Prevalence and Associated Risks Factors of Bovine Trypanosomosis in Guba District of the Benishagul Gumuz Region, Western Ethiopia

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Abstract: This study was carried out in Guba district of the Benishangul Gumuz Region, Western Ethiopia from February to March, 2017 to determine the prevalence and associated risk factors of bovine trypanosomosis and its vector density. Blood samples collected from 301 randomly sampled cattle (Bos indicus) was examined using parasitological (buffy coat technique) and haematological (Measurement of packed cell volume) procedures. An overall, 37 (12.29%) prevalence of trypanosomosis was recorded. The infection was caused mainly by T. congolense 22/37 (59.46%), T. vivax 9/37 (24.32%) and mixed infection with T. congolense and T. vivax 6/37 (16.22%) and the infection rate was statistically significant among different trypanosome species (P<0.05). Mean packed cell volume (PCV) value of infected animals was lower (18.24% ± 5.68) than non-infected animals (27.52% ±3.82) and the variation was found statistically significant (P<0.05). Similarly, higher prevalence (17.19%) of trypanosome infection was registered in animals with poor body condition when compared to animals with medium (6.67%) and good (4.41%) body condition and the difference was statistically significant (P<0.05). Moreover, prevalence of trypanosomosis was statistically significant among study sites and age categoriesof study animals (P< 0.05). In contrast sex of study animals and trypanosome infection did not show significant association (P>0.05). Glossina fuscipes and Glossina tachinoide were the tsetse fly species caught and their mean apparent density measured as flies/trap/day was 3.725. In addition, other mechanical vectors such as tabanids and haematopota were captured with flies/trap/day 2.1 and 0.375, respectively. T0 conclude, the result of the present finding reveals moderately high prevalence of trypanosomosis in the study district implying the need for strategic and participatory approach to control the vector and to minimize the impact of the disease in the study district.
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1. Introduction

African animal trypanosomosis is indisputably a great problem on the African scene, but the perspective alters when it is examined against a global background, even when taking into account mechanically and venereal transmitted African trypanosomes which have spread to other continents (Trypanosoma evansi causing surra, mechanically transmitted T. vivax and T. equiperdum the causal agent of the venereal disease dourine). The cost of the development of new compounds has increased enormously in keeping with inflation and increasingly exacting and expensive requirements of the licensing authorities on the absence of chronic toxicity, carcinogenicity and residue problems. At the same time, the market for recovering the enormous investments needed to develop new compounds to the commercial stage is limited and mostly poor. Logically, the economic stimulus required by the private industry for attracting their investment in research on new drug development is sadly lacking (Uilenberg, 1998).

Bovine trypanosomosis is a major animal disease constraint to livestock production in sub-Saharan Africa. It is estimated that some 46 million cattle are at risk of contracting African animal trypanosomosis in sub- Saharan Africa (Nonga and Kambarage, 2009).

Trypanosomosis is a parasitic disease caused by species of flagellated protozoa belonging to the genus Trypanosoma which inhabit the blood plasma, various body tissues and fluids of vertebrate host. The disease is transmitted cyclically by tsetse flies (Glossina species), and none cyclically by other biting flies (Tadesse et al., 2011).

This parasite restricts animal production, besides causing economic losses by the clinical signs of the infection such as restricted growth, abortion, anemia, treatment cost, and death of the affected animals (Batista et al., 2012).

Trypanosomosis induces loss of body condition in pregnant animals leading to birth of offspring‘s, with low birth weights fetal and neonatal losses, besides production losses in lactating animals. The consequences of trypanosomiasis are less severe in

better-nourished animals but good nutrition does not by itself provide protection. Adequate energy, protein and vitamin nutrition enhances the ability of trypanosome-infected animals to withstand the adverse effects of infection (Pathak, 2009).

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, 2005). 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, 1999).

Tsetse flies in Ethiopia are confined to southern and western regions between longitude of 33 ^{0} and 38^0 East and latitude of 5^0 and 12^0 North which amounts to be about 200,000 Km2. Tsetse infested areas lies in the low lands and also in the river valleys of Blue Nile, Baro Akobo, Didessa, Ghibe and Omo. 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 (*Glossina morsitans submorsitans*, *G. Pallidipes, G. tachnoides, G. f. fuscipes and G. longipennis*) have been registered in Ethiopia (Keno, 2005).

