Epidemiological Study of Bovine Trypanosomosis and Associated Risk Factors in Odabildiglu District of the Benishangul Gumuz Regional State, Western Ethiopia

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Abstract: A cross-sectional study was carried out in Odabildiglu district of the Benishangul Gumuz Regional State, western Ethiopia from Sptember to October, 2016 to determine the prevalence of trypanosomosis, prevailing species of trypanosomes, associated risks and its vector density. Blood samples collected from (n=530) randomly sampled cattle (*Bos indicus*) was examined using parasitological (buffy coat technique) and hematological (measurement of packed cell volume) procedures. An overall, 38 (7.17%) prevalence of trypanosomosis was recorded. The infection was caused mainly by *Trypanosoma congolense 35/38 (*92.1%), *Trypanosoma vivax* 2/38 (5.2%) & to less extent by *Trypanosoma brucei* 1/38 (2.6%). The infection rate was statistically significant among the different trypanosome species (P<0.05). Mean packed cell volume (PCV) value of parasitaemic animals was lower (21.23% ± 3.66) than aparasitaemic animals (26.87% ± 2.23) and the variation was statistically significant (P<0.05). Higher prevalence (14.05%) was registered in animals with poor body condition when compared with animals with medium (3.42%) and good (4.23%) body condition and the difference was statistically significant (p<0.05). The infection rate was higher in animals > 2 years (9.25%) when compared with animals < 2 years (1.42%) and the variation was statistically significant (P<0.05). While prevalence of trypanosomosis was not statistically significant across study sites (p>0.05). *Glossina* morsitans submorsitans was the only tsetse fly species caught and its mean apparent density measured as f/t/d was 0.3375. In addition, other mechanical vectors such as, stomoxys, tabanids and haematopota with f/t/d of 0.3625, 0.2 and 0.1875 were recorded respectively. To conclude, the result of the present finding shows moderately high prevalence of trypanosomosis in the study sites indicating the need for strategic and integrated approach to control the vector and to minimize the impact of the disease in the study district.
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Key words: Odabildiglu District, PCV, Risk factor, Trypanosome, Trypanosomosis, Tsetse fly

1. Introduction

Trypanosomiasis is a devastating disease of livestock caused by protozoal parasites of the genus trypanosoma that inhabits blood and other tissues of vertebrates including animals, wildlife and human (Adam *et al.,* 2003; Gupta *et al*., 2009; Bal *et al*., 2014). It is a vector borne disease that is transmitted biologically by tsetse flies and mechanically by other biting flies (FAO, 2002; OIE, 2009). It is a major constraint contributing to direct and indirect economic losses to crop and livestock production (Abebe, 2005) and has a significant negative impact on economic growth in many parts of the world (Taylor *et al*., 2007; Sharma *et al*., 2013), particularly in sub-Saharan Africa (Cecchi *et al*., 2008).

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 and *Trypanosoma equiperdium* in horses (Abebe, 2005). The influence of tsetse on African agriculture through the transmission of trypanosomosis continues to be a major constraint to the development of national economies and their achievement of self sufficiency in basic food production. The general distribution of tsetse flies is determined principally by climate and influenced by altitude, vegetation, and presence of suitable host animals (Leak, 1999). Tsetse flies in Ethiopia are confined to southern and western regions between longitude of 33 ^{0} and 38^0 East and latitude of 5^0 and 12^0 North which amounts to be about 200,000 Km^2. Tsetse infested areas lies in the low lands and also in the river valleys of Blue Nile, Baro Akobo, Didessa, Ghibe and Omo. Benishangul Gumuz is one of the five regions of Ethiopia infested by more than one species of tsetse flies (Keno, 2005). Five species of Glossina (*G. m. submorsitans*, *G. Pallidipes, G. tachnoides, G. f. fuscipes and G. longipennis*) have been registered in Ethiopia (Keno, 2005). In the study region of Benishangul Gumuz regional state, four glossina species namely, *G. tachinoides, G. m.*

submorsitances, G. pallidipes and *G.fuscipes* were identified (ARVDSMSL, 2015). Apart from the cyclical transmission of trypanosomosis by Glossina species, it is highly considered that mechanical transmission is a potential threat to livestock production and productivity in some parts of Ethiopia (Abebe, 2005).

