Study on Cattle Trypanosomosis, Associated risk factors, and Vector density in Bullen District, Western Ethiopia

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Abstract: A cross- sectional study was carried out in Bullen District of Benishangul Gumuz Regional State, Western Ethiopia from September to January, 2017 to estimate the prevalence of trypanosomosis in cattle and the prevailing species of trypanosomes, associated risks and its vector density. Blood samples were collected from (n=384) randomly sampled cattle (*Bos indicus*) and examined using parasitological (buffy coat technique) and hematological (measurement of packed cell volume) procedures. An overall, 113/384 (29.43%) prevalence was recorded. The infection was caused by *T. congolense 96/130 (*73.84%), *T. vivax* 21/130 (16.15%), T. brucei 6/130(4.62%) and mixed infection was found to be 7/130 (5.4%). The infection rate was found statistically significant (P<0.000) among trypanosome species. Mean packed cell volume (PCV) value of infected animals was lower (21.2% ± 3.85) than non- infected animals (26.41 % ± 1.86) and the variation was statistically significant (P<0.000). Non - significant difference was recorded within study sites, sex and age categories of animals (P>0.05), where as significant association was observed in body conditions. *Glossina tachinoides* was the only tsetse fly caught and its mean apparent density measured as f/t/d was 3.53. In addition, other mechanical vectors such as Stomoxys, Haematopota, and Tabanids with f/t/d of 1.67, 0.3 and 0.33 were recorded respectively. In conclusion, the result of the current study showed the economical importance of trypanosomosis in the study area signaling for devising strategic control efforts.

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1. Introduction

Animal trypanosomiasis is disease of domestic animals resulting from infection with parasitemic protozoa of the genus *Trypanosoma* transmitted primarily by tsetse fly and also by other haematophagus flies (Urquhart, 1996). Trypanosomiasis is the most serious in animal production mainly in sub Saharan Africa and prevents the keeping of cattle over millions of square kilometers of potentially productive land (Radiostitis, 2007).

Trypanosomosis is a disease complex caused by several species of blood and tissue dwelling protozoal parasites of the genus Trypanosoma (Singla *et al*., 2004). It is a disease of domestic livestock that causes a significant negative impact on food and economic growth in many tropical and subtropical countries of the world including sub-Saharan Africa. The course of the disease may run from an acute and rapidly fatal to a chronic long lasting one depending on the vector-parasite-host interactions. It is characterized mainly by intermittent fever, progressive anemia and loss of condition of susceptible hosts which if untreated leads to high mortality rates (Aulakh *et al.,* 2005).

Glossina species are an important African fly that act as the true vector of trypanosomiasis. Tsetse fly transmitted trypanosomiasis is commonly grouped together under the name 'nagana'. Their distribution lies within the tsetse fly belts of Africa, which extend from 14° N to 20°S in south west Africa and 29°N in Mozambique, covering an area of 10 million km. Many species of wild animals are symptom less carries of nagana trypanosomiasis and provide asylvatic reservoir of infection in which the trypanosomes are cyclically transmitted naturally from host to host by tsetse flies. The principal carrier of these trypanosomes are wild bovids and suids. Cattle are infected when they come in contact with these wild animal carries and bitten by infected tsetse fly as a result (Andrews., 2004).

The country has been infested with five tsetse fly species (*Glossina pallidipes, G. tachinoides, G. morsitans submorsitans, G. fusixipes fuscipes* and *G. longipennis*) that act as vectors for 5 trypanosome species (*T. vivax, T. congolense, T. brucei, T. evansi* and *T. rhodendiense*) out of six trypanosome species existing in Ethiopia (Abebe, 2005).

Western and southern river basins of Ethiopia are the most severely affected areas by trypanosomosis in the country. In the area specifically in the western part a wide diversity of tsetse and trypanosome species and strains co-exist (Abebe, 2005). These various species of Glossina and trypanosoma invade about 31,000 km^2 (62.13%) of fertile land in the Benishangul-Gumuz

regional state western parts of the country (NTTICC, 1996).

