



Prevalence of Bovine Trypanosomosis in Bambasi District, Assosa Zone, Benishangul Gumuz Regional State, Western Ethiopia.

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Abstract: A cross-sectional study was carried out in Bambasi district of Benishangul Gumuz Regional State, Western Ethiopia from November 2017 to May 2018 to determine the prevalence of trypanosomosis, identification of circulating trypanosome species, identification of the vectors and associated risk factors. Blood samples were collected from a total of 400 cattle and examined using buffy coat technique. Overall 25 (6.25%) trypanosomosis prevalence was recorded. The major species of Trypanosoma identified include: Trypanosoma Congolense (56%), Trypanosoma vivax (24%), Trypanosoma brucei (12%) and mixed infection accounted for 8%. Mean packed cell volume (PCV) value of the infected animals was lower ($21.08\% \pm 2.06$) than uninfected animals ($26.06\% \pm 2.6$) and the variation was statistically significant ($P < 0.05$). Overall anemia prevalence of 30.5% (122/400) was recorded and it was significantly higher (72%) in infected cattle than in non-infected (27.73%). Significant difference was not observed between sex groups and age categories ($p > 0.05$) but there was significant difference in the prevalence of trypanosomosis among study sites and body conditions ($P < 0.05$). Glossina morsitans sub morsitans was the only tsetse fly caught and its mean apparent density measured as fly/trap/day was 0.325. In addition, mechanical vectors of trypanosomosis such as Stomoxys (0.15 f/t/d), Tabanus (0.1f/t/d) and Haematopota (0.04 f/t/d) were identified. In conclusion, although the result of the current study showed relatively low prevalence of bovine Trypanosomosis, the impact of this disease on production and the role of these animals as potential risk of transmission to other livestock is not underestimated. Therefore, appropriate intervention measures need to be taken.

[Mubarik Kedir Haile Worku. **Prevalence of Bovine Trypanosomosis in Bambasi District, Assosa Zone, Benishangul Gumuz Regional State, Western Ethiopia.** *Researcher* 2020;12(8):45-51]. ISSN 1553-9865 (print); ISSN 2163-8950 (online). <http://www.sciencepub.net/researcher>. 8. doi:[10.7537/marsrsj120820.08](https://doi.org/10.7537/marsrsj120820.08).

Key words: Bambasi, Trypanosomosis, Tsetse fly, prevalence, Risk factors

1. Introduction.

The livelihoods of more than 85% of the people of Ethiopia depend on the agricultural sector. This sector mainly possesses crop production, livestock production and mixed farming. Since people are dependent on this sector, the presence of livestock is one of the necessities to this sector. This fact has made Ethiopia to be one of the richest countries in livestock production in Africa (Azage and Alemu, 1997). Official figures gives a National Ethiopia animal population of 40.9 million cattle, 25.5 million sheep, 23.4 million goats, 2.7 million horses, 0.63 million mules, 5.2 million donkeys and 1.07 million camels (CSA, 2003).

Tsetse fly infest 10 million km² potentially productive land of Africa between 14° N and 29° S (Radiostits, 2006). There are 23 different species of tsetse fly and they exist in 37 countries of Africa. Five of them namely *G.m.submorsitans*, *G.pallidipes*, *G.tachinoides*, *G.fuscipes* and *G.longipennis* are reported in Ethiopia. Several reports made in Ethiopia

revealed that tsetse fly occupy over 66,000 km² areas (Ford *et al.*, 1976) based on 1500 masl, breeding limits in the south and southwestern valley of the country. Langridge (1976) has reported that some 98,000km² areas 1600 masl breeding limits in the southern and southern western of Ethiopia. The tsetse flies in Ethiopia are confined to the southern and western regions between longitude 33° and 38° E and latitude 5° and 12° N which amounts to about 200,000 km². Out of this 31,000 km² or (62%) Regional land area of Benishangul-Gumuz is infested with Tsetse fly (NTTICC, 1996). Tsetse flies are hard to control and the tsetse fly infestation is becoming more and more serious in Africa. The clearing of large forest tracks some time cause the flies to spread to more populated areas and the deforest land covered with savannah grass consequently newly invade by morsitans group (Jordan, 1986).

Tsetse flies are enormous health risks in part of Africa. They can transmit a disease

trypanosomosis. African trypanosomosis is a hemoparasitic disease considered as the main obstacle to animal production development (Getachew A. and Yilma J., 1996). It is the wasting disease; affected animals are chronically unproductive in terms of milk, meat, manure, traction. The number of trypanocidal drugs available for treating and preventing infections in endemic areas is limited, and about 6 million doses are administered yearly in Africa. The drugs have been in the market for over 30 years, their range of therapeutic safety is small. The disease in Africa costs livestock producers and consumers an estimated US \$ 1340 million each year (Radostits, 2006).

