



## BOVINE TRYPANOSOMOSIS AND ASSOCIATED RISK FACTORS IN KAMASHI DISTRICT OF BENISHANGUL GUMUZ REGIONAL STATE, WESTERN ETHIOPIA

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**ABSTRACT:** A cross sectional study was carried out in Kamashi District of Benishangul Gumuz Regional State, western Ethiopia from September to February, 2020 to determine 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, 87/384 (22.65 %) prevalence was recorded. The infection was caused by *T. congolense* 76/98 (77.5%), *T. vivax* 16/98 (16.3%), *T. brucei* 3/98(3.06%) and mixed infection was found to be 3/98 (3.06 %). The infection rate was found statistically significant ( $P<0.000$ ) among trypanosome species. Mean packed cell volume (PCV) value of infected animals was lower (20.7%) than non- infected animals (24.81%) and the variation was statistically significant ( $P<0.000$ ). Non - significant difference was recorded within study sites, sex and age categories of animals ( $P>0.05$ ), whereas 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 2.56. In addition, other mechanical vectors such as Stomoxys, Haematopota, and Tabanids with f/t/d of 1.69, 0.30 and 0.28 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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**Key words:** *Blood, biting flies Trypanosome, Tsetse fly, risk factor*

### 1. Introduction

Trypanosomiasis is caused by protozoa in the family Trypanosomatidae genus *Trypanosoma*. African animal trypanosomosis is disease complex caused by tsetse-fly-transmitted *T. congolense*, *T. vivax* or *T. brucei* or simultaneous infection with one or more of these trypanosomes. It is a protozoan disease of both human and animals caused by different species of the genus trypanosome and the most important in terms of economic loss in domestic livestock (Getachew, 2005). It is major cause of disease in cattle in west Africa, but can cause serious losses in goats, and sheep, horse, pigs and camels and also in domestic dogs, chronic infection often results in a carrier state (OAU, 2001). The modern classification of trypanosomiasis is arranged in to two sections, the Stercoraria which is non pathogenic to man and animals with few exceptions and the Salivaria which is pathogenic to human & other animals (Kassa, 2005). Infection of cattle by one or more of the three African animal trypanosomes results in subacute, acute, or chronic

disease. The disease is characterized by intermittent fever, anaemia, occasional diarrhea, and rapid loss of condition, lymphadenopathy, splenomegally and cachexia often followed by death in untreated cases. In southern Africa the disease is widely known as nagana, which is derived from a Zulu term meaning "to be in low or depressed spirits", a very apt description of the disease (Kuzoe, 1991; Brown *et al.*, 1990).

In Ethiopia, tsetse transmitted animal trypanosomosis is a serious constraint to livestock production and agricultural development, exorcising farmers and livestock keepers out of areas having very high potential for growth, and forcing them to live on a highly degraded highlands of the country (Langridge, 1976; Abebe, 2005). The problem caused by tsetse and trypanosomosis is not only limited to inflicting diseases but also leading to significant negative impacts such as losses due to mortality and morbidity in domestic animals, cost of livestock treatment and tsetse control, and getting rid of draught animals from their infestation areas (Juyal *et al.*, 2005).

Tsetse flies in Ethiopia are confined to southern and western regions between longitude of 33<sup>o</sup> and 38<sup>o</sup> East and latitude of 5<sup>o</sup> and 12<sup>o</sup> North which amounts to be about 200,000 Km<sup>2</sup>. 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). The tsetse flies (vectors), *G. fusca*; the bush fly, *G. morsitans*, which inhibit principally savannah area and *G. palpalis*; a riverine species, effectively prevent the rearing of the cattle over the large area of the Africa (Blood *et al.*, 1989).

Trypanosomes are microscopic, elongated and flattened cell which move with the help of single flagella directed towards, at the base of which is found characteristic structure, the kinetoplast (Jemere, 2004). The distribution of trypanosomosis is depending on the three factors: the distribution of vectors, the virulence of the parasite and the response of the host. Epidemiologically trypanosomes are distributed in the tropical Africa in the latitude of 14<sup>o</sup>c and 29<sup>o</sup>c where they are associated with their vectors, Glossina, the tsetse fly (Urquhart *et al.*, 1996).