Trypanasomosis control is a long-term fight and therefore requires the involvement of decision makers, researchers and farmers. Until now, the use of trypanocidal drugs to treat or to prevent susceptible livestock against trypanosomosis remains the only control measure for most of the farmers. Very limited trypanocidal compounds are available and they have been used for many years. This long-term use of the same molecules selected drug resistant strains of trypanosomes in many African countries (Geerts *et al*., 2001).

In order to improve the welfare and security of rural communities, particularly Ethiopia, rapid method for assessing risk and diagnosing urgent problems are needed for the control of animal diseases. In Bullen district trypanosomosis was found to be one of the most important factors that hampered livestock rearing in almost all peasant associations. Hence, a study on the status of the disease and investigating the vectors and their relative abundance is crucial for a successful prevention and control in the area. The present study was, therefore, conducted in the district with the objectives of determining the prevalence of trypanosomosis, identifying the species of Trypanosoma and assessing of risk factors of the disease.

2. Materials And Methods

Study Area: The study was conducted from September to January, 2017 in Bullen district of Benishangul Gumuz Regional State, western part of Ethiopia. It was conducted in seven peasant associations including Bullen town, Chilako, Emange, Dobi, Addis Alem, Doshe and Benoshe. The district has 19 kebeles covering an area of 3252.397 km2 with human population of 46,920. Area lies at latitude of 10° and 36'15.1 N and longitude of 036° and 04'52.1'' E at an altitude of 1465 meter above sea level. Annual average temperature of area is 29.5°C and its rainfall range is 900 to 1100 mm (NMSA, 2007). Mixed agriculture is a common practice with livestock population of 47218 cattle, 6367 sheep, 16392 goats, 5211 equines, 51089 poultry and 1420 beehives (CSA, 2015).

Study Design and Study Animals: The study design used was cross-sectional to determine the prevalence of trypanosomosis in cattle and apparent density of tsetse and other biting flies that are involved in the transmission of trypanosomosis. Zebu cattle (*Bos indicus*), that are usually kept under 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 the study animal was scored as good, medium and poor (Nicholson and Butterworth, 1986). Concurrently, their age was determined based on De-Lahunta and Habel (1986) principles as young (≤ 3 years old), matured (4-7 years old) and adult (> 7 years old).

***Sampling method and Sample Size Determination*:** The study sites was selected purposively as convenient. Animals were sampled randomly involving both sexes, all age groups, and all types of body conditions. The desired sample size was calculated according to the formula given by Thrusfield (2007). The sample size was determined based on the previous prevalence of 6.0 %, confidence level of 95%, and 5% desired absolute precision. As result a total of 87 cattle were calculated but it increased to (n=384) to increase precision and these cattle were sampled at their communal grazing area using simple random sampling.

2. Study methodology

***Packed cell volume (PCV) determination*:** Blood samples were obtained by puncturing the marginal ear vein with lancet and collected directly into a pair of heparinised capillary tubes. The tubes were then sealed at one end with crystal seal and 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 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 on to a glass slide, and covered with cover slip. The slide was examined under x40 objective and x10 eye piece for movement of parasites (Murray, 1991). Trypanosome species were identified according to their morphological descriptions as well as movement in wet film preparations (OIE, 2008).

