

Prevalence of Bovine Trypanosomosis and Apparent Density of Tsetse Flies in Eastern Part of Dangur District, North Western Ethiopia

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Abstract

Trypanosomosis is a parasitic disease that causes serious economic losses in livestock, in sub-Saharan African countries. A cross sectional study was conducted from October 2011 to March 2012 in the eastern part of Dangur district, Benishangul-Gumuz regional state, Ethiopia to determine the prevalence of bovine trypanosomosis and apparent density of tsetse flies. For prevalence study, a total of 543 blood samples were collected from randomly selected animals. Packed Cell Volume (PCV) was determined and samples were examined for the presence of trypanosomes using the buffy coat technique. In total, 46 (8.5%) of the samples were tested positive for trypanosomes. The majority of the infections were caused by *Trypanosoma congolense* (95.7%), and the remaining was caused by *Trypanosoma vivax*. The difference between prevalence of trypanosomes among study sites was statistically significant ($p < 0.05$). The mean PCV value of parasitemic animals (22.6%) was significantly lower ($p < 0.05$) than that of aparasitemic animals (27.0%). A total of 528 tsetse flies were caught by deploying 78 monopyramidal traps. Of these tsetse flies, 71.8% were *Glossina tachinoides* and the remaining were *G. morsitans submorsitans*. The overall apparent density of tsetse flies was 3.4 flies per trap per day (F/T/D). In conclusion, this study revealed that trypanosomes and their vectors are prevalent and pose a huge threat to cattle production in the area. Therefore, proper intervention strategies should be put in place and implemented to minimize the burden of the disease.

Keywords: Tsetse; Apparent density; Cattle; Dangur; Prevalence; Trypanosomosis

Introduction

Trypanosomosis is a disease complex caused by several species of blood and tissue dwelling protozoan parasites of the genus *Trypanosoma* [1-3]. 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 [4]. 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 anaemia and loss of condition of susceptible hosts which if untreated leads to high mortality rates [5,6].

The disease is distributed over approximately 10 million km² of Sub Saharan Africa between latitudes 14°N and 29°S which directly coincide with distributions of tsetse flies [2,7]. In Ethiopia, the most important tsetse born trypanosomes inflicting economic losses in domestic livestock are *T. congolense*, *T. vivax*, and *T. brucei* [8]. The distribution of tsetse flies is determined principally by climate and influenced by altitude, vegetation and presence of suitable hosts [9]. Five species of tsetse flies, *G. m. submorsitans*, *G. pallidipes*, *G. tachinoides*, *G. f. fuscipes* and *G. longipennis* have been recorded in Ethiopia. Tsetse infested areas lie in lowlands and in the valleys of Abay (Blue Nile), Baro, Akobo, Didessa, Ghibe and Omo Rivers. The infestation is confined to the southern and western regions of Ethiopia between 33°-38°E and 5°-12° N which amounts to about 200,000 km². Out of the nine administrative regions of Ethiopia, five (Amhara, Benishangul-Gumuz, Gambella, Oromia and Southern Nations and Nationalities and People Regional State (SNNPRS)) are infested with more than one species of tsetse fly [8].

Although few studies were conducted in Northwestern Ethiopia, no study was conducted in Dangur district. Owing to the fact that, tsetse and trypanosomosis fronts in many places in Ethiopia are unstable

and tsetse animal interface is constantly moving [10], studies on the epidemiology of trypanosomosis are crucial to plan and implement evidence based interventions. Aiming at filling the information gap in Dangur district, this study was conducted to determine the prevalence of trypanosomosis, identify trypanosome and tsetse species in cattle in the study areas.

