Epidemiology of bovine Trypanosomosis in Kamashi District of Benishangul Gumuz Regional State, Western Ethiopia: Prevalence, Vector density and associated risks

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Abstract: A cross sectional study was carried out in Kamashi district of Benishangul Gumuz Regional State, western Ethiopia between April and May, 2015 to determine the prevalence of trypanosomosis, prevailing species of trypanosomes, associated risks and its vector density. Blood samples collected from (n= 413) randomly sampled cattle (*Bos indicus*) was examined using parasitological (buffy coat technique) and hematological (measurement of packed cell volume) procedures. An overall, 37 (8.96%) prevalence was recorded. The infection was caused mainly by *Trypanosoma congolense (*73%), *Trypanosoma vivax* 5/37 (13.5%), to less extent by *Trypanosoma brucei* 1 (2.7%), mixed infection with *T. congolense* and *T. vivax* 2 (5.4%), and *T. congolense and T. brucei* 2 (5.4%). The infection rate was statistically significant among difference trypanosome species (P<0.05). Mean packed cell volume (PCV) value of parasitaemic animals was lower (26.89% ± 7.51) than aparasitaemic animals (30.12% ± 6.53) and the variation was not statistically significant (P>0.05). Higher prevalence (25.93%) was registered in poor body conditioned animals when compared with medium (8.65%) and good body conditioned animals (6.32%) and the difference was not statistically significant (p>0.05). Similarly, prevalence of trypanosomosis was not statistically significant across study sites, among age categories and between sex groups (P> 0.05). *G. tachinoides* was the only tsetse fly caught and its mean apparent density measured as f/t/d was 2.68. In addition, other mechanical vectors such as stomoxys, tabanids and haematopota with f/t/d of 2.84, 1.54 and 0.92 were recorded respectively. Therefore, the result of the present finding shows moderately high prevalence of trypanosomosis in the study sites indicating the need for strategic and participatory integrated approach to control the vector and to minimize the impact of the disease in the study district.

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Key words: Kamashi district, PCV, Risk factor, Trypanosome, Trypanosomosis, tsetse fly

1. Introduction

Trypanosomiasis is a devastating disease of livestock caused by protozoal parasites of the genus trypanosoma that inhabits blood and other tissues of vertebrates including animals, wildlife and human (Adam *et al.,* 2003; Gupta *et al*., 2009; Bal *et al*., 2014). It is a vector borne disease that is transmitted biologically by tsetse flies and mechanically by other biting flies (FAO, 2002; OIE, 2009). It is a major constraint contributing to direct and indirect economic losses to crop and livestock production (Abebe, 2005) and has a significant negative impact on economic growth in many parts of the world (Taylor *et al*., 2007; Sharma *et al*., 2013), particularly in sub-Saharan Africa (Cecchi *et al*., 2008).

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 Km^2. 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). 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). 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).

Kamashi is one the five districts of Kamashi zone in the Benishangul Gumuz regional state, western Ethiopia 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 obstacles of livestock production and productivity, there is no previous study conducted in the district to show the situation of the disease and to integrate all efforts towards combating the disease and reducing its economic impact. Therefore, the present study is designed to determine the epidemiology of bovine trypanosomosis and to assess associated risk factors and to suggest actions towards the control measure.

2. Materials And Methods

Study Area: The study was conducted from April to May, 2015 in Kamashi district of Kamashi zone, Benishangul Gumuz Regional State, Western Ethiopia. It was carried out in five kebeles hereafter called sites namely: Kamashi, Chicha, Mirmita, Kobi Badessa and Daguba. The district has 15 kebeles covering an area of 1,598 km^2 with human population of 21,354. It has an altitude of 1,351 meter above sea level. Its annual average temperature is 32.5^{0}c (28-37^{0}c) and its rainfall range is 900-1350 mm (NMSA, 20014). The livelihood of the people in the district largely depends on mixed livestock and crop production having livestock population of 6,577 cattle, 1,289 sheep, 7,000 goats, 528 equines, 12,224 poultry and 1,420 beehives (CSA, 2013/14).