Odabildiglu is one the seven districts of Asossa 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 obstacles of livestock production and productivity, there is no previous 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, to assess associated risk factors and to suggest actions towards the control measure.

2. Materials and Methods

2.1. Study Area:

The study was conducted from September to October, 2016 in Odabildiglu district of Asossa zone, Benishangul Gumuz Regional State, Western Ethiopia. It was carried out in four kebeles hereafter called sites namely: Bildiglu 01 & 2, Tuli, Buchikobe & Daleti. The district has 25 kebeles covering an area of 1518km^2 with human population of 74175. It has an altitude of 815 meter above sea level. Its annual average temperature is 33^{0}c (28-38^{0}c) and its rainfall range is 900-1400 mm (NMSA, 2014). The livelihood of the people in the district largely depends on mixed livestock and crop production having livestock population of 10581 cattle, 1271 sheep, 16711 goats, 3143 equines, and 26317 poultry (CSA, 2014).

2.2. Study Design and Study Animals

Cross sectional study design was used. A local zebu cattle (*Bos indicus*), which are mainly 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). Similarly, their age was determined based on (De-Lahunta and Habel, 1986) principles as < 2 years old, and > 2 years old.

2.3. Sampling Techniques and Sample Size Determination

The study sites were purposively selected as convenient. Study 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 a result a total of 384 cattle were calculated but increased to (n=530) to increase precision and these cattle were sampled at their communal grazing area using simple random sampling.

3. Study Methodology

3.1. 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. PCV was measured in a micro-haematocrit centrifuge (Hermmle 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 samples 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).

3.2. Buffycoattechnique

Heparinised 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 (Murray and Dexter, 1988). Trypanosome species were identified according to their morphological descriptions as well as movement in wet film preparations (OIE, 2008).

3.3. Data Analysis

During the study period, data were collected using the sample collection format and entered into Microsoft Excel. Hematological and parasitological data were managed very carefully. Then, the data from the Microsoft excel sheet were processed and analyzed by using a statistical soft ware program. Chi-square was used to compare the prevalence of trypanosomosis

in different variables and to determine the relationship between variables and the result. Data collected on PCV values were analyzed by ANOVA to compare the mean PCV values of parasitaemic animals against that of aparasitaemic animals. In all cases the difference between parameters were tested for significance at probability level of 0.05 or less. The prevalence of cattle trypanosomosis was calculated as the number of parasitologically positive animals examined by buffy coat method to the total animals examined (Thrusfield, 2007).

4. Result

4.1. Distribution of trypanosomes infection

Out of the total animals examined, 38(7.17%) were infected with trypanosomes. The trypanosome species responsible for the infection were *T. congolense, T. vivax* and *T. brucei*. As indicated in table 1, the proportional prevalence of each species of trypanosome was 35(92.1%) for *T. congolense*, 2*(5.2%)* for *T. vivax* and *1(*2.6%*)* for *T. brucei* as observed in the fresh blood examined during the study period and the proportional prevalence of trypanosome species was found to be statistically significant (P<0.05) (table 1).

The highest and the lowest prevalence of trypanosomosis were recorded in Tuli 17 (9.65%) and Bulidgelu 2(3.33%) study sites respectively. However, there was no statistically significant difference among the study sites (p >0.05) (Table 3).

The Prevalence of trypanosomosis varies in both sexes; the infection in female animals is slightly higher 23/271 (8.48 %) than male 15/259 (5.79%) and the association was not statistically significant (P>0.05) (Table 3).

4.2. Packed Cell Volume

The mean PCV values for all examined animals were 24.05 ± 2.95 SD. However, the mean PCV values for non infected animals were 26.87 ± 2.23 SD and the mean PCV value of the infected animals was 21.23 ± 3.66 SD. There was statistically significant difference in the mean PCV value between non infected and infected animals (P<0.05) (Table 2).

4.3. Trypanosomosis and associated risks

In the present study animals examined were categorized in to different age groups as < 2 years and >2 years. Out of the total sampled animals, 141 and 389 were < 2 years and > 2 years old respectively and the prevalence was found to be 2(1.42 %) and 36(9.25%) for tested animals < 2 years and > 2 years respectively and the difference in the prevalence was statistically significant (p <0.05) (table 3).