Materials and Methods

Study area description

The study was conducted in 5 Kebeles (lowest administrative units in Ethiopia): Kitili, Burji, Gublak, Ipuwuwa and Beles 2 of Dangur district, located 563 kms west of Addis Ababa in the Benishangul-Gumuz administrative region. Mixed agriculture is the mainstay of the livelihood of the society where crop and livestock production play integral roles. The district (Figure 1) is situated at 11°18' N and 36°14' E with a total area of 838,700 hectares. It has an elevation that varies from 800 to 2000 meters above sea levels (masl) and has 70% plains, 8% valley and 22% mountainous topographic feature. The average annual low and high temperatures are 30°C and 38°C respectively and the mean annual rainfall ranges from 900 to 1400 ml. The dominant vegetations in the

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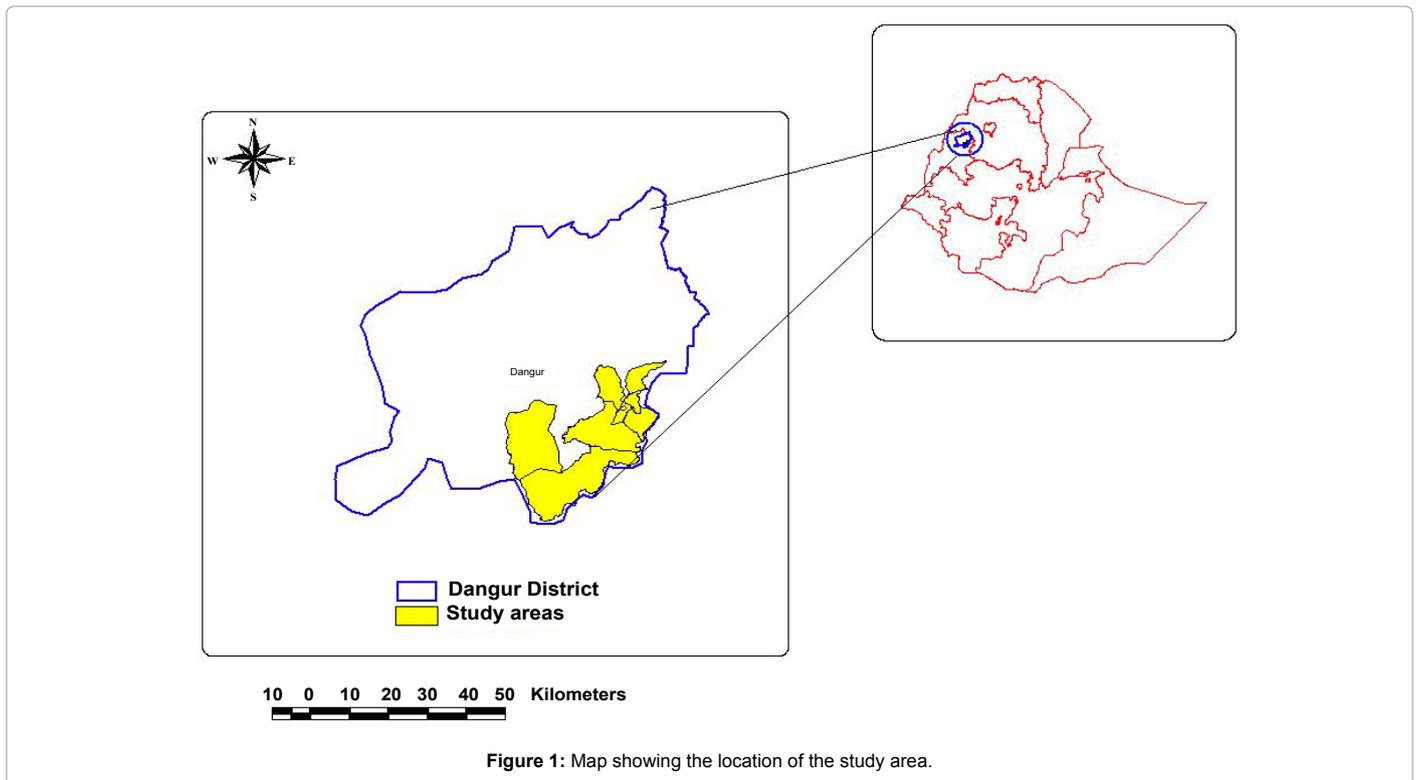


