Survey On Bovine Trypanosomosis In Mandura District Of Benishangul Gumuz Regional State, Western Ethiopia: Prevalence And Associated Risk Factors

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Abstract: This study was conducted in Mandura district of Benishangul Gumuz Regional State, Western Ethiopia between January and April, 2017 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 101/384 (26.30%) were trypanosomes positive. *Trypanosoma congolense* 88/101 (87.13%), *Trypanosoma vivax* 6/101(5.94%), *Trypanosoma brucei* 2/101(1.98%) were detected with their mixed infections 5/101(4.95%). Trypanosomes infection rate was statistically significant (P<0.0001). Mean packed cell volume (PCV) value of parasitaemic animals was lower (22.01% +3.81) than that of aparasitaemic animals (27.03% + 0.65) and the variation was statistically significant (P<0.0001). Among the examined animals, 45.83% (176/384) were found anaemic. Anaemia distribution was significantly higher 31.25% in infected cattle than in non-infected 14.58%. Study sites (p>0.05) and age categories were demonstrated significant risk factors, however, sex groups were found non- significant (P> 0.05). But body conditions has significant difference (P<0.003). During the survey, *Glossina tachinoides* was found in the area (5.64 f/t/d) along with other mechanical vectors such as stomoxys (4.24 f/t/d), haematopota 0.72 f/t/d) and tabanid (1.06 f/t/d). To summarize, the present 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

1. Introduction

In Ethiopia, tsetse transmitted animal trypanosomosis is a serious constraint to livestock production and agricultural development (Abebe, 2005).

Tsetse and trypanosomosis have kept farmers and livestock Keepers out of areas having very high potential for agricultural development by various species of trypanosomes is not only limited to inflicting diseases but also leading to significant negative impact on natural resource conservation and its sustainable utilization (Juyal *et al*., 2005).

This is especially true when one considers losses due to mortality and morbidity in domestic animals, cost of livestock treatment and the cost of tsetse control, inaccessibility to sufficient animal draught power and forcing the farming community to make their living on highly degraded highlands of the country (NTTICC, 1996; Awoke, 2000). Various species of trypanosomes are also held responsible for causing immunosppression resulting into poor immune response to bacterial and viral antigens (Singla *et al.,* 2010).

Ethiopia lies in the Eastern African tsetse belt and about 200,000 Km2 its area is believed to be infested by tsetse flies and new areas are becoming infested due to the constant advancement of tsetse flies (NTTICC, 1996). These areas are primarily situated along the larger river valleys in the country comprising Abay basin, Baro/Akobo, Omo/Ghibe and Didessa rivers whereby abundant rainfall is present with fertile soils, though, the arable land remains uncultivated throughout the year for tsetse and trypanosomosis impact in. In this area, an estimated total of 14.8 million cattle, 6.12 million sheep and goats, 1 million camels and 1.23 million equines are at risk of contracting trypanosomosis. These areas follow the Baro/Akobo, Omo/Ghibe and Abay/Didessa valleys of the large rivers in the country (NTTICC, 1996).

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. In line with this, various fragmented surveillance activities have been witnessed during the past. These, however, were limited to certain geographical areas and did not cover much of potentially risk areas of western region of the

country. Indeed for the effective way of controlling tsetse-transmitted trypanosomosis, knowledge of insect biology and ecology, and the status of the disease prevalence is of paramount importance (Leak, 1999).

Benishangul-Gumuz regional state pertains to the area of Northwest part of the country and nearly 31,000 km2 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 published baseline data in the mandura district. The aims of the present study were, therefore, to assess the epidemiology of trypanosomosis and its 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. Five kebeles hereafter called sites namely: Mandura town, Dihanzibagune, Esitsa, Ejenta and Adida were covered during the study. It is located at the edge of the Blue Nile Valley between 11^0 09'18.3"N and $036^0$19' 55.8"E with altitudinal range of 1000-1400 meters above sea level. It has 28^0c 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, 2016).

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 (≤3 years old), (4-7 years old) and adult (> 7years old) (De-Lahunta and Habel, 1986).

The sample size was determined based on the prevoius prevalence of 13 %, confidence level of 95% and 5% desired absolute precision, and computed according to Thrusfield (2007) principles. As result, a total of 174 cattle were calculated but increased to (n=384) to increase precision.

Study protocol

Measuring PCV

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).

