

Epidemiological Study on the Prevalence of Bovine Trypanosomosis in Pawi District of the Benishngul Gumuz Region, North Western Ethiopia

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Abstract: A cross sectional study was carried out in Pawi district of the Benishangul Gumuz Regional State, North-western Ethiopia from November 2016 to February 2017 to determine the prevalence of bovine trypanosomosis, prevailing species of trypanosomes, associated risks of the disease. Blood samples collected from (n=300) randomly sampled cattle (*Bos indicus*) was examined using parasitological (buffycoat technique) and hematological (measurement of packed cell volume) procedures. An overall, 22 (7.33%) prevalence was recorded. The infection was caused by *Trypanosoma congolense* 10/22 (45.45%), *Trypanosoma vivax* 6/22(27.27%), *Trypanosoma vivax*, 3/22(33.64%), *Trypanosoma brucei* and 3/22 (13.64%) mixed infection with *Trypanosoma congolense* and *Trypanosoma vivax* was recorded. The infection rate amongst trypanosomes species was found statistically significant ($P<0.05$). Mean packed cell volume (PCV) value of the parasitaemic animals was lower ($19.16\% \pm 4.51$) than aparasitaemic animals ($27.92\% \pm 3.01$) and the variation was statistically significant ($P<0.05$). An overall 176/300 (58.67 %) prevalence of anemia was recorded and it was significantly higher (90.90%) in infected cattle than in non-infected (9.9 %). Higher prevalence 14/117(11.97 %) was registered in animals with poor body condition when compared to animals with medium 5/113(4.42%) and good 3/70(4.29%) body condition and it was found statistically significant ($P<0.05$). Significant association was not recorded within study sites, age categories and between sex groups ($P> 0.05$) of study animals. In conclusion, the result of the current study showed that the disease is economically important in the study district calling for devising strategic and participatory control efforts. [Muleta K., Birhanu E. and Akenaw W. **Epidemiological Study on the Prevalence of Bovine Trypanosomosis in Pawi District of the Benishngul Gumuz Region, North Western Ethiopia.** *Rep Opinion* 2017;9(11):53-59]. ISSN 1553-9873 (print); ISSN 2375-7205 (online). <http://www.sciencepub.net/report>. 6. doi:[10.7537/marsroj091117.06](https://doi.org/10.7537/marsroj091117.06).

Key words: Pawi District, PCV, Risk Factor, Trypanosome, Trypanosomosis.

1. Introduction

Ethiopia is one of the richest countries in livestock population. Central statistical Authority (2014) 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 complex disease caused by unicellular flagellate protozoa called trypanosomes and found in the blood and other tissue fluids of vertebrates including cattle and man (Tesfaye, 2002; Uilenberg, 1998). Three elements influences the epidemiology of the disease namely, the distributions of the vectors, the virulence of the parasites (trypanosomes) and response of the host to tsetse fly bite. Trypanosome species affecting livestock in Ethiopia are *T. congolense*, *T. Vivax* and *T. brucei* in cattle, sheep, and goats, *T. evansi* in camels and *T. equiperdium* in horses (Getachew, 2005).

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).

The presence of trypanosomiasis is a major constraint to the introduction of highly productive exotic dairy cattle and draught oxen to lowland settlement and resettlement areas of Ethiopia for utilization of large land resource (Abebe and Wolde, 2010). In Ethiopia, the overall economic loss due to trypanosomiasis was estimated to be between US\$ 1408 and 1540 million per annum (NTTICC, 1996). Baseline data collection and regular investigation on the prevalence of the parasite is essential to know the burden of the disease at different geographic locations and to enable the measurement of the impact of any control options that will be introduced. For the determination of trypanosomes infection status in rural Africa settings, microscopy-based techniques using direct observation of wet blood films, microscopic examination of Giemsa stained blood smears and concentration technique (HTC) are the most common parasites detection methods available (Gonzales *et al.*, 2003)

There are different efforts at different sites and time to control tsetse and trypanosomiasis in the country. The efforts are mainly directed at the parasites in the host through trypanocidal drugs. On the other hand several efforts applied were targeted at vector control include odour-baited, and insecticide impregnated targets and traps and insecticide application on the back of the the host animal (Silngenbergh, 1992). Western and southern river basins of Ethiopia are the most severely affected areas by trypanosomiasis 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 km² (62.13%) of fertile land in the Benishangul-Gumuz regional state western parts of the country (NTTICC, 1996).