Mortality and the morbidity rate can be high. There is a direct association between increase prevalence and proximity herd pens watering points distance but no association of herd pens to grazing point distances which suggests that hydrological network played an important part in trypanosomosis (Enwezor *et al.*, 2009). The disease distribution over 10 million km² of potentially productive land of Africa. The risk falls between 15^o N and 29^o S latitudes. As the result a total of 14.8 million cattle 6.12 million sheep and goats, 1 million camels and 1.23 million equines are at risk of contracting the disease (NTTICC, 2001) in Ethiopia. Therefore, the main objectives of the present study were to determine prevalence of bovine trypanosomosis and associated risk factors; to identify the species of trypanosomes in the study areas and to forward possible control measures.

2. Materials and Methods

2.1 Study area

Bambasi district is located in Benishangul Gumz Regional State, west Ethiopia and it is 616 km away from Addis Ababa. The study was conducted from November 2017 to May 2018 in 3 PAs namely nebar keshmando, keshmando kutr hulet and shobora of Bambasi district. Bambasi district is located 09°45' N and 34°45' east. The altitude of the area ranges from 1100-4500 m.a.l bordering the Dabus river system to east direction. Topography of the area is marked by hill, steep slopes and flat surface of the land. The district has a sub-humid climate with a hot temperature, with less variation in average temperature between day and night time. It receives high and reliable annual rain fall. The rain fall in the area is bimodal. The mean annual rain fall is recorded to be 1375mm ranging from 1350-1400mm. The long dry season lasts from December to May. The area experiences an average temperature of about 32°C. The highest average monthly temperature occurs in May (29-32°C). Where the mean maximum temperature is 35°C. The coldest month is August, when the average

monthly maximum temperature is 21 °c (NMSA, 2008).

Livestock population in the study area comprises: Cattle 40,152 Sheep 3,439, Goat 12,452, Equines 5,560 & Poultry 50,832, according to the recent information from Bambasi district agricultural office. They provide with vast range of products and services such as milk, meat, skin, hair, horns, bones, and manure etc. The commonly encountered animal diseases in the area were trypanosomosis, pasteurellosis, black leg, CBPP, PPR, LSD, external parasites and internal parasite (BG.BARD, 2004).

2.2. Study population

A total of 400 blood samples were collected from cattle during the study period. To assess the socio-economic impact of trypanosomosis animal owners of the study group were interviewed about the husbandry practices, the farming practices, treatment cost (expenses against trypanosomosis control) and other trypanosomosis and its vector related questions. The different variables such as, species, sex and age groups were involved in the study as risk factors. Age categorization was as described by Gatenby into Young (< 1 year) and Adult (> 1 year).

2.3. Study design and sample size determination

A cross sectional epidemiological study design was employed for this study. The sample size for the study was determined with 50% expected prevalence to increase the precision of the data and calculated using a formula: $n = (1.96)^2 \times P_{exp} (1 - P_{exp}) / d^2$ (Thrustfield, 2005).

Where

n = the required sample size for the district

P_{exp} = expected prevalence (50% in this case)

d^2 = desired absolute precision (5% in this case)

Therefore; $1.96^2 \times 0.50 (1 - 0.50) / (0.05)^2 = 384$

2.4 Study Methodology

2.4.1 Fly survey

During the study one type of traps was deployed: 60 monoconical, traps. Every trap was odor baited with acetone, Octanol and cow urine. The underneath of each trap pole was smeared with grease in order to prevent the ants climbing up the pole towards the collecting cage that could damage the tsetse flies. The trap deployment time was 48 hours. After the flies captured 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 (Langridge, 1976; 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, as a result male flies easily identified by enlarged hypopygium.

2.4.2. Sample Storage and Processing

The sampling informed formats were documented at the laboratory for future information. The capillary tubes were centrifuge immediately after arrival of the laboratory to prepare Buffy coat to detect the parasites based on their movements, to provided screening the case and PCV evaluation. Before staining blood films need to be fixed with acetone free methyl alcohol (methanol) for some minutes in order to prevent hemolysis while staining them with aqueous (water –based) stain such as Giemsa. The slides were stored at slide storing boxes until staining and examination.

2.4.2. Hematological examination

The blood samples were centrifuge at high speed (12,000 rpm) for 5 minutes. Finally the packed cell volume (PCV) value were read by micro-hematocrit reader which could be adjusted individually for the length of the blood column in each tube to get value indication presence, absence and degree of anemia (Uilenberg,1998).

