Across sectional Study on Bovine Trypanosomosis and Apparent Vector density in Bambasi District of Benishangul Gumuz Regional State, Western Ethiopia: prevalence and Vector density

Asmamaw Aki and Mengistu Godeso

Regional veterinary Diagnostic, Surveillance, Monitoring and Study Laboratory, P.O. Box: 326, Asosa, Ethiopia.
Email: asmamawaki@gmail.com; Telephone: 251 577751541

Abstract: Across-sectional study was carried out in Bambasi woredas of Asossa zone of Benishangul Gumuz Regional State, Western Ethiopia from October to Febuarary, 2016 to determine trypanosomosis prevalence, anemia association with Trypanosomosis, prevailing trypanosomes species, associated risks and vector density. Blood samples collected from (n= 514) randomly sampled cattle (*Bos indicus*) was examined using buffy coat technique and hematological procedures. An overall, 47 (9.14%) trypanosomosis prevalence was recorded. The infection was caused by *Trypanosoma congolense 37/47 (*78.72%). *Trypanosoma vivax* 6/47(12.76%), *Trypanosoma brucei* 1/47 (2.13%) and mixed infection 2/47(4.25%). The infection rate difference between trypanosomes was statistically significant (P< 0.05). Mean packed cell volume (PCV) value of the infected animals was lower (23.63% ± 2.42) than uninfected animals (26.24% ± 1.38) and the variation was statistically significant (P< 0.05). Overall, anemia prevalence of 40.07% (206/514) was recorded and it was significantly higher (63.82%) in infected cattle than in non-infected (37.68%). Significant association was not recorded among study sites, sex groups, age categories and body conditions (p>0.05). *Glossina moristans submorsitans* was the only tsetse fly caught and its mean apparent density measured as fly/trap/day was 3.92. In addition, other mechanical transmitters of trypanosomosis such as stomoxys (1.76 f/t/d), tabanid (0.2 f/t/d) and haematopota (0.35 f/t/d) were recorded. Taken as a whole, the present work evidenced that tsetse and trypanosomosis has continued to pose a considerable threat to cattle of the study area warranting an integrated control to safeguard cattle production and productivity.
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Key words: Bambasi, PCV**,** Trypanosomosis, *Glossina morsitans submorsitans*, Risk factors

1. Introduction

Agricultural development is essential for growth across sub-Saharan Africa, employing 65% of the labour force and accounting for 32% of gross domestic product (Majekodunmi *et al*., 2013). Diseases of livestock reduce agricultural output by up to 30% in developing countries (twice the impact as in developed countries) (FAO, 1990). The majority of the disease burden faced is from infection with endemic diseases, in particular African Animal Trypanosomiasis (AAT), tick borne diseases and helminthiases, all of which decrease production and increase morbidity and mortality. The presence of African Animal Trypanosomosis is estimated to reduce cattle density by 37 –70%, reduce off take by 50%, reduce the calving rate and increase calf mortality by 20% (Swallow BM, 2000).

Trypanosomes can infect all domesticated animals; clinical cases have been described in cattle, water buffalo, sheep, goats, camels, horses, donkeys, alpacas, llamas, pigs, dogs, cats and other species. In parts of Africa, cattle are the main species affected, due to the feeding preferences of tsetse flies; in effect, they can shield other domesticated animals such as goats and pigs from the effects of trypanosomiasis (OIE, 2009).

The host preferences of each trypanosome species may differ, but *T. congolense, T. vivax* and *T. brucei* have a wide host range among domesticated animals. *T. godfreyi* and *T. suis* occur in pigs. *T. simiae* appears to be most important in pigs, but it has also been reported by PCR in camels, horses and cattle(OIE, 2009).

Trypanosomiasis, a disease of humans and animals caused by several species of trypanosomes and spread by tsetse flies is a major constraint to livestock production in 37 countries within the Sub-Saharan region. An estimated 45-50 million cattle are at risk of infection in the region, with an estimated economic loss of up to US $ 1.3 billion in cattle production (Kristjanson PM., 1999).

In Ethiopia, tsetse flies are confined to the South west and North western region between longitude 33^0 and38^0 E and latitude 5^0 S and 12^0 N and covers an area of 220,000 km^2. According to (NTTICC) tsetse infested area of Benishangul Gumuz regional state is about 31,000 km^2. The presence of animal trypanosomosis in the area where more then 90% of crop production is dependant on animal drought power

mainly on ploughing oxen is a major constraints to utilize large land resource which worsen insuring food security (Shimelis *et al*, 2011).

