Epidemiology of Bovine Trypanosomosis and Apparent Density of Tsetse and Biting flies in selected Districts of Benishangul Gumuz Regional State, Western Ethiopia

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Abstract: A cross-sectional study was carried out in seven Districts of Benishangul Gumuz Regional State, Western Ethiopia from September to January, 2016 to determine bovine trypanosomosis prevalence, prevailing trypanosomes species, vector density and associated risks. Blood samples collected from (n= 1645) randomly sampled cattle (*Bosindicus*). Dark phase contrast buffy coat procedures were used for determining prevalence. Whereas, haematocrit method was used for packed cell volume (PCV) values determination. Furthermore, traps were deployed for the purpose of entomological survey. Out of total 1645 samples, 162 (9.85%) were found trypanosome positive. *Based on Predominant trypanosome species among recorded were Trypanosome congolense 124/162(*76.54%), *Trypanosome vivax* 30/162(18.63%), *Trypanosome brucei* 4/162(2.48%) and mixed infection 4/162(2.48%). There were statistically significant differences concerning existing trypanosome species (P< 0.05). Mean packed cell volume (PCV) value of the parasitic animals was lower (23.84% $\pm$ 1.85) than a parasitic animals (25.50% $\pm$1.14) and the variation was statistically significant (P< 0.05). Among the examined animals, 49.30% (811/1645) were found anaemic. Anaemia distribution was significantly higher (68.52%) %) in infected cattle than in non-infected (47.20%). Sex groups, age categories and body conditions (P< 0.0001) were demonstrated significant risk factors, however study districts was found non- significant(p>0.05). During the survey, *Glossina moristans submorsitans* was found in the area (2.49 f/t/d) along with other mechanical vectors such as *stomoxys* (1.66f/t/d), *haematopota* (0.31 f/t/d) and *tabanus* (0.12 f/t/d). In conclusion, the current study showed high trypanosomosis prevalence in the area reflecting the need for strategic control measures.
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1. Introduction

Trypanosomosis is a complex disease caused by unicellular parasites called trypanosomes which harbor in the blood and other tissues of vertebrates including livestock, wild life and people (Radostitis et al., 2007). The disease can be expressed as chronic or acute which could cause sudden death in susceptible hosts if left untreated. Its distinguishing factors include intermittent fever, progressive anaemia, and emaciation (Connor and Bossche, 2004).

Different trypanosomes of veterinary importance were reported in Ethiopia in various hosts. Langridge (1976) reported trypanosomes in cattle, sheep and goats comprising of Trypanosoma congolense, Trypanosoma vivax and Trypanosoma brucei, and Trypanosoma evansi in camels. The findings of Dagnachew, et al. (1981) also showed Trypanosoma equiperdium in horses. Prior research works indicated that trypanosomosis is transmitted by tsetse flies and other biting flies such as stomoxys, tabanus, Haematopota and chrysops (Leak, 1999).

Trypanosomosis is a main limitation to agricultural production impeding the national economy in Ethiopia (Langridge, 1976). Swallow (1998) showed that trypanosomosis increases calf mortality and calving intervals; however, it reduces milk production off-take and draught efficiency. In addition to its direct adverse effects on affected cattle, it can also preclude rearing of animals in endemic areas leaving large amount of arable lands uncultivated (Awoke, 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. The host preferences of each trypanosome species may differ, but *Trypanosoma 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).

In Ethiopia, tsetse flies are confined to the South west and North western region between longitude

33^0w and 38^0E and latitude 5^0 S and 12^0 N covering an area of 220,000 km^2. According to (NTTICC,1996) 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 dependent on animal drought power mainly on ploughing oxen is a major constraint to utilize large land resource which worsens insuring food security (Shimelis *et al.,* 2011).

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. So, in selected districts, trypanosomosis was found to be one of the factors that hampered livestock rearing in most peasant associations. Therefore, the objectives of the present study were to determine the trypanosomosis prevalence and its contribution to anemia, associated risk factors and the apparent density of tsetse and biting flies ascribed in the transmission of trypanosomosis, so as to forward the effective control and prevention measures in study area.

2. Materials And Methods

Study Area and period: The study was conducted in Selected seven Districts of Asosa zone of Benishangul Gumuz Regional State from September to January, 2016. It was surveyed in seven districts here after called namely: Asossa, Bambasi, Oda bildugilu, Homesha, Kurmuk, Mange and Sherkole. Asossa Zone has 214 peasant association, stretching over an area of 18,340.55 kilometer square, with human population of 270,980. The region is located between latitude of $8^0$30'' and 40^0 21'' N and longitude of 34^0 21'' and 39^0 1'' E and its altitude range is 700-1560 meter above sea level. Annual rain fall is between 900-1500 mm with uni modal type of rain fall that occurs between April and October. Annual temperature ranges between 25- 35^0c. The livelihood of the society largely depends on mixed livestock and crop production having livestock population of 77,688 Cattle, 167281 Goat, 9651 Sheep, 27638 Equines, 279098 Poultry and 66019 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 MJ, and Butterworth MH (1986). Concurrently, their age was categorized in years (< 2, 2-5, >5) based on De-Lahunta A, and Habel RE (1986).