Similarly, during the study, animals were categorized in to different body conditions as good, medium and poor. From the total 530 animals examined 118, 234 and 178, were animals with good, medium and poor body condition respectively and out of which 5 (4.23%), 8 (3.42%), and 25 (14.05 %) prevalence of trypanosomosis was recorded for animals with good, medium and poor body condition respectively. Trypanosome infection and body condition scores of study animals were found statistically significant (p <0.05) (Table 3).

4.4. Entomological Survey

The present survey of tsetse flies depicted that Glossina morsitans submorsitans & Glossina pallidipes is the species of tsetse fly responsible for cyclical transmission of trypanosomosis in the study area. Tsetse fly survey was carried out in four kebeles of the study district by deploying a total 40 geo-referenced traps (23 mono-conical, 10 mono-pyramidial and 7 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 is 11, 29, 21 and16 for Bildiglu 01 & 02, Tuli, Buchikobe and Daleti respectively. The mean apparent density of Glossina morsitans submorsitans & Glossina pallidipes in the survey sites was investigated as 1.01and 0.8 f/t/d while the mean apparent density of mechanical vectors such as stomoxys (1.2 f/t/d), tabanids (0.6 f/t/d) and haematopota (0.39 f/t/d) were recorded (table 4).

Table 1: Prevalence of Trypanosomosis in Odabildiglu District

Trypanosomes	**No. positive**	**Prevalence (%)**	**X^2**	**(p-value)**
T. congolense	35	92.1	485.1994	0.000
T. vivax	2	5.2		
T. brucei	1	2.6		
Total	**38**	**100**		

Table 2: Mean PCV Comparison between Parasitaemic and Aparasitaemic Animals in Odabildiglu District

Status	**Frequency**	**Mean PCV (%)**	**SDs**	**Overall PCV**	**X^2**	**p- value**
Infected	38	21.23	3.66	806.90	14.88	0.000
Non infected	492	26.87	2.23	13218.66		
Total	**530**	**24.05**	**2.95**	**12323**		

Table 3: Prevalence of Bovine Trypanosomosis and Its Association with Various Risk Factors in Odabildiglu District

Risk factors	No. examined	No. positive	Prevalence (%)	χ^2	p-value
Sites					
Bildiglu 01 & 02	60	2	3.33	3.1864	0.364
Tuli	176	17	9.65		
Buchi kobe	170	11	6.46		
Daleti	124	8	6.45		
Total	**530**	**38**	**7.17**		
Sex					
Male	259	15	5.79	1.4458	0.229
Female	271	23	8.48		
Total	**530**	**38**	**7.17**		
Age (years)					
< 2	141	2	1.42	9.5506	0.008
> 2	389	36	9.25		
Total	**530**	**38**	**7.17**		
Body conditions					
Good	118	5	4.23	19.1124	0.000
Medium	234	8	3.42		
Poor	178	25	14.05		
Total	**530**	**38**	**7.17**		

Table 4: Flies Caught in Different Areas of Survey Sites of Odabildiglu District

Sites	Total flies caught	No. of traps	Tsetse flies caught					Biting flies		
			No.	Species	M	F	*F/T/D	Stomoxys	Tabanid	Haematopota
Bildiglu 01 & 02	16	10	5	G.m	2	3	0.0625	7	3	1
Tuli	24	10	8		3	5	0.1	5	6	5
Buchi kobe	22	10	7		3	4	0.0875	6	5	4
Daleti	25	10	7		2	5	0.0875	11	2	5
Total No	**87**	**40**	**27**		**10**	**17**	**27**	**29**	**16**	**15**
Total/F/T/D							**0.3375**	**0.3625**	**0.2**	**0.1875**

F/T/D=fly per trap per day, G.m = Glossina morsitans submorsitans, M=male, F=female