2.4.2. Parasitological examination

More sensitive technique utilizes centrifugation in micro-heamatocrit the followed by microscopic examination of the interface between the Buffy coat and plasma. Capillary tubes containing blood after centrifugation were cut with diamond pointed pen

1mm below the Buffy coat to include the upper most layer of red blood cell and 3mm above to include the plasma so that the contents were gently expressed onto a slide, mixed and covered with cover slip (22mm x22mm). The preparation were then examined using a 10x eye piece in combination with 40x objective to get optimum views allowing large visual field and sufficient magnification for easy identification of trypanosomes based on their movement (Murray, 1977)

2.5. Data Analysis

All data recorded in this study was entered in to Microsoft excel and subsequently analyzed using STATA version 7 soft ware. Chi-square test was used to determine the variation in Trypanosomes between sex, age, body condition, PCV and species.

Results

Out of total animals examined (n=400), 25/400 (6.25%) were infected with trypanosomes. The prevalence in terms of trypanosome species was 3.5 % *T.congolense* and 1.5% *T.vivax*, 0.75% *T. brucei* and 0.5% mixed infection. The proportion of trypanosome species was 14/25(56 %) *T. congolense*, 6/25(24%) *T. vivax*, 3/25(12%) *T.brucei* and 2/25(8%) mixed (Table 1).

3.1. Prevalence of trypanosomosis species identified

Table 1: The species of Trypanosoma identified from the study sites

Peasant association (PA)	No of examined	Parasites identified				Total	X ²	p- value
		T.congolense	T.vivax	T.brucei	mixed			
Nebar keshmando	145	7	3	2	1	13	182.75	0.0001
Keshmando kutre hulet	130	5	2	1	1	9		
shobora	125	2	1	0	0	3		
Tatal	400	14	6	3	2	25		
Prevalence	%	3.5	1.5	0.75	0.5	6.25		

Table 2: Prevalence of trypanosomosis by peasant associations

Peasant association (PA)	No of examined	No of positives	Prevalence(%)	X ²	P-value
Nebar keshmand	145	13	8.96	0.049	11.101
Keshmando kutre hulet	130	9	6.92		
shobora	125	3	2.40		
Total	400	25	6.25		

3.1. Mean PCV Distribution and Anemia in Studied Area

The mean PCV value for whole examined animals was 25.75 ± 2.58 SE. However, the mean PCV value for uninfected animals was 26.06 ± 2.6 SE and mean PCV value of the infected animals was 21.08± 2.06 SE. The mean PCV values of cattle were significantly ($\square = 0.000$) influenced by trypanosome infection as 21.08 % and 26.06 % PCV values in trypanosome positive and trypanosome negative animals were registered, respectively (Table 4). The

overall anemia prevalence in the studied district was 30.5 % (122/400). The anemia prevalence was significantly higher in trypanosome infected cattle (72%) than in non-infected cattle (28%) ($\square < 0.000$). Of 30.5 % anemia prevalence, 4.5% (18/400) was trypanosome infected animals. However, large number of animals 26% (104/400) had anemia (PCV < 24) without having trypanosome infection. Some animals 1.75% (7/400) were infected by trypanosome but their PCV was found normal (Table 3).

Table 3: Mean PCV value between infected and uninfected Bovine of bambasi district

Status	Frequency	Mean PCV (%)	SE	Overall PCV	X ²	p-value
Infected	25	21.08	2.06	527	25.37	0.000
Uninfected	375	26.06	2.6	9,772		
Total	400	25.75	2.58	10,299		

Table 4: Proportion of anemia in infected and uninfected Bovine population of bambasi district

Status	anemia	Frequency	Percent/%	Percent share per strata
Infected	Anemic	18	4.5	72
	Non-anemic	7	1.75	28
Non-infected	Anemic	104	26	27.73
	Non-anemic	271	67.75	72.27

3.2. Prevalence of Trypanosomosis by Age, Sex, Sites and body Condition

The highest trypanosomosis prevalence (7.14%) was recorded in >7 years old animals whilst the lowest prevalence (5.13%) were <2years old. Slightly higher prevalence was registered in males 6.5% than in females 6.1 %, which was statistically non-significant. Trypanosomosis was recorded across the study sites with the highest (8.96 %) prevalence in Nebar keshmando PA and the lowest 2.4 % in Shobora PA. Trypanosomosis prevalence was statistically significant among study sites. There was a significant difference ($P < 0.005$) in the prevalence of trypanosomosis between good and poor body

conditioned animals with highest prevalence in poor body condition category.