The negative impacts of tsetse flies and trypanosomiasis in the Benishangul Gumuz Regional State are so vast and it should be underlined that no other development scheme or policy can generate the evidence of success in guaranteeing sustainable livestock development and alleviation of poverty in the rural areas of the region without addressing the problems posed by tsetse flies and trypanosomiasis. Hence, intervening in the prevention and control of tsetse flies and trypanosomiasis is very vital in the region.

Pawi district is one of the twenty districts of the Benishangul Gumuz regional State with a serious problem of trypanosomiasis. 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 major obstacles of livestock production and productivity in the district, the most recent study on the prevalence of cattle trypanosomiasis was carried out by Asmamaw and Getachew, 2016 indicating an overall prevalence of 5.58% and no further control intervention has been made since then and cattle trypanosomiasis is still becoming the most serious disease impairing the production and productivity of the animals in the district. To overcome this problem, knowing the status of the disease and its associated risk factors is crucial for a successful prevention and control trypanosomiasis in the district. Therefore, the present study was designed to determine the prevalence and host related risk factors of bovine trypanosomiasis and to recommended constructive suggestion that enable the control and prevention of the disease in the district.

2. Materials and Methods

2.1. Study Area

The study was conducted from November 2016 to February 2017 in Pawi district of Benishangul Gumuz Regional State, Western part of Ethiopia. It was conducted in three kebeles/villages/ here after called sites namely: village 7, village 14 and village 23/45. The district has 20 kebeles/villages/ covering an area of 3,682.89 km² with human population of 82,920. The district is located at a distance of 572 km away from Addis Ababa city at 11°30'N longitude 30'E latitude at an elevation of 1100 m.a.s.l. The climate of the district is characterized by a tropical hot humid with uni-modal rainfall distribution concentrated in one season from May to October. The total amount of rainfall during crop production season on average is 1640 mm. The mean maximum and minimum temperatures are 32.4°C and 16.7°C respectively (NMSA, 2015). The livestock population of the district is 64,304 Cattle, 5,996 Goats, 5,970 Sheep, 955 Equines, and 35,889 Poultry and the livelihood of the society largely depends on mixed livestock and crop production (CSA, 2014).

2.2. Study Design and Study Animals

The study design used was cross-sectional to determine the prevalence of trypanosomiasis in cattle and host related risk factors for the occurrence of the disease in the district. 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. During sample taking, the body condition of each 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 (< 3 years old), matured (3-7 years old) and adult (> 7 years old).

2.3. Sampling Techniques and Sample Size Determination

The study sites (village 7, village 24 and village 23/45) were selected purposively as convenient.

The type of sampling methods was simple random sampling involving both sexes, all age groups, and all types of body conditions to establish the prevalence the disease and host related risk factors of trypanosome infection in the study area. The desired sample size was determined using the formula given by (Thrusfield, 2007) as shown below:

$$n = 1.96^2 p_{exp} (1-p_{exp}) / d^2$$

where: n = require sample size

p_{exp} = expected prevalence

d = desire absolute precision

1.96^2 = z-value for the 95% confidence level

The most recently reported prevalence of bovine trypanosomosis in Pawi district was 5.58% by (Asmamaw and Getachew, 2016). Therefore, an expected prevalence of 5.58% was taken to estimate the sample size. Taking 95% confidence level, 5% precision and 5.58% expected prevalence 81 animals were needed to establish the prevalence. However, 300 cattle were sampled to increase the level of precision and randomness.

3. Study Methodology and Procedure

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. Data management and Analysis

Raw data were entered into a Microsoft Excel spreadsheet and descriptive statistics was used to summarize the data. STATA® version 7.0 statistical software programs were used to analyze the data. The 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 (χ^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 trypanosomes infection

Out of the total animals examined (n=300), 22/300(7.33%) were found to be infected with trypanosomes (table 1). The prevalence in terms of trypanosome species was 3.33 % for *T. congolense*, 2 % for *T. vivax*, 1 % for *T. brucei* and 1% was found to be mixed infection with *T. congolense* and *T. vivax*. The proportion of trypanosome species was 10/22(45.45%) for *T. congolense*, 6/22(27.27%) for *T. vivax*, 3/22(13.64%) for *T. brucei* and 3/22 (13.64%) for mixed infection and the infection rate was found to be statistically significant (P<0.05) among trypanosome species (Table 1).