Dark contrast 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) 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.

Fly survey

During the study period, three types of traps were used comprising of 14 monopyramidal, 23 monoconical and 16 biconical. They were deployed at 200-250 meters 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 was entered into Microsoft excel spread sheet. Then, it was processed and analyzed using a STATA version 11.0. Trypanosomosis association with the variables was computed using Pearson's chi-square ($\chi 2$). Mean PCV values of uninfected and infected animals were compared using ANOVA. Prevalence was calculated as the percentage of infected animals as indicated by dark contrast buffy coat procedures out of the total animals examined. Considering a confidence level of 95%, P-value of less than 0.05 at 5% level of significance was considered as statistically significant.

3. Results

Trypanosomes prevalence

In this cross - sectional study, 101/384(26.30%) cattle were infected with different species of trypanosomes. Amongst the total cattle examined, 22.92 % were infected with *T. congolense, 1.56 % T. vivax*, 0.52 *T. brucei* and 0.78 % was mixed infections of *T. congolense and T. vivax and 0.52 % T. congolense and T. brucei. Trypanosoma congolense* was the most prevalent trypanosomes species in the study area. The relative prevalence of trypanosome species showed 88/101(87.2%) *T. congolense, 6/101(5.94 %) T. vivax, and* 2/101(1.98%) *T. brucei.* mixed infections of *T. congolense and T. vivax* was also encountered accounting for 3/101(2.97%), and 2/101 (1.98 %) for *T. congolense* and *T.brucei*, as indicated below (Table 1). The infection rate difference between the trypanosome species was statistically significant (P<0.000, df=1; $\chi 2$=382.00).

Packed cell volume (PCV) and Anaemia status

The study indicated an overall mean PCV value of 29.4 ± 3.43 SE. It was demonstrated that parasitaemic animals had lower PCV (22.01 %+3.81 SE) than that of aparasitaemic animals (27.03 %+0.65 SE). The variation was statistically significant ($\chi 2$ =164.75; P=0.000, df=1) as depicted in table 2. The overall anemia prevalence in the studied district was 45.83% (176/384). The anemia prevalence was significantly higher in trypanosome infected cattle 120/384 (31.25%) than in non-infected cattle 56/384 (14.58%) (p < 0.000; $\chi 2$=164.75, df=1) (Table 3).

Trypanosomosis associated with risk factors

Trypanosomosis was found across the study sites and varies significantly. The highest (36.84%) prevalence was recorded in Dihazibagune whilst the lowest (19.48 %) in Mandura town. It was also revealed that the infection rate of trypanosomosis was higher (29.45 %) in males than in females (24.47 %), though, the difference was not statistically significant. The infection rate of trypanosomes was slightly higher (33.33 %) in older animals (>7 years of age) than (26.75 %) in matured animals (4-7 years of age). However, the variation was not statistically significant (P>0.05). The highest (37.86 %) prevalence was registered in animals with poor body conditions and the least prevalent was observed in medium body conditions (18.79 %). However, it was observed statistically significant (p<0.003). The significance of age, sex, body condition and study sites are summarized in table 4 below.

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

Trypanosomes	**No. positive**	**Prevalence (%)**	$\chi 2$	**p-value**
T. congolense	88	87.13	382.00	0.0001
T. vivax	6	5.94		
T. brucei	2	1.98		
Mixed (*T. congolense* & *T. vivax*)	3	2.97		
Mixed *(T. congolense* & *T. brucei*)	2	1.98		
Total	**101**	**100**		

(chi2=382.00, p=0.0001, df=4)

Table 2: Mean PCV comparison between parasitaemic and aparasitaemic animals

Status	**Frequency**	**Mean PCV (%)**	SE	$\chi 2$	**p- value**
Infected	146	22.01	3.81	164.75	0.0001
Non infected	238	27.03	0.65		
Total	**384**	**29.43**	3.43		

(Chi2=164.75, df=1, pr=0.0001)

Table 3: Proportion of anemia in parasitaemic and aparasitaemic cattle population

Status	Anemia	Frequency	Percent	Percent Share Per Strata
Infected	Anemic	120	31.25	82.19
	non anemic	26	6.77	17.80
Non infected	Anemic	56	14.58	23.52
	Non anemic	182	47.39	76.47

Table 4: Prevalence of bovine trypanosomosis and its association with various risk factors in mandura district

