Prevalence of Cattle Trypanosomosis, Vector density and associated risks in Dangur District of Benishangul Gumuz Regional State, Northwest Ethiopia

Asmamaw Aki Jano

Regional Veterinary Diagnostic, Surveillance, Monitoring and Study Laboratory, P.O.Box:326, Asossa, Ethiopia; email address: asmamawaki@gmail.com; Telephone: +251922232353

Abstract: Cross - sectional study was conducted in Dangur district of Benishangul Gumuz Regional State between March and June, 2017 to determine the prevalence of cattle trypanosomosis, the prevailing species of trypanosomes and to identify associated risks. Parasitological (buffy coat technique) and haematological (measuring packed cell volume) procedures were employed to analyze the blood samples collected from (n=382) randomly selected cattle (*Bos indicus*). The overall prevalence of trypanosomosis was 87(22.77%). The infection was mainly caused by *T. congolense* 68 (78.16%), *T.vivax* 12 (13.79%), *T.brucei* 2(2.29%) and mixed infection of *T. Congolense* and *T. vivax* 3(3.44%) and *T. Congolense* and *T.brucei* 2(2.29%). The variation in prevalence was statistically significant (chi2=316.44, p<0.000). The mean packed cell volume (PCV) value of infected animals was statistically significantly (p <0.000) lower (21.59%) than that of non-infected animals (30.4%). The prevalence showed no significant difference in susceptibility among study sites, between sex categories, age groups and body conditions. During the survey, *Glossina tachinoides* was found in the area (6.06 f/t/d) along with other mechanical vectors such as stomoxys (4.15 f/t/d), haematopota 0.93 f/t/d) and tabanid (1.36f/t/d). The study revealed that trypanosomosis is an important disease of cattle in the study area signifying the need to devise control strategies towards the diseases to alleviate its adverse impact.

[Asmamaw Aki Jano. **Prevalence of Cattle Trypanosomosis, Vector density and associated risks in Dangur District of Benishangul Gumuz Regional State, Northwest Ethiopia.** *Biomedicine and Nursing* 2017; 3(1): 91-97]. ISSN 2379-8211 (print); ISSN 2379-8203 (online). http://www.nbmedicine.org. 13. doi: 10.7537/marsbnj030117.13.

Key words: Cattle, Dangur, PCV, Prevalence, Risk factor, Trypanosome, Trypanosomosis

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. 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 G, 2005).

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 diagnosis of trypanosome infection is based on clinical signs (Sekoni *et al.,* 2004); but the clinical signs of the African Animal trypanosomosis are indicative but are not sufficiently pathognomonic. Therefore, standard methods have been developed and applied practically to diagnose the disease in animals. The methods include: direct microscopic examination of blood, either by the wet film method; but it is insensitive (Nantulya, 1990). Stained thin and thick smear techniques permit detailed morphological studies and identification of different *Trypanosoma* species by light microscopy (Borden, 2005). Sensitivity can be improved through parasitological buffy coat techniques of concentration of the parasites by centrifugation and blood inoculating into susceptible laboratory animals.

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 with more than one species of tsetse flies (Keno, 2005). Five species of Glossina (*Glossina morsitans 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.morsitant submorsitances, G. pallidipes* and *G.fuscipes* were found (ARVDSMSL, 2016). 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).

Dangur is one the seven districts of Metekel 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 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 based on previous study to determine the prevalence of bovine trypanosomosis and to assess associated risk factors and to suggest actions towards the control measure.

2. Materials and Methods:

Study Area: The study was conducted from March to June, 2017 in Dangur district of Metekel zone, Benishangul Gumuz Regional State, Western Ethiopia. It was carried out in six kebeles hereafter called sites namely: Beles No2, Borenja, Dangur town, Misreta Pa, Dusabi kokele, Jurayesis. Dangur district is located at the edge of the Blue Nile Valley between 11^0 17'47.6"N and $036^0$14' 36.7"E with altitudinal range of 910-3300 meters above sea level, covering an area of 8387 km^2. And also its annual temperature is (21 - 36^0c) and its rainfall range is 1100-1400 mm (NMSA, 2016). The livelihood of the people in the district largely depends on mixed livestock and crop production having livestock population of 35,211 cattle, 11,133 sheep, 29,548 goats, 6458 equines, 67, 393 poultry and 14, 940 beehives. The major livestock disease of the area are Trypanosomosis, PPR, Sheep and goat pox, LSD and NCD (CSA, 2015/16).