5. Discussion

The present study revealed an overall 38(7.17 %) prevalence of trypanosomosis caused by different species of trypanosomes. This finding was in agreement with the previous findings of (Dano *et al*., 2014) in neighbournig districts of Oromia region who reported an overall prevalence of 7.81% in Guto Gida district of eastern wollega zone and (Tefese *et al*., 2012) whose finding was 8.55% in Sasiga and Diga districts of eastern wollega zone. Similarly, it was in concordance with studies carried out by (Yehunie *et al*., 2012) who reported an overall prevalence of 7.81% in Wemberma district of west Gojjam zone, Northwest Ethiopia. In contrast, the present finding was lower when compared with previous reports of 26.3% in around Assosa district by (Mulaw *et al*., 2011) and 24.7% in Mao-komo special district by (Daud and Molalegne, 2011) of Benishangul Gumuz regional state, western Ethiopia. The relatively low prevalence of trypanosomosis in the present study might be due to the differences in agro-ecology and climatic conditions of the localities.

Of the total cases registered, 35(92.1%), 2(5.2%), and 1(2.6%) were found to be caused by *T. congolense, T. vivax* and *T. brucei* respectively. This indicated statistically significant difference among the distribution of trypanosome species ($p< 0.05$). This finding was in consistent with the previous findings of (Duguma *et al*., 2015) who reported T. congolense (76.0 %), T. vivax (18.1 %), T. brucei (3.6 %) during their study in south-western Ethiopia.

Among the study sites, the highest and the lowest prevalence of trypanosomosis were recorded in Tuli PA 17(9.65 %**)** and Bildiglu PA 2(3.33%) respectively. However there was no significant difference ($p > 0.05$) in the prevalence of

trypanosomosis and the study sites. According to (Adale and Yasine, 2013), there is difference in prevalence of trypanosomosis in different study sites and the difference among kebeles is due to difference in vegetation cover; reproduction and development of flies are highly influenced by climatic conditions.

The prevalence of trypanosome infection was slightly higher in female animals (8.48%) than males (5.79%), although it was not statistically significant ($p>0.05$). This finding was in agreement with the previous findings of (Feyissa *et al*., 2011); (Tasew and Duguma, 2012). Similarly, (Bogale *et al*., 2012) found higher infection rate in females animals than males in some parts of Ethiopia. The possible reason for this difference might be due to physiological difference between male and female animals (Feyissa *et al*., 2011) because female animals are more exposed to physiological stresses than males.

Higher prevalence of trypanosomosis was observed (14.05 %) in animals with poor body condition when compared to medium (3.42%) and good (4.23%) body condition and the association was statistically significant ($p <0.05$) and this finding was in agreement with study carried out by (Lelisa *et al*., 2015); (Teka *et al*., 2012) and (Ayana *et al*., 2012) who recorded higher trypanosome infection rate in animals with poor body condition than in animals with medium and good body condition. Slightly higher prevalence was registered in animals > 2 years (9.25%) when compared with animals < 2 years (1.42%) and the association was statistically significant ($p < 0.05$) and the finding was in agreement with the previous finding of (Seyoum and Abera, 2016) who reported slightly higher prevalence (5.8%) in adults than young (4.6%) animals.

The overall mean PCV value of all examined animals was 24.05 ± 2.95 SD. The mean PCV of non infected cattle was higher (26.87%± 2.23 SD) than that of infected animals (21.23%± 3.66 SD) and the association was statistically significant. This finding was in agreement with the previous work of (Denu *et al.,* 2012). Additionally, (Daud and Molalegne, 2011) and (Molalegne *et al*., 2010) reported lower mean PCV value in infected animals than in the non-infected animals.

Glossina morsitans submorsitans was the only tsetse fly species caught in the study area and its mean apparent density measured as f/t/d was 0.3375. It accounts for 27(31.03%) out of the total flies caught. In addition, other mechanical transmitters of trypanosomosis such as stomoxys 29 (33.33%), tabanus 16 (18.39%) and haematopota 15(17.24%) were recorded. The apparent density for these biting flies expressed as f/t/d was found to be 0.3625, 0.2, and 0.1875 for stomoxys, tabanids and haematopota respectively. The current findings was slightly lower than the previous finding of (Aki and Godeso, 2016) at Bambasi district of the Benishangul Gumuz region, western Ethiopia that was reported to be 3.92 f/t/d, 1.76 f/t/d, 0.2 f/t/d, and 0.35 f/t/ for tsetse, stomoxys tabanids and hematopota respectively. The difference might be due to the difference in agro-ecology and climatic conditions in the localites.

