

Trypanosomosis in Cattle Population of Pawe District of Benishangul Gumuz Regional State, Western Ethiopia: Anemia, Vector Density and Associated Risks

¹Asmamaw Aki and ^{2*}Getachew Dinede

¹Regional veterinary Diagnostic, Surveillance, Monitoring and Study Laboratory, P.O. Box: 326, Asossa, Ethiopia

²Ministry of Livestock and Fishery, Epidemiology Directorate, P.O. Box: 1084, Addis Ababa, Ethiopia

Telephone: +251116676953; Email: dinedegech@gmail.com

Abstract: A cross-sectional study was carried out in Pawe district of Benishangul Gumuz Regional State, Western Ethiopia from January to March, 2016 to determine trypanosomosis prevalence, trypanosomosis association with anemia, prevailing trypanosomes species, associated risks and vector density. Blood samples collected from (n= 519) randomly sampled cattle (*Bos indicus*) was examined using buffy coat technique and hematological procedures. An overall, 29 (5.58%) trypanosomosis prevalence was recorded. The infection was caused by *Trypanosoma congolense* 22/29 (75.86%) and *Trypanosoma vivax* 7/29(24.14%). The infection rate difference between trypanosomes was statistically significant ($P < 0.05$). Mean packed cell volume (PCV) value of the infected animals was lower ($22.79\% \pm 4.51$) than uninfected animals ($25.81\% \pm 5.53$) and the variation was statistically significant ($P < 0.05$). Overall, anemia prevalence of 35.06% (182/519) was recorded and it was significantly higher (58.6%) in infected cattle than in non-infected (33.67%). Significant association was not recorded with study sites, sex groups, age categories and body conditions ($P > 0.05$). *Glossina tachinoides* was the only tsetse fly caught and its mean apparent density measured as fly/trap/day was 5.03. In addition, other mechanical transmitters of trypanosomosis such as stomoxys (1.62 f/t/d), tabanus (0.41f/t/d) and haematopota (0.22 f/t/d) were recorded. In conclusion, the result of the current study showed the economical importance of trypanosomosis in the area calling for devising strategic control efforts.

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Key words: Anaemia; Pawe; PCV; Risk factor; Trypanosomosis; Tsetse fly

1. Introduction

Trypanosomosis is a complex disease caused by unicellular parasites found in the blood and other tissues of vertebrates including livestock, wild life and people (Radostitis *et al.*, 2007). 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 (Langridge, 1976) and *Trypanosoma equiperdium* in horses (Dagnachew, *et al.*, 1981).

The course of the disease may run from a chronic long lasting to an acute and rapidly fatal depending on the vector-parasite-host interactions. The disease is mainly characterized by intermittent fever, progressive anemia, and loss of condition of susceptible hosts which if untreated leads to heavy mortalities (Bourn, *et al.*, 2001). Its effects are not only due to direct losses but also indirect losses including exorcising of livestock and animal power from the trypanosomosis prevalent areas (Awoke, 2000).

The epidemiology of trypanosomosis is influenced by the distribution of the vectors, trypanosomes' virulence and host immune (Urquhart

et al., 1996). Ethiopia is located at the East end of the African tsetse belt and tsetse flies are confined to Western, South-western and Southern regions (Abebe and Jobre, 1996; Abebe, 2005) between longitude 33° and 38°E and latitude 5° and 12°N of an area covering 220,000 km² (NTTCI, 1996). These areas are located in the low lands and along the country's larger rivers such as the Blue Nile/Abay, Baro/Akobo, Didessa, Ghibe and Omo. Five species of Glossina namely: *Glossina morsitans submorsitans*, *Glossina pallidipes*, *Glossina tachinoides*, *Glossina fuscipes fuscipes*, and *Glossina longipennis* were recorded in Ethiopia (Getachew, 2005).

The knowledge of the status of the disease prevalence, its health impact on animals affected, its vector distribution and the associated risks are very important for understanding the epidemiology of the disease and to devise suitable control measures. Therefore, the aims of the present study were to determine trypanosomosis prevalence, trypanosomosis association with anemia, prevailing trypanosomes species, associated risks and vector density.

2. Materials and Methods

Study Area

The study was conducted from January to March, 2016 in Pawe district of Metekel zone, Benishangul Gumuz Regional State. It was conducted in six kebeles hereafter called sites namely: Hidase pawi, Abay Ber pawi, Mender-11, Mender-12, Mender-28 and Mender-29. The district has 20 kebeles covering an area of 64,300 hectare with human population of 42,000. It 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. Its annual average temperature is 32°C and its rainfall range is 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 and 3076 beehives (CSA, 2015).

