



Prevalence of Bovine Trypanomosis and Associated Risk Factors in Pawi special District of Benishangul Gumuz region, North Western Ethiopia.

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Abstract: A cross sectional study was carried out in pawe special district of Benishangul Gumuz Regional State, North West Ethiopia from January to March 2019 to determine the prevalence of trypanosomosis, prevailing species of trypanosomes, associated risks and its vector density. Blood samples collected from 384 randomly sampled cattle (*Bos indicus*) was examined using parasitological (buffy coat technique) and haematological (Measurement of packed cell volume) procedures. An overall, 10.94% (42/384) prevalence of trypanosomosis was recorded. The infection was caused mainly by *Trypanosoma congolense* 31/42 (73.8%), *Trypanosoma vivax* 11/42 (26.8%), and the infection rate was statistically significant among different trypanosome species ($P < 0.05$). Mean packed cell volume (PCV) value of infected animals was lower (19.4 ± 1.3) than non-infected animals (22.03 ± 1.73) and the variation was found statistically significant ($P < 0.05$). Similarly, higher prevalence (17.8%) of trypanosomosis infection was registered in animals with poor body condition when compared to animals with medium (13.46%) and good (4.76%) body condition and the difference was statistically significant ($P < 0.05$). In contrast, prevalence of trypanosomosis was not statistically significant among study sites, age categories and sex groups of study animals ($P > 0.05$). *Glossina morsitans submorsitans*, and *Glossina tachinoedes* were the tsetse fly species caught and their mean apparent density measured as flies/trap/day was 0.78. In addition, other mechanical vectors such as stomoxys and tabanids were captured with flies/trap/day 0.12 and 0.8, respectively. To wrap up, the result of the current finding reveals moderately high prevalence of trypanosomosis in the study district signaling the need for strategic and participatory approach to control the vector and to minimize the impact of the disease in the study district.

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Key words: Pawi, Trypanosomosis, Tsetse fly, prevalence, Risk factors.

1. Introduction

Trypanosomes are extracellular protozoan parasites that cause debilitating diseases called trypanosomosis in animals and sleeping sickness in humans and have great socio-economic impact adversely affecting food production and economic growth in many parts of Africa, particularly in Sub-Saharan Africa (SSA), (Taylor, 2015). The disease in animal is transmitted cyclically by the genus *Glossina*, but it can also be transmitted mechanically by other biting flies among which *Tabanus* and *Stomoxys* are presumed to be the most important, as exemplified by their presence in South and Central America as well as in some areas of Africa free or cleared of *Glossina* such as Ethiopia, Chad, Senegal, Sudan *etc* (Truc *et al.*, 2013).

The distribution of the disease coincides with the habitat of the tsetse fly vector and is called the tsetse fly “belt” or it is sometimes referred to as “green desert” because ~10 million km² of potential fertile land is rendered to be unsuitable for cultivation (Shaw *et al.*, 2014). Within this area, the majority of the *Glossina* infested countries are underdeveloped, poor, heavily indebted and food-deficit due to lack of productive animals as far as meat/milk production and draft power are concerned, resulting in an annual economic loss of about 5 billion US\$ (Giordani *et al.*, 2016; Yaro *et al.*, 2016).

In Ethiopia, the most important trypanosome spp affecting livestock include *T. congolense*, *T. vivax* and *T. brucei* in cattle, sheep and goats; *T. evansi* in camels and *T. equiperdium* in horses (Abebe, 2005).

Western and southern river basins of Ethiopia are the most severely affected areas by trypanosomosis in the country. In area specifically in the western part, a wide diversity of *Glossina* and trypanosome spp and strains co-exist (Abebe, 2005). Therefore, the current study was conducted to assess the prevalence of trypanosomosis; to determine distribution and apparent density of tsetse fly in the study area and to recommend possible control and prevention measures.

