

EPIDEMIOLOGY OF BOVINE TRYPANOSOMOSIS: PREVALENCE AND ASSOCIATED RISK FACTORS IN KAMASHI DISTRICT, 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 January, 2022 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, 98/384 (25.52 %) prevalence was recorded. The infection was caused by *T. congolense* 75/98 (76.53%), *T. vivax* 14/98 (14.28%), *T. brucei* 3/98(3.06%) and mixed infection was found to be 6/98 (6.12 %). The infection rate was found statistically significant ($P<0.000$) among trypanosome species. Mean packed cell volume (PCV) value of infected animals was lower ($20.7\% \pm 3.75$) than non- infected animals ($24.81\% \pm 1.43$) 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 2.41. In addition, other mechanical vectors such as Stomoxys, Haematopota, and Tabanids with f/t/d of 1.66, 0.29 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.

[Endalkachew Mekonen and Asmamaw Aki. **EPIDEMIOLOGY OF BOVINE TRYPANOSOMOSIS: PREVALENCE AND ASSOCIATED RISK FACTORS IN KAMASHI DISTRICT, WESTERN ETHIOPIA.** *Researcher* 2025;17(6):22-30]. ISSN 1553-9865 (print); ISSN 2163-8950 (online). <http://www.sciencepub.net/researcher>. 04. doi:[10.7537/marsrsj170625.04](https://doi.org/10.7537/marsrsj170625.04)

Key words: Kamashi district; PCV; Risk factor; Trypanosome; Trypanosomosis; Tsetse fly

1. INTRODUCTION

Trypanosomiasis, a disease of humans and animals caused by several species of trypanosomes and spread by tsetse flies is a major constraint to livestock production in 37 countries within the Sub-Saharan region. An estimated 45-50 million cattle are at risk of infection in the region, with an estimated economic loss of up to US \$ 1.3 billion in cattle production (Gage KL, 2008). Its public health importance has led to attempts to control the disease nationally and regionally with initiatives as Pan Africa tsetse and trypanosomosis eradication program (PATTEC) (Schofield CJ, 2008). These attempts rely on repeated large-scale epidemiological studies and environmental surveys, guiding the design and implementation of intervention strategies. The accuracy of these surveys is limited by use of parasitological diagnostic techniques as microscopy due to low sensitivity (Picozzi K, 2002), and the difficulty in incorporating climatic and environmental data known to influence tsetse distribution, and as a result disease spread (Hendrickx G, 2000; Gage KL, 2008).

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 a sylvatic 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)

Tsetse flies in Ethiopia are confined to southern and western regions between longitude 33^o and 38^o East and latitude 5^o and 12^o North which amounts to about 200,000 Km². Tsetse infested areas lied in the low lands and also in the river valleys of Blue Nile, Baro Akobo, Didessa, Ghibe and Omo. Out of the nine regions of Ethiopia five (Amhara, Benishangul Gumuz, Gambella, Oromia and Southern Nation

Nationalities and peoples) are infested with more than one species of tsetse flies (Keno M, 2005). To date five species of *Glossina* (*Glossina morsitans submorsitans*, *G. Pallidipes*, *G. tachnoides*, *G. f. fuscipes* and *G. longipennis*) have been recorded from Ethiopia ((Keno M, 2005). Apart from the cyclical transmission of trypanosomosis by the *Glossina* species, it is highly considered that mechanical transmission is a potential threat to livestock productivity in some parts of Ethiopia (Abebe G, 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 km² (62.13%) of fertile land in the Benishangul-Gumuz regional state western parts of the country (NTTICC, 1996- 2004).

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.

Kameshi 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 constraints of livestock production and productivity, there is few 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 September to January, 2022 in Kamashi district of Benishangul Gumuz Regional State, western part of Ethiopia. It was conducted in seven peasant associations including Kamashi town, Dimetu, Miremita, Kobi badesa, Demeska oda, Jalo and Gunda Derba. The district has 15 kebeles covering an area of 1,598 km² with human population of 21,354. It has an altitude of 1,351 meter above sea level. Its

annual average temperature is 32.5^oc (28-37^oc) 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).