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.

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 but increased to (n=519) 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 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 (Hermmler 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. 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 (Paris, *et al.*, 1982). Trypanosome species were identified according to their morphological descriptions as well as movement in wet film preparations (OIE, 2008).

Fly survey

During the study four types of traps were deployed: 25 Monopyramidal, 18 monoconical, 4 biconical, and 5 engu traps. Every trap was odor baited with acetone and cow urine. 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. The trap deployment time was 48 hours. After the flies captured 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 (Langridge, 1976; 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, as a result male flies easily identified by enlarged hypophageum.

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. Result

Trypanosomes infection prevalence

Out of total animals examined, 29/519(5.58%) were infected with trypanosomes. The prevalence in terms of trypanosome species was 4.24% *T.congolense* and 1.35% *T.vivax*. The proportion of trypanosome species was 22/29 (75.9%) *T. congolense* and 7/29(24.1%) *T. vivax* (Table 1). During study period mixed infection was not detected. The infection rate difference between trypanosomes was statistically significant (P<0.0001).

Cattle PCV Distribution and Anemia in Studied Area

The mean PCV value for whole examined animals was 25.64 ± 5.61 SD. However, the mean PCV value for uninfected animals was 25.81 ± 5.53 SD and mean PCV value of the infected animals was 22.79 ± 4.51 SD. The mean PCV values of cattle were significantly (□ = 0.0011) influenced by trypanosome infection as 22.79% and 25.81% PCV values in trypanosome positive and trypanosome negative animals were registered, respectively (Table 2).

The overall anemia prevalence in the studied district was 35.06% (182/519). The anemia prevalence was significantly higher in trypanosome infected cattle (58.6%) than in non-infected cattle (33.67%) (□ <0.05). Of 35.06% anemia prevalence, 3.28% (17/519) was trypanosome infected animals. However, large number of animals 31.8% (165/519) had anemia

(PCV < 24) without having trypanosome infection. Some animals 2.3% (12/519) were infected by trypanosome but their PCV was found normal (Table 3).

Prevalence of Trypanosomosis according to Age, Sex, sites and Body Condition

The highest trypanosomosis prevalence 333 (64.16%) was recorded in 2-5 years old animals whilst the lowest prevalence 58(11.17%) was in animals ≤ 2 years old. Slightly higher prevalence was registered in females19 (6.10 %) than in males10 (4.80 %). Trypanosomosis was recorded across the study sites with the highest (10.25%) prevalence in Mender-12 and the lowest (4.05% in Mender-29. Trypanosomosis prevalence was statistically non-significant between age categories, sex groups and across study sites. The highest prevalence (8.71%) was found in poor body condition animals while the least (3.31%) 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.

Entomological Findings

A total of 757 tsetse and biting flies were caught during the study period from different site. Out of the total, 523 (69.09%) were belonging to tsetse of the species *Glossina tachinoides*, followed by 168 (22.19%) stomoxys, 43 (5.68%) tabanus and 23 (3.04%) haematopota. Only *G. tachinoides* were identified in the survey site with the overall apparent density of 5.03 F/T/D (fly/trap/day). The highest fly density 313 (22.36 F/T/D) were observed in Mender-29 and the lowest (0.5 F/T/D) recorded in Mender-12 (Table 5).

Table 1. The prevalence of single and mixed infection of trypanosomes in Pawe district

Trypanosomes	No. positive	Prevalence (%)	X ²	(p-value)
<i>T. congolense</i>	22	75.86	254.04	0.000
<i>T. vivax</i>	7	24.14		
Total	29	100		

Table 2. Mean PCV comparison between infected and uninfected animals

Status	Frequency	Mean PCV (%)	SDs	Overall PCV	X ²	p-value
Infected	29	22.79	4.51	661	4.69	0.030
Uninfected	490	25.81	5.53	12,647		
Total	519	25.64	5.61	13,308		

Table 3. Proportion of anemia in infected and uninfected cattle population

Status	Anemia	Frequency	Percent	Percent share per strata
Infected	anemic	17	3.28	58.6
	non-anemic	12	2.3	41.38
Uninfected	anemic	165	31.8	33.67
	non-anemic	325	62.6	66.33

Table 4. prevalence of bovine trypanosomosis and its association with various risk factors in Pawe district