Trypanosomosis is transmitted by tsetse and other biting flies through the transfer of blood from one animal to another and/or by tsetse flies cyclically (biologically) - species such as *T.congolense*, *T. vivax* and *T. brucei* ; non cyclically (mechanically) by other biting flies of the genus *Tabanus*, *Stomoxis*, *Haematopota*, *hiperosia* and *chrysops* flies and by other means like venereal, Iatrogenic and by coitus of transmission (Awoke, 2000; Urquhart *et al.*, 1996). *T. vivax* and *T.brucei* have spread beyond the tsetse fly belts where transmission by biting flies; with single exception of *T. equiperdium* of equines which is venereal disease (FAO, 1998).

In the Abay basin areas of northwest Ethiopia, tsetse transmitted trypanosomosis is one of the most economically important diseases impeding the development of livestock and agricultural farming activity which requires development of proper surveillance and control strategy. Benishangul-Gumuz regional state pertains to the area of North west part of the country and nearly 31,000 km<sup>2</sup> or 62% of the region's total land area is believed to be infested with tsetse fly (NTTICC, 1996). Despite this fact, very scant information is available about the disease epidemiology and its vector with baseline data in the Kamashi district. The aims of the present study were, therefore, to assess the epidemiology of trypanosomosis and its vector density in six kebeles of Kamahi district.

Therefore, the objectives of the study were

- To determine the prevalence of bovine trypanosomosis.
- To assess associated risk factor
- To determine apparent density of tsetse and other biting flies

## 2. Materials and Methods

### 2.1 Study area

The study was conducted from September to February, 2020 in Kamashi district of Benishangul Gumuz Regional State, western part of Ethiopia. It was conducted in six peasant associations including kamashi town, Dimetu, kobi badesa, miremita, Demeska oda, and Gunda Derba. The district has 15 kebeles covering an area of 1,598 km<sup>2</sup> with human population of 21,354. It has an altitude of 1,351 meter above sea level. Its annual average temperature is 32.50c (28-370c) and its rainfall range is 900-1350 mm (NMSA, 2016). 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, 2016).

### 2.2 Study Design and Study Animals

Cross sectional study design was used. A local zebu cattle (*Bos indicus*), which 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 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).

### 2.3 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 (Thrusfield, 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, and these cattle were sampled at their communal grazing area using simple random sampling.

### 2.4 Study methods

#### 2.4.1 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 (Hermmlle 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).

#### 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 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).

#### 2.4.3 Entomological survey

A total of 86 odour baited traps of monoconical, biconical and mono pyramidal 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).

#### 2.5 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 ( $\chi^2$ ) was used to evaluate the association of different variables with the prevalence of trypanosome infection. In all of the statistical analysis, a confidence level of 95% is used and P-value of less than 0.05 (at 5% level of significance) was considered as statistically significant.

### 3. RESULT

#### 3.1 Parasitological findings

Out of the total animals examined (n=384), 87/384(22.65%) were found to be infected with trypanosomes (Table2). The prevalence in terms of trypanosome species was 19.79% for *T. congolense*, 4.16% for *T. vivax*, 0.78% for *T. brucei* and 0.78% was found to be mixed infection. The proportion of trypanosome species was 76/98(77.5%) for *T. congolense*, 16/ 98(16.3%) for *T. vivax*, 3/98(3.06%) for *T. brucei* and 3/98 (3.06%) 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 trypanosomosis at Kameshi district

Trypanosomes	No. positive	Positive (%)	X <sup>2</sup> (p-value )
<i>T. congolense</i>	76	77.5	323.46 (P<0.000)
<i>T. vivax</i>	16	16.3	
<i>T. brucei</i>	3	3.06	
Mixed ( <i>T. congolense</i> & <i>T. vivax</i> )	3	3.06	
Total	98	100	

### 3.2 Haematological findings

The mean PCV value for all examined animals was 23.05. However, the mean PCV value for non-infected and infected animals was 24.81 and 20.7 respectively. The mean PCV values of cattle were significantly ( $P < 0.000$ ) influenced by trypanosome infection as 20.7 % and 24.81 % PCV values in trypanosome positive and negative animals were registered, respectively (Table 4).