2. Materials and Methods

2.1 Study area

The study was conducted from January to March, 2019 in Pawe special district of Metekel zone, Benishangul Gumuz Regional State. The study was conducted in five villages namely: Almu, Mender-14, Mender-17, Mender-5 and Mender-7. The district has 20 kebeles covering an area of 64,300 hectare with human population of 42,000. The study area is located at latitude of 11° and 15° 24.7'N and, longitude of 36° and 23° 10'E. It has an altitude of 1064m above sea level. The mean annual average temperature of the district is 32°C and the mean annual rainfall range from 900-1400 mm (NMSA, 2007). The livelihood of the society largely depends on mixed livestock and crop production having livestock population of 58,810 Cattle, 5440 Goat, 5523 Sheep, 843 Equines, 29378 Poultry (CSA, 2015). The district lies at Dabus and Beles rivers water shed system which is one of the tributary of Nile River.

2.2. Study Design and Study Animals

Cross sectional study design was used. A local zebu cattle (*Bos indicus*), which are usually kept under an extensive husbandry system grazing the communally owned pasture land throughout the year were randomly sampled. They grazed together during the day time and returned to their individual owner's farmstead each evening. The body condition of each of the study cattle was scored as good, medium and poor (Nicholson and Butterworth, 1986). Concurrently, their age was categorized in years (< 2, 2-5 and > 5) based on De-Lahunta and Habel (1986) principles.

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, (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 and cattle were sampled at their communal grazing area using simple random sampling method.

2.4. Study Methodology

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 (Hermle 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 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. PCV was read and recorded for each sample. 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 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. Trypanosome species were identified according to their morphological descriptions as well as movement in wet film preparations (OIE, 2008).

2.4.3. Entomological Survey

To assess the apparent densities, distributions and species of tsetse flies and other biting flies the entomological data were collected in late rain season (May to Jun 2019) along the suitable tsetse habitats. These include livestock grazing areas, watering points, wild game reserve areas, rivers, savanna grass land and area of dense river side forests in the Districts. Monopyramidal traps baited with acetone, octanol and cow urine Taylor., *et al.*, (2007), were deployed at an interval of about 100 meters along watershed and vegetation to assess the fly density. The coordinates of each trap position were recorded with a Global Positioning System (GPS).

In all study sites a total of 50 Monoconical traps were deployed early in the morning and maintained in position for 48 hrs at five different peasant associations (PAs) in 5 trapping sites. The underneath of each trap pole was smeared with grease in order to prevent the ants climbing up the pole towards the collecting cage that could damage the tsetse flies and the cages from these traps were emptied to caught tsetse flies and other biting flies were counted,

recorded and identified and sexed for the tsetse fly, other biting flies according to their morphological characteristics such as size, color, wing venation structure and proboscis at the genus level Taylor, *et al.*, (2007). Sexing was done for tsetse fly just by observing the posterior end of the ventral aspect of abdomen by hand lens, as a result male flies easily identified by enlarged hypophageum.

2.5. Data Analysis

All the collected raw data and the results of parasitological and hematological examination data were entered into a Microsoft excel spread sheets program and then was transferred to Intercool STATA version 10.0 for analysis. The prevalence of trypanosome infection was calculated as the number of positive animals as examined by buffy coat method divided by the total number of animals examined at the particular time. Data collected on PCV values were analyzed by ANOVA to compare the mean PCV values of infected animals against that of uninfected animals. Pearson's chi-square (χ^2) was used to evaluate the association of different variables with the

prevalence of trypanosome infection. In all of the statistical analysis, a confidence level of 95% is used and P-value of less than 0.05 (at 5% level of significance) was considered as statistically significant.

3. Results

3.1. Prevalence of Trypanosome species in Pawi District

Out of total animals examined, 10.94% (42/384), were infected with trypanosomes. The prevalence in terms of trypanosome species was 73.8% *T. congolense* and 26.8 % *T. vivax*. The proportion of trypanosome species was 31/42 (73.8%) *T. congolense* and 11/42 (26.2%) *T. vivax*. The maximum and minimum Trypanosome infection rate in examined peasant association of pawe district was 17 % and 5 % recorded at mender 17 and mender 5 peasant associations (Table 1). The infection rate difference between trypanosomes was statistically significant ($P < 0.0001$).