Apart from the cyclical transmission of trypanosomosis by Glossina species, it is highly considered that mechanical transmission is a potential threat to livestock production and productivity in some parts of Ethiopia (Abebe, 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.m.submorsitans*, *G. Pallidipes, G. tachinoides, G. f. fuscipes and G. longipennis)* have been registered in Ethiopia (Keno, 2005). In the study region of Benishangul Gumuz regional state, four glossina species namely, *G. tachinoides, G.morsitant submorsitances, G. pallidipes* and *G.fuscipes* were found (ARVDSMSL, 2015).

Guba is one of the twenty districts of the Benishangul Gumuz regional State with a serious problem of trypanosomosis. Controlling this economically important disease in this area could have a number of benefits to improve the livelihood of the poor people of the district by increasing milk, meat, surplus capital from the sale of livestock and livestock products and improving the availability of draft power (oxen). Although the disease is one of the major obstacles of livestock production in the district, there is no study conducted to know prevalence and situation the disease to take action in the control of the disease. Therefore, the present study is designed to determine prevalence and associated risk factors of bovine trypanosomosis and to suggest possible control measures of the disease in the district.

2. Materials and Methods

2.1. Study Area

The study was conducted in Guba district of the Benishangul Gumuz Regional State, Western Ethiopia, from February to March, 2016. It was carried out in three kebeles hereafter called sites namely: Mankush, Jadia and Babizenda. The district has 17 kebeles covering an area of 756,943 hectare with human population of 19,416 (CSA, 2015). Guba is located at a distance of 645 Km away from Addis Ababa, the capital city of Ethiopia and is located at an altitude of 500-900 meter above sea level. Its annual average temperature is 35^{0}c (27-43^{0}c) and its rain fall range is 900-1050 mm (NMSA, 2015). The livelihood of the people in the district largely depends on mixed livestock and crop production having livestock population of 15,291 cattle, 4,963 sheep, 22602 goats, 2428 equines (CSA, 2015).

2.2. *Study Design and Study Animals*

Cross sectional study design was used. A local zebu cattle (*Bos indicus*), which are mainly kept under an extensive management system grazing the communally owned pasture land throughout the year were randomly sampled. Study animals were herded together during the day time and returned to their individual owner's farmstead every evening. The body condition of each of the study animal was scored as good, medium and poor (Nicholson and Butterworth, 1986). Similarly, their age was determined based on (De-Lahunta and Habel, 1986) principles as young (<2 years old), matured (2-5 years old) and adult (> 5 years old).

2.3. *Sampling Techniques and Sample Size Determination*

The study sites were selected randomly. The study was conducted on 301 animals that were sampled randomly involving both sexes, all age groups, and all types of body conditions to determine prevalence and associated risk factors of bovine trypanosomosis in the study district.

2.4. Study Methodology

2.4.1. Packed cell volume (PCV) determination

Blood samples were obtained by puncturing the marginal ear vein with sterile lancet and collected directly into a pair of heparinized capillary tubes (75mm x1.2mm). The tubes were then sealed at one end with crystal seal. 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 samples 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).

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 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 (Murray and Dexter, 1988). Trypanosome species were identified according to their morphological descriptions as well as movement in wet film preparations (OIE, 2008).

2.5. Data Analysis

During the study, data were collected using the data collection format and entered into Microsoft Excel. Hematological and parasitological data were managed very carefully. Then, the data from the Microsoft excel sheet were processed and analyzed by using a statistical soft ware program. Chi square was used to compare the prevalence of trypanosomosis in different variables and to determine the relationship between variables and the result. Data collected on PCV values were analyzed by ANOVA to compare the mean PCV values of infected animals against that of non infected animals. In all cases the difference between parameters were tested for significance at probability level of 0.05 or less. The prevalence of bovine trypanosomosis was calculated as the number of parasitologically positive animals examined by buffy coat method to the total animals examined (Thrusfield, 2007).