### 3.3 Identified risk factors

The highest prevalence (23.07%) of trypanosomosis was recorded in animals 3-5 years old whilst the lowest prevalence (22.28 %) was recorded in animals >5 years of old (adult) and the association was not found statistically significant among the age groups

(Table 3). Slightly, higher prevalence was registered in female animals (23.76 %) than in male animals (21.42 %), which was not found to be statistically significant ( $p > 0.05$ ) (Table 3). Trypanosomosis was recorded across the study sites with the highest and lowest prevalence of (29.26%) and (11.36 %) in Demeska oda and kobi bedessa respectively and prevalence of trypanosomosis was not significant across the study sites (Table 2). The highest prevalence of trypanosomosis (38.63%) was found in animals with poor body condition while the lowest (14.66%) was recorded in animals with good body conditions respectively, and the difference was significant ( $p < 0.000$ ). The effect of age, sex, sites and body condition on prevalence of trypanosomosis is summarized in Table 2 and Table 3.

**Table 2:** Origin based prevalence of bovine trypanosomosis at Kamashi District

Sites	No. examined	No. positive	(%) positive	$\chi^2$ (p-value)
Kamashi town	72	17	23.6	8.19 (P>0.05)
Dimetu	64	17	26.56	
Kobi badesa	44	5	11.36	
Miremita	81	13	16.04	
Demeska Oda	41	12	29.26	
Gunda Derba	82	23	28.04	
Total	384	87	22.65	

**Table 3:** Prevalence of Trypanosomosis infection with different potential risk factors

Risk factors	No. examined	No. positive	(%) positive	$\chi^2$ (p-value)
<b>Sex</b>				0.29(P>0.58)
Male	182	39	21.42	
Female	202	48	23.76	
Total	384	87	22.65	
<b>Age(years)</b>				0.03(P>0.98)
≤ 2	88	20	22.72	
3 – 5	130	30	23.07	
> 5	166	37	22.28	
Total	384	87	22.65	
<b>Body conditions</b>				18.45(P<0.000)
Good	150	22	14.66	
Medium	146	37	25.3	
Poor	88	43	38.63	
<b>Total</b>	384	87	<b>22.65</b>	

**Table 4:** Mean PCV comparison of parasitaemic and aparasitaemic animals

Status	Frequency	Mean PCV (%)	X <sup>2</sup> ( p-value)
Infected	87	20.70	83.73 (p<0.000)
Non- infected	297	24.81	
<b>Total</b>	384	23.05	

PCV: packed cell volume

#### 4.4 Entomological findings

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 six kebeles of the study district by deploying a total of 86 geo-referenced traps (30 mono-conical, 30 mono-pyramidal and 26 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 after 48 hour is 96, 67, 90, 41, 76, 71 for Kamashi town, Dimetu, Miremita, Kobi bedesa, Demeska oda, and Gunda Derba respectively. A total

of 834 tsetse and biting flies were caught from different sites during the study period. Out of the total, 441/834 (52.87%) were belong to tsetse fly of the genus glossina, followed by stomoxy 292 (35.01%), tabanid 49(5.87%) and haematopota 52 (6.23%). The mean apparent density of *G. tachnoides* in the survey sites was investigated as 2.56 f/t/d while the mean apparent density of mechanical vectors such as stomoxys (1.69 f/t/d), tabanids (0.28 f/t/d) and haematopota (0.30 f/t/d) were recorded. The highest fly density was observed in Miremita peasant association 176 (5.86 f/t/d) and the lowest was recorded in kobi 83 ( 2.96 f/t/d) (Table 5).