Table 1: Prevalence of Trypanosome by peasant association

No	PA's	No-Sample taken	No-positive	Prevalence %	Trypanosome species	
					T.congo	T.vivax
1	Mender 5	80	4	5 %	3	1
2	Mender 14	100	17	17 %	11	6
3	Mender 17	80	12	15 %	10	2
4	Almu	44	3	6.8 %	2	1
5	Mender 7	80	6	7.5 %	5	1
	Total	384	42	10.94%	31	11

Table 2: Prevalence of Trypanosomosis by species of the parasites.

Trypanosome	No positive	Prevalence %
<i>T. congolense</i>	31	73.8%
<i>T. vivax</i>	11	26.2%

3.2. Cattle PCV Distribution and Anemia in Studied Area

The mean PCV value for whole examined animals was 25.5 ± 1.73 SD. However, the mean PCV value for uninfected animals was 22.03 ± 1.73 SD and mean PCV value of the infected animals was 19.4 ± 1.3 SD. The mean PCV values of cattle were significantly ($P = 0.0011$) influenced by trypanosome infection as 22.79% and 25.81% PCV values in trypanosome positive and trypanosome negative animals were registered, respectively. The overall

anemia prevalence in the studied district was 49.5% (190/384). The anemia prevalence was significantly higher in trypanosome infected cattle (56.8%) than in non-infected cattle (43.2%) ($P < 0.05$). Out of 49.5% anemia prevalence, 10.94% (42/384) was trypanosome infected animals. However, large number of animals 38.5% (148/384) had anemia ($PCV < 24$) without having trypanosome infection. Some animals 3.6% (14/384) were infected by trypanosome but their PCV was found normal.

Table 3: Prevalence of Trypanosomosis according to different risk factors

Status	frequency	Mean Pcv	SE	Overall pcv	X^2	p-value
Infected	42	19.4	1.3	824	194.2868	<0.001
Non infected	348	22.03	1.52	8462		
Total	384	25.5	1.73	9784		

The highest trypanosomosis prevalence 218 (12.8%) was recorded in >5 years old animals whilst the lowest prevalence 48 (4.34%) was in animals < 2 years old. Slightly higher prevalence was registered in females 24 (11.32 %) than in males 18 (10.5 %). Trypanosomosis was recorded across the study sites with the highest (17%) prevalence in Mender-14 and the lowest (5% in Mender-5). Trypanosomosis

prevalence was statistically non-significant between age categories, sex groups and across study sites. The highest prevalence (17.8%) was found in poor body condition animals while the least (4.76%) in good body conditions. This difference was statistically significant. The effect of age, sex, sites and body condition on trypanosomosis prevalence is summarized in table 4 and 5.

Table 4: Prevalence of Trypanosomosis with age category in pawi special district.

Variable	No-of examined	No-positive	Prevalence %
Age			
<2	46	2	4.34%
2-5	130	12	9.23%
>5	218	28	12.8%

Table 5: Prevalence of Trypanosomosis according to Body condition of pawi district.

Variable	No-of examined	No-positive	Prevalence %
Body condition			
Good	42	2	4.76%
Medium	104	14	13.46%
Poor	146	26	17.8%
Total	384	42	10.93%

Table 6: Prevalence of Trypanosomosis according to sex

Variable	No-of examined	No-positive	Prevalence %
Sex			
Male	172	18	10.5%
Female	212	24	11.32%
Total	384	42	10.9%