3. Result

3.1. Prevalence of trypanosomes infection

Out of the total animals examined, 37(12.29%) were infected with trypanosomes. The trypanosome species responsible for the infection were *T. congolense, T. vivax* and mixed infection by *T. congolense and T. congolense and T.vivax.* As indicated in table 1, the proportional prevalence of each species of trypanosome was 22(59.46%) for *T. congolense*, 9*(*24.32%*)* for *T. vivax* and 6(16.22%) mixed infection by *T. congolense* & *T. vivax* was observed in the fresh blood examined during the study period and the proportional prevalence of trypanosome species was found to be statistically significant (P<0.05) (Table 1).

Table 1: Prevalence of single and mixed infection of trypanosomes in Guba district

Trypanosomes	No. positive	Prevalence (%)	X^2	(P-value)
T. congolense	22	59.46	169.3508	0.000
T. vivax	9	24.32		
Mixed (*T. congolense* & *T. vivax*)	2	16.22		
Total	**37**	**100**		

3.2. Trypanosomosis and associated risks

The highest and the lowest prevalence of trypanosomosis were recorded in Babizenda 35/37 (94.59%) and Mankush 2/37(5.41%) study sites respectively, where infection of trypanosome was recorded. However, there was no animal infected by trypanosome in Jadia study site. Statistically significant was registered among the study sites and infection of animals by trypanosomes (p <0.05) (Table 2).

The Prevalence of trypanosomosis varies in both sexes; the infection in female is slightly higher 25/164(15.24 %) than in male 12/137(8.76%) and the association was not found statistically significant (P>0.05) (Table 2).

Study animals were categorized in to different age groups as young (< 2 years), matured (2-5 years) and adult (>5 years). Out of the total sampled animals; 96, 120 and 85 were < 2 years, 2-5 years and > 5 years old respectively and the prevalence was found to be 22(22.91 %) for animals < 2 years, 10(8.33%) for animals 2-5 years and 5(5.88%) for animals > 5 years and the difference in the prevalence among the different age group was statistically significant (P<0.05) (table 2).

Similarly, during the study period, animals were categorized in to different body conditions as good, medium and poor. From a total of 301 animals examined; 68, 105 and 128 were registered as animals with good, medium and poor body condition respectively, out of which 3(4.41%) prevalence of trypanosomosis was recorded in animals with good body condition. The rest 7 (6.67%), and 27 (21.09%) prevalence of trypanosomosis were registered in animals with medium and poor body condition respectively. Trypanosome infection and body condition scores of study animals revealed statistically significant association (p < 0.05) (Table 2).

Table 2: Prevalence of bovine trypanosomosis and its association risk factors in Guba district

Risk factors	No. examined	No. positive	Prevalence (%)	χ^2	P-value
Sites					
Mankush	147	2	1.36	115.8755	0.000
Jadia	82	0	0		
Babizenda	72	35	48.61		
Total	**301**	**37**	**12.29**		
Sex					
Female	164	25	15.24	2.9115	0.088
Male	137	12	8.76		
Total	**301**	**37**	**12.29**		
Age(years)					
< 2	96	22	22.92	15.0347	0.001
2-5	120	10	8.33		
> 5	85	5	5.88		
Total	**301**	**37**	**12.29**		
Body conditions					
Good	68	3	4.41	16.1961	0.000
Medium	105	7	6.66		
Poor	128	22	17.19		
Total	**301**	**37**	**12.29**		

3.3. Packed Cell Volume

The mean PCV values for all examined animals were 23.96 ± 4.75 SE. However, the mean PCV values for non infected animals were 27.52 ± 3.82 SE and the mean PCV values of infected animals were 18.24 ± 5.68 SE. There was statistically significant difference in the mean PCV values of non infected and infected animals ($P<0.05$) (Table 3).