***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=1645) to increase precision and these cattle were sampled at their communal grazing area using simple random sampling.

Study Procedures and protocol

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 microhematocrits 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 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).

Fly survey: A total of 261 traps including 55 Monopyramidal, 156 monoconical, 40 biconical, and 10 NGU traps were deployed. Every trap was deployed with 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. Results

Trypanosomes distribution

In this study, 162/1645(9.84%) cattle were infected with different species of trypanosome. Amongst the total cattle examined, 7.54% % were infected with *T.congolense*, 1.82% *T.vivax*, 0.24% *T.brucei*, and 0.24% *T. congolense* & *T.vivax* mixed infection. *Trypanosoma congolense* was the most prevalent trypanosomes species in the study area. The relative prevalence of trypanosome species showed, 124/162 (76.54%) *T. congolense*, 30/162 (18.52%) *T. vivax*, 4/162 (2.47%) *T.brucei* and 4/162 (2.47%) mixed as indicated below (Table 1). The infection rate difference between the trypanosome species was statistically significant (P<0.0001).

Packed cell volume (PCV) and Anaemia Status

The mean PCV value for whole examined animals was 25.34 ± 2.03 SE. However, the mean PCV value for non-infected animals was 25.50 ± 1.14 SE and mean PCV value of the infected animals was 23.84 ± 1.85 SE. The mean PCV values of cattle were significantly (<0.0001) influenced by trypanosome infection as 23.84% and 25.50% PCV values in trypanosome positive and trypanosome negative animals were registered, respectively (Table 2). Of the total cattle examined, 49.30% (811/1645) were found anaemic. The anaemic distribution was higher in infected cattle (68.52%) than in the non-infected ones (47.20%) (<0.05) as indicated in (Table 3).

Trypanosomosis association with risk factors

The highest trypanosomosis prevalence 12.65% was recorded in 2-5 years old animals while the lowest prevalence 4.84 % was in animals < 2 years old. Slightly higher prevalence was registered in males 55 (9.98 %) than in females 105 (9.59 %). Trypanosomosis was recorded across the study Districts with the highest (21.87%) prevalence in Bambasi district and the lowest (3.22%) in Sherkole woreda. Trypanosomosis prevalence was statistically significant between age categories, sex groups (p<0.0001) but it was non- significant across study districts (p>0.05). The highest prevalence (12%) was found in poor body condition animals while the least (5.50%) in good body conditions. This difference was statistically significant. The effect of age, sex, districts and body condition on trypanosomosis prevalence is summarized in table 4.

Entomological Survey

A total of 2392 tsetse and biting flies were caught during the study period from different seven woredas. Out of total, 1303/2392 (54.47%) were belonging to tsetse species *Glossina moristan submoristans*, followed by 872/2392 (36.37%) *stomoxys*, 159/2392 (6.64%) *haematopota* and 60/2392 (2.51%) *tabanus*. Only *G. moristans submorsitans tsetse group* was identified in the survey site with the overall apparent density of 2.49 F/T/D (fly/trap/day). The highest fly density 748 (5.34 F/T/D) were observed in Bambasi district and the lowest (2.71 F/T/D) recorded in Oda bildugilu (Table 5).

Table 1: The prevalence of single and mixed infection of trypanosomes in Selected Seven Woredas

Trypanosomes	No. positive	Prevalence (%)	X^2	(p-value)
T. congolense	124	76.54		
T. vivax	30	18.63		
T.brucei	4	2.48	1178.28	0.0001
Mixed(*T.congolense &T.vivax*)	4	2.48		
Total	162	100		

Table 2: Mean PCV comparison between infected and uninfected animals of Selected Seven Woredas

Status	Frequency	Mean PCV (%)	SE	Overall PCV	X^2	p-value
Infected	162	23.84	1.85	3,863	74.61	0.0001
Uninfected	1483	25.50	1.14	37,825		
Total	1645	25.34	2.03	41,688		

Table 3: Proportion of anemia infected and uninfected cattle population of Selected Seven Woredas

Status	Anemia	Frequency	Percent	Percent share per strata
Infected	Anemic	111	6.74	68.52
	non-anemic	51	3.10	31.48
Uninfected	Anemic	700	42.55	47.20
	non-anemic	945	57.44	63.72

Table 4: prevalence of bovine trypanosomosis and its association with various risk factors in Selected Seven Woredas

Risk factors	No. examined	No. positive	Prevalence (%)	χ^2	p-value
Woredas'				6.00	0.306
Kurmuk	34	7	20.58		
Sherkole	31	1	3.22		
Mange	119	23	19.32		
Oda-Buldigilu	333	37	11.11		
Asossa	642	33	5.14		
Bambasi	422	47	11.14		
Homosha	64	14	21.87		
Total	1645	162	9.85		
Sex				45.44	0.0001
Male	551	55	9.98		
Female	1094	105	9.59		
Total	**1645**	**162**			
Age(years)				44.42	0.0001
< 2	495	24	4.84		
2-5	640	81	12.65		
>5	510	57	11.17		
Total	1645	162	9.84		
Body conditions				50.98	0.0001
Good	545	30	5.50		
medium	500	56	11.2		
Poor	600	76	12.66		
Total	**1645**	**162**	**9.84**		