Figure 1: Map showing the location of the study area.

area are: *Arundodonax*, *Arundinaria*, *Strykinosspynosa*, *Acacia abyssinica*, *Ficussycomonus*, *Prunusafricana* and *Piliostigmathonningi* trees together with wooded grasslands. The main crop types cultivated in this area are teff, sesame, maize, peanut and sorghum. The commonly found wild animals are buffalos, antelopes, monkeys, leopard, lion, hyena and elephants [11].

Study animals

The study population constituted of indigenous zebu cattle managed under smallholder mixed crop-livestock farming system. The animals are kept under traditional extensive husbandry system with communal grazing and watering points [11]. Animal population of the district consists of 23,610 cattle, 7,945 sheep, 17,201 goats, 18 horses, 63 mules, 6194 donkeys and 43,448 poultry [11].

Sampling and sample size determination

A cross-sectional study design was conducted in dry season (November 2011-March 2012) to estimate the prevalence of trypanosomosis in cattle in the area. The study sites (kebeles) were selected based on their accessibility to transport. Sample size allocation was done based on the cattle population of the respective kebeles. Cattle owners were informed one day a head of sample collection to gather their animals at one place and simple random sampling technique was employed to select the study animals from the population. The sample size required was calculated at 50% prevalence with level of precision at 5% and 95% confidence interval using the formula described by Thrusfield [12]. As the actual prevalence was unknown, 50% was used to produce the largest sample size possible. Hence, a total of 384 animals were needed to be sampled. However, 543 animals were sampled to increase the precision of the study. Age, sex and body condition score of the studied animals were recorded during sampling. The age was estimated by means of their dentition [13]. The

body condition status of selected animals was assessed and ranked as good, medium and poor [14].

Parasitological and hematological data

Blood samples were collected from superficial ear veins using sterilized lancet and heparinized micro-haematocrit capillary tubes. Immediately after blood collection, the tubes were sealed on one side with Cristaseal (Hawksley Ltd, Lancing, UK). The capillary tube was then transferred to a hematocrit centrifuge and spun for 5 min at 1200 revolutions per minute. The centrifuged capillary tube was measured on a hematocrit reader to estimate the Packed Cell Volume (PCV) as an indicator of anaemia. Then, the capillary tube was cut using a diamond tipped pen 1 mm below the buffy coat to include the uppermost layers of the red blood cells. The content of the capillary tube was expressed on to slide, homogenized onto a clean glass slide and covered with a 22 × 22 mm cover slip. The slide was examined using the 40 × objective for the movement of parasites [15]. Then Packed Cell Volume (PCV) was calculated using micro-haematocrit reader. For the purpose of species identification, thin blood smears were made and fixed with methanol for 3 minutes, stained with Giemsa stain for 30 minutes and examined under a microscope using the oil immersion 100 x objectives [16].

Entomological data

A total of 78 monopyramidal traps including 16, 20, 15, 6 and 21 were deployed in the riverside and wooded grassland areas of Kitili, Burji, Gublak, Ipuwuwa and Beles 2 kebeles, respectively. The density and species of tsetse flies were assessed using odour-baited monopyramidal traps deployed at 200-250 m intervals. The odour baits used contained acetone, octanol and cow urine with appropriate apertures in order to release the necessary amounts of attractants. After 48 hours of trapping, the trap cage was collected [17]. The species and sex of the captured flies were identified based on morphological characteristics [18]. The

apparent density of tsetse flies was determined based on the daily mean number of flies captured in baited traps and recorded as fly per trap per day (F/T/D) [19].

Data analysis

Raw data were entered into a Microsoft Excel spreadsheet and descriptive statistics were 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 (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.

