

## Epidemiology of Cattle Trypanosomosis and Associated Anaemia in Mandura District

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**Abstract:** This study was conducted in Mandura district of Benishangul Gumuz Regional State, Western Ethiopia between May and June, 2015 to determine trypanosomosis status, anemia association with trypanosomosis, trypanosomes species and to identify associated risks. Dark phase contrast buffy coat procedures were used for determining prevalence. Whereas, haematocrit method was used for packed cell volume (PCV) values determination. Furthermore, traps were deployed for the purpose of entomological survey. Of the total animals diagnosed 52/391(13.3%) were trypanosomes positive. *Trypanosoma vivax* 48/52(92.30%) and *Trypanosoma congolense* 2/52(3.85%) were detected with their mixed infections 2/52(3.85%). Trypanosomes infection rate was statistically significant ( $P<0.0001$ ). Mean packed cell volume (PCV) value of parasitaemic animals was lower ( $20.94\% \pm 3.02$ ) than that of aparasitaemic animals ( $24.62\% \pm 1.89$ ) and the variation was statistically significant ( $P<0.002$ ). Among the examined animals, 44.24% (173/391) were found anaemic. Anaemia distribution was significantly higher (63.5%) in infected cattle than in non-infected (41.29%). Study sites ( $p<0.0001$ ) and age categories were demonstrated significant risk factors, however, sex groups and body conditions were found non-significant ( $P> 0.05$ ). During the survey, *Glossina tachinoides* was found in the area (0.91 f/t/d) along with other mechanical vectors such as stomoxys (6.72 f/t/d), haematopota (5.32 f/t/d) and tabanus (0.08 f/t/d). To summarize, the current study showed high trypanosomosis prevalence in the area reflecting the need for strategic control measures.

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**Key words:** Anaemia; Mandura; PCV; Risk factor; Trypanosomosis; Tsetse fly

### Introduction

Trypanosomosis is a complex disease caused by unicellular parasites called trypanosomes which harbor in the blood and other tissues of vertebrates including livestock, wild life and people Radostitis *et al.*, 2007). The disease can be expressed as chronic or acute which could cause sudden death in susceptible hosts if left untreated. Its distinguishing factors include intermittent fever, progressive anaemia, and emaciation (Connor and Bossche, 2004).

Different trypanosomes of veterinary importance were reported in Ethiopia in various hosts. Langridge (1976) reported trypanosomes in cattle, sheep and goats comprising of *Trypanosoma congolense*, *Trypanosoma vivax* and *Trypanosoma brucei*, and *Trypanosoma evansi* in camels. The findings of Dagnachew, *et al.* (1981) also showed *Trypanosoma equiperdium* in horses. Prior research works indicated that trypanosomosis is transmitted by tsetse flies and other biting flies such as stomoxys, *tabanus*, *Haematopota* and *chrysops* (Leak, 1999)

Trypanosomosis is a main limitation to agricultural production impeding the national economy in Ethiopia (Langridge, 1976). Swallow (1998) showed that trypanosomosis increases calf mortality and calving intervals; however, it reduces milk production,

off-take and draught efficiency. In addition to its direct adverse effects on affected cattle, it can also preclude rearing of animals in endemic areas leaving large amount of arable lands uncultivated (Awoke, 2000). In Ethiopia, an area stretching between 33°and 38°E longitude, and 5°and 12°N latitude which is about 220,000 km<sup>2</sup> in the Western, South-western and Southern regions is tsetse infested making agricultural production difficult (NTTICC, 1996).

Despite its wide-range economical significance on agricultural activity, very limited research works had been conducted on it in the study area. A baseline data on the epidemiology of the disease is very important to draw effective control measures to alleviate its negative consequences. Hence, the objectives of the present study were to determine trypanosomosis prevalence and its contribution to anemia, and to determine the vector density in the study area.

### 2. Materials and Methods

#### The Study Area

The study was conducted in Mandura district of Metekele zone, Benishangul Gumuz Regional State. Three kebeles hereafter called sites namely: Esitsa, Adida and Mandura town were covered during the study. It is located at the edge of the Blue Nile Valley

between 110°09'18.3"N and 036°19'55.8"E with altitudinal range of 1000-1400 metres above sea level. It has 28°C and 1000-1600mm annual average temperature and rainfall range respectively (NMSA, 2007). Its community relies largely on mixed farming system having livestock population of 35275 cattle, 11580 sheep, 20580 goats, 1046 equines, 15389 poultry and 5044 beehives (CSA, 2014).