3.3 Entomological Findings

A total of 74 Tsetse and biting flies were caught during the study period from different sites. Out of the total, 39 (52.7 %) were belonging to tsetse of the genus Glossina, followed by 18 (24.32%) Stomoxys, 12(16.22 %) Tabanid and 5 (6.76%) Haematopota. Only Glossina morsitans submorsitans were identified in the survey site with the overall apparent density of 0.325 F/T/D (fly/trap/day). The highest fly density were observed in Nebar keshmando peasant association 21 (0.525 F/T/D) and the lowest recorded in Shobora 7 (0.175 F/T/D) (Table 6).

Table 5: prevalence of bovine trypanosomosis against the various risk factors in Bambasi district

Risk factors	No. examined	No. positive	Prevalence (%)	χ^2	p-value
Sex					
Male	154	10	6.5	0.85	0.35
Female	246	15	6.1		
Total	400	25	6.25		
Age group (years)					
< 2	78	4	5.13	0.110	0.946
2 – 7	280	18	6.43		
> 7	42	3	7.14		
Total	400	25	6.25		
Body conditions					
Good	155	6	3.87	32.75	0.000
Medium	167	10	5.99		
Poor	78	9	11.54		
Total	400	25	6.25		

Table 6. Vectors of trypanosomosis identified from the study sites

Kebele	Tsetse fly			Biting fly					
	Glossina m.sub moristance			stomoxys		Tabanus		Hematopota	
	M	F	ftd	T	ftd	T	ftd	T	ftd
Nebar keshmando	6	15	0.525	9	0.225	5	0.125	3	0.075
Keshmando kutre hulet	3	8	0.275	5	0.125	4	0.1	2	0.05
shobora	2	5	0.175	4	0.1	3	0.075	0	0
Total	11	28	0.325	18	0.15	12	0.1	5	0.04

4. Discussion

The present study revealed an overall prevalence of 25/400 (6.25%) in the study area. This finding was lower than earlier works of (Mekuria, S et al., 2011) who reported 20.74% from Metekel zone who studied Survey on bovine trypanosomosis and its vector in Metekel and Awi zones of North west Ethiopia and, the result of the reported bovine trypanosomosis prevalence of 24.7% from neighboring Mao- Komo special district (Ali, D. et al., 2011). The lower prevalence of Trypanosomosis recorded in Bovine in this study may be attributed to the establishment of Assosa Tsetse fly and Trypanosomosis Control and Surveillance Center under National Institute for Control and Eradication of Tsetse fly and Trypanosomosis, which practice on application of control measures such as; pour on by Deltamethrin 1% on the back of Animals, deployment of traps and targets and treatment of sick animals.

The study showed that the infection was predominantly caused by *T. congolense* 14/25 (56%), *T. vivax* 6/25(24%), *T. brucei* 3/25(12%) and mixed 2/25(8%). This result was in agreement with prior reports of (Mekuria, S et al., 2011) who studied prevalence of major trypanosomes affecting cattle in the neighboring Assosa district of Benishangul Gumuz Regional State, Western Ethiopia, and *T. congolense* proportional prevalence of 66. 7%; (Abraham Z. et al., 2012) was found worked on the prevalence of bovine trypanosomosis in selected district of Arba Minch, Southern Ethiopia and reported *T. congolense* proportional prevalence of 61.4%; (Biyazen, H. et al., 2014) and reported *T. congolense* proportional prevalence 63.64% during his work on trypanosomosis and anemia in cattle population of Dale Wabera district of Kellelem Wollega Zone, Western Ethiopia; Bayisa, K et al., 2015 demonstrated *T. congolense* proportional prevalence of 85% during his research on cattle trypanosomosis prevalence in Asossa district, Benishangul Gumuz Regional State, Western Ethiopia. The high proportion infection rate of *T. congolense* in cattle might be attributable to the high number of serodemes of *T. congolense* relative to *T. vivax*. It could also be due to the possible development of better immune response to *T. vivax* by the infected animals as demonstrated by (Leak, S 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 (Langridge WP., 1976; Leak, S et al., 1999). Different studies (Leak, S et al., 1993; G. J. Rowlands et al., 1995) have indicated that *T. vivax* is highly susceptible to

treatment while the problems of drug resistance are higher in *T. congolense*, and *T. congolense* is mainly confirmed in the blood, while *T. vivax* and *T. brucei* also invade the tissues (L. E. Stephen., 1986).