Table 5: Flies caught in different areas of survey sites at kamashi district

Sites	Total flies caught	No. of traps	Tsetse flies caught					Biting flies		
			No.	species	M	F	*F/T/D	Stomoxys	tabanid	Haematopota
Kamshi town	169	15	96	GT	30	66	3.2	55	8	10
Dimetu	116	14	67		28	39	2.39	38	5	6
Miremita	176	15	90		31	59	3.00	64	10	12
Kobi badesa	83	14	41		12	29	1.46	26	7	9
Demeska oda	152	14	76		22	54	2.71	55	11	10
Gunda Derba	138	14	71		22	49	2.54	54	8	5
<b>Total</b>	<b>834</b>	<b>86</b>	<b>441</b>		<b>145</b>	<b>296</b>	<b>2.56</b>	<b>292</b>	<b>49</b>	<b>52</b>

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

#### 5. DISCUSSION

Overall, 22.65% of Cattle trypanosomosis prevalence were reported 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 Mandura district. In contrast, 8.96% bovine trypanosomosis prevalence was reported by Aki A *et al.* (2015) in Kamashi District, which was low as compared to the current study. This research showed that the infection was predominantly caused by *T. congolense* 76/98 (77.5%),

*T. vivax* 16/98(16.3%), and *T. brucei* 3/98(3.06%) and mixed infection 3/98(3.06%). This result is in line with the reported proportions of *T. congolense* (75.5 %) followed by *T. vivax* (14.28%) 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 serodemes 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 23.05. The mean PCV value of infected animals was significantly lower (20.7) than that of non infected animals (24.81). This result is in alignment with previous works of (Ali *et al.*, 2011; Mulaw, 2011).

Entomological findings indicated that, *G. tachinoides* was the only tsetse fly caught and its mean apparent density measured as f/t/d was found to be 2.56. It accounts for 52.87 % (441/834) out of the total flies caught. In addition, other mechanical transmitters of trypanosomosis such as stomoxys, haematopota and tabanid account for 35.01% (292), 6.23% (52) and 5.87% (49) of total flies caught with f/t/d of 1.69, 0.30 and 0.28 respectively. The highest fly density was observed in Miremita peasant association 176 (5.86 f/t/d) and the lowest was recorded in kobi 83 (2.96 f/t/d). The current finding is concord with the previous findings of (NTTICC, 2012-2014) at neighboring 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. Similarly, this finding was in line with the previous findings of (Aki A *et al.*, 2015) in Kamshi Distirict, which was reported to be 2.54, 2.84, 1.54, and 0.92 for *G. tachinoides*, *stomoxys*, *tabanids* and *haematopota* respectively.

## 6. CONCLUSION AND RECOMMENDATIONS

The high prevalence of Cattle trypanosomosis (22.65%) were remains a major problem that hinders livestock production and productivity in the district. The most widely distributed and dominant species of trypanosomes in the study sites are *T. congolense* (77.5%) followed by *T. vivax* (16.3%), and to some extent *T. brucei* (3.06 %) which was mainly transmitted by *Glossina tachinodes* and other biting flies with f/t/d/ of 2.56, 1.69, 0.30 and 0.28 for *G. tachinoides*, *stomoxys*, *haematopota* and *tabanid* respectively. Parameters of study animals such as sex and age were not found to be a risk factor for trypanosomosis whereas body conditions and trypanosome species were risk factors. 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. Based on the current findings, the following recommendations are forwarded:-

- Particular attentions towards the identified trypanosome species are essential to control the impact of the disease on cattle that are potential reservoir of the infections.
- Development of control options that could minimize the tsetse fly and biting flies in the study area should be introduced in a wholistic approach.
- Proper and strict follow up of trypanocidal drug distribution, therapeutic strategies and alternative control measures should be implemented by concerned stake holders.
- The farmer in the area should be trained how to control the vector of the disease and provided with materials

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