**Study Animals:** During the study, the local zebu cattle (*Bos indicus*) of all ages, sexes and body conditions were sampled at their communal grazing area during day time.

#### **Study Design and Sampling techniques**

Cross-sectional study was used with purposive selection of the study sites. Following this, the cattle were randomly sampled. Body condition of the sampled cattle was scored as good, medium and poor (Nicholson and Butterworth, 1986) with simultaneous age classification into young ( $\leq 2$  years old) and adult ( $> 2$  years old) (De-Lahunta and Habel, 1986). The sample size was determined based on the expected prevalence of 50%, confidence level of 95% and 5% desired absolute precision, and computed according to Thrusfield (2007) principles. As result, a total of 384 cattle were calculated but increased to (n=391) to increase precision.

#### **Study Procedures**

##### **Packed cell volume (PCV) determination**

Blood samples were directly collected into a pair of heparinised capillary tubes from a marginal ear vein after puncturing with a lancet. The tubes were then sealed at one end with crystal seal. Subsequently, they were loaded symmetrically to ensure good balance in microhaematocrit centrifuge with sealed end outermost. Following this, the rotary cover was screwed and the centrifuge lid was closed to centrifuge the specimens at 12,000 rpm for 5 minutes. After centrifugation, the capillary tubes were placed in a haematocrit reader and the length of the packed red blood cells column was read to determine PCV. Animals having PCV less than 24% were classified anaemic (OIE, 2008).

##### **Buffy coat technique**

The centrifuged 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. Then, the content of the capillary tube was dropped onto a glass slide, and covered with cover slip. Finally, the slide was examined under x40 objective and x10 eye piece for movement of parasite (Paris *et al.*, 1982). Trypanosome species were identified depending on their morphological features as well as movement in wet film preparations (OIE, 2008).

##### **Fly survey**

A total of 11 traps including 5 monopyramidals, 4 monoconical and 2 biconical were deployed. Acetone

and cow-urine were used as fly attractants. The traps pole was smeared with grease to avoid ants climb. The traps were deployed for 48 hours and then the caught flies were collected and sorted depending on species and sex. Morphological characteristics such as colour, size, proboscis and wing venation were employed as distinguishing features for flies (Fischer and Say, 1989). Enlarged hypophageum was utilized to differentiate between male and female tsetse flies.

#### **Data Analysis**

All data were fed into Microsoft excel spread sheets with subsequent transfer to Intercool STATA version 10.0. Trypanosomosis prevalence was worked out as the proportion of positive animals to the total number of animals diagnosed at a particular time. Association between variables were analyzed using Pearson's chi-square ( $\chi^2$ ). During statistical analysis, a confidence level of 95% was used and P-value of less than 0.05 (at 5% level of significance) was considered statistically significant.

### **3. Results**

#### **Trypanosomes distribution**

In this study, 52/391(13.3%) cattle were infected with different species of trypanosomes. Amongst the total cattle examined, 12.3% were infected with *T. vivax*, 0.5 % *T. congolense*, and 0.5 % was mixed infections of *T. congolense* and *T. vivax*. *Trypanosoma vivax* was the most prevalent trypanosomes species in the study area. The relative prevalence of trypanosome species showed 48/52(92.3%) *T. Vivax* and 2/52(3.85%) *T. congolense*. Mixed infections of *T. congolense* and *T. Vivax* was also encountered accounting for 2/52(3.5%) as indicated below ( Table 1). The infection rate difference between the trypanosome species was statistically significant ( $P<0.05$ ).

#### **Packed cell volume (PCV) and Anaemia status**

It was demonstrated that parasitaemic animals had lower PCV (20.94%±3.02) than that of aparasitaemic animals (24.62%±1.89). The variation was statistically significant ( $\chi^2=9.461$ ;  $P=0.002$ ) as depicted in table 2. Of the total cattle examined, 137/391(44.2%) were found anaemic. The anaemic distribution was higher in infected cattle 63.46% than in the non-infected ones (41.29%) as indicated in table 3.