Table 1: Prevalence of single and mixed infection of cattle with trypanosomes in Pawi district

Trypanosomes	No. positive	Prevalence (%)	X ²	P-value
<i>T. congolense</i>	10	45.45	77.3655	0.000
<i>T. vivax</i>	6	27.27		
<i>T. brucei</i>	3	13.64		
Mixed (<i>T. congolense</i> & <i>T. vivax</i>)	3	13.64		
Total	22	100		

4.2. Haematological survey results

The mean PCV value for all examined animals was 23.64 ± 3.76 SE. However, the mean PCV value for non infected and infected animals was 27.92 ± 3.01 SE and 19.16 ± 4.51 SE respectively. The mean PCV values of cattle were significantly ($P = 0.001$) influenced by trypanosome infection as 19.16 % and 27.92 % PCV values in trypanosome positive and

negative animals were registered, respectively (Table 2). The overall prevalence of animals with anemia in the study district was 176/300 (58.67 %). The prevalence of animals with anemia was statically significant in trypanosome infected cattle (90.90%) than in non-infected cattle (9.10) ($P < 0.001$) (Tables 2 & 3).

Table 2: Mean PCV comparison of parasitaemic and aparasitaemic animals in Pawi district

Status	Frequency	Mean PCV (%)	SE	Overall PCV	X ²	P-value
Parasitaemic	22	19.16	4.51	421.52	10.1780	0.001
Aparasitaemic	278	27.92	3.01	7761.76		
Total	300	23.64	3.76	7002		

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

Status	Anemia	Frequency	Percent	Percent Share Per Strata
Infected	Anemic	20	7.00	90.90
	non anemic	2	0.10	9.10
Non infected	Anemic	156	52.00	56.12
	Non anemic	122	40.80	43.88

4.3. Trypanosomosis association with risk factors

The highest prevalence 10/86(11.63 %) of trypanosomosis was recorded in animals > 7 years old (old) whilst the lowest prevalence 6/118(5.08 %) was recorded in animals 3-7 years of old (adult) and the association was not found statically significant ($P > 0.05$) among the age groups (table 4). Although it was not found statically significant ($P > 0.05$) higher prevalence was registered in female animals 13/163 (7.98 %) than in male animals 9/137(6.57%) (table 4). Trypanosome infection was recorded across the study

sites with the highest 14/125(11.20%) and lowest prevalence 2/60 (3.33 %) in village 14 and village 7 respectively and prevalence of trypanosomosis was not statically significant ($P > 0.05$) across the study sites (table 4). The highest prevalence of trypanosomosis 14/117 (11.97%) was found in animals with poor body condition while the lowest prevalence 3/70 (4.29 %) was recorded in animals with good body conditions and the difference was statistically significant ($p < 0.05$). The effect of age, sex, sites and body condition on prevalence of trypanosomosis is summarized in table 4.

Table 4: Prevalence of trypanosome infection and associated risk factors in cattle in Pawi district

Risk factors	No. examined	No. positive	Prevalence (%)	X ²	p-value
Sites					
Village 14	125	14	11.20	4.9205	0.085
Village 23/45	115	6	5.22		
Village 7	60	2	3.33		
Total	300	22	7.33		
Sex					
Male	137	9	6.57	0.2166	0.642
Female	163	13	7.98		
Total	300	22	7.33		
Age (years)					
< 3	96	6	6.25	3.3775	0.185
3 – 7	118	6	5.08		
> 7	86	10	11.63		
Total	300	22	7.33		
Body conditions					
Good	70	3	4.29	6.0582	0.048
Medium	113	5	4.42		
Poor	117	14	11.97		
Total	300	22	7.33		

