Prevalence of Cattle Trypanosomosis, Apparent vector density and Associated Risk Factors in Debate District, Western Ethiopia

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Abstract: A cross sectional study was carried out in Dibate District of Benishangul Gumuz Regional State, Western Ethiopia from October to November, 2016 to determine the prevalence of trypanosomosis in cattle and the prevailing species of trypanosomes, associated risks and its vector density. Blood samples were collected from (n=384) randomly sampled cattle (*Bos indicus*) and examined using parasitological (buffy coat technique) and hematological (measurement of packed cell volume) procedures. An overall, 75/384 (19.53 %) prevalence trypanosomosis was recorded. The infection was caused by *T. congolense 49/75 (*65.33%), *T. vivax* 20/75(26.7%), to less extent *T. brucei* 2/75(2.7%) and mixed infection was found to be 4/75 (5.33%). The infection rate was found statistically significant (P<0.0001) among trypanosome species. Mean packed cell volume (PCV) value of parasitaemic animals was lower (20.22% ± 3.38) than aparasitaemic animals (25.91 % ± 2.29) and the variation was statistically significant (P<0.0001). Higher prevalence (34.37 %) of trypanosomosis was registered in poor body conditioned animals when compared with animals with good body condition (12.17%) and the difference was found statistically significant (p<0.0001); moreover, significant difference was registered in age categories of animals, while, non - significant difference was recorded within study sites and age categories of animals (P>0.05). *Glossina tachinoides* was the only tsetse fly caught and its mean apparent density measured as f/t/d was 3.29. In addition, other mechanical vectors trypanosomes such as stomoxys, haematopota, and tabanids with f/t/d of 0.83, 0.15 and 0.14 were recorded respectively. In conclusion, the result of the current study showed high prevalence and economic importance of trypanosomosis in the study area signaling the need for strategic and participatory approach to mitigate the impacts.
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1. Introduction

Ethiopia is one of the richest countries in livestock population. Centeral statistical Authority (2013/14) report shows that the country has about 55 million heads of cattle, 55.5 million shoats and 9.3 million equines which is the highest in Africa. This sector of production is a determinant component for the overall farming systems serving as a source of draft power for the majority of rural population besides supplying products (milk and meat), by-products (manure, skin and hides) and cash income from the sale of livestock and their products (Ahmed, 2001).

Although the country is the first in livestock population in Africa, the productivity of these animal is very low due to a number of factors among which qualitative and quantitative deficiencies of feed, poor performance of the animal, lack of knowledge on the dynamics of farming system existing in the country and the presence of livestock diseases throughout the country can be mentioned as some (Getachew, 2005).

Parasitic diseases of production animals are distributed throughout the world. The effects of parasitism can be separated in two categories: sub-clinical (asymptomatic) and clinical (symptomatic). The sub-clinical effects include losses in animal productivity such as mild production, reduced weight gain, altered carcass composition and conception rate, where as visible disease symptoms like diarrhea, anemia, associated edema and roughness of coat are clinical effects (Eysker and Ploeger, 2000).

Trypanosomosis is a disease complex caused by several species of blood and tissue dwelling protozoal parasites of the genus Trypanosoma (Singla *et al.,* 2004). It is a disease of domestic livestock that causes a significant negative impact on food and economic growth in many tropical and subtropical countries of the world including sub-Saharan Africa. The course of the disease may run from an acute and rapidly fatal to a chronic long lasting one depending on the vector-parasite-host interactions. It is characterized mainly by intermittent fever, progressive anemia and loss of

condition of susceptible hosts which if untreated leads to high mortality rates (Aulakh *et al.,* 2005).

Anemia is a major classical sign following infection with pathogenic trypanosomes in cattle and other domestic animals (Murray *et al.,* 1988). Packed cell volume (PCV) usually gives an indication of anemia and disease status of trypanosome infected animal and is correlated with animal production and reproduction performance (Trail *et al*., 1993).

PCV is expected to decrease with increasing prevalence of trypanosomosis (Van den Bossche *et al.,* 2001). Hence, the relationship between prevalence of trypanosome infection and herd mean PCV could be a useful tool in the control and management of trypanosomosis. However, this relationship has not been quantified in Benishangul Gumuz Regional State of Dibate district, Western part of Ethiopia. The country has been infested with five tsetse fly species (*Glossina pallidipes, G. tachinoides, G. morsitans submorsitans, G. fusixipes fuscipes* and *G. longipennis*) that act as vectos for 5 trypanosome species (*T. vivax, T. congolense, T. brucei, T. evansi* and *T. rhodendiense*) out of six trypanosome species existing in Ethiopia (Abebe, 2005)

Western and southern river basins of Ethiopia are the most severely affected areas by trypanosomosis in the country. In the area specifically in the western part a wide diversity of tsetse and trypanosome species and strains co-exist (Abebe, 2005). These various species of Glossina and trypanosoma invade about 31,000 km^2 (62.13%) of fertile land in the Benishangul-Gumuz regional state western parts of the country (NTTICC, 1996).

In Dibate district trypanosomosis was found to be one of the most important factors that hampered livestock rearing in almost all peasant associations. Hence, a study on the status of the disease and investigating the vectors and their relative abundance and associated risk factors is crucial for a successful prevention and control in the area. Therefore, the present study is designed to determine the prevalence of trypanosomosis in cattle and apparent density of tsetse and other biting flies that are involved in the transmission of trypanosomosis from diseased to healthy animals.

2. Materials And Methods

2.1. Study Area

The study was conducted from October to November, 2016 in Dibate district of Benishangul Gumuz Regional State, Western part of Ethiopia. It was conducted in five kebeles/PA/ here after called sites namely: Berber, Legabuna, Bechati, Jane and Donben. The district has 29 kebeles/PA/ covering an area of 3,682.89 km2 with human population of 82,920. Dibate lies at latitude of $10^0$30'27.2" N and longitiude of 036°10'47.3" E. It has an altitude range of 1200-1665 meter above sea level. Its annual temperature ranges from 23^0c - 39^0c with annual rainfall range of 1500-1700 mm and extends from May to September with peak rainy periods from June to August (NMSA, 2015). The livestock population of the district is 111,312 Cattle, 56,094 Goats, 13,318 Sheep, 5574 Equines, 88,859 Poultry & 16,826 beehives and the livelihood of the society largely depends on mixed livestock and crop production (CSA, 2015).

2.2. Study Design and Study Animals

The study design used was cross-sectional to determine the prevalence of trypanosomosis in cattle and apparent density of tsetse and other biting flies that are involved in the transmission of trypanosomosis. Zebu cattle (*Bos indicus*), that are usually kept under 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 the study animal was scored as good, medium and poor (Nicholson and Butterworth, 1986). Concurrently, their age was determined based on (De-Lahunta and Habel, 1986) principles as young (< 2 years old), matured (2-6 years old) and adult (> 6 years old).

2.3. Sampling Techniques and Sample Size Determination

The study sites (Berber, Legabuna, Jane, Donben, and Bechati) was selected purposively as convenient. Animals were sampled randomly involving both sexes, all age groups, and all types of body conditions. The desired sample size was calculated according to the formula given by Thrusfield (2007). The sample size was determined based on the expected prevalence of 50%, confidence level of 95%, and 5% desired absolute precision. As result a total of 384 cattle were calculated 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 and placed in microhaematocrit centrifuge with sealed end outermost. Then the tube was loaded symmetrically to ensure good balance. After screwing the rotary cover and closing the centrifuge lid, the specimens were allowed to centrifuge at 12,000 rpm for 5 minutes.

After centrifugation, the capillary tubes were placed in a haematocrit reader. The length of the packed red blood cells column is expressed as a percentage of the total volume of blood. Animals with PCV less than 24% were considered to be anemic (OIE, 2008).

3.2. Buffy coat technique

Heparinized microhaematocrit capillary tubes, containing blood samples were centrifuged for 5 minutes at 12,000 rpm. After 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 on to a glass slide, and covered with cover slip. The slide was examined under x40 objective and x10 eye piece for movement of parasites (Murray et al., 1988). Trypanosome species were identified according to their morphological descriptions as well as movement in wet film preparations (OIE, 2008).

3.3. Entomological survey

A total of 71 odour-baited traps (22 Monopyramidal, 33 monoconical and 16 biconical) were deployed at 200-250 m 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.,* 1987).

3.4. Data management and Analysis

Raw data were entered into a Microsoft Excel spreadsheet and descriptive statistics was used to summarize the data. STATA® version 11.0 statistical software programs were used to analyze the data. The point prevalence was calculated for all data as the number of infected individuals divided by the number of individuals examined and multiplied by 100. The association between the prevalence of trypanosome infection and risk factors were assessed by chi-square test ($\chi 2$), whereas the two sample student's t-test was used to assess the difference in mean PCV between trypanosome positive and negative animals. The test result was considered significant when the calculated p-value was less than 0.05 at 95% confidence interval (Thrusfield, 2007).

4. Result

4.1. Prevalence of trypanosome infection

Out of the total animals examined (n=384), 75/384(19.53%) were found to be infected with trypanosomes(table 2). The prevalence in terms of trypanosome species was 12.76 % for *T. congolense*, 5.20 % for *T. vivax, 0.52 % for T. brucei and 1.04% was found to be mixed infection.* The proportion of trypanosome species was 49/75(65.3%) for *T. congolense*, *20/75(*26.70%) for *T. vivax, 2/75(2.70%) for T. brucei* and 4/75 (5.33%) for mixed inection and the infection rate was found to be statistically significant (P<0.0001) among trypanosome species (Table 1).

Table 1: Prevalence of single and mixed infection of cattle with trypanosomes at Dibate district

Trypanosomes	**No. positive**	**Prevalence (%)**	**X^2**	**p-value**
T. congolense	49	65.33		
T. vivax	20	26.66		
T. brucei	2	2.66	258.212	0.0001
Mixed	4	5.33		
Total	**75**	**100**		

4.2. Haematological survey results

The mean PCV value for all examined animals was 23.06 ± 3.99 SE. However, the mean PCV value for non infected and infected animals was 25.91 ± 2.29 SE and 20.22 ± 3.38 SE respectively. The mean PCV values of cattle were significantly (= 0.0001) influenced by trypanosome infection as 20.22 % and 25.11 % PCV values in trypanosome positive and negative animals were registered, respectively (Table 3). The overall prevalence of anemia in the study

district was 168/384 (43.75 %). The prevalence of anemia was statically significant in trypanosome infected cattle (51.14%) than in non-infected cattle (37.5) (< 0.0001) (Table 3).

4.3. Trypanosomosis association with risk factors

The highest prevalence (23.89 %) of trypanosomosis was recorded in animals < 2 years old (young) whilst the lowest prevalence (15.87 %) was recorded in animals > 6 years of old (adult) and the association was not found statistically significant among the age groups (table 1). Higher prevalence was registered in female animals (25.76 %) than in male animals (10.32%), which was found to be statistically significant (p< 0.0001) (table 1). Trypanosomosis was recorded across the study sites with the highest and lowest prevalence of (21.88 %) and (16.12 %) in Berber and Jane respectively and prevalence of trypanosomosis was not statistically significant across the study sites (table 1). The highest prevalence of trypanosomosis (34.37%) was found in animals with poor body condition while the lowest (12.17 %) was recorded in animals with good body conditions and the difference was statistically significant (p<0.0001). The effect of age, sex, sites and body condition on prevalence of trypanosomosis is summarized in table 2.

Table 2: Prevalence of trypanosomosis infection and its associated risk factors in cattle at Dibate district

Risk factors	No. examined	No. positive	Prevalence (%)	χ^2	p-value
Sites					
Berber	96	21	21.88		
Legabuna	122	25	20.49		
Jane	62	10	16.12	1.00	0.90
Bechati	58	11	18.96		
Donben	46	8	17.39		
Total	**384**	**75**	**19.53**		
Sex					
Male	155	16	10.32	14.037	0.0001
Female	229	59	25.76		
Total	**384**	**75**	**19.53**		
Age(years)					
< 2	159	38	23.89		
2 – 6	162	27	16.66	3.3126	0.191
> 6	63	10	15.87		
Total	**384**	**75**	**19.53**		
Body conditions					
Good	115	14	12.17		
Medium	172	27	15.70	19.1738	0.0001
Poor	96	33	34.37		
Total	**384**	**75**	**19.53**		

4.4. Entomological survey results

A total of 603 tsetse and biting flies were caught from different sites during the study period. Out of the total, 444 (73.63%) were belong to tsetse of the genus glossina, followed by stomoxy 118 (19.57%), Haematopota 21 (3.48%) and tabanus 20 (3.32%). Among tsetse species, only G. tachinoide was identified in the survey sites with the overall apparent density of 3.29 F/T/D (fly/trap/day). The highest fly density was observed in Berber peasant association 188 (4.73 F/T/D) and the lowest was recorded in Jane 68 (1.96 F/T/D) (Table 5).

Table 3: Mean PCV comparison of parasitaemic and aparasitaemic animals

Status	Frequency	Mean PCV (%)	SE	Overall PCV	X^2	p-value
Parasitaemic	176	20.22	3.38	3538		
Aparasitaemic	208	25.91	2.29	5389	14.27	0.0001
Total	**384**	**23.06**	**3.99**	**8927**		

Table 4: Proportion of anemia in parasitaemic and aparasitaemic cattle population

Status	Anemia	Frequency	Percent	Percent Share Per Strata
Infected	Anemic	90	23.43	51.14
	non anemic	86	22.39	48.86
Non infected	Anemic	78	20.31	37.5
	Non anemic	130	33.85	62.5

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

Sites	Total flies caught	No. of traps	Tsetse flies caught					Biting flies		
			No.	Species	M	F	*F/T/D	Stomoxys	Tabanid	Haematopota
Berber	188	15	142	GT	47	95	4.73	35	6	5
Legabuna	182	15	121		45	76	4.03	44	8	9
Jane	68	14	55		16	39	1.96	9	2	2
Bechati	99	14	67		25	42	2.39	26	2	4
Donben	66	13	59		21	38	2.27	4	2	1
Total	**603**	**71**	**444**		**154**	**290**	**3.29**	**118**	**20**	**21**

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

5. Discussion

The current study revealed an overall prevalence of 75/384 (19.53%) trypanosomosis infection in the study area. This finding was in agreement with the study conducted by (Bayisa *et al.,* 2015) who reported 22.38% prevalence in Assosa district of the Benishagul Gumuz region, Western Ethiopia. The present finding is slightly higher than the study carried by (Getachew and Asmamaw, 2016) in the neighboring Mandura district who reported 13.3% and the difference in the prevalence might be due to the difference in climatic conditions of the areas and study season.

This study indicated that the infection was predominantly caused by *T. congolense 49/75 (*65.33%), *T.vivax* 20/75(26.7%), and to less extent *T. brucei* 2/75(2.7%) and mixed infection 4/75(5.33%). This result is in consonance with the reported proportions of *T.congolense* (77.6%) followed by *T.vivax* (14.9%) from Metekel and Awi zones (Mekuria and Gadissa*,* 2011). This result was also in consistent with prior reports of (Mulaw *et al.,* 2011) who studied on prevalence of major trypanosomes affecting cattle in Assosa district of Benishangul Gumuz Regional State, Western Ethiopia and who found proportional prevalence of *T. congolense to be* 66.7%; (Abraham *et al*., 2012) conducted their study on prevalence of bovine trypanosomosis in selected sites of Arba Minch district, Sothern Ethiopia whose result showed proportional prevalence of *T. congolense* to be 61.4%; (Biyazen *et al*., 2014) reported proportional prevalence of *T. congolense to be* 63.64% during their work on trypanosomosis and anemia in cattle population of Dale Wabera district of Kellem Wollega Zone, Western Ethiopia.

The high proportional infection rate of *T. congolense* in cattle might be attributable to the high number of serodems of *T. congolense* relative to other species of trypanosomes. It could also be due to the possible development of better immune response to *T. vivax* by infected animals as demonstrated by (Leak *et al*., 1993). Further, it might be attributed to the efficient transmission of *T.congolense* by cyclical vectors than *T.vivax* in tsetse-infested areas. Previous reports indicated that *T. congolense* and *T.vivax* are the most prevalent trypanosomes that infect cattle in tsetse infested and tsetse free areas of Ethiopia respectively (Leak, 1999). Studies carried out by (Leak *et al*., 1993 and Rowland *et al*., 1995) have indicated that *T. vivax* is highly susceptible to treatment while the problem of drug resistance is higher in *T. congolense.*

The effect of different risk factors such as sex, age categories, study sites and body conditions on prevalence of cattle trypanosomosis was studied and, statistically significant associations were not observed in age groups and study sites ($p>0.001$) while sex groups and body condition were found to be statistically signifcant (<0.001). This result is in agreement with previous reports of (Lelisa *et al.*, 2015, Bayisa *et al.,* 2015, Shemelis, 2010, Seyoum and dessie, 2015).

The overall prevalence of anemia in the study district was 43.71 % (168/384). The prevalence of anemia was significantly higher in trypanosome infected cattle (51.14%) than in non-infected cattle (37.5%) (<0.0001). This is in concordance with

previous results from different researchers (Bekele *and Nasir*, 2011; Biyazen *et al*., 2014). Out of the total animals with anemia 23.44% (90/384) was found to be trypanosome infected. In addition to trypanosome infected animals, 20.31% (78/384) of non-infected animals were found to be anemic (PCV < 24). This result indicated the fact that other factors such as internal and external parasitism, nutritional deficiencies, and other vector-borne diseases could affect the PCV value of cattle (Van den Bossche *et al*., 2001).

This study indicated that 22.39% (86/384) of cattle were infected by trypanosome; however, their PCV was laid in the normal range. This might be attributed to the capability of infected cattle to maintain their PCV within the normal range for a certain period of time. It could also be possibly due to inadequacy of the detection method used (Murray *et al.,* 1988), furthermore, the occurrence of positive animals with PCV of greater than 24% might be thought of as recent infections of animals (Van den Bossche *et al*., 2001).

The overall mean PCV value for examined animals was 23.06 ± 3.99 SE. The mean PCV value of infected animals was significantly lower (20.22 ± 3.38 SE) than that of non-infected animals (25.91 ± 2.29 SE). This result is in alignment with previous works of (Ali *and Bitew,* 2011; Mulaw *et al*., 2011).

In the entomological survey, *Glossina tachinoides* was the only tsetse fly caught and its mean apparent density measured as f/t/d was found to be 3.29. It accounts for 73.63% (444/603) out of the total flies caught. In addition, other mechanical transmitters of trypanosomosis such as stomoxys, haematopota, and tabanid account for 19.57% (118), 3.48% (21) and 3.32% (20) of total flies caught with f/t/d of 0.83, 0.15 and 0.14 respectively. The current finding is in consistent with the previous findings of (NTTICC, 2012-2014) at neigbouring Mandura district of Western Ethiopia which was reported to be 3.59 f/t/d, 1.38 f/t/d, 0.33 f/t/d and 0.014 f/t/d respectively for tsetse fly, *stomoxys, haematopota*, and *tabanus.*

5. Conclusion

The overall high prevalence of trypanosmosis obtained in cattle of Dibate District indicated the importance of the problem and its contribution to hampering the productivity, work performance and general health status of these animals. The most widely distributed and dominant species of trypanosome in the study sites are *T. congolense (65.33%) followed by T.vivax (26.66%), and to less extent T.brucei (2.66%)* which was mainly transmitted by tsetse fly (*Glossina tachinodes*) and other biting flies (stomoxys, tabanid, haematopota) with f/t/d/ of 3.29, 0.83, 0.15 and 0.14 for Glossina tachinoides, stomoxys, haematopota and tabanide respectively. Since the District lies within the tsetse belt area, the result of the present study (19.53%) shows the fact and expected prevalence. Significant association was not recorded within study sites, and age groups of animals (p> 0.0001) while there was significant association between sex groups and body condition categories (P< 0.0001). This study showed that trypanosome infection and other factors such as (nutritional, seasonal; concurrent disease) was found to negatively affect the PCV values of animals. These all show that Dibate district is favorable for the successive breeding of tsetse and other biting flies that play a major role in the transmission of trypanosomes to susceptible hosts and hence, designing and implementing control strategies of trypanosomosis focusing on vectors and against the parasites will be under take in the study area and farmers of the district have to be educated about the impact of trypanosomosis on the health and productivity of animals as to implement participatory approach in the control of the parasites and vectors.

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References

1. Abebe, G. (2005): Review article: Trypanosomiasis in Ethiopia. *Ethiopian Journal of Biomedical Science*, 4(1): 75-121.
2. Abraham Z.A, and Zeryehun T. (2012): Prevalence of Bovine Trypanosomosis in Selected District of Arba Minch, Snnpr, Southern Ethiopia, Global Veterinaria 8(2): 168-173, 2012, DOI: 10.5829/idosi.gv.2012.8.2.61312.
3. Ahmed, M. (2001): Raw hides and skin improvement in Ethiopia. A paper presented on "technical workshop on good practice for the Ethiopian hides and skin industry. Imerial Hotel conference Hall, Addis Ababa, Ethiopia, Dec 4-7/2001.
4. Ali D, and Bitew M. (2011): Epidemiological study of bovine trypanosomosis in Mao-Komo special district, Benishangul Gumuzn Regional State, Western Ethiopia. *Global Veterinaria*, 6: 402-408.
5. Aulakh G.S., Singla L.D., Singh J. (2005): Bovine trypanosomosis due to Trypanosoma evansi: clinical, haematobiochemical and

therapeutic studies. In: New Horizons in Animal Sciences. Sobti R.C, Sharma V.L (eds.), Vishal Publishing and Co., Jalandhar, India, pp: 137-144.

6. 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(4): 171-175, 2015, DOI: 10.5829/idosi.ejas.2015.7.4.101128.
7. Bekele M, and Nasir M.(2011): "Prevalence and host related risk factors of bovine trypanosomosis in Hawagelan district, West Wellega zone, Western Ethiopia," *African Journal of Agricultural Research*, vol. 6, no. 22, pp. 5055–5060.
8. 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.*
9. Central Statistical Authority, (2015): Agricultural Sample Survey, Statistical Bulletin, Ethiopia, Addis Ababa, pp. 39-47.
10. De-Lahunta A, and Habel R.E. (1986): Teeth. Applied veterinary Anatomy. USA. W. B. Sounders. Company, pp: 4-16.
11. Eysker, M, and Ploeger, H.W. (2000): Value of present diagnostic methods for GI nematode infection in ruminants, Division of parasitology and Tropical veterinary medicine Utrecht University, The Netherlands Pp 109-116.
12. Fisher M.S, Say R. (1989): Manual of Tropical Veterinary Parasitology. UK: CAB International publication. Pp.100-278.
13. Getachew A. (2005): Review article: Trypanosomosis in Ethiopia. *Ethiopian Journal of Biological Society*, 4: 75-121.
14. Getachew D, and Asmamaw A.(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.
15. Leak S.G.A. (1999): Tsetse biology and ecology: Their role in the Epidemiology and control of trypanosomosis. Wallingford, UK, CABI Publishing and ILRI, p. 152-210.
16. Leak S.G.A., Mulatu W., Authie E., D'Ieteren., G.D.M, Peregrine, A.S.(1993): Epidemiology of bovine trypanosomosis in the Gibe valley, Southern Ethiopia. Tsetse challenge and its relationship to trypanosome prevalence in cattle. *Acta Tropica*, 53, 1221-1234.
17. Leak S.G.A., Woume K.A., Colardeue C., Duffera W., Feron A, et al. (1987): Determination of tsetse challenge and its relationship with trypanosomosis prevalence in trypanotolerant livestock at sites of the African trypanotolerant livestock network. The African Trypanotolerant Livestock Network, Nairobi, Kenya, pp: 43-52.
18. 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. *J Veterinar Sci Technol* 6: 229.
19. Mekuria S, and Gadissa F. (2011): Survey on bovine trypanosomosis and its vector in Metekel and Awi zones of northwest Ethiopia. Acta Tropica, 117: 146-151.
20. 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, 2011.
21. Murray M., Dexter T.M. (1988): Anaemia in bovine African Trypanosomiasis: a review. *Acta Trop.*, 1988, 45, 389-432.
22. Murray M., Murray P.K, and McIntyre W.I.M. (1988): An improved parasitological technique for the diagnosis of African trypanomiasis. *Transaction of the Royal Soci-ety of Tropical Medicine and Hygien*, 71, 325-326.
23. Nicholson M.J, and Butterworth M.H, (1986): A guide to condition scoring of zebu cattle, International Livestock Center for Africa (ILCA), Addis Ababa, Ethiopia. pp: 45-48.
24. NMSA (National Meteorological Services Agency), (2015): Monthly report on temperature and Rainfall distribution for Metekel Zone, Regional Metrological Office, Assosa, Ethiopia, pp: 17-19.
25. NTTICC (1996): National Tsetse and Trypanosomosis Investigation and Control Center Annual report, Bedelle, Ethiopia.
26. NTTICC. (2012 - 2014): National Tsetse and Trypanosomosis Investigation and Control Center Annual report, Bedelle, Ethiopia.
27. OIE. (2008): "Standardized techniques for the diagnosis of tsetse transmitted trypanosomosis," in *OIE Terrestrial Manual*, p. 49, Rome, Italy.
28. Rowlands G.J, Mulatu W.S, Nagda M, Dolan R.B, and d'Ieteren G.D.M. (1995): "Genetic variation in packed red cell volume and frequency of parasitaemia in East African Zebu cattle exposed to drug-resistant trypanosomes," Livestock Production Science, vol. 43, no. 1, pp. 75–84.

29. Seyoum Z., and Dessie., A.(2015): Prevalence of bovine trypanosomosis in Chilga District, Northwest Ethiopia: Using Aldehyde and Parasitological tests, *Academia Journal of Microbiology Research* 4(4): 072-077, April 2016 DOI: 10.15413/ajmr.2016.0108.
30. Shemelis M. (2010): Prevalence of Bovine Trypanosomosis in and around Assosa District of Benishangul Gumuz., North West Ethiopia. DM Thesis in Jimma University.
31. Singla L.D., Aulakh G.S., Juyal P.D., Singh J. (2004): Bovine trypanosomosis in Punjab, India. Proceeding of The 11th International Conference of the Association of Institutions for Tropical Veterinary Medicine and 16th Veterinary Association Malaysia Congress, 23-27 August 2004, Petaling Jaya, Malaysia, pp: 283-285. 4.
32. Trail JCM., D'Ieteren G.D.M., Murray M., Ordner G., Yangari G., Maille J.C., Viviani P., Colardelle C., Sauveroche B. (1993): Measurements of trypanotolerance criteria and their effect on reproductive performance of N'Dama cattle. *Vet. Parasitol.*, 1993, 45, 241-255.
33. Van den Bossche P., Rowlands G.J, (2001): The relationship between the parasitological prevalence of trypanosomal infections in cattle and herd mean packed cell volume. *Acta Trop.*, 2001, 78, 163-170.

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