Entomological survey: A total of 70 odour-baited traps (18 Monopyramidal, 35 monoconical and 17 biconical) were deployed at 200-250 m intervals to assess the density and species of tsetse flies during the study. Each and every trap was odour baited with acetone and cow urine. The underneath of each trap

pole was smeared with grease in order to prevent ants climbing up the pole towards the collecting cage that could damage the tsetse flies. The trap deployment time was 48 hours. After capturing the flies in the collecting cage, they were then sorted by sex and species and recorded. The species of tsetse was identified based on the characteristic morphology. Other biting flies were also separated according to their morphological characteristics such as size, color, proboscis and wing venation structures at the genus level (Fischer and Say, 1989). Sexing was done for tsetse fly just by observing the posterior end of the ventral aspect of abdomen by hand lens, accordingly, male flies were easily identified by enlarged hypophageum. The apparent density of tsetse flies was determined based on the daily mean number of flies captured in odour-baited traps and recorded as fly per trap per day (F/T/D) (Leak *et al.,* 2009).

Data management and Analysis: Raw data were entered into a Microsoft Excel spreadsheet and descriptive statistics was used to summarize the data. STATA® version 12.0 statistical software programs were used to analyze the data. The point prevalence was calculated for all data as the number of infected individuals divided by the number of individuals examined and multiplied by 100. The association between the prevalence of trypanosome infection and risk factors were assessed by chi-square test ($\chi 2$), whereas the two sample student's t-test was used to assess the difference in mean PCV between trypanosome positive and negative animals. The test result was considered significant when the calculated p-value was less than 0.05 at 95% confidence interval (Thrusfield, 2005).

3. Result

Trypanosomes prevalence: Out of the total animals examined (n=384), 113/384(29.43%) were found to be infected with trypanosomes (table 2). The prevalence in terms of trypanosome species was 25 % for *T. congolense*, 5.47 % for *T. vivax, 1.56 % for T. brucei and 1.82 % was found to be mixed infection.* The proportion of trypanosome species was 96/130(73.84%) for *T. congolense*, *21/ 130(*16.15%) for *T. vivax, 6/130(4.61%) for T. brucei* and 7/130 (5.38%) for mixed infection and the infection rate was found to be statistically significant (P<0.000) among trypanosome species (Table 1).

Table 1. Species based prevalence of bovine trypanasomosis at Bullen district

Trypanosomes	**No. positive**	**(%) positive ± SE**	**95 % CI**	**X^2 (p-value)**
T. congolense	96	73.84± 0.19	0.923-0.998	333.09 (P<0.000)
T. vivax	21	16.15± 0.49	0.124-0.320	
T. brucei	6	4.61± 0.62	0.061-0.304	
Mixed (*T.congolense & T.vivax*	7	5.38± 0.042	0.047-0.213	
Total	130	100		

Table 2. Origin based prevalence of bovine trypanasomosis at Bullen District

Sites	**No. examined**	**No. positive**	**(%) positive ± SE**	**95 % CI**	**χ^2 (p-value**
Bullen town	56	17	30.35±0.62	0.18-0.43	1.92 (P>0.93)
Chilako	48	15	31.25±0.68	0.198-0.468	
Emange	68	20	29.41±0.56	0.185-0.403	
Dobi	51	12	23.52±0.59	0.118-0.353	
Addis Alem	56	16	28.57±0.61	0.166-0.405	
Doshe	42	12	28.57±0.68	0.127-0.396	
Benoshe	63	21	33.33±0.60	0.216-0.451	
Total	384	113	29.42±52	0.196-0.398	

Haematological survey results: The mean PCV value for all examined animals was 24.06 ± 1.96 SE. However, the mean PCV value for non infected and infected animals was 26.41 ± 1.86 SE and 21.2 ± 3.85 SE respectively. The mean PCV values of cattle were significantly ($P < 0.000$) influenced by trypanosome infection as 21.2 % and 26.41 % PCV values in trypanosome positive and negative animals were registered, respectively (Table 3).