5. Discussion

The current study revealed an overall prevalence 22/300 (7.33%) of trypanosomiasis in the study district. The present finding was comparable with the study conducted by (Dano *et al.*, 2014) who reported an overall prevalence of 7.81 in their study on prevalence of bovine trypanosomiasis in Guto Gida district of East Wollega Zone, Oromia Regional State, (Asmamaw *et al.*, 2016) who reported 8.96 % in their study on Epidemiology of bovine Trypanosomiasis in Kamashi District of Benishangul Gumuz Regional State, Western Ethiopia, (Belete, 2014) whose finding showed 6% prevalence in his study on prevalence of bovine and host related risk factors in the neighboring Jawi district of the Amhara regional state south west of Ethiopia, (Asmamaw and Getachew, 2016) whose report showed 5.58 % prevalence in their study on trypanosomiasis in Cattle Population of Pawi District of Benishangul Gumuz Regional State, Western Ethiopia and (Lelisa *et al.*, 2015) who reported 5.43% prevalence in their study on prevalence of bovine trypanosomiasis and Apparent Density of Tsetse and Other Biting Flies in Dangur district of the Benishagul Gumuz region, western Ethiopia. However, the present finding was slightly lower than the studies made by (Bayisa *et al.*, 2015) in the neighboring Dangur district who reported 11.27% in their study on trypanosomiasis and its associated risks in cattle population, (Getachew and Asmamaw, 2016) who reported 13.30% in their study on Epidemiology of Cattle Trypanosomiasis and Associated Anaemia in Mandura District of the Benishangul Gumuz regional state, west Ethiopia.

This study indicated that the infection was predominantly caused by *T. congolense* 10/22 (45.45%), *T. vivax* 6/22(27.27%) and to less extent *T. brucei* 3/22(13.64%) and mixed infection 3/22(13.27%). 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 (Shemelis *et al.*, 2011) who studied 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 and Zeryehun, 2012) who conducted their study on prevalence of bovine trypanosomiasis 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 *T. congolense* to be 63.64% during their work on trypanosomiasis 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 serodemes 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 the 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 *et al.*, 1999). Different studies (Leak *et al.*, 1993; Rowland *et al.*, 1995) have indicated that *T. vivax* is highly susceptible to treatment while the problems of drug resistance are higher in *T. congolense*.

The effect of different risk factors such as sex, age categories, study sites and body conditions on prevalence of cattle trypanosomiasis was studied and, statistically significant associations were not observed in between sexes, age groups and study sites ($p > 0.05$) while body condition were found to be statistically significant ($P < 0.05$). This result is in agreement with previous reports (Lelisa *et al.*, 2015). This showed that animals with poor body condition are highly susceptible to trypanosomiasis infection when compared with animals with good body condition.

The overall prevalence anemia in the studied district was 176/300 (58.67 %). The prevalence of anemia was significantly higher in trypanosome infected cattle (90.90%) than in non-infected cattle (9.10%) ($P < 0.05$). This is in concordance with previous results from different researchers (Mihret and Mamo, 2007; Tasew and Duguma, 2012; Bekele and Nasir, 2011; Biyazen, 2014). Out of 59 % prevalence of anemia, 20/300 (7%) was trypanosome infected animals. Nonetheless, 156/300 (52%) of non-infected animals were found to be anemic (PCV < 24). This indicates the fact that other factors such as gastrointestinal parasitism, nutritional deficiencies, fasciolosis and vector-borne diseases could affect the PCV value of cattle (Van den Bossche and Rowlands, 2001).

This study revealed that 2/300 (0.10%) of the 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 value 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), other anemia causing diseases (Van den Bossche and Rowlands, 2001), or delayed recovery of the anemic situation after current treatment with trypanocidal drugs. Furthermore, the occurrence of positive animals with PCV of greater than 24% might

be thought of as recent infections of the animals (Van den Bossche and Rowlands, 2001).

The overall mean PCV value for examined animals was 21.90 ± 3.76 SE. The mean PCV value of the infected animals was significantly lower (19.16 ± 4.51 SE) than that of non infected animals (24.64 ± 3.01 SE). This result is in alignment with previous works (Ali and Bitew, 2011; Shimels et al., 2011).

6. Conclusson

Animal trypanosomosis is a major problem to livestock production and productivity in Pawi district of the Benishangul Gumuz regional state, western Ethiopia. Since the district lies within the tsetse belt area of the country, the expected prevalence of trypanosomosis in the area would be high. The most common trypanosomes species in the study district was *T.congolense* followed by *T.vivax*. Parameters such as sex, study sites and age were not found to be a risk factor for trypanosomes infection; however, body conditions of study animals were identified to be risk factor for the occurrence of trypanosomosis in the district. The mean PCV value of infected animals was significantly lower than that of non infected animals indicating the adverse effect of trypanosomosis on the PCV value of cattle in the district. The disease was not detected in some anemic cattle indicating the occurrence of other causes of anemia in the area. Hence, proper control strategies have to be designed and implemented to minimize the effect of the disease on livestock production and productivity in the study district.