Risk factors	No. examined	No. positive	Prevalence (%)	p-value	χ^2
Sites					
Hidase pawe	145	6	4.14	0.52	4.21
Abay Ber pawe	101	5	4.95		
Mender-11	68	4	5.88		
Mender-12	78	8	10.25		
Mender-28	53	3	5.66		
Mender-29	74	3	4.05		
Total	519	29	5.58		
sex					
Male	208	10	4.80	0.53	0.400
Female	31	19	6.10		
Total	519	29	5.58		
Age(years)					
≤ 2	58	2	3.45	0.39	1.84
2 - 5	333	22	6.61		
> 5	128	5	3.91		
Total	519	29	5.58		
Body conditions					
Good	151	5	3.31	0.05	5.88
Medium	173	7	4.04		
Poor	195	17	8.71		
Total	519	29	5.58		

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

Sites	Total flies caught	No. of traps	Tsetse flies caught					Biting flies		
			Number	species	M	F	*F/T/D	Stomoxys	tabanid	Haematopota
Hidase pawe	172	14	109	GT	26	83	3.89	39	21	3
Abay Ber pawe	49	10	7		3	4	0.35	23	6	13
Mender-11	107	7	69		18	51	4.93	24	9	5
Mender-12	7	7	2		1	1	0.14	3	0	2
Mender-28	109	7	71		31	40	5.07	34	4	0
Mender-29	313	7	265		86	179	18.93	45	3	0
Total	757	52	523		165	358	5.03	168	43	23

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

4. Discussions

The present study revealed an overall prevalence of 29/519 (5.58%) in the study area. This finding was in agreement with earlier works of Lelisa *et al.* (2015) who reported 5.43% prevalence in the neighboring Mandura district; Tilahun *et al.* (2014) studied the prevalence of cattle trypanosomosis, its vector density and distribution in Dale Sadi District, Kellem Wollega Zone and reported a prevalence of 6.34%.

The study showed that the infection was predominantly caused by *T. congolense* 22/29 (75.86%) followed by *T. vivax* 7/29(24.14%). This result was in consistent with prior reports of Mulaw, *et al.* (2011) who studied prevalence of major trypanosomes affecting cattle in the neighboring Asossa district of Benishangul Gumuz Regional State, Western Ethiopia and found *T. congolense* proportional prevalence of 66. 7%; Zacharias and

Zeryehun, (2012) worked on the prevalence of bovine trypanosomosis in selected district of Arba Minch, Southern Ethiopia and reported *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.

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 (Langridge, 1976; 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 (Stephen, 1986).

The prevalence of bovine trypanosomosis was studied between sex categories, age groups and body conditions, though; significant association was not observed ($\chi^2 > 0.05$). 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; Teka, *et al.*, 2012; Ayele, *et al.*, 2012; Lelisa, *et al.*, 2015).

The overall anemia prevalence in the studied district was 35.06% (182/519). The anemia prevalence was significantly higher in trypanosome infected cattle (58.6%) than in non-infected cattle (33.67%) ($\chi^2 < 0.05$). This is in concordance with previous results from different researchers (Mihret and Mamo, 2007; Bekele and Nasir, 2011; Biyazen, *et al.*, 2014). Out of 35.06% anemia prevalence, 3.28% (17/519) was trypanosome infected animals. Nonetheless, 31.8% (165/519) 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 (Bossche and Rowlands, 2001).

This study revealed that 2.3% (12/519) 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 (Bossche and Rowlands, 2001), 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 (Bossche and Rowlands, 2001).

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 (Ali and Bitew, 2011; Mulaw, *et al.*, 2011; Bayisa, *et al.*, 2015).

Glossina tachinoides was the only tsetse fly caught and its mean apparent density measured as f/t/d was 5.03. It accounts for 523 (69.09%) out of the total flies caught. In addition, other mechanical transmitters of trypanosomosis such as stomoxys 168 (22.19%), tabanus 43 (5.68%) and haematopota 23(3.04%) 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 was *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* was the only tsetse fly species discovered in this study. Other mechanical transmitters of trypanosomosis such as stomoxys, tabanus and haematopota were recorded in the area. In wrapping up, trypanosomosis is an economically important disease threatening the health and productivity of cattle in Pawe district. Therefore, proper strategies have to be designed and implemented to minimize its effect on livestock production in the studied district.

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Corresponding Author:

Dr. Getachew Dinede
Epidemiology Directorate
Ministry of Livestock and Fisheries
Addis Ababa, Ethiopia
Telephone: +251116676953
Email: dinedegech@gmail.com

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