Trypanosomosis associated with risk factors: The highest prevalence (39.13%) of trypanosomosis was recorded in animals >7 years old (adult) whilst the lowest prevalence (26.15 %) was recorded in animals ≤ 3 years of old (young) and the association was not found statistically significant among the age groups (table 2). Higher prevalence was registered in male animals (33.14 %) than in female animals (26.32%), which was not found to be statistically significant (p> 0.05) (table 2). Trypanosomosis was recorded across the study sites with the highest and lowest prevalence of (33.3%) and (23.52 %) in Benoshe and Dobi respectively and prevalence of trypanosomosis was

not statistically significant across the study sites (table 2). The highest prevalence of trypanosomosis (48.96%) was found in animals with poor body condition while the lowest (22.44% and 22.46%) was recorded in animals with medium and good body conditions respectively, and the difference was statistically significant (p<0.000). The effect of age, sex, sites and body condition on prevalence of trypanosomosis is summarized in table 2.

Table 2. Cont

Risk factors	**No. examined**	**No. positive**	(%) positive ± SE	**95 % CI**	**χ^2 (p-value)**
Sex					
Male	175	58	33.14±0.36	0.261-0.401	2.14(P>0.14)
Female	209	55	26.32±0.31	0.203-0.323	
Total	384	113	29.42±0.46	-0.159-0.023	
Age (years)					
≤ 3	130	34	26.15±0.38	0.186-0.337	5.49(P>0.06)
4 – 7	162	43	26.54±0.35	0.197-0.334	
> 7	92	36	39.13±0.51	0.291-0.492	
Total	384	113	29.42±0.30	0.00-0.121	
Body conditions					
Good	138	31	22.46±0.36	0.154-0.294	29.41(P<0.000)
Medium	156	35	22.44±0.033	0.158-0.290	
Poor	96	47	48.96±0.53	0.418-0.625	
Total	**384**	**113**	**29.42**±0.29	0.077-0.194	

Table 3. Mean PCV comparison of parasitaemic and aparasitaemic animals

Status	**Frequency**	**Mean PCV (%)**	**SE**	**X^2(p-value)**
Infected	164	21.20	3.85	108.83 (p<0.000)
Non- infected	220	26.41	1.86	
Total	**384**	**24.06**	**1.96**	

SE: Standard Error

***Entomological survey results*:** A total of 816 tsetse and biting flies were caught from different sites during the study period. Out of the total, 494 (60.54%) were belong to tsetse of the genus glossina, followed by stomoxy 234 (28.67%), tabanid 46 (5.63%). Haematopota 42 (5.14%) and Among tsetse species, only G. tachinoide was identified in the survey sites with the overall apparent density of 3.53 F/T/D (fly/trap/day). The highest fly density was observed in Benoshe peasant association 149 (1.064 F/T/D) and the lowest was recorded in Dobi 84 (0.6 F/T/D) (Table 5).

Table 5. Flies caught in different areas of survey sites at Bullen district

Sites	**Total flies caught**	**No. of traps**	**Tsetse flies caught**					**Biting flies**		
			No.	**species**	**M**	**F**	***F/T/D**	**Stomoxys**	**tabanid**	**Haematopota**
Bullen town	140	10	79	GT	24	55	3.95	45	9	7
Chilako	105	10	65		19	46	3.25	24	5	11
Emange	96	10	71		22	49	3.55	18	4	3
Dobi	84	10	54		16	38	2.7	16	6	8
Addis Alem	132	10	68		23	45	3.4	54	8	2
Doshe	110	10	66		27	39	3.05	33	5	6
Benoshe	149	10	91		32	59	4.55	44	9	5
Total	**816**	**70**	**494**		**163**	**331**	**3.53**	**234**	**46**	**42**

F/T/D=fly per trap per day, Gt=*Glossina tachinoidess*, M=male, F=female

4. Discussion

The current study revealed an overall prevalence of 113/384 (29.42%) trypanosomosis infection in the study area. This finding was in line with the study conducted by (Bayisa *et al.,* 2015) who reported 22.38% prevalence in Assosa district of the Benishagul Gumuz region, Western Ethiopia. Similarly, 26.30% trypanosomosis prevalence was

reported by Aki A *et al*. (2017) in neighbor Mandura district.