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 9.0 %, confidence level of 95%, and 5% desired absolute precision. As result a total of 126 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 100 odour-baited traps of mono conical, 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).

Data management and Analysis: Raw data were entered into a Microsoft Excel spreadsheet and descriptive statistics was used to summarize the data. STATA® version 11.0 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 (χ^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, 2007).

3. RESULT

Parasitological findings : Out of the total animals examined (n=384), 98/384(25.52%) were found to be infected with trypanosomes (table 1). The prevalence in terms of trypanosome species was 19.53 % for *T. congolense*, 3.64 % for *T. vivax*, 0.78 % for *T. brucei* and 1.56% was found to be mixed infection. The proportion of trypanosome species was 75/98(76.53%) for *T. congolense*, 14/ 98(14.29%) for *T. vivax*, 3/98(3.06%) for *T. brucei* and 6/98 (6.12%) 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 Kamashi district

Trypanosomes	No. positive	(%) positive ± SE	95 % CI	X ² (p-value)
<i>T. congolense</i>	75	76.53± 0.21	0.917-1.00	323.4 (P<0.000)
<i>T. vivax</i>	14	14.28± 0.50	0.266-0.465	
<i>T. brucei</i>	3	3.06± 0.83	0.086-0.414	
Mixed(<i>T.congolense</i> & <i>T.vivax</i>)	6	6.12± 0.43	0.111-0.281	
Total	98	100		

Haematological findings : The mean PCV value for all examined animals was 23.05 ± 2.54 SE. However, the mean PCV value for non infected and infected animals was 24.81 ± 1.43SE and 20.7 ± 3.75 SE 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 3).

Identified risk factors: The highest prevalence (26.47%) of trypanosomosis was recorded in animals ≤ 3 years old (young) whilst the lowest prevalence (24.2 %) was recorded in animals 4-7 years of old (mature- adult) and the association was not found statistically significant among the age groups (table 2). Slightly, higher prevalence was registered in male animals (25.53 %) than in female animals (25.51 %), 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 (30.9%) and (20.37 %) in Demeska oda and Jalo respectively and prevalence of trypanosomosis was not statistically significant across the study sites (table 2). The highest prevalence of trypanosomosis (46.59%) was found in animals with poor body condition while the lowest (15.33%) was recorded in animals with 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 and table 3.

Table 2. Origin based prevalence of bovine trypanosomosis at Kamashi District

Sites	No. examined	No. positive	(%) positive \pm SE	95 % CI	χ^2 (p-value)
Kamashi town	54	12	22.2 \pm 0.57	0.11-0.334	2.04 (P>0.91)
Dimetu	54	14	25.92 \pm 0.60	0.141-0.38	
Jalo	54	11	20.37 \pm 0.55	0.095-0.312	
Kobi badesa	55	14	25.45 \pm 0.59	0.138-0.37	
Miremita	54	14	25.92 \pm 0.60	0.141-0.38	
Demeska Oda	55	17	30.90 \pm 0.63	0.186-0.43	
Gunda Derba	58	16	27.58 \pm 0.59	0.159-0.39	
Total	384	98	25.52 \pm 0.01	-0.01-0.033	

Table 3. Prevalence of trypanosomosis infection with different potential risk factors

Potential Risk factors	No. examined	No. positive	(%) positive \pm SE	95 % CI	χ^2 (p-value)
Sex					0.00(P>0.99)
Male	188	48	25.53 \pm 0.32	0.193-0.318	
Female	196	50	25.51 \pm 0.31	0.194-0.316	
Total	384	98	25.52 \pm 0.45	-0.088-0.087	
Age(years)					0.25(P>0.88)
≤ 3	136	36	26.47 \pm 0.38	0.192-0.342	
4 – 7	157	38	24.20 \pm 0.34	0.175-0.309	
> 7	92	24	26.08 \pm 0.46	0.171-0.351	
Total	384	98	25.52 \pm 29	0.062-0.053	
Body conditions					29.12(P<0.00)
Good	150	23	15.33 \pm 0.30	0.095-0.211	
Medium	146	34	23.28 \pm 0.35	0.164-0.302	
Poor	88	41	46.59 \pm 0.53	0.361-0.571	
Total	384	98	25.52\pm0.28	0.094-0.203	