Table 3: Mean PCV comparison between infected and non infected animals in Guba district

Status	Frequency	Mean PCV (%)	SEs	Overall PCV	X^2	P- value
Infected	37	18.24	5.68	674.88	7.9469	0.005
Non infected	264	27.52	3.82	7265.28		
Total	**301**	**23.96**	**4.75**	**7211.96**		

3.4. Entomological Survey results

The present survey of tsetse flies depicted that *G. fuscipes and G. tachinoide* are species of tsetse fly responsible for cyclic transmission of trypanosomosis in the study district. Tsetse fly survey was carried out in three sites of the study district by deploying a total of 40 geo-referenced mono-conical traps in the river border and on grazing fields of cattle; the number of tsetse flies captured in each study site is Mankush (0), Jadia (0), and Babizenda (298). The species of tsetse flies investigated in the current study were *G. fuscipes* and *Glossina tachinoide*. The mean apparent density of tsetse flies in the survey sites was investigated as 3.725 f/t/d and mean apparent density of mechanical vectors such as tabanus (2.1 f/t/d) and haematopota (0.375 f/t/d) were also recorded (table 4).

Table 4: Flies caught in different areas of survey sites of Mao Guba district

Sites	Total flies caught	No. of traps	Tsetse flies caught					Other biting flies	
			No.	Species	M	F	*F/T/D	Tabanus	Haematopota
Mankush	-	10							
Jadia	-	10							
Babizenda	492	20	298	*G.fuscipes*	5	14	0.475	168	26
				G. tachinoide	80	199	6.975		
Total	**492**	**40**	**298**		**85**	**213**	**3.725**	**168**	**26**

*F/T/D=fly per trap per day, M=male, F=female, *G.fuscipes= Glossina fuscipes, G. tachinoide= Glossina tachinoide*

4. Discussion

The present study revealed an overall prevalence of 37/301 (12.29%) trypanosomosis infection in the study districet. This finding was in agreement with the findings of (Dinede and Aki, 2016) in Mandura district of the Benishangul Gumuz Region, Western Ethiopia who reported 13.30% prevalence in their study on epidemiology of cattle trypanosomosis and associated anaemia, (Kenaw and Dinede, 2015) who reported 11.70% in their study on trypanosomosis and its associated risks in cattle population of Dangur district of the Benishangul Gumuz region, Western Ethiopia, (Aki and Godesso, 2016) who reported 9.14% intheir study on a cross sectional Study on Bovine Trypanosomosis and Apparent Vector density in Bambasi District of Benishangul Gumuz Regional State, Western Ethiopia, (Gemeda, 2015) who reported 9% prevalence in his study on Prevalence of Bovine Trypanosomosis in and around Nekemte Areas, East Wollega Zone, Ethiopia. However, the current finding was lower than the previous findings of (Kenaw *et al.,* 2015) who reported 22.38% prevalence in Assosa district of the Benishagul Gumuz regional State, Western Ethiopia and (Mulaw *et al.,* 2011) whose result indicated 28.10% in their study on the prevalence of major trypanosomes affecting bovine in tsetse infested Assosa district of the Benishangul Gumuz Regional State, Western Ethiopia, (Daud and Molalegne, 2011) whose finding showed an overall prevalence of 24.7% in Mao-Komo special districtof the Benishangul Gumuz region, Western Ethiopia and (Eticha and Aki, 2016) whose finding revealed an overall prevalence of 19.53% in their study on Prevalence of Cattle Trypanosomosis, Apparent vector density and Associated Risk Factors in Debate District, Western Ethiopia. The difference in the prevalence of trypanosomosis in the previous and the current findings might be due to the difference in agro ecology and climatic conditions of the areas and partly it might be the difference in seasons the study period.

The current finding indicated that the infection was mainly caused by *T. congolense 22/37 (*59.46%), *T.vivax* 9/37(24.32%) and mixed infection with T. congolense and T. vivax 6/37(16.22%). This result was in consonance with the reported proportions of *T. congolense by* (Abraham *et al*.,2012) who conducted their study on prevalence of bovine trypanosomosis in selected sites of Arba Minch district, Sothern Ethiopia, whose result showed proportional prevalence of *T. congolense* to be 61.4%; (Biyazen *et al*., 2014) whose finding showed proportional prevalence of *T. congolense to be* 63.64% during their work on trypanosomosis and anemia in cattle population of Dale Wabera district of Kellem Wollega Zone, Western Ethiopia. This result was also inconsistent with prior report of (Eticha and Aki, 2016) whose finding revealed proportional prevalence of *T. congolense* to be 65.33%. The high proportional infection rate of *T. congolense* in cattle might be attributable to the high number of serodems 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 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, 1999). Studies carried out by (Rowland *et al*., 1995) have indicated that *T. vivax* is highly susceptible to treatment while the problem of drug resistance is higher in *T. congolense* The effect of different risk factors such as sex, age categories, study sites and body conditions on prevalence of bovine trypanosomosis was assessed and, statistically significant associations were observed in age categories, study sites and body condition scores ($P<0.05$) while sex was not found to be statistically significant ($\chi^2 > 0.05$). This result was in agreement with previous reports of (Mulaw *et al*., 2011) whose finding indicated that sex did not show statistically significant association with trypanosomosis infection, (Worku, *et al.,* 2016) whse finding showed that body condition score of study animals has statisticacaly significant association with trypanosomosis infection. It was also in consistent with the previous work of (Eticha and Aki, 2016) in the neighbouring Debate district whose result revealed significant association between body condition score and and trypanosome infection; however, in contrast to the present result their finding showed that no statistically significant association was seen among age, study sites and trypanosome infection of study animals. The present finding was also in consistent with the previous work of (Yehunie *et at*., 2012) in that their finding indicated that there was statistically significant association between body condtion score of study animals and trypanosomosis infection in the study sites.

The overall mean PCV values for all examined animals were 23.96 ± 4.75 SE. The mean PCV values of infected animals was lower (18.24 ± 5.68 SE) than that of non infected animals (27.52 ± 3.82 SE) and there was statistically significant association between PCV values and trypaonosome infection of study animal. This result was in alignment with previous works of (Gemeda, 2015; Lelisa *et al*., 2015 and Aki

and Godesso, 2016) whose findings concide with the present result in that they all reported statistically significant association between PCV values and trypanosome infection of study animals.

In the entomological survey, *G. fuscipes and G. tachinoide* were tsetse fly species captured and their mean apparent density measured as f/t/d was found to be 3.725. Tsetse flies account for 298/492(60.57%) out of the total flies caught. In addition, other mechanical transmitters of trypanosomosis such as tabanus and haematopota were registered and they account for 168/492(34.17%) and 26/492 (5.28%) of the total flies caught with f/t/d of 2.1 and 0.375 respectively. The present finding was in agreement with the previous reports of (Aki et al., 2016) in Kamashi district of the Benishangul Gumuz regional state, Western Ethiopia, in that the previous and the current findings showed that the f/t/d of tsetse fly, tabanus and haematopota was 2.54, 1.54 and 0.92 respectively indicating that tsetse flies and other mechanical transmitter of trypanosomosis flies are responsible for the transmission of trypanosomosis from diseased to healthy animals.

5. Conclusion

The overall moderately high prevalence of trypanosmosis obtained in cattle of Guba district indicated the importance of the problem and its contribution to hampering the product, productivity, work performance and general health status of these animals. The most widely distributed and dominant species of trypanosome in the study sites are *T. congolense (59.46%)* followed by *T. vivax (24.32%) and* mixed infection with *T. congolense and T. vivax (16.22%)* which was mainly transmitted by tsetse flies (*G. fuscipes and G. tachinoide*) and other biting flies (tabanus and haematopota) with f/t/d/ of 3.725, 2.1, and 0.375 for the different species of tsetse flies, tabanus, and haematopota respectively. Since the district lies within the tsetse belt area, the result of the present study (12.29%) showed the fact and expected prevalence. Significant association was not observed between sex and trypanosome infection (P> 0.05) while there was statisitically significant association among body condition scores, age, study sites and PCV values of study animals and trypanosome infection (P< 0.05). These all revealed that Guba district is favorable for the successive breeding of tsetse and other biting flies that play a major role in the transmission of trypanosomes to susceptible hosts and hence, designing and implementing control strategies of trypanosomosis focusing on vectors control and against the parasites will be undertaken in the study area and farmers of the district have to be educated about the impact of trypanosomosis on the health and productivity of animals so as to implement participatory approach in the control of the vectors and parasites.

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