References

1. Abebe R and Wolde A., (2010): A cross-sectional study of trypanosomosis and its vectors in donkeys and mules in Northwest Ethiopia, *Parasitology Research*, 106: 911-916
2. 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.
3. Ali D, and Bitew M., (2011): Epidemiological study of bovine trypanosomosis in Mao-Komo special district, Benishangul Gumuz Regional State, Western Ethiopia. *Global Veterinaria*, 6: 402-408.
4. Asmamaw A, and Getachew D., (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.
5. Asmamaw A, Shimelis T, Teshome K, Birhanu E. and Yesharg A., (2016): 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.
6. 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.
7. 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).
8. 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.
9. Belete A., (2014): Study on the prevalence of bovine trypanosomosis in Jawi district of Awi Zone of Amhara region; DVM thesis, Wollo University, unpublished.
10. 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*.
11. Central Statistical Authority, (2014): *Agricultural Sample Survey, Statistical Bulletin*, Ethiopia, Addis Ababa, pp. 39-47.
12. Dano T, Benti D, and Mukarim A., (2014): Prevalence of Bovine Trypanosomosis in Guto Gida District of East Wollega Zone, Oromia Regional State, Ethiopia. *Global Journal of Medical Research: G Veterinary Science and Veterinary Medicine* Volume 14 Issue 2.
13. De-Lahunta A, and Habel R.E., (1986): *Teeth*. *Applied veterinary Anatomy*. USA. W. B. Saunders. Company, pp: 4-16.
14. 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.
15. Getachew A., (2005): Review article: Trypanosomiasis in Ethiopia. *Ethiopian Journal of Biomedical Science*, 4(1): 75-121.

16. Getachew D. and Asmamaw A., (2016): Epidemiology of Cattle Trypanosomosis and Associated Anaemia in Mandura District. *Nat Sci* 2016;14(5):85-90. ISSN 1545-0740 (print); ISSN 237167 (online). <http://www.sciencepub.net/nature>. doi:10.7537/marsnsj14051612.
17. Gonazles, J.L., Jones, T.W., Picozzy, K. and Cuellar, R.H., (2003): Evaluation of polymerase chain reaction assay for the diagnosis of bovine trypanosomosis and epidemiological surveillance in Bolivia. *Kinetoplastid, Biology and Disease*, 2: 1-14.
18. Leak S.G.A., (1999): Tsetse biology and ecology: Their role in the Epidemiology and control of trypanosomosis. Wallingford, UK, CABI Publishing and ILRI, pp. 152-210.
19. 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.
20. 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.
21. 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.
22. Mihret and G. Mamo, (2007): "Bovine trypanosomosis in three districts of East Gojjam Zone bordering the Blue Nile River in Ethiopia," *Journal of Infection in Developing Countries*, 2007; vol. 1, no.3, pp. 321-325.
23. Shemelis M., 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.
24. 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.
25. 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.
26. 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.
27. NMSA (National Meteorological Services Agency), (2015): Monthly report on temperature and Rainfall distribution for Metekel Zone, Regional Metrological Office, Assosa, Ethiopia, pp: 17-19.
28. NTTICC, (1996): National Tsetse and Trypanosomosis Investigation and Control Center Annual report, Bedelle, Ethiopia.
29. OIE., (2008): "Standardized techniques for the diagnosis of tsetse transmitted trypanosomosis," in *OIE Terrestrial Manual*, p. 49, Rome, Italy.
30. 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.
31. Slingenbergh, J., (1992): Tsetse control and agricultural development in Ethiopia. *World Anim Rev.*, 70(71): 30-36.
32. Tasew S., and Duguma R., (2012): Cattle anaemia and trypanosomiasis in western Oromia State, Ethiopia. *Revue Méd. Vét.* 12: 581-588.
33. Tesfaye, M., (2002): Report of trypanosome infection rate, in G.m.submoristance and G.tachinoides in Dedessa Valley from July 29 to September 26, Bedele.
34. Thrusfield M., (2007): *Veterinary Epidemiology*. 3rd ed., UK, Blackwell Science Ltd. pp: 233-250.
35. Uilenberg, G., (1998): A field guide for diagnosis, treatment and prevention of Africa animal trypanosomosis adapted from the original edition by Boyt, W.P. Food and Agricultural Organization of United Nations (FAO), Rome, Pp: 209-253.
36. 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.