This study indicated that the infection was predominantly caused by *T. congolense 96/130 (*73.84%), *T.vivax* 21/130(16.2%), and *T. brucei* 6/130(4.61%) and mixed infection 7/130(5.4%). This result is in line with the reported proportions of *T.congolense* (77.6%) followed by *T.vivax* (14.9%) from Metekel and Awi zones (Mekuria *et al.,*2011). This result was also in consistent with prior reports of (Mulaw *et al.,*2011) who studied on prevalence of major trypanosomes affecting cattle in Assosa district of Benishangul Gumuz Regional State, Western Ethiopia and who found proportional prevalence of *T. congolense to be* 66.7%; (Abraham *et al*., 2012) conducted their study on prevalence of bovine trypanosomosis in selected sites of Arba Minch district, Southern Ethiopia whose result showed proportional prevalence of *T. congolense* to be 61.4%; (Biyazen *et al*., 2014) reported proportional prevalence of *T. congolense to be* 63.64% during their work on trypanosomosis and anemia in cattle population of Dale Wabera district of Kellem Wollega Zone, western Ethiopia.

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 the infected animals as demonstrated by (Leak *et al*., 1993). Further, it might be attributed to the efficient transmission of *T. congolense* by cyclical vectors than *T. vivax* in tsetse-infested areas. Previous reports indicated that *T. congolense* and *T. vivax* are the most prevalent trypanosomes that infect cattle in tsetse infested and tsetse free areas of Ethiopia respectively (Leak *et al*., 1999). Different studies (Leak *et al*., 1993; Rowland *et al*., 1995) have indicated that *T. vivax* is highly susceptible to treatment while the problems of drug resistance are higher in *T. congolense*.

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

The overall mean PCV value for examined animals was 24.06 ± 1.96 SE. The mean PCV value of infected animals was significantly lower (21.20 ± 3.85 SE) than that of non infected animals (26.41 ± 1.86 SE). This result is in alignment with previous works of (Ali *et al*., 2011; Mulaw, 2011).

In the entomological survey, *Glossina tachinoides* was the only tsetse fly caught and its mean apparent density measured as f/t/d was found to be 3.53. It accounts for 60.54 % (494/816) out of the total flies caught. In addition, other mechanical transmitters of trypanosomosis such as stomoxys, haematopota and tabanid account for 28.67% (234), 5.14% (42) and 5.63% (46) of total flies caught with f/t/d of 1.67, 0.3 and 0.33 respectively. The current finding is in consistent with the previous findings of (NTTICC, 2012-2014) at neigbouring mandura district of western Ethiopia which was reported to be 3.59 f/t/d, 1.38 f/t/d, 0.33 f/t/d and 0.014 f/t/d, for tsetse fly, *stomoxys, haematopota*, and *tabanus* respectively.

5. Conclusion

The high prevalence of Trypanosmosis was reported in cattle of Bullen District which indicated impact of the disease, associated risk factors and its contribution to hampering the productivity, work performance and health status of animals. The most widely distributed and dominant species of trypanosomes in the study sites are *T. congolense (73.84%) followed by T.vivax (16.2%), and* to some extent *T. brucei (4.61%)* which was mainly transmitted by *Glossina tachinodes* and other biting flies with f/t/d/ of 3.53, 1.67, 0.30 and 0.33 for G. tachinoides, stomoxys, haematopota and tabanid respectively. Since the district lies within the tsetse belt area, the result of the present study (29.42%) shows the fact and expected prevalence. Significant association was not recorded within study sites, sex and age groups of animals ($p> 0.05$) while there was significant association among trypanosomes species and body condition categories ($P< 0.05$). This study showed that trypanosome infection and other factors such as (nutritional, seasonal, concurrent disease) was found to negatively affect the PCV values of animals. Therefore, Bullen 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 and against the parasites will be under take in the study area.

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