3.3. Entomological survey result

The entomological survey showed that the apparent densities of different flies across the rainy seasons in study area with total mean catches per trap per day were 0.78 for tsetse flies, also numeral number of *Stomoxys* and *Tabanus* were recorded. In Districts tsetse flies have highly infested and identified in selected Peasant association. The fly species were identified *G.m.submorsitans*, and *G.tachinoides* and it was coughed in mender 5, 14 and 17 PA around Beles river. In this study the distribution of *G.tachinoides*, was found to be along the large rivers of Beles and its tributaries, which cross most of zone

the region and has covered a vast area with even distribution in all PAs from low to high apparent densities in the District. A total of 78 tsetse fly were cached during the study period from different site. Out of the total, 60 (76.8%) were belonging to tsetse of the species *Glossina tachinoides*, and 18 (23%) were belonging to tsetse of the species *Glossina morsitans submorsitans*. In addition to tsetse fly, other biting fly such as 12(0.12) stomoxys and 8(0.8) tabanus were identified. The highest fly density 27 (1.35 F/T/D) were observed in Mender 14 and the lowest 9 (0.45 F/T/D) recorded in Almu and detail of the each District summarized in table below.

Table 7: Tsetse fly distribution of pawi district

No	Kebele	No of trap deployed	No of Caught fly	FTD	Sex	
					M	F
1	Mender 5	10	9	0.45	3	6
2	Mender 14	10	27	1.35	10	17
3	Mender 17	10	20	1	6	14
4	Almu	10	10	0.5	4	6
5	Mender 7	10	12	0.6	3	9
	Total	50	78	0.78	25	53

Table 8: Vectors of trypanosomosis identified from the study sites

No	Kebele	No of Caught fly	Species of tsetse fly		Biting fly	
			G.tachnoides	G.m.submorsitans	Stomoxoy	tabanas
1	Mender 5	9	7	2	3	1
2	Mender 14	27	21	6	1	2
3	Mender 17	20	16	4	2	2
4	Almu	10	8	2	2	1
5	Mender 7	12	8	4	4	2
	Total	78	60	18	12	8

4. Discussions

The present study revealed an overall prevalence of 42/384 (10.94%) in the study area. This finding was in agreement with earlier works of Bayisa and Getachew who reported 11.7% prevalence in the neighboring Mandura district; Asmamaw, (2017) worked on the prevalence of bovine trypanosomosis in mandura district and reported prevalence of 13.10%, Bayisa and Getachew who reported 11.7% prevalence in the neighboring Dangur district; however present study was lower than works of Bayisa and Getachew who reported 22.38% prevalence in the neighboring Asossa district; Asmamaw and Getachew (2017), worked on the prevalence of bovine trypanosomosis in Asossa district and reported prevalence of 28.10%, Dawud and Molalegne (2011), worked on the prevalence of bovine trypanosomosis in Asossa district and reported prevalence of 24.7%, Mulaw et al, worked on the prevalence of bovine trypanosomosis in Asossa district and reported prevalence of 25.8%.

The difference in the prevalence of trypanosomosis in the previous and the current findings might be due to the difference in agro ecology and climatic conditions of the areas and partly it might be the difference in seasons in the study period and Assosa District from the regionis heavily infested with tsetse fly and have high prevalence of trypanosomosis. *T. congolense* proportional prevalence of 61.4%; Biyazen, et al. (2014) reported *T. congolense* proportional prevalence 63.64% during his work on trypanosomosis and anemia in cattle population of Dale Wabera district of Kellem Wollega Zone, Western Ethiopia; Bayisa et al. (2015) demonstrated *T. congolense* proportional prevalence of 85% during his research on cattle trypanosomosis prevalence in Asossa district, Benishangul Gumuz Regional State, Western Ethiopia. Mulaw et al reported *T. congolense* proportional prevalence 66.7% during his work on trypanosomosis and anemia in cattle population of Dale Wabera district of Kellem Wollega Zone, Western Ethiopia Abraham and zerihun, (2012) reported *T. congolense* proportional prevalence 61.4% during his work on bovine trypanosomosis and anemia in selected sites in Arba minch district, southern

Ethiopia.