Risk factors	No. examined	No. positive	Prevalence (%)	$\chi 2$	df	P-value
Sites						
Mandura town	77	15	19.48			
Dihazibagune	76	28	36.84			
Esitsa	91	20	21.97	7.17	4	0.13
Ejenta	90	25	27.77			
Adida	50	13	26			
Total	384	101	26.30			
Sex						
Male	146	43	29.45	1.15	1	0.28
Female	237	58	24.47			
Total	384	101	26.30			
Age(years)						
≤ 3	130	27	20.76			
4-7	157	42	26.75	4.50	2	0.105
> 7	96	32	33.33			
Total	384	101	26.30			
Body conditions						
Good	132	34	25.75			
Medium	149	28	18.79	11.82	2	0.003
Poor	103	39	37.86			
Total	384	101	26.30			

Entomological survey result

Overall, 1236 flies were captured during the study period from different sites. Tsetse flies account for 598 (48.4 %) of the total whereas other biting flies covers 51.62 % comprising of 450 (36.40%) stomoxys, 112 (9.06 %) tabanus and 76 (6.14%) haematopota. Of the 598 tsetse flies captured, 56.18 % were females. Only *G. tachinoides* were identified in the survey site with the overall apparent density of 5.64 (fly/trap/day). The highest fly density were observed in Adida 15.05 F/T/D and the lowest recorded in Dihanzibaguna 7.8 F/T/D (Table 5).

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

Sites	Total flies caught	No. of traps	Tsetse flies caught					Biting flies		
			No.	Species	M	F	*F/T/D	Stomoxys	Tabanid	Haematopota
Mandura town	163	10	84	GT	32	52	4.2	59	12	8
Dihanzibaguna	156	10	66		27	39	3.3	55	23	12
Esitsa	274	11	154		66	88	7.0	78	23	19
Ejenta	342	12	159		76	83	6.63	134	29	20
Adida	301	10	135		61	74	6.75	124	25	17
Total	**1236**	**53**	**598**		**262**	**336**	**5.64**	**450**	**112**	**76**

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

4. Discussion

This study showed an overall cattle trypanosomosis of 26.30%. Comparable research works were reported in various parts of Ethiopia, for example, Shimelis *et al.* (2011) revealed Cattle trypanosomosis prevalence of 28.1 % in Assosa district of Benishangul Gumuz region, Western Ethiopia. In addition, Bayisa *et al.* (2015) indicated a prevalence of 22.8 % during his research activity on prevalence of bovine trypanosomosis and apparent density of tsetse and other biting flies in Assosa district. And the difference in the disease distribution was due to the difference in climatic conditions of the areas and seasonal variation. Also, 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.

In this research, the majority of trypanosomosis infection was due to *Trypanosoma congolense*. The relative prevalence of trypanosome species showed 88/101 (87.13%) *T. congolense, 6/101(5.94%) T. vivax, and 2/101(1.98%) T.brucei.* Mixed infections of *T. congolense and T. vivax* was 3/101 (1.98%) and also *T. congolense* and *T.brucei* (1.98%). This result was in agreement with earlier works of Aki A *et al*., (2016) demonstrated *T. congolense* proportional prevalence of 75.86% and proportional prevalence *trypanosome vivax* of 24.14% during his research on cattle trypanosomosis in Pawe district, Benishangul Gumuz Regional State, Western Ethiopia; (Bayisa K *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 current study revealed an overall mean (ẍ) PCV value of 29.43% + 3.43. The PCV value of the infected animals was statistically significantly ($\chi 2$ =164.75; P=0.000) lower (22.01% +3.81) than that of uninfected animals (27.03 % + 0.65). This result was consistent with earlier reports (Bitaw *et al*., 2011; Mulaw *et al*., 2011; Bayisa, *et al*., 2015).

The overall anemia prevalence in the studied district was 176/384 (45.83). However, anaemia distribution was higher 120/384 (31.25%) in infected cattle population than in the non-infected ones 56/384 (14.58%). 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, and sex groups were not observed significant for susceptibility of animals to trypanosomosis. These findings were lining up with earlier works (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. Body conditions has significant difference for cattle trypanosomosis ($P<0.003$, $\chi 2=11.82$, df=2).