Study Design and Study Animals: Cross sectional study design was used. A local zebu cattle (*Bos indicus*), which are mainly 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 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).

Sampling Techniques and Sample Size Determination: The study sites were purposively selected as convenient. Study 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 Thrusfield (2007). 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 but increased to (n=413) to increase precision and these cattle were sampled at their communal grazing area using simple random sampling.

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. 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 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).

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).

Data Analysis: During the study period, data were collected using the sample 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 parasitaemic animals against that of aparasitaemic animals. In all cases the difference between parameters were tested for significance at probability level of 0.05 or less. The prevalence of cattle trypanosomosis was calculated as the number of parasitologically positive animals examined by buffy coat method to the total animals examined (Thrusfield, 2007).

3. Result

Distribution of trypanosomes infection: Out of the total animals examined, 37 (8.96%) were infected with trypanosomes. The trypanosome species responsible for the infection were *T. congolense, T. vivax* and *T. brucei*. As indicated in table 1, the proportional prevalence of each species of trypanosome was 27 (72.97%) for *T. congolense*, *5 (*13.51%*)* for *T. vivax*, *1 (*2.70%*)* for *T. brucei* and 4 (10.81%) mixed infections with (*T. congolense* & *T. vivax*=2 and *T. congolense* & *T. brucei*=2) were 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).

The highest and the lowest prevalence of trypanosomosis were recorded in Miremita 12 (12.5%) and Kamashi 6 (6.45%) study sites respectively. However, there was no significant difference among the study sites (p >0.05) (Table 3).

The Prevalence of trypanosomosis varies in both sexes; the infection in female is slightly higher 17/188 (9.04 %) than male 20/225 (8.88%) and the association was not statistically significant (P>0.05) (Table 3).

Packed Cell Volume: The mean PCV values for all examined animals were 29.83 ± 6.61 SD. However, the mean PCV value for non infected animals were 30.12 ± 6.53 SD and the mean PCV value of the infected animals was 26.89 ± 7.51 SD. There was no significant difference in the mean PCV value between non infected and infected animals (P>0.05) (Table 2).

Trypanosomosis and associated risks: In the present study animals examined were categorized in different age groups as < 2 years, 2-5 years and >5 years. Out of the total sampled animals, 71, 210 and 132, were < 2 years, 2-5 years and > 5 years old respectively and the prevalence was found to be 7 (9.86 %), 19 (9.05%) and 11 (8.33%) for tested animals < 2 years, 2-5 years and > 5 years respectively and the difference in the prevalence was not statistically significant (p >0.05) (table 3).

Similarly, during the study, animals are categorized in to different body conditions as good, medium and poor. From the total 413 animals examined 174, 185 and 54, were good, medium and poor body conditioned respectively and out of which 11 (6.32%), 16 (8.65%), and 10 (18.52 %) prevalence of trypanosomosis were recorded for animals with good, medium and poor body condition respectively. Trypanosome infection and body condition scores of study animals were not found statistically significant (p > 0.05) (Table 3).

Entomological Survey: The present survey of tsetse flies depicted that *G. tachinoides* is the only species tsetse fly responsible for cyclical transmission of trypanosomosis in the study area. Tsetse fly survey was carried out in five kebeles of the study district by deploying a total 25 geo-referenced traps (8 mono-conical, 10 mono-pyramidial and 7 biconical traps) in the river border, open wood land (savanna grass land) and on grazing fields of cattle, the number of tsetse flies captured in each study site is 27, 19, 11, 35, 35 for Kamashi, Chicha, Kobi Badessa, Miremita, and Daguba respectively. The mean apparent density of *G. tachnoides* in the survey sites was investigated as 2.54 f/t/d while the mean apparent density of mechanical vectors such as stomoxys (2.84 f/t/d), tabanids (1.54 f/t/d) and haematopota(0.92 f/t/d) were recorded (table 4).