6. Conclusion

Trypanosomosis caused by *T. congolense, T. vivax* and *T. brucei* with higher prevalence of *T. congolense* remains a major problem that hinders livestock production and productivity in the district. Glossina morsitans submorsitans and Glossina pallidipes were tsetse fly species captured in the study sites. Other mechanical transmitters of trypanosomosis such as stomoxys, tabanus and haematopota were registered in the study area. Parameters of study animals such as age and body condition were found to be a risk factor for trypanosomosis infection, while other parameters such as sex was not found as risk factor for trypanosomosis infection. To wrap up, the result of the present finding showed moderately high prevalence of trypanosomosis in the study sites indicating the need for strategic and holistic approach to control the vector and to minimize the impact of the disease in the study district.

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References

1. Abebe G (2005): Current situation of Trypanosomosis. In: review article on: Trypanosomosis in Ethiopia. Ethiop. J Biol Sci 4: 75-121.
2. Adale E, Yasine A (2013): Prevalence of bovine trypanosomiasis in Wolaita Zone Kindo Koish District of Ethiopia. Afr. J. Agr. Res. 8(49): 6383-6387.
3. Adam KMG, Paul J, Zaman V (2003): Medical and Veterinary Protozoology. Churchill living stone Edinburgh and London.
4. Aki A, and Godeso M (2016). Across sectional Study on Bovine Trypanosomosis and Apparent Vector density in Bambasi District of Benishangul Gumuz Regional State, Western Ethiopia: prevalence and Vector density. Researcher 2016;8(7):32-39]. http://www.sciencepub.net/researcher. 5. doi:10.7537/marsrsj080716.05.

5. Asossa, Regional Veterinary Diagnostic, Surveillance, Monitoring and Study Laboratory, (2015); laboratory annual report.
6. Ayana M, Tesfaheywet Z, Getnet F (2012): A cross-sectional study on the prevalence bovine trypanosomiasis in Amhara region, Northwest Ethiopia. Livestock Res. Rural Dev. 24 (8).
7. Bal MS, Sharma A, Ashuma Bath BK, Kaur P and Singla LD (2014). Detection and management of latent infection of Trypanosoma evansi in a cattle herd. Ind. J. Anim. Res. 48(1): 31-37.
8. Bogale B, Kebede W, Mersha C (2012): Occurrence and Identification of Bovine Trypanosomiasis in Genji District, Western Ethiopia. Acta Parasit. Glob. 3(3): 38-42.
9. Cecchi G, Mattioli RC, Slingenbergh J, de la Rocque S (2008). Land cover and tsetse fly distributions in sub-Saharan Africa. Med. Vet. Entom. 22: 364-373.
10. CSA (2014): Central Statistical agency, Federal Democratic Republic of Ethiopia, Agricultural Sample Survey volume 2. 573 Statistical bulletin, pp.39-49, 71.
11. Daud A, and Molalegne, B (2011): Epidemiological study of Bovine Trypanosmosis in Mao-komo Special District, Benishangul Gumuz Regional State, Western Ethiopia. G lobal Veterinaria, 6: 402-408.
12. De-Lahunta A, and Habel R.E (1986): Teeth. Applied veterinary Anatomy. USA. W. B. Sounders. Company, pp: 4-16.
13. Denu T.A. Y, Asfaw and Y. H. Tolossa (2012). Bovine trypanosomosis in three districts of Southwest Oromia, Ethiopia. Ethiop. Vet. J., 16: 23-39.
14. Duguma R, Tasew S, Olani A, Damena D, Alemu D, Mulatu T, Alemayehu Y, Yohannes M, Bekana M, Antje H, Abatih E, Habtewold T, Vincent D, Luc D (2015). Spatial distribution of Glossina sp. and Trypanosoma sp. in south-western Ethiopia; Parasites & Vectors 8:430 DOI 10.1186/s13071-015-1041-9.
15. FAO (2002): Food, Agriculture and food Security: The Global Dimension, WFS02/Tech/ Advance Unedited Version. FAO. Rome. pp: 19-28.
16. Feyissa B, Samson A, Mihreteab B (2011). Bovine Trypanosomiasis in Selected Villages of Humbo District, Southern Ethiopia. Glob. Veterinaria. 7(2): 192-198.
17. Gupta MP, Kumar H and Singla LD (2009): Trypanosomiasis concurrent to tuberculosis in black bucks. Ind. Veter. J. 86: 727-728.
18. Keno M. (2005): The current situation of tsetse and trypanosomosis in Ethiopia, Ministry of Agriculture and Rural Development, Veterinary service department, in proceeding of 28th meeting of International Scientific Council for Trypanosomosis Research and Control.
19. Leak S.G (1999): Tsetse biology and ecology: The role in the epidemiology and control of trypanosomosis. CAB International. Wallingford (UK), pp. 152-210.
20. Lelisa K, Damena D, Kedir M, Feyera T (2015) Prevalence of Bovine Trypanosomosis and Apparent Density of Tsetse and Other Biting Flies in Mandura District, Northwest Ethiopia. J Veterinar Sci Technol 6: 229. doi:10.4172/2157-7579.1000229.
21. Molalegne B, Yshitila A, Asmamaw A (2010): Prevalence of Bovine trypanosomosis in Selected Areas of Jabi Tehenan District, West Gojjam of Amhara Regional State, North western Ethiopia Global Veterinaria 5 (5): 243-247.
22. Mulaw S, Addis M, and Fromsa A (2011): Study on the Prevalence of Major Trypanosomes Affecting Bovine in Tsetse Infested Asossa District of Benishangul Gumuz Regional State, Western Ethiopia. *Global Veterinaria* 7 (4): 330-336.
23. Murray M and Dexter TM (1988): Anemia in Bovine in African Animal Trypanosomosis. Acta. Top-45: 389-432.
24. Nicholson MJ and Butterworth MH (1986): A guide to condition scoring of zebu cattle. ICCA, Addis Ababa, Ethiopia.
25. NMSA (National Meteorological Services Agency), (2014): Monthly report on temperature and Rainfall distribution for Kamashi Zone, Regional Metrological Office, Assosa, Ethiopia, pp: 17-19.
26. OIE (2008). "Standardized techniques for the diagnosis of tsetse transmitted trypanosomosis," in OIE Terrestrial Manual,; pp. 49, Rome, Italy.
27. OIE (2009). Manual of standards for diagnostic tests and vaccines for terrestrial animals, 6th ed. Paris. pp: 813-2008.
28. Seyoum Z, and Abera D (2016). Prevalence of bovine trypanosomosis in Chilga District, Northwest Ethiopia: Using Aldehyde and Parasitological tests; Academia Journal of Microbiology Research 4(4): 072-077.
29. Sharma A, Singla LD, Ashuma, Batth BK, Kaur P, Javed M, Juyal PD (2013): Molecular prevalence of Babesia bigemina and Trypanosoma evansi in dairy animals from Punjab, India by duplex PCR: A step forward to detection and management of concurrent latent infections. Biomed. Res. Int. Article ID 893862, 8 pages.

30. Tasew S, Duguma R (2012). Cattle anaemia and trypanosomiasis in western Oromia State, Ethiopia. Revue Méd. Vét. 12: 581-588.
31. Taylor MA, RL coop and RL wall (2007): Veterinary Parasitology 3rd ed. Block Well publishing Ltd, oxford. Uk, Pp 96-102, 212-214.
32. Tefese W, Melaku, A. and Fentahun T (2012): 'Prevalence of bovine trypanosomosis and its vectors in two districts of East Wollega Zone, Ethiopia; Onderstepoort Journal of Veterinary Research 79(1):385.
33. Teka W, Terefa D, Wondimu A (2012). Prevalence study of bovine trypanosomiasis and tsetse density in selected villages of Arbaminch, Ethiopia. J. Vet. Med. Anim. Health. 4 (3): 36-41.
34. Thrusfield M (2007). Veterinary Epidemiology. 3rd ed., UK, Blackwell Science Ltd. pp: 233-250.
35. Yehunie B, Wudu T, Nuria Y, Sefinew A (2012). Prevalence of bovine trypanosomosis in Wemberma district of West Gojjam zone, North West Ethiopia. Ethiop. Vet. J., 2012, 16 (2), 41-48.

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