Table 5: Flies caught in different areas of survey in Selected Seven Woredas

Woredas'	Total flies caught	No. of traps	Tsetse flies caught					Biting flies		
			Number	species	M	F	*F/T/D	Stomoxys	Tabanid	Haematopota
Kurmuk	138	11	10	GM	1	9	0.45	97	3	28
Sherkole	161	15	22		3	19	0.73	108	10	21
Mange	172	25	43		10	33	0.86	84	8	37
Oda-Buldigilu	217	40	67		19	48	0.83	130	5	15
Asossa	787	79	603		225	378	3.82	151	12	21
Bambasi	748	70	544		204	340	3.88	160	11	33
Homosha	169	21	14		5	9	0.33	140	11	4
Total	2392	261	1303		467	836	2.49	870	60	159

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

4. Discussions

The present study revealed an overall prevalence of 162/1645 (9.85%) 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 and 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 (Tasew S *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 also 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.

In this study, the majority of trypanosomosis infection was due to *Trypanosoma congolense.* The relative prevalence of trypanosome species showed, *124/162(*76.54%) *T. congolense,* 30/162(18.63%) *T.vivax*, and 4/162(2.48%) *T. brucei*. Mixed infections of *T. congolense* and *T. Vivax* was also encountered accounting for 4/162(2.48%). 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, Sothern 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 SGA *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 SGA, 1999). Different studies (Leak SGA *et al.,*1993; Rowland W *et al.,* 1995) have indicated that *T. vivax* is highly susceptible to treatment while the problems of drug resistance is 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, significant association was observed (□<0.0001). This might be because of high 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 current study revealed an overall mean (ẍ) PCV value of 25.34 ± 2.03 SE. The mean PCV value of the infected animals was significantly lower (23.84 ± 1.85 SE) than that of uninfected animals (25.50 ± 1.14 SE). This result was consistent with earlier reports (Ali D *et al.,* 2011; Mulaw et al., 2011; Bayisa, et al., 2015).

Of the total cattle examined, 49.30% (811/1645) were found anaemic. Amongst these anaemic cattle only (6.72%) of them were from infected group, and (42.55%) % were from non-infected category. However, anaemia distribution was higher (68.52%) in infected cattle population than in the non-infected ones (37.20 %). The fact that infected animals were found non-anaemic might be due to their ability to manage their PCV in normal range or because of recent infection which is not yet progressed to lower PCV. It is well documented that anaemia is the best indicators of trypanosomosis (Stephen, 1986); however, this study indicated that a large proportion of non-infected animals were found anaemic. This might be attributed to their recent recovery from the disease. It could also be for the inadequate sensitivity of buffy coat examination techniques which consider animals uninfected while they actually have the parasite (Murray et al., 1977). Furthermore, Bossche and Rowlands (2001) showed that other diseases such as fasciolosis, gastro-intestinal parasitism and malnutrition could induce anaemia.

During the survey, *Glossina moristans sub moristans* was found in the area (2.49 f/t/d) along with other mechanical vectors such as *stomoxy* (1.66 f/t/d), *haematopota* (0.31 f/t/d) and *tabanus* (0.12 f/t/d). These results were in agreement with previous works of Tilahun et al., (2014) who reported G. tachnoides with apparent density of 0.11 fly/trap/day, and he also indicated other findings such as 0.05, 2.42, 3.89, 1.29 fly/trap/day for *Glossina morsitance sub-morsitans*,

Glossina pallidipes, *Stomoxys* and *Tabanus* respectively.

It was also in consistent with previous works of (Solomon M *et al*., 2010) in 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. Similarly, the study was in agreement with findings of (NTTICC, 2004) in 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.

5. Conclusion

Animal trypanosomosis is a major problem to livestock production and productivity in Selected Seven Districts. Over all bovine trypanosomosis prevalence was 162 (9.85%). Non- significant association was recorded among study sites ($p>0.05$). There was significant risk factor between age categories, sex groups, and body conditions ($p<0.0001$). The mean PCV value of infected animals was significantly lower than that of non-infected animals indicating the adverse effect of trypanosomosis on the PCV profile of cattle. The *most* widely distributed and dominant species is *T. congolense 76.54% followed by T.vivax 18.63 %*, which was mainly transmitted by tsetse fly, *Glossina morsitans sub morsitans* (2.49 f/t/d/) and biting flies (1.66 f/t/d, *Stomoxys*, *tabanid* 0.11 f/t/d and *haematopota* 0.30 f/t/d) respectively. The disease was found to cause substantial economic losses through cattle mortality, drug purchases and draft power loss of infected oxen. 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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