#### **Identified risk factors**

Trypanosomosis was found across the study sites and varies significantly. The highest (26.35%) prevalence was recorded in Esitsa whilst the lowest (4.72%) in Mandura town. It was also revealed that the infection rate of trypanosomosis was higher (15.2%) in males than in females (12.41%), though, the difference was not statistically significant. The infection rate of trypanosomes was slightly higher (13.5%) in young animals ( $\leq 2$  years of age) than (13.08%) in older

animals (> 2 years of age). However, the variation was not statistically significant. The highest (16.78%) prevalence was registered in animals with good body conditions. Nonetheless, it was not observed statistically significant. The significance of age, sex, body condition and study sites are summarized in table 4 below.

During the survey, a total of 287 tsetse and biting flies were captured. The survey result showed that stomoxys, haematopota, *Glossina tachinoides* and tabanus were prevalent in the area with proportions of 148(51.56%), 117(40.76%), 20 (6.96%) and 2(0.69%) respectively. The overall apparent density (fly/trap/day) of *G.tachinoides* was 0.91 as shown in table 5.

**Entomological Findings**

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

Trypanosomes species	No. positive	Prevalence (%)	p-value	$\chi^2$
<i>T. congolense</i>	2	3.85	0.000	200.05
<i>T. vivax</i>	48	92.30		
Mixed infections	2	3.85		

Table 2: Mean PCV in infected and uninfected cattle in Mandura district

Status	No. examined	Mean PCV (%)	p-value	$\chi^2$
Infected	52	20.94±3.51	0.002	9.461
Uninfected	339	24.14±5.53		

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

Status	Anemia	Frequency	Percent	Percent share per strata
Infected	anemic	33	8.44	63.46
	non-anemic	19	4.86	36.54
Uninfected	anemic	140	35.81	41.29
	non-anemic	199	50.89	58.70

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

Variables	No. examined	No. positive	Prevalence (%)	p-value	$\chi^2$
<b>Age, years</b>				0.014	0.905
<2	200	27	13.5		
>2	191	25	13.08		
<b>Total</b>	<b>391</b>	<b>52</b>	<b>13.3</b>		
<b>Sex</b>				0.448	0.574
Male	125	19	15.2		
Female	126	33	12.41		
<b>Total</b>	<b>391</b>	<b>52</b>	<b>13.3</b>		
<b>Body condition</b>				0.138	3.963
Good	143	24	16.78		
Medium	136	12	8.82		
Poor	112	16	14.28		
<b>Total</b>	<b>391</b>	<b>52</b>	<b>13.3</b>		
<b>Sites</b>				0.0001	26.359
Mandura town	127	6	4.72		
Esitsa	135	34	26.35		
Adida	129	12	9.30		
<b>Total</b>	<b>391</b>	<b>52</b>	<b>13.3</b>		

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

Sites	Total flies caught	No. of traps	Tsetse flies caught				Biting flies			
			Number	species	M	F	*F/T/D	Stomoxys	tabanid	Haematopota
Mandura town	106	5	4	GT	1	3	0.4	50	1	51
Esitsa	107	3	14		5	9	2.33	53	0	40
Adida	74	3	2		0	2	0.33	45	1	26
Total	287	11	20		6	14	0.91	148	2	117

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

#### 4. Discussions

Overall, 13.3% of the examined cattle had trypanosomosis infections. This finding was in consistent with previous research results in Ethiopia. Trypanosomosis prevalence of 12% was reported by Shimels *et al.*, (2005) during his study on epidemiology of tsetse transmitted trypanosomosis in Dembecha and Jabitehenan of Abay (Blue Nile) basin of Northwest Ethiopia. In addition, 12.41% prevalence was shown by Mekuria and Gadisa, (2011) while conducting survey on bovine trypanosomosis and its vector density in Metekele and Awi zones of Northwest Ethiopia. The agreement of these works might be attributed to the similarities of the study areas in their ecological set up such as altitude, ambient temperature, vegetation cover and vector abundance. However, Lelisa *et al.* (2015) reported significantly lower prevalence of 5.43% in Mandura district, the study area. This could possibly be because of the reduction in the fly population subsequent to the integrated control measures conducted in the district from 2012 to 2014. The higher prevalence in the present study might be for the re-invasion of the area by biting flies.