Results

Parasitological results

A total of 543 animals were sampled including 123, 129, 70, 119 and 102 from Kitili, Burgi, Gublak, Ipuwuwa and Beles 2 kebeles, respectively. Out of these, 46 (8.5%) were infected with trypanosomes. The majority (95.5%) of trypanosome species identified was *T. congolense* and the remaining 4.5% was *T. vivax*. Statistically different variation was observed in the infection status among different sites (Table 1, $p=0.01$). The prevalence of trypanosome in different age groups was 7.6%, 8.7% and 6.7% in young, adult and old animals respectively (Table 2). The prevalence of trypanosomosis was not significantly different among age and sex groups. Of the 543 cattle examined, 97 (17.9%), 247 (45.5%) and 199 (36.7%) were in poor, medium and good body conditions, respectively. Higher proportion (18.3%) of cattle ranked as having poor body conditions were significantly more infected compared to those ranked as medium (8.1%) and good (4%) body conditions (Table 3, $p=0.001$).

The mean PCV value of the parasitemic animals (22.6%) was lower compared to the mean PCV value of aparastemic animals (26.9%) as indicated in Table 4. There was a statistically significant difference in mean PCV among parasitemic and aparasitemic animals ($p=0.02$). Besides, a total of 528 tsetse flies were caught (Table 5). The majorities

Kebele	No. of animals examined	Prevalence N (%)	95% CI
Kitili	123	12 (9.8)	5.1-16.4
Burgi	129	19 (14.7)	9.1-22
Gublak	70	9 (12.9)	6-23
Ipuwuwa	119	3 (2.5)	0.5-7
Beles 2	102	3 (2.9)	0.6-8
Overall prevalence	543	46 (8.5)	6.3-11.1

Table 1: Prevalence of trypanosomes in different sites (Kebeles) in eastern part of Dangur district.

Variables		Number examined	Prevalence (%)	95% CI
Sex	Male	302	9.9	6.8-13.88
	Female	241	6.6	3.8-10.56
Age group	Young (<1 years)	150	7.6	5.5-12.75
	Adult (1-3 years)	200	8.7	5.9-13.50
	Old (>3 years)	193	6.7	3.9-10.87

Table 2: Prevalence of trypanosomosis in association with sex and age groups in eastern part of Dangur district.

Body condition	No. of animals examined	Prevalence N (%)	95% CI
Good	199	8 (4)	1.8-7.8
Medium	247	20 (8.1)	5-12
Poor	97	18 (18.6)	11.4-27.7
Total	543	46 (8.5)	6.3-11.1

Table 3: Relationship between infection and body condition of cattle in eastern part of Dangur district.

Infection status	No. of animals examined	Mean PCV (%)	t-test	P-value
Aparasitemic	497	27.0	6.4	0.00001
Parasitemic	46	22.6		

Table 4: Association between trypanosome infection and PCV value of cattle in eastern part of Dangur district.

Kebele	Altitude (masl)	Number of traps	Glossina species caught				Total	F/T/D
			G. m. submorsitans		G. tachinoides			
			Male	Female	Male	Female		
Kitili	1228	16	3	7	26	28	64	2
Burji	1316	20	43	60	27	30	160	4
Gublak	1300	15	4	6	27	23	60	2
Ipuwuwa	1240	6	17	9	6	4	36	3
Beles 2	1245	21	0	0	123	85	208	4.95
Total	—	78	67	82	209	170	528	3.38

Table 5: Species and sex of tsetse flies caught in 5 kebeles of eastern part of Dangur district.

(71.8%) of the flies were *G. tachinoides* and the remaining were *G. m. submorsitans*.

Discussion

In this study, the overall prevalence of bovine trypanosomosis was 8.5% (95% CI=6.3-11.1). Similar findings were reported from different parts of Ethiopia. Earlier studies indicated the prevalence of bovine trypanosomosis ranging from 8.6 to 9% and from 6.6 to 11.3% in southwestern and north western parts of the country respectively [20-25].