The influence of tsetse on African agriculture through the transmission of trypanosomosis continues to be a major constraint to the development of national economies and their achievement of self sufficiency in basic food production. The general distribution of tsetse flies is determined principally by climate and influenced by altitude, vegetation, and presence of suitable host animals (Leak SG.,1999).

In Bambasi districts, trypanosomosis was found to be one of the factors that hampered livestock rearing in most peasant associations. And also a study on the status of the disease and investigating the vectors and their relative abundance is crucial for a successful control in the area. The knowledge of the status of the disease prevalence, its health impact on animals affected, its vector distribution and the associated risks are very important for understanding the epidemiology of the disease and to devise suitable control measures. Therefore, the present work aimed at determining the prevalence of bovine trypanosomosis and apparent density of tsetse and other biting flies ascribed in the transmission of trypanosomosis.

2. Materials And Methods

Study Area and Period: The study was conducted from October to Feburary, 2016 in Bambasi districts of Asosa zone of Benishangul Gumuz Regional State. It was conducted in six kebeles here after called sites name Mender-49, Mender-55, Mender-16, Keshmando, Jamatsa and Mender-65. Bambasi district has 38 kebeles stretches over an area of 2210.16 k.m.square with human population of 62693. The region is found in the north west of the country between latitude of 9 and 11^0N and longitude of 34 and 35^0E and its altitude range is 1500-1900 meter above sea level. Annual rain fall is between 1350-1400 mm with uni modal type of rain fall that occurs between April and October. Annual temperature ranges between 21^0c - 35^0c. The livelihood of the society largely depends on mixed livestock and crop production having livestock population of 36,735 Cattle, 10732 Goat, 3739 Sheep, 4467 Equines, 41438 Poultry and 23423 beehives (CSA, 2015).

***Study Design and Study Animals*:** Cross sectional study design was used. A local zebu cattle (*Bos indicus*), 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 Nicholson. M.J and M. H. Butterworth (1986). Concurrently, their age was categorized in years (<2, 2-5, >5) based on De-Lahunta, A., and R.E. Habel (1986) principles.

Sampling Techniques and Sample Size Determination: The study sites were selected purposively as convenient. 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 (Thrusfied, 2005). The sample size was determined based on the expected prevalence of 50%; confidence level of 95%, and 5% desired absolute precision. As result a total of 384 cattle were calculated, however; it was increased to (n=514) to increase precision and these cattle were sampled at their communal grazing area using simple random sampling.

Study methodology and procedures

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-haematocrit centrifuge (Hermmle Labortechnik, type Z, Germany). The capillary tubes were placed in microhaematocrit 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 haematocrit 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).

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 expressed 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).

Fly survey: During the study four types of traps were deployed: 30 Monopyramidal, 22 monoconical, 20 biconical, traps and 8 NGU traps. Every trap was odor baited with acetone 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 hypophageum.

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 10.0 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 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.

3. Result

Trypanosomes Survey result: A total of 514 cattle were examined. The overall prevalence of trypanosomosis was 9.14%. The prevalence in terms of trypanosome species was 7.19% *T. congolense*, 1.16% *T.vivax, 0.19% T.brucei, and 0.38% mixed (T.congolense & T.vivax)*. The proportion of trypanosome species was 37/47 (78.72%) *T. congolense,* 6/47 (12.76%) *T. vivax, 1/47(2.13%) T.brucei and 2/47(4.25%) mixed infection* (Table 1). The infection rate difference between trypanosomes was statistically significant (P<0.000).

Haematological Survey results: The mean PCV value for whole examined animals was 26 ± 2.59 SE. However, the mean PCV value for uninfected animals was 26.24 ± 1.38 SE and mean PCV value of the infected animals was 23.63 ± 2.42 SE. The mean PCV values of cattle were significantly (= 0.006) influenced by trypanosome infection as 23.63% and 26.24 % PCV values in trypanosome positive and trypanosome negative animals were registered, respectively (Table 2). The overall anemia prevalence in the studied district was 40.07% (206/514). The anemia prevalence was significantly higher in trypanosome infected cattle (63.82%) than in non-infected cattle (37.68%) (<0.05). Of 40.07% anemia prevalence, 5.84 % (30/514) was trypanosome infected animals. However, large number of animals 34.24% (176/514) had anemia (PCV < 24) without having trypanosome infection. Some animals 3.31 % (17/514) were infected by trypanosome but their PCV was found normal (Table 3).