In this study, the majority of trypanosomosis infection was due to *Trypanosoma vivax*. The relative prevalence of trypanosome species showed 48/52(92.3%) *T. Vivax* and 2/52(3.85%) *T. congolense*. Mixed infections of *T. congolense* and *T. Vivax* was also encountered accounting for 2/52(3.5%). This result was in agreement with earlier works of Bishaw, *et al.* (2012) who reported 80% *T. vivax* for tsetse infested areas of West Gojjam zone, Northwest Ethiopia. Lelisa *et al.*(2015) reported *T.vivax* proportional prevalence of 81.82% in the study area, Mandura district of western Ethiopia. The dominance of *T. vivax* could most likely be attributed to the presence of major mechanical vectors and more efficient transmitters of *T. vivax*.

The current study revealed an overall mean ( $\bar{x}$ ) PCV value of 24.14%. The PCV value of the infected animals was statistically significantly ( $\chi^2 = 9.461$ ;  $P=0.002$ ) lower (20.94%  $\pm 3.51$ ) than that of uninfected animals (24.62%  $\pm 4.43$ ). This result was consistent with earlier reports (Bitaw *et al.*, 2011; Mulaw *et al.*, 2011; Bayisa, *et al.*, 2015).

Of the total cattle examined, 173/391(44.25%) were found anaemic. Amongst these anaemic cattle only 8.44% of them were from infected group, and 35.81% were from non-infected category. However, anaemia distribution was higher (63.46%) in infected cattle population than in the non-infected ones (41.29%). The fact that infected animals were found non-anaemic might be due to their ability to manage their PCV in normal range or because of recent infection which is not yet progressed to lower PCV. It

is well documented that anaemia is the best indicators of trypanosomosis (Stephen, 1986); however, this study indicated that a large proportion of non-infected animals were found anaemic. This might be attributed to their recent recovery from the disease. It could also be for the inadequate sensitivity of buffy coat examination techniques which consider animals uninfected while they actually have the parasite (Murray, *et al.*, 1977). Furthermore, Bossche and Rowlands (2001) showed that other diseases such as fasciolosis, gastro-intestinal parasitism and malnutrition could induce anaemia.

In this study, animal parameters like age categories, sex groups and body conditions were not observed significant for susceptibility of animals to trypanosomosis. These findings were lining up with earlier works (Bacha *et al.*, 2013; Ayele *et al.*, 2015; Lelisa, *et al.*, 2015; Regasa, *et al.*, 2015). The fact that trypanosomosis do not depend on gender could possibly be hypothesised that both male and female animals have virtually equal chance of being in contact with flies and ultimately developing the disease.

This survey revealed the highest (26.35%) trypanosomosis prevalence in Esitsa and the lowest (4.72%) in Mandura town. The variation was statistically significant. This might be attributed to the relative ecological pattern variation such as microclimate of the sites, distance between herds, animal herd density, and other factors which, in turn, influences tsetse fly and/or other biting flies' population and type present in each study sites (Sinshaw, *et al.*, 2006). Similarly, Bayisa *et al* (2015) indicated the significant variation of trypanosomosis prevalence among the study sites in Asossa district.

During the survey, *Glossina tachinoides* was found in the area (0.91 f/t/d) along with other mechanical vectors such as stomoxys (6.72 f/t/d), haematopota (5.32 f/t/d) and tabanus (0.08 f/t/d). These results were in agreement with previous works of Tilahun *et al.* (2014) who reported *G.tachnoides* with apparent density of 0.11 fly/trap/day, and he also indicated other findings such as 0.05, 2.42, 3.89, 1.29 fly/trap/day for *Glossina morsitance sub-morsitans*, *Glossina pallidipes*, Stomoxys and Tabanus respectively.

#### 5. Conclusions

The present study indicated that *T.vivax* was the predominant trypanosome species to cause trypanosomosis in the area. Also, it was revealed that trypanosomosis causes anaemia in cattle lowering the PCV values. Moreover, animal level parameters like sex categories, age groups and body condition were not found to be associated risks. Further, it was showed that trypanosomosis is a prevailing disease

and a potential threat that adversely affects livestock industry. Therefore, appropriate control measures have to be designed to lessen the undesirable impact of the disease in the studied area.

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