However, our finding was higher than previous reports from districts in southern part of Ethiopia that showed the diseases prevalence ranging from 4.2 to 4.4% [26,27]. In these districts tsetse control has been carried out by the southern tsetse and trypanosomosis control project for many years which significantly reduced the prevalence. On the other hand, the current finding is lower than other reports of earlier studies in Ethiopia where the prevalence ranging from 17.3% to 28.1% were reported [28-30]. These variations could be attributed to seasonal differences during sampling periods and methods employed for the studies. The present study showed that the majority (95.5%) of the infections is caused by *T. congolense* and the remaining 5% is caused by *T. vivax*. The predominance of *T. congolense* in tsetse infested areas of Ethiopia has been reported by many authors. In Southwest Ethiopia, Abebe and Jobre reported an infection rate of 58%, 31.2% and 3.5% for *T. congolense*, *T. brucei* and *T. Vivax*, respectively [10]. Another study in south western Ethiopia recorded an infection rate of 37% for *T. congolense* [31]. The present finding is also supported by earlier works done in which 82.4% *T. congolense* and 5.9% *T. vivax* infections in Arbaminch, southern Ethiopia has been reported [26]. The predominance of *T. congolense* infection in cattle may be due to the high number of serodemes of *T. congolense* as compared to *T. vivax* and the development of better immune response to *T. vivax* by the infected animal [9].

The trypanosome infection in male animals is slightly higher than in the female animals, but the variation was not statistically significant ($p=0.21$); showing that both male and female cattle were equally susceptible to trypanosomosis infection. This is in line with previous studies in Ethiopia [22,23].

There was a significant difference in trypanosome prevalence between the study kebeles ($p=0.001$). The high prevalence of the disease in Burji might have been attributed to the presence of relatively more suitable habitats (denser grassland and bush coverage) for the vectors compared to other areas. However in Beles 2 lower disease prevalence was observed despite a dense tsetse fly population. This could be attributed to the proximity of this Kebele to a veterinary clinic where the community has more easily access to animal health care compared to other areas. Treating animals with prophylactic drugs against the disease minimizes the prevalence of trypanosomosis in high tsetse fly population densities [32].

This study also showed that there is strong association between the body condition of cattle and trypanosome infection. The occurrence of infection was 4%, 8.1% and 18.6% in cattle with good, medium and poor body conditions, respectively. Thus, the majority of the infected animals manifest poor body conditions because of the effect of the disease. However, poor body condition could also be the consequence of other pathogens and nutritional stress [33]. The finding agrees with the reports of earlier studies in Ethiopia [21,28]. In this study, strong associations existed between the mean PCV value of the animals and occurrence of parasitaemia. The mean PCV value of aparastaemic (27.0%) was significantly higher than that of parastaemic animals (22.6%). The lower mean PCV value in parasitaemic animals than that of aparastaemic ones was well recorded in previous studies in Ethiopia [34,35]. Another study conducted in southwestern Ethiopia indicated that in an increase in PCV value, the proportion of positivity decreases and hence mean PCV is a good indicator for the health status of the herd in endemic areas [36]. As anaemia is the classical sign of the disease pathogenicity [16], the low PCV in parasitaemic animals could have contributed in reducing the mean PCV of the cattle.

The 8.5% overall prevalence of bovine trypanosomosis recorded in this study might not fully express the true extent of the disease burden because of the very low sensitivity and high variability of the parasite detection methods. Even though relatively high in acute state of infection, the sensitivity of buffy coat technique decreases over the course of the infection and becomes very low in chronic state of the disease [37]. A study conducted in Zambia also indicated that the buffy coat method fails to detect 66% of the infected animals [38]. However; the authors suggested that, the PCV-value of an individual animal is a good indicator of the presence of a trypanosomal infection. Therefore, the apparent parasitological prevalence of trypanosomosis is a little or much lower than the true parasitological prevalence in endemic areas. Hence, in endemic areas, it is necessary to complement the parasitological detection methods with PCR/RFLP and other sensitive molecular techniques to better understand the epidemiology of trypanosomosis and institute appropriate interventions.