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

Trypanosomes	No. positive	Prevalence (%)	X^2	(p-value)
T. congolense	27	73	9.483	0.024
T. vivax	5	13.5		
T. brucei	1	2.7		
Mixed (*T. congolense* & *T. vivax*)	2	5.4		
Mixed *(T. congolense* & *T. brucei*)	2	5.4		
Total	**37**	**100**		

Table 2: Mean PCV comparison between parasitaemic and aparasitaemic animals

Status	Frequency	Mean PCV (%)	SDs	Overall PCV	p- value	X^2
Infected	37	26.89	7.51	995	0.029	7.06
Non infected	376	30.12	6.53	11328		
Total	**413**	**29.83**	**6.61**	**12323**		

SD: standard deviation, **PCV**: packed cell volume

Table 3: prevalence of bovine trypanosomosis and its association with various risk factors in Kamash district

Risk factors	No. examined	No. positive	Prevalence (%)	p-value	χ^2
Sites					
Kameshi	93	6	6.45		
Chicha	109	9	8.27		
Kobi Badessa	68	6	8.82	0.199	6.00
Miremita	96	12	12.5		
Daguba	47	4	8.51		
Total	**413**	**37**	**8.96**		
Sex					
Male	225	20	8.88	0.063	3.42
Female	188	17	9.04		
Total	**413**	**37**	**8.96**		
Age(years)					
< 2	71	7	9.86		
2-5	210	19	9.05	0.468	0.527
> 5	132	11	8.33		
Total	**413**	**37**	**8.96**		
Body conditions					
Good	174	11	6.32		
Medium	185	16	8.65	0.025	5.023
Poor	54	10	18.52		
Total	**413**	**37**	**8.96**		

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

Sites	Total flies caught	No. of traps	Tsetse flies caught					Biting flies		
			No.	Species	M	F	*F/T/D	Stomoxys	Tabanid	Haematopota
Kameshi	94	5	27	GT	9	18	2.7	39	19	9
Chicha	99	5	19		6	13	1.9	45	23	12
Kobi Badessa	48	4	11		4	7	1.375	18	11	8
Miremita	83	6	35		12	23	4.42	29	9	10
Daguba	68	5	35		11	24	2.92	11	15	7
Total	**392**	**25**	**127**		**42**	**85**	**2.54**	**142**	**77**	**46**

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

4. Discussion

The present study revealed an overall 37(8.96 %) prevalence of trypanosomosis caused by different species of trypanosomes. This finding was in agreement with the previous studies conducted in neighbournig districts of Oromia region by (Dano *et al*., 2014) who reported an overall prevalence of 7.81% in Guto Gida district of eastern wollega zone, (Tefese *et al*., 2012) whose finding was 8.55% in Sasiga and Diga districts of east Wollega and (Feyera, 2015) who reported 9% prevalence in and around Nekemte areas of east wollega zone. Similarly, it is in concordance with studies carried out by (Yehunie *et al*., 2012) who reported an overall prevalence of 7.81% in Wemberma district of west Gojjam zone, Northwest Ethiopia. In contrast, the present finding is low when compared with previous reports, (26.3%) in around Assosa district (Mulaw *et al*., 2011) and 24.7% in Mao-komo special district (Daud and Molalegne, 2011) of Benishangul Gumuz regional state, western Ethiopia. The relatively low prevalence of trypanosomosis in the present study might be due to the differences in agro-ecology and climatic conditions of the localities.

Of the total cases registered, 27(72.97%), 5(13.51 %), 1(2.70%) and 4(10.81%) were found to be caused by *T. congolense, T. vivax, T. brucei* and mixed infection respectively. This indicates statistically significant difference among the distribution of trypanosome species ($p< 0.05$). This finding was in consistent with the previous finding of (Biyazen *et al*., 2014) who reported 63.64%, 27.27%, and 9% for trypanosome species of *T. congolense, T. vivax, and T.*

brucei respectively during their study in Dale Wabera district of Kellem Wollega Zone, Western Ethiopia.

Among the study sites, the highest and the lowest prevalence of trypanosomosis were recorded in Miremita PA 12(12.5%) and Kamashi PA 6(6.45 %**)** respectively. However there was no significant difference ($p > 0.05$) in the prevalence of trypanosomosis and the study sites. According to (Adale and Yasine, 2013), there is difference in prevalence of trypanosomosis in different study sites and the difference among kebeles is due to difference in vegetation cover; reproduction and development of flies are highly influenced by climatic conditions.

The prevalence of trypanosome infection was slightly higher in female animals (9.04%) than males (8.88%), although it was not statistically significant ($p>0.05$). This finding was in agreement with the previous findings of (Feyissa *et al*., 2011); (Tasew and Duguma, 2012). Similarly, (Bogale *et al*., 2012) found higher infection rate in females than males in some parts of Ethiopia. The possible reason for this difference might be due to physiological difference between male and female animals (Feyissa *et al*., 2011) because female animals are more exposed to physiological stresses males.

Higher prevalence of trypanosomosis was observed (18.52%) in poor body conditioned animals when compared with medium (8.5%) and good (6.32%) body conditioned animals even if the association was not statistically significant ($p >0.05$) and this finding was in agreement with study carried out by (Lelisa *et al*., 2015); (Teka *et al*., 2012) and (Ayana *et al*., 2012) who recorded higher trypanosome infection rate in poor body conditioned animals than in good and medium body conditioned animals. Similarly, slightly higher prevalence was registered in animals aged < 2 years (9.86%) when compared with animals 2-5 years (9.05%) and > 5 years (8.33%) and statistically significant associations were not observed ($p > 0.001$) and the finding was in agreement with previous workers (Cherenet *et al*., 2004); (Tasew and Duguma, 2012) and (Terefe *et al*., 2014), who reported comparable results on trypanosome infection across different age categories.

The overall mean PCV value of all examined animals was 29.83 ± 6.61 SD. The mean PCV of non infected cattle was higher (30.12%) than that of infected animals (26.89%) and the association was not statistically significant. This finding was in agreement with the previous work of (Denu *et al.,* 2012). Additionally, (Daud and Molalegne, 2011) and (Molalegne *et al*., 2010) reported lower mean PCV value in infected animals than in the non-infected animals.

Glossina tachinoides was the only tsetse fly species caught in the study and its mean apparent density measured as f/t/d was 2.54. It accounts for 127 (32.4%) out of the total flies caught. In addition, other mechanical transmitters of trypanosomosis such as stomoxys 142 (36.22%), tabanus 77 (19.64%) and haematopota 46(11.73%) were recorded. The apparent density for these biting flies expressed as f/t/d was found to be 2.84, 1.54, and 0.92 for stomoxys, tabanids and haematopota respectively. The current findings was lower when compared with previous 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 for tsetse and stomoxys respectively. The difference might be due to the difference in agro-ecology and climatic conditions in the localites.

5. Conclusion

Trypanosomosis caused by *T. congolense, T. vivax* and *T. brucei* with higher prevalence of *T. congolense* remains a major problem that hinders livestock production and productivity in the district. *G. tachinoides* was the only tsetse fly species captured in the study sites. Other mechanical transmitters of trypanosomosis such as *stomoxys, tabanus* and *haematopota* were registered in the area. Parameters of study animals such as sex, age and body condition were not found to be a risk factor for trypanosomosis infection. To wrap up, the result of the present finding shows moderately high prevalence of trypanosomosis in the study sites indicating the need for strategic and holistic approach to control the vector and to minimize the impact of the disease in the study district.

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