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 young (≤3 years old), matured (4-7 years old) and adult (> 7 years old).

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 previous prevalence of 11 %, confidence level of 95% and 5% desired absolute precision. As result a total of 150 cattle were calculated but increased to (n=382) to increase precision and these cattle were sampled at their communal grazing area using simple random sampling.

3. Methodology:

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 micro haematocrit 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).

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 (Murray and Dexter, 1988). Trypanosome species were identified according to

their morphological descriptions as well as movement in wet film preparations (OIE, 2008).

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 software 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 parasitological positive animals examined by buffy coat method to the total animals examined (Thru field, 2007).

4. Result:

Trypanosomes survey results

Out of the total animals examined, 87/382(22.77%) were infected with trypanosomes. The trypanosome species responsible for the infection were *T. congolense, T. vivax* and *T. brucei*. The proportional prevalence of each species of trypanosome was 68(78.16%) for *T. congolense*, *12(*13.79%*)* for *T. vivax*, *2(*2.29%*)* for *T. brucei* and 5(5.74 %) mixed infections with (*T. congolense* & *T. vivax*=3 and *T. congolense* & *T. brucei*=2) were observed in the fresh blood examined during the study period and the proportional prevalence of trypanosome species was found to be statistically significant (P<0.000, chi2=316.44)(table -1).

Haematological survey result:

The mean PCV values for all examined animals were 26.49 ± 2.54 SE. However, the mean PCV value for non - infected animals were 30.4 ± 1.70 SE and the mean PCV value of the infected animals was 21.59 ± 3.82 SE. There was significant difference in the mean PCV value between non- infected and infected animals (P<0.000, chi2=71.81) (Table -2).

Trypanosomosis and associated risks:

In the present study animals examined were categorized in different age groups as ≤ 3 years, 3-7 years and >7 years old. Out of the total sampled animals 26.70%, 45.81%, 27.48% were ≤ 3 years, 4-7 years and > 7 years old respectively and The lowest trypanosomosis prevalence 21/102(20.58%) was observed in ≤3 years of age and the highest (26.66%) was seen in >7 years of age and the difference in the prevalence was not statistically significant (p >0.05) (table - 3). The highest and the lowest prevalence of trypanosomosis were recorded in Jureysis pa 31.74% and Borenja 11.47% study sites respectively. However, there was no significant difference among the study sites (p >0.05) (Table -3). The prevalence of trypanosomosis varies in both sexes; the infection in female is slightly higher 48/206 (23.30 %) than male 39/176 (22.16%) and the association was not statistically significant (P>0.05) (Table 3).

Similarly, during the study, animals are categorized in to different body conditions as good, medium and poor. From the total 382 animals examined 36.64%, 36.64% and 26.70%, were good, medium and poor body conditioned respectively. The infection rate was highest (27.45%) in poor body condition and lowest (20%) in good body conditions. Trypanosome infection and body condition scores of study animals were not found statistically significant (p > 0.05) (Table 3).

Entomological Survey result:

The present survey of tsetse flies depicted that *G. tachinoides* is the only species tsetse fly responsible for cyclical transmission of trypanosomosis in the study area. Overall, 901 flies were captured during the study period from different sites. Tsetse flies account for 437 (48.50 %) of the total whereas other biting flies covers 51.49 % comprising of 299 (33.18%) stomoxys, 98(10.87 %) tabanus and 67 (7.43%) haematopota. Of the 437 tsetse flies captured, 59.26 % were females. *G. tachinoides* were identified in the survey site with the overall apparent density of 6.06 (fly/trap/day) while the mean apparent density of mechanical vectors such as stomoxys (4.15 f/t/d), tabanids (1.36 f/t/d) and haematopota(0.93 f/t/d) were recorded (table 4). The highest fly density were observed in Jureysis 17.75 F/T/D and the lowest recorded in Borenja 5.66 F/T/D (Table 5).