This survey revealed the highest (36.84 %) trypanosomosis prevalence in Dihazibagune and the lowest (19.48 %) in Mandura town. The variation was not statistically significant ($p>0.05$; $\chi 2= 7.17$, df=4). 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.

In entomological survey, overall, 1236 flies were captured during the study period from different sites. Tsetse flies account for 598 (48.4 %) of the total whereas other biting flies covers 51.62 % comprising of 450 (36.40%) stomoxys, 112(9.06 %) tabanus and 76 (6.14%) haematopota. Of the 598 tsetse flies captured, 56.18 % were females. *Glossina tachinoides* found in the area was (5.64 f/t/d) along with other mechanical vectors such as stomoxys (4.24 f/t/d), haematopota (0.72 f/t/d) and (1.06 f/t/d) tabanid. These results were similar with previous works of Aki A *et al***.** (2015) at Kameshi district of Benishangul Gumuz Regional state, western Ethiopia, who reported *G. tachnoides* with apparent density of 2.68 fly/trap/day, and he also indicated other findings such as 2.84, 1.54, 0.92 fly/trap/day for Stomoxys, Tabanus and haematopota respectively. It was also in agreement with findings of (Aki A and Dinede G, 2015) at Pawe district of Benishangul Gumuz Regional state, western Ethiopia, which was reported to be 5.03 f/t/d, 1.62 f/t/d,0.41 and 0.22 f/t/d for *G. tachinoides, Stomoxys Tabanus* and heamatopota respectively.

5. Conclusions

The present study indicated that *T. congolense* 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 peasant associations, sex categories and age groups were not found to be associated risks ($p>0.05$). However, body conditions has been indicated significant difference ($p<0.003$). 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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References

1. Langridge, W.P. (1976): Tsetse and trypanosomosis survey of Ethiopia. London, UK, Ministry of Overseas Development, p. 1-40.
2. P. van den Bossche and G. J. Rowlands, (2001): "The relationship between the parasitological prevalence of trypanosomal infections in cattle and herd average packed cell volume," *Acta Tropica*, vol. 78, no. 2, pp. 163–170. doi:10.1016/S0001-706X(00)00182-0.
3. Aki A. and Dinede G, (2016): Cattle Trypanosomosis in Pawe District, Benishangul Gumuz Regional State, Western Ethiopia: Prevalence; vector density and Associated Risk Factors, *European Journal of Applied Sciences* 8(3): 60-66, 2016, DOI: 10.7537/marsrsj08031609.
4. Aki A et al., (2015): Epidemiology of bovine Trypanosomosis in Kamashi District of Benishangul Gumuz Regional State, Western Ethiopia: Prevalence, Vector density and associated risks; *Biomedicine and Nursing* 2016; 2(4): 48-54]. http://www.nbmedicine.org. 8. doi:10.7537/marsbnj020416.08.
5. Leak, S.G.A., (1999): Tsetse biology and ecology; their epidemiology and control of Trypanosomosis, CABIpublishing in association with the ILRI, pp: 152-210.
6. Awoke, K., (2000). Study of trypanosomosis and its Vectors in Humbo and Merabo Warheads. Ethiop. Vet. J., 4: 1-61.
7. NTTICC (National Tsetse and Trypanosomosis Investigation and Control Centre), (1996): Annual Report on Tsetse and Trypanosomosis, Survey, Bedele, Ethiopia. Pp.11-15.
8. NMSA (National Meteorological Services Agency), (2007): Monthly report on temperature and Rainfall. Distribution for Asossa Zone, Regional Metrological Office, Asossa, Ethiopia.
9. CSA (Central Statistical Authority), (2016): Agricultural Sample Survey, Statistical Bulletin, Ethiopia, Addis Ababa.
10. Nicholson, M.J. and M.H. Butterworth, (1986): A guide to condition scoring of zebu cattle. ILRI/FAO, Addis. Ababa, Ethiopia.
11. De-Lahunta, A. and R.E. Habel, (1986): Teeth. Applied veterinary Anatomy. USA. W. B. Sounders. Company, pp: 4-16.
12. Thrusfield M (2007): Veterinary Epidemiology, 3rd edition, Blackwell Science Ltd, Oxford, UK.
13. OIE, (2008): "Standardized techniques for the diagnosis of tsetse transmitted trypanosomosis," in *OIE Terrestrial Manual*, p. 49, Rome, Italy.
14. Paris J, Murray M, and Mcodimba, F., (1982): A comparative evaluation of the parasitological technique currently available for the diagnosis of African Trypanosomosis in cattle, Acta Trop., 39: 307-316.
15. Fisher MS, Say R (1989): Manual of Tropical Veterinary Parasitology. UK: CAB International publication. Pp.100-278.
16. Shimelis, D., A. K. Sangwan and A. Getachew, (2005): "Epidemiology of tsetse transmitted trypanosomosis in Abay (Blue Nile) basin of Northwest Ethiopia," *Revue d''Elevage etde M´edecine V´et´erinaire des Pays Tropicaux*, vol. 58, pp. 151–157.
17. Lelisa, K., Damena, D., Kedir, M., and Feyera, T., (2015)**:** Prevalence of Bovine Trypanosomosis and Apparent Density of Tsetse and Other Biting Flies in Mandura District, Northwest Ethiopia, Veterinary Science & Technology, 6:3.
18. Y. Bishaw, W. Temesgen, N. Yideg, S. Alemu, (*2012):* Prevalence of bovine trypanosomosis in Wemberma district of West Gojjam zone, North West, *Ethiop. Vet. J., 16 (2), 41-48.*
19. Bitaw M, Amedie Y, Abebe A, Tolosa T (2011): Prevalence of bovine trypanosomosis in selected areas of Jabi Tehenan district, West Gojam of Amhara regional state, North western Ethiopia. Africa Journal of Agricultural Research, 6**:**140-144.
20. Mulaw, S., M. Addis and A. Fromsa, (2011): Study on the Prevalence of Major Trypanosomes Affecting Bovine in Tsetse Infested Asosa District of Benishangul Gumuz Regional State, Western Ethiopia. *Global Veterinaria* 7 (4): 330-336.
21. Bayisa, K., Getachew, D., Tadele, T. (2015): Bovine Trypanosomosis in Asossa District,

Benishangul Gumuz Regional State, Western Ethiopia: Prevalence and Associated Risk Factors, European Journal of Applied Sciences 7.
22. Abebe, G., (2005): Review article: Trypanosomosis in Ethiopia. Ethiopian Journal of Biomedical Science, 4(1): 75-121.
23. M. Murray, P. K. Murray, and W. I. M. Mclntyre, "An improved technique for the diagnosis of African trypanosomosis," *Transactions of the Royal Society of Tropical Medicine and Hygiene*, vol. 71, no. 4, pp. 325–326, 1977.
24. Bacha, B., Z. Beyene, M. Woyessa and A. Hunde, (2013): Prevalence of Small Ruminants Trypanosomosis in Assosa District of Benishangul Gumuz Regional State, Western Part of Ethiopia. *Acta Parasitologica Globalis* 4:99-104.
25. Ayele T., Ephrem D., Elias K., Tamiru B., Gizaw D., Mebrahtu G., Mebrat E. (2015): Prevalence of Bovine Trypanosomosis and its Vector Density in Daramallo District, South Western Ethiopia. J Vet Adv 2012, 2(6): 266-272.
26. Regasa, T., Bedada, M., Workin, M., Beyera, M., Terefe, S., Kebede, A.,(2015): Study on Spatial Distribution of Tsetse Fly and Prevalence of Bovine Trypanosomosis and Other Risk Factors: Case Study in Bedele Woreda, Ilu Aba Bora Zone, South Western Ethiopia, Acta Parasitologica Globalis 6 (3): 174-181, DOI: 10.5829/idosi.apg.2015.6.3.95194.
27. Sinshaw A, Abebe G, Desquesnes M, Yoni W (2006): Biting flies and Trypanosoma vivaxinfection in three highland districts bordering Lake Tana, Ethiopia. Vet. Parasitology 142: 35-46.
28. L. E. Stephen, (1986): *Trypanosomiasis, A Veterinary Perspective*, Pergamon Press, Oxford, UK.
29. Juyal PD, Singla LD, Kaur P (2005): Management of surra due to Trypanosoma evansi in India: an overview. Infectious diseases of domestic animals and zoonosis in India. 75:109-120.
30. Dinede G, and Aki A (2016): Epidemiology of Cattle Trypanosomosis and Associated Anaemia in Mandura District. Nat. Sci. 14(5):85-90 pdf. http://www.sciencepub.net/nature/ns140516/12_30384nsj140516_85 90.

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