There was a significant difference ($p < 0.05$) in the prevalence of trypanosomosis among the study sites and body condition. This result is in agreement with previous reports (Mihreteab, B et al., 2011; 29-31, Ayele, T., et al., 2012; Lelisa, K et al., 2015). The overall anemia prevalence in the studied district was 30.5% (122/400). The anemia prevalence was significantly higher in trypanosome infected cattle (72%) than in non-infected cattle (27.73%) ($p < 0.05$). This is in concordance with previous results from different researchers (Mihret et al., 2007; M. Bekele et al., 2011, Biyazen, H et al., 2014). Out of 30.5% anemia prevalence, 4.5% (18/400) was trypanosome infected animals. Nonetheless, 26% (104/400) of non-infected animals were found to be anemic (PCV < 24). This indicates the fact that other factors such as gastrointestinal parasitism, nutritional deficiencies, fasciolosis and vector-borne diseases could affect the PCV value of cattle (P. van den Bossche et al., 2001)

This study revealed that 1.75% (7/400) of the cattle were infected by trypanosome; however, their PCV was laid in the normal range. This might be attributed to the capability of infected cattle to maintain their PCV within the normal range for a certain period of time. It could also be possibly due to inadequacy of the detection method used (Murray, M et al., 1988), other anemia causing diseases (P. van den Bossche et al., 2001), or delayed recovery of the anemic situation after current treatment with trypanocidal drugs. Furthermore, the occurrence of positive animals with PCV of greater than 24% might be thought of as recent infections of the animals (P. van den Bossche et al., 2001).

The overall mean PCV value for examined animals was 25.75 ± 2.58 SE. The mean PCV value of the infected animals was significantly lower (21.08 ± 2.06 SE) than that of uninfected animals (26.06 ± 2.6 SE). This result is in alignment with previous works (Ali, D. Eta., 2011, Mulaw, S et al., 2011, Bayisa, K et al., 2015). *Glossina morsitans sub morsitans* was the only tsetse fly caught and its mean apparent density measured as f/t/d was 0.325. It accounts for 39 (52.7%) out of the total flies caught. In addition, other mechanical transmitters of trypanosomosis such as stomoxys 18 (24.32%), Tabanid 12 (16.22%) and Haematopota 5(6.76%) were recorded. The current findings were not in consistent with previous works of (Solomon, M et al., 2010) at Metekel Awi zones of Northwest Ethiopia, who reported 6.49 f/t/d and 0.65 f/t/d for tsetse and biting flies, respectively. It was also lower than findings of (NTTICC, (2004) at Bure

Iluababor zone of Western Ethiopia which was reported to be 7.23 f/t/d, 3.13 f/t/d and 0.06 f/t/d for tsetse fly, *Stomoxys* and *Tabanus*, respectively.

This result was also consistent with the previous findings of (NTTICC, 2012-2014) at neighboring Mandura districts of western Ethiopia which was reported to be 3.59 & 1.16 f/t/d; 0.15, 0.20 & 4.5 f/t/d; 0.02, 0.05 & 0.33 f/t/d; 0.014, 1.38 & 4.5 f/t/d) for tsetse fly, *stomoxys*, *tabanus* and *haematopota*, respectively. Similarly, It w also in consistent with the previous findings of (NTTICC, 2014) at Dangur districts of western Ethiopia which was reported to be 1.14 f/t/d; 4.04 & 0.09 f/t/d; 3.84 & 0.04 f/t/d; 0.4 & 0.6 f/t/d) for tsetse fly, *stomoxys*, *tabanus* and *haematopota*, respectively.

5. Conclusion

The most common trypanosomes species was *T.congolense* followed by *T.vivax*. The animal parameters such as sex and age were not found to be a risk factor; however, study site and body conditions were identified as risk factors. The mean PCV value of infected animals was significantly lower than that of uninfected animals indicating the adverse effect of trypanosomosis on the PCV profile of cattle. Trypanosomes were not detected in some anemic cattle indicating the occurrence of other causes of anemia in the area. *G.moristans sub morsitans* was the only tsetse fly species discovered in this study. Other mechanical transmitters of trypanosomosis such as *stomoxys*, *tabanus* and *haematopota* were recorded in the area. In wrapping up, trypanosomosis is an economically important disease threatening the health and productivity of cattle in Bambasi district. Therefore, proper control strategies have to be designed and implemented to minimize its effect on livestock production in the studied district.

Acknowledgements:

The authors would like to extend their gratitude to individuals who provide unreserved help for identification of the parasites and multi-directional cooperation during the study and preparation of the manuscript.

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8/25/2020