The entomological survey revealed that tsetse fly species in the study area are *G. m. submorsitans* and *G. tachinoides*. In the study area, there is a typical habitat pattern for riverine species (*G. tachinoides*) along the rivers surrounded by savannah habitats suitable for *G. m. submorsitans*. Both of the identified fly species in the present study are among the five Glossina species recorded in Ethiopia [8]. The overall apparent density of tsetse flies was 3.38 F/T/D. Earlier studies in the western part of the country, reported the apparent density of Glossina species

ranging from 0.3 to 24.4 F/T/D [26,39,40]. Such wide variations could have been resulted from differences in season and density of vegetation cover and types of traps deployed, type and volume of odour attractants utilized during the studies. The low density of tsetse in the study area may have been due to the expansion of settlements and farmlands in the area. It may also be explained by the migration of the game as a result of climate and habitat changes [9]. The relative abundance of *G. tachinoides* (71.8%) than *G. m. submorsitans* (28.2%) might have been due to ability of this species to adapt to unsuitable habitats. Riverine flies appear to be largely unaffected by human population density and can even adapt to human-made environments [41].

Conclusion

In conclusion, *T. congolense* is the predominant species of trypanosome in the study area although *T. vivax* was also present. Tsetse fly species caught in the study area were *G. m. submorsitans* and *G. tachinoides*. This study also indicated that infection with trypanosomosis negatively affects the body condition and PCV profile of animals. Taken together, tsetse borne trypanosomosis is posing a considerable threat to cattle production in Dangur district, western Ethiopia. Therefore, it is imperative to extend and strengthen the national tsetse and trypanosomosis control scheme in tsetse infested areas in Ethiopia to minimize the burden of the disease.

Competing Interests

The authors have declared that no competing interests exist.

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References

1. Taylor KA (1998) Immune responses of cattle to African trypanosomes: protective or pathogenic? Int J Parasitol 28: 219-240.
2. Uilenberg G (1998) A field guide for diagnosis, treatment and prevention of African animal trypanosomosis. Food and Agricultural Organization, Rome, pp: 43-135.
3. Singla LD, Aulakh GS, Juyal PD, 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. d'Ieteren GD, Authié E, Wissocq N, Murray M (1998) Trypanotolerance, an option for sustainable livestock production in areas at risk from trypanosomosis. Rev Sci Tech 17: 154-175.
5. Bourn D, Reid R, Rogers D, Snow B, Wint W (2001) Environmental change and the autonomous control of tsetse and trypanosomosis in sub-Saharan Africa: case histories from Ethiopia, Gambia, Kenya, Nigeria and Zimbabwe. p: 175.
6. Aulakh GS, Singla LD, Singh J (2005) Bovine trypanosomosis due to *Trypanosoma evansi*: clinical, haematobiochemical and therapeutic studies. In: New Horizons in Animal Sciences. Sobti RC, Sharma VL (eds.), Vishal Publishing and Co., Jalandhar, India, pp: 137-144.
7. Radostitis OM, Gay C, Constable PD (2007) Veterinary Medicine: A text book of diseases of cattle, horses, sheep, pigs and goats. 10th edn. Elsevier, London. pp: 1531-1540.
8. Keno M (2005) The current situation of tsetse and trypanosomiasis in Ethiopia. Ministry of Agriculture and Rural development, Veterinary service department, in proceedings of the 28th meeting of International Scientific Council for Trypanosomiasis Research and Control (ISCTRC), Addis Ababa, Ethiopia.
9. Leak SGA (1999) Tsetse biology and ecology: their role in the epidