*, 2011) who studied on prevalence of major trypanosomes affecting cattle in Assosa district of Benishangul Gumuz Regional State, western Ethiopia and who found proportional prevalence of *T. congolense* to be 66.7% ; (Abraham *et al.*, 2012) conducted their study on prevalence of bovine trypanosomosis in selected sites of Arba Minch district, Southern Ethiopia whose result showed proportional prevalence of *T. congolense* to be 61.4%; (Biyazen *et al.*, 2014) reported proportional prevalence of *T. congolense* to be 63.64% during their work on trypanosomosis and anemia in cattle population of Dale Wabera district of Kellelem Wollega Zone, western Ethiopia.

The high proportional infection rate of *T. congolense* in cattle might be attributable to the high number of serodemes of *T. congolense* relative to other species of trypanosomes. It could also be due to the possible development of better immune response to *T. vivax* by the infected animals as demonstrated by (Leak *et al.*, 1993). Further, it might be attributed to the efficient transmission of *T. congolense* by cyclical vectors than *T. vivax* in tsetse-infested areas. Previous reports indicated that *T. congolense* and *T. vivax* are the most prevalent trypanosomes that infect cattle in tsetse infested and tsetse free areas of Ethiopia respectively (Leak *et al.*, 1999). Different studies (Leak *et al.*, 1993; Rowland *et al.*, 1995) have indicated that *T. vivax* is highly susceptible to treatment while the problems of drug resistance are higher in *T. congolense*.

The effect of different risk factors such as sex, age categories, study sites, PCV status, trypanosome

species, and body conditions on prevalence of cattle trypanosomosis was studied and, statistically significant associations were observed in, PCV status, body conditions and trypanosomes species ($p < 0.05$) while sex groups, age categories and study sites were not found to be statistically significant ($\square > 0.05$). This result is in agreement with previous reports of (Lelisa *et al.*, 2015 and Bayisa *et al.*, 2015).

The overall mean PCV value for examined animals was 23.02. The mean PCV value of infected animals was significantly lower (19.06) than that of non - infected animals (26.01). This result is in alignment with previous works of (Ali *et al.*, 2011; Mulaw, 2011).

Entomological survey result indicated that, *G. morsitans sub morsitans* was the only tsetse fly caught and its mean apparent density measured as f/t/d was found to be 2.99. It accounts for 54.73 % (335/612) out of the total flies caught. The highest fly density was observed in komoshiga 25 peasant association (7.55 f/t/d) and the lowest was recorded in amba8 (3.4 f/t/d). 2.99 (f/t/d) Tsetse fly, 1.77(f/t/d) stomoxys, 0.26 (f/t/d) tabanidae, 0.25 (f/t/d) hematopota were investigated in this research. The current finding is concord with the previous findings of (NTTICC, 2012-2014) at neighboring mandura district of western Ethiopia which was reported to be 3.59 f/t/d, 1.38 f/t/d, 0.33 f/t/d and 0.014 f/t/d, for tsetse fly, *stomoxys*, *haematopota*, and *tabanus* respectively. Similarly, this finding was in line with the previous findings of (Aki A *et al.*, 2015) in Kamshi District, which was reported to be 2.54, 2.84, 1.54, and 0.92 for *G. tachinoides*, *stomoxys*, *tabanids* and *haematopota* respectively.

6. CONCLUSION AND RECOMMENDATIONS

The high prevalence of Cattle trypanosomosis (10.15 %) were remains a major problem that hinders livestock production and productivity in the district. The most widely distributed and dominant species of trypanosomes in the study sites are *T. congolense* (74.41%) followed by *T. vivax* (13.95%), and to some extent *T. brucei* (4.65 %) which was mainly transmitted by *G. morsitans sub morsitans* and other biting flies with f/t/d/ of 2.99, 1.77, 0.26 and 0.25 for *G. tachnoides*, *stomoxys*, *tabanids* and *haematopota* respectively. Parameters of study animals such as sex, sites, and age were not found to be a risk factor for trypanosomosis whereas body conditions and trypanosome species were risk factors. This study showed that trypanosome infection and other factors such as (nutritional, seasonal,

concurrent disease) was found to negatively affect the PCV values of animals.

Based on the current findings, the following recommendations are forwarded:-

- Particular attentions towards the identified trypanosome species are essential to control the impact of the disease on cattle that are potential reservoir of the infections.
- Development of control options that could minimize the tsetse fly and biting flies in the study area should be introduced in a wholistic approach.
- Proper and strict follow up of trypanocidal drug distribution, therapeutic strategies and alternative control measures should be implemented by concerned stake holders.
- The farmer in the area should be trained how to control the vector of the disease and provided with materials
- Further study on the trypanosomosis, tsetse fly investigation and also on possible factors should be carried out by laboratory experts to give the best strategic control and prevention measures in the study area.

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