Table 1: Prevalence of single and mixed infection of trypanosomes in Dangur district

Trypanosomes	**No. positive**	**Prevalence (%)**	**X^2**	**(p-value)**
T. congolense	68	78.16	316.44	0.000
T. vivax	12	13.79		
T. brucei	2	2.29		
Mixed (*T. congolense* & *T. vivax*)	3	3.44		
Mixed *(T. congolense* & *T. brucei*)	2	2.29		
Total	**87**	**100**		

Table 2: Mean PCV comparison between parasitaemic and aparasitaemic animals

Status	Frequency	Mean PCV (%)	SE	p- value	X^2
Infected	169	21.59	3.82	0.000	71.86
Non infected	213	30.4	1.70		
Total	382	26.49	2.54		

Table 3: prevalence of bovine trypanosomosis and its association with various risk factors in Dangur district

Risk factors	No. examined	No. positive	Prevalence (%)	p-value	χ^2
Sites				0.166	7.83
Dangur town	58	13	22.41		
Boreja	61	7	11.47		
Beles no2	72	15	20.83		
Miserate pa	60	15	25		
Duseb Kokele	68	17	25		
Jureyesis	63	20	31.74		
Total	**382**	**87**	**22.77**		
Sex				0.791	0.07
Male	176	39	22.16		
Female	206	48	23.30		
Total	**382**	**87**	**22.77**		
Age(years)				0.52	1.29
≤ 3	102	21	20.58		
4-7	175	38	21.71		
> 7	105	28	26.66		
Total	**382**	**87**	**22.77**		
Body conditions				0.384	1.91
Good	140	28	20		
Medium	140	31	22.14		
Poor	102	28	27.45		
Total	**382**	**87**	**22.77**		

Table 4: Flies caught in different areas of survey sites of Dangur district

Sites	Total flies caught	No. of traps	Tsetse flies caught					Biting flies		
			No.	Species	M	F	*F/T/D	Stomoxys	Tabanid	Haematopota
Dangur	136	6	47	GT	19	28	3.91	59	19	11
Boreja	68	6	21		8	13	1.75	28	11	8
Beles no2	231	7	131		54	77	13.1	65	23	12
Miserate pa	123	5	55		22	33	3.92	39	9	20
Duseb Kokele	130	6	75		31	44	6.25	33	15	7
Jureyesis	213	6	108		44	64	9.0	75	21	9
Total	**901**	**36**	**437**		**178**	**259**	**6.06**	**299**	**98**	**67**

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

5. Discussion:

The present study revealed an overall, 87 (22.77 %) prevalence of trypanosomosis caused by different species of trypanosomes. This finding was comparable with previous report of 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. Similarly, the present finding is concordance with previous reports, (26.3%) in around Assosa district (Mulaw *et al*., 2011) and 24.7% in Mao-komo special district (Daud and Molalegne, 2011) of Benishangul Gumuz regional state, western Ethiopia. The relatively 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 recorded, 68 (78.16%), 12(13.79 %), 2(2.29%) and 5(5.74%) were found to be caused by *T. congolense, T. vivax, T. brucei* and mixed infection respectively. This indicates statistically significant difference among the distribution of trypanosome species ($p< 0.0001$). This finding was in consistent with the previous finding of (Biyazen *et al*., 2014) who reported 63.64%, 27.27%, and 9% for

trypanosome species of *T. congolense, T. vivax, and T. brucei* respectively during their study in Dale Wabera district of Kellem Wollega Zone, Western Ethiopia. This result was also 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.

Among the study sites, the highest and the lowest prevalence of trypanosomosis were recorded in Jureysis 31.74 % and Borenja 11.47 % respectively. However, there was no significant difference ($p > 0.05$) in 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 (23.30 %) than males (22.16%), although it was not statistically significant ($p>0.05$). This survey result 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 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 males.

Higher prevalence of trypanosomosis was observed (27.45 %) in poor body conditioned animals when compared with good (20.0%) body conditioned animals even if the association was not statistically significant ($p >0.05$) and this result 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 poor body conditioned animals than in good and medium body conditioned animals. Similarly, slightly higher prevalence was registered in animals aged > 7 years old (26.66%) when compared with animals 4-7 years (21.71 %) and $\leq$3 years (20.58%) and statistically significant associations were not observed ($p > 0.05$) and the finding was in agreement with previous workers (Cherenet *et al*., 2004); (Tasew and Duguma, 2012) and (Terefe *et al*., 2014), who reported comparable results on trypanosome infection across different age categories.

The overall mean PCV value of all examined animals was 26.49 ± 2.54 SE. The mean PCV of non infected cattle was higher (30.4%) than that of infected animals (21.59 %) and the association was statistically significant($p<0.000$). 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.

In fly survey, overall, 901 flies were captured during the study period from different sites. Tsetse flies account for 437 (48.50 %) of the total whereas other biting flies covers 51.49 % comprising of 299 (33.18%) stomoxys, 98(10.87 %) tabanus and 67 (7.43%) haematopota. Of the 437 tsetse flies captured, 59.26 % were females. *Glossina tachinoides* found in the area was (6.06 f/t/d) along with other mechanical vectors such as stomoxys (4.15 f/t/d), haematopota (0.93 f/t/d) and (1.36 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.

6. Conclusion:

Trypanosomosis caused by *T. congolense, T. vivax* and *T. brucei*. Higher prevalence of *T. congolense* remains a major problem that hinders livestock production and productivity in the district. *G. tachinoides* was the only tsetse fly species captured in the study sites. Other mechanical transmitters of trypanosomosis such as stomoxys, tabanus and haematopota were reported in the area. Parameters of study animals such as study sites, sex, age and body condition were not found to be a risk factor for trypanosomosis infection. To summarize, the result of the present finding shows 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.

Acknowledgement

The author is very much indebted to the Asossa Regional Veterinary Diagnostic, Surveillance, Monitoring and Study Laboratory for funding the

study. We are also grateful to the staffs for their unlimited support during the study.

Reference

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. 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).
5. 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.
6. Biyazen H, Duguma R, and Asaye M (2014): Trypanosomosis, Its Risk Factors, and Anaemia in Cattle Population of Dale Wabera District of Kellem Wollega Zone, Western Ethiopia, *Journal of Veterinary Medicine.*
7. 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.
8. 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.
9. Cherenet T, Sani RA, Panandam JM, Nadzr S, Speybroeck N, Van Den Bossche P (2004). Seasonal prevalence of bovine trypanosomiasis in a tsetse-infested zone and a tsetse-free zone of the Amhara region, north-west Ethiopia. Onderstepoort *J. Vet. Res.* 71(4): 307-312.
10. CSA (2015/16): 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. FAO (2002): Food, Agriculture and food Security: The Global Dimension, WFS02/Tech/ Advance Unedited Version. FAO. Rome. pp: 19-28.
15. Feyissa B, Samson A, Mihreteab B (2011). Bovine Trypanosomiasis in Selected Villages of Humbo District, Southern Ethiopia. Glob. Veterinaria. 7(2): 192-198.
16. Firaol T, Bizunesh M, Rajeeb K. R, Waktole T (2014): Post Control Survey on Prevalence of Bovine Trypanosomosis and Vector Distribution in Ameya District, South West Shewa, Ethiopia. Global Journal of Medical research: k Interdisciplinary, Volume 14 Issue 3.
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.
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), (2016): Monthly report on temperature and Rainfall distribution for Metekel Zone,

Regional Metrological Office, Assosa, Ethiopia, pp: 17-19.
26. NTTICC (2004). National Tsetse and Trypanosomosis Investigation and control center. Report for the period 7th June 2003 to 6th July 2004. Bedele, Ethiopia, pp.21-24.
27. OIE (2009). Manual of standards for diagnostic tests and vaccines for terrestrial animals, 6th ed. Paris. pp: 813-2008.
28. OIE. "Standardized techniques for the diagnosis of tsetse transmitted trypanosomosis," in OIE Terrestrial Manual, 2008; pp. 49, Rome, Italy.
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. Terefe E, Haile A, Mulatu W, Dessie T, Mwai O (2014). Phenotypic characteristics and trypanosome prevalence of Mursi cattle breed in the Bodi and Mursi districts of South Omo Zone, southwest Ethiopia. Trop. Anim. Health Prod. 46: 8.
35. Thrusfield M (2007). Veterinary Epidemiology. 3rd ed., UK, Blackwell Science Ltd. pp: 233-250.
36. 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.
37. Asossa, Regional Veterinary Diagnostic, Surveillance, Monitoring and Study Laboratory, (2016); laboratory annual report.
38. Bayisa, K., Getachew, D., Tadele, and T. (2015): Bovine Trypanosomosis in Asossa District, Benishangul Gumuz Regional State, and Western Ethiopia: Prevalence and Associated Risk Factors, European Journal of Applied Sciences 7.
39. 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.
40. 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.

3/25/2017