Prevalence of Trypanosomosis according to Age, Sex, sites and Body Condition: The highest trypanosomosis prevalence 11.26% was recorded in >5 years old animals whilst the lowest prevalence 5.88% was in animals < 2 years old. Slightly higher prevalence was registered in males 24 (11.16 %) than in females 23 (7.69 %). Trypanosomosis was recorded across the study sites with the highest (15.83%) prevalence in Mender-55 and the lowest (4.16 %) in Mender-49 peasant associations. Trypanosomosis prevalence was statistically non-significant across study sites, between age categories and sex groups (p>0.05). The highest prevalence (12.06 %) was found in poor body condition animals while the least (10.38%) in good body conditions. This difference was statistically non-significant. The effect of age, sex, sites and body condition on trypanosomosis prevalence is summarized in table 4.

Entomological Survey results: A total of 1000 tsetse and biting flies were caught during the study period from different sites of study. Out of the total, 628 (62.8%) were belonging to tsetse of the species *Glossina moristan submoristans*, followed by 283 (28.3%) Stomoxy, 57(5.7%) Haematopota and 32 (3.2%) Tabanid. Only *Glossina moristans submorsitans* were identified in the survey site with the overall apparent density of 3.92 fly/trap/day). The highest fly density 236 (11.8 f/t/d) were observed in Mender-55 peasant association and the lowest 141 (4.7 f/t/d) recorded in Mender-49 kebele in Bambasi district. Large number of Musca Species were also caught but discarded because they have no role in the transmission of trypanosomosis. (Table 5).

Table 1: The prevalence of single and mixed infection of trypanosomes in Bambasi

Trypanosomes	**No. positive**	**Prevalence (%)**	**X^2**	**(p-value)**
T. congolense	37	78.72	396.15	0.000
T. vivax	6	12.76		
T.brucei	1	2.13		
Mixed (*T.congolense & T.vivax*)	2	4.25		
total	47	100		

Table 2: Mean PCV comparison between infected and uninfected animals of Bambasi

Status	Frequency	Mean PCV (%)	SE	Overall PCV	X^2	p-value
Infected	47	23.63	2.42	1111	7.47	0.006
Uninfected	467	26.24	1.38	12253		
Total	514	26	2.59	13,364		

NB: SE- standard Error, PCV- packed cell volume

Table 3: Proportion of anemia infected and uninfected cattle population of Bambasi district

Status	Anemia	Frequency	Percent	Percent share per strata
Infected	anemic	30	5.84	63.82
	non-anemic	17	3.31	36.17
Uninfected	anemic	176	34.24	37.68
	non-anemic	291	56.61	62.31

Table 4: prevalence of bovine trypanosomosis and its association with various risk factors in Bambasi

Risk factors	No. examined	No. positive	Prevalence (%)	χ^2	p-value
Sites				10.54	0.061
Mender-49	120	5	4.16		
Mender-55	120	19	15.83		
Mender-16	108	8	7.40		
Keshmando	74	7	9.45		
Jamatsa	27	2	7.40		
Mender-44	65	6	9.23		
Total	514	47	9.14		
sex				1.81	0.178
Male	215	24	11.16		
Female	299	23	7.69		
Total	**514**	**47**	**9.14**		
Age(years)				1.27	0.53
< 2	17	1	5.88		
2-5	346	29	8.38		
>5	151	17	11.26		
Total	514	47	9.14		
Body conditions				3.037	0.219
Good	77	8	10.38		
medium	321	25	7.78		
Poor	116	14	12.06		
Total	**514**	**47**	**9.14**		

Table 5: Flies caught in different areas of survey Bambasi woreda

Sites	Total flies caught	No. of traps	Tsetse flies caught					Biting flies		
			Number	species	M	F	*F/T/D	Stomoxys	tabanid	Haematopota
Mender-49	141	15	84	GM	33	51	2.8	45	4	8
Mender-55	236	10	153		46	107	7.65	63	7	13
Mender-16	130	10	110		47	63	5.5	12	5	3
Keshmando	189	15	116		40	76	3.86	56	6	11
Jamatsa	135	10	52		19	33	2.6	63	7	13
Mender-44	169	15	113		52	61	3.76	44	3	9
total	1000	80	628		237	391	3.92	283	32	57

F/T/D=fly per trap per day, GM=*Glossina morsitan submorsitans*, M=male, F=female

4. Discussions

The present study revealed an overall prevalence of 47/514 (9.14%) in the study area. This finding was in agreement with earlier works of (Kebede N. *et al*., 2009) who reported 10.1 % from Awi zone, 9.63% from Awi & 20.74% from Metekel zones (Mekuria,S *et al*., 2011); studied the overall prevalence of cattle trypanosomosis from selected districts, north western Ethiopia. This finding is also agree with the previous works of (S.Tasew *et al*., 2012) who reported 8.57% from Oromia; studied Cattle anaemia and trypanosomosis prevalence in Western Oromia State, Ethiopia. And also this findings is agree with work of (Dawit T. *et al*., 2012) who reported 12.1% from Metekel zone; studied Economic burden of bovine trypanosomosis in three villages of Metekel zone, Northwest Ethiopia.