The high proportion infection rate of *T. congolense* in cattle might be attributable to the high number of serodemes of *T. congolense* relative to *T. vivax*. It could also be due to the possible development of better immune response to *T. vivax* by the infected animals as demonstrated by Leak, 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, 1999). Different studies (Leak, et al., 1993; Rowlands, et al., 1995) have indicated that *T. vivax* is highly susceptible to treatment while the problems of drug resistance are higher in *T. congolense*, and *T. congolense* is mainly confirmed in the blood, while *T. vivax* and *T. brucei* also invade the tissues.

The prevalence of bovine trypanosomosis was studied between sex categories, age groups and body conditions, though; significant association was not observed (> 0.05). This might be because of an equal chance of exposure to the parasite. This result is in agreement with previous reports (Mihreteab and Mubarek, 2011; Lelisa, et al., 2015). The overall anemia prevalence in the studied district was 35.93% (138/384). The anemia prevalence was significantly higher in trypanosome infected cattle (58.6%) than in non-infected cattle (43.%) (< 0.05). This is in concordance with previous results from different researchers (Mihret and Mamo, 2007; Biyazen, et al., 2014).

Out of 49.5% anemia prevalence, 10.94% (42/384) was trypanosome infected animals. Nonetheless, 38.5% (148/384) of non-infected animals were found to be anemic (PCV < 24). This indicates the fact that other factors such as gastrointestinal parasitism, nutritional deficiencies, fasciolosis and vector-borne diseases could affect the PCV value of cattle (Rowland, et al., 1995).

This study revealed that 3.6% (14/384) of the cattle were infected by trypanosome; however, their PCV was laid in the normal range. This might be attributed to the capability of infected cattle to

maintain their PCV within the normal range for a certain period of time. It could also be possibly due to inadequacy of the detection method used (Murray, et al., 1997), other anemia causing diseases (Rowland, et al., 1995), or delayed recovery of the anemic situation after current treatment with trypanocidal drugs. Furthermore, the occurrence of positive animals with PCV of greater than 24% might be thought of as recent infections of the animals (Rowland, et al., 1995). The overall mean PCV value for examined animals was 25.64 ± 5.61 SD. The mean PCV value of the infected animals was significantly lower (22.79 ± 4.51 SD) than that of uninfected animals (25.81 ± 5.53 SD). This result is in alignment with previous works (Mulaw, et al., 2011; Bayisa, et al., 2015).

Glossina tachinoides *Glossina m.submorsitans* was tsetse fly caught and their mean apparent density measured as f/t/d was 0.78. In addition, other mechanical transmitters of trypanosomosis such as *Stomoxys* 12 (0.12%), *Tabanus* 8 (0.8%) were recorded. The current findings were in consistent with previous works of Solomon and Fitta, (2010) at Metekel Awi zones of Northwest Ethiopia, who reported 6.49 f/t/d and 0.65 f/t/d for tsetse and biting flies, respectively. It was also in agreement with 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 and 0.06 f/t/d for tsetse, *Stomoxys* and *Tabanus*, respectively.

5. Conclusions

The most common trypanosomes species identified were *T.congolense* followed by *T.vivax*. The animal parameters such as sex, age and body condition were not found to be a risk factor. The mean PCV value of infected animals was significantly lower than that of uninfected animals indicating the adverse effect of trypanosomosis on the PCV profile of cattle. Trypanosomes were not detected in some anemic cattle indicating the occurrence of other causes of anemia in the area. *G.tachinoides* and *G.m.submorsitans* was the tsetse fly species discovered in this study. Other mechanical transmitters of trypanosomosis such as *Stomoxys*, and *Tabanus* were recorded in the area. In wrapping up, trypanosomosis is an economically important disease threatening the health and productivity of cattle in Pawi district. Therefore, proper strategies have to be designed and implemented to minimize the impact of the disease on livestock production in the studied district.

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