Table 4. Mean PCV comparison of parasitaemic and aparasitaemic animals

Status	Frequency	Mean PCV (%)	SE	X ² (p-value)
Infected	98	20.70	3.75	104.6 (p<0.000)
Non- infected	286	24.81	1.43	
Total	384	23.05	2.54	

SE: Standard Error, PCV: packed cell volume

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 seven kebeles of the study district by deploying a total of 100 geo-referenced traps (32 mono-conical, 40 mono-pyramidal and 28 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 99, 66, 91, 40, 88, 27, 71 for Kamashi town, Dimetu, Miremita, Kobi bedesa, Demeska oda, Jalo and Gunda Derba respectively. A total of 929 tsetse and biting flies were caught from different sites during the study period. Out of the total, 482/929 (51.88%) were belong to tsetse fly of the genus glossina, followed by stomoxys 332 (35.73%), tabanid 56 (6.02%) and haematopota 59 (6.35%). The mean apparent density of *G. tachinoides* in the survey sites was investigated as 2.41 f/t/d while the mean apparent density of mechanical vectors such as stomoxys (1.66 f/t/d), tabanids (0.28 f/t/d) and haematopota (0.29 f/t/d) were recorded. The highest fly density was observed in Demeska oda peasant association 183 (6.53 f/t/d) and the lowest was recorded in Jalo 58 (2.07 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	174	15	99	GT	34	65	3.3	55	8	12
Dimetu	114	14	66		29	37	2.35	36	5	7
Miremita	180	15	91		32	59	3.03	64	12	13
Kobi badesa	82	14	40		12	28	1.42	26	7	9
Demeska oda	183	14	88		33	55	3.14	74	11	10
Jalo	58	14	27		8	19	0.96	23	5	3
Gunda Derba	138	14	71		22	49	2.54	54	8	5
Total	929	100	482		170	312	2.41	332	56	59

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

4. DISCUSSION

Overall, 25.5% 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* 75/98 (75.53%), *T.vivax* 14/98(14.28%), and *T. brucei* 3/98(3.06%) and mixed infection 6/98(6.12%). 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 ± 2.54 SE. The mean PCV value of infected animals was significantly lower (20.7 ± 3.75 SE) than that of non infected animals (24.81 ± 1.4 SE). 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 2.41. It accounts for 51.88 % (482/929) out of the total flies caught. In addition, other mechanical transmitters of trypanosomosis such as stomoxys,

haematopota and tabanid account for 35.73% (332), 6.35% (59) and 6.02% (56) of total flies caught with f/t/d of 1.66, 0.29 and 0.28 respectively. The highest fly density was observed in Demeska oda peasant association 183 (6.53 f/t/d) and the lowest was recorded in Jalo 58 (2.07 f/t/d). The current finding is concord with the previous findings of (NTTICC, 2012-2014) at neighbouring 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 inline 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.

5. CONCLUSION

The high prevalence of Cattle trypanosomosis (25.52%) 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* (76.53%) followed by *T. vivax* (14.28%), and to some extent *T. brucei* (3.06 %) which was mainly transmitted by *Glossina tachinodes* and other biting flies with f/t/d/ of 2.41, 1.66, 0.29 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. Therefore, Kameshi 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.

ACKNOWLEDGEMENT

The author would like to acknowledge the Asossa Regional Veterinary Diagnostic, Surveillance, Monitoring and Study Laboratory management staffs for funding the study and for their unreserved cooperation during the entire activities of the study

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