The study showed that the infection was predominantly caused by *T. congolense 37/47 (*78.72%), *T. vivax* 6/47 (12.76%), *T. brucei* 1/47 (2.13%) and *T.congolense* and *T.vivax* mixed 2/47 (4.25%). This result is in consonance with the reported proportions of *T.congolense* (77.6%) followed by *T.vivax* (14.9%), *T.brucei* (6.0%) and *T.congolense* and *T.vivax* mixed (1.5%) from Metekel and Awi zones (Mekuria, S *et al*., 2011). This result was also in consistent with prior reports of (Mulaw *et al.*, 2011) who studied prevalence of major trypanosomes affecting cattle in the neighboring Asossa district of Benishangul Gumuz Regional State, Western Ethiopia and found *T. congolense* proportional prevalence of 66. 7%; (Abraham Z *et al.,* 2012) 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) reported *T. congolense* proportional prevalence 63.64% during his work on trypanosomosis and anemia in cattle population of Dale Wabera district of Kellem 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 serodems 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.G.A *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 (Langridage WP.1976; Leak S.G.A. 1999). Different studies (Leak S.G.A *et al*.,1993; Rowland, W *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 (Stephen, 1986).

The prevalence of bovine trypanosomosis was studied between sex categories, age groups and body conditions, non- significant association was observed (>0.05). This might be because of low chance of exposure to the parasite. This result is in agreement with previous reports (Mihreteab.B *et al*., 2011, Teka. W *et al*., 2012; Lelisa, K *et al*., 2015).

The overall anemia prevalence in the studied district was 40.07% (206/514). The anemia prevalence was significantly higher in trypanosome infected cattle (63.82%) than in non-infected cattle (37.68 %) (<0.05). This is in concordance with previous results from different researchers (Mihret *et al.,* 2007, M. Bekele *et al*., 2011; Biyazen, H., 2014). Out of 40.07 % anemia prevalence, 5.84% (30/514) was trypanosome infected animals. Nonetheless, 34.24% (176/514) 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 (Van den Bossche *et al*., 2001).

This study revealed that 3.31% (17/514) 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 (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 (Van den Bossche *et al*., 2001).

The overall mean PCV value for examined animals was 26 ± 2.59 SE. The mean PCV value of the infected animals was significantly lower (23.63 ± 2.42 SE) than that of uninfected animals (26.24 ± 1.38 SE). This result is in alignment with previous works (Ali, D *et al*., 2011, Mulaw S., 2011; Bayisa K *et al*., 2015).

Glossina moristans sub moristans was the only tsetse fly caught and its mean apparent density measured as f/t/d was 3.92. It accounts for 628 (62.8%) out of the total flies caught. In addition, other mechanical transmitters of trypanosomosis such as

stomoxys 283 (28.3%), tabanus 32 (3.2%) and haematopota 57(5.7%) were recorded. The current findings were 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 in agreement with 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 neigbouring 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 was also in consistent with the previous findings of (NTTICC, 2012 & 2014) at neigbouring 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. Conclusions

Animal trypanosomosis is a major problem to livestock production and productivity in Bambasi Districts. Since the Districts lies within the tsetse belt area, the expected prevalence of trypanosomosis prevailing in the area should be high. The overall bovine trypanosomosis prevalence was 47 (9.14 %). Non-significant association was recorded among study sites, between age categories, sex groups, and body conditions ($p>0.05$). And also the study result of trypanosomosis and other factors such as (nutritional, seasonal; concurrent disease) was found to be negatively affects the PCV values of affected animals. The most widely distributed and dominant species is *T. congolense 78.72 % followed by T.vivax 12.76 %,* which was mainly transmitted by tsetse fly, Glossina morsitans submorsitans (3.92 f/t/d/) and biting flies (stomoxys 1.76 f/t/d, tabanid 0.2 f/t/d and haematopota 0.35 f/t/d) respectively. The disease was found to cause substantial economic losses through cattle mortality, drug purchase, and draft power loss of infected oxen. The farmers in the area were spending a significantly ($p < 0.05$) higher amount of money for the treatment of trypanosomosis than all other diseases combined. Many of the farmers prioritized losses of draft power as the most important impact of the disease. Thus, tsetse suppression activities that involve the local community can be an important tool towards minimizing the economic burden of the disease in the area. In conclusion, the result of the current study showed the economical importance of trypanosomosis in the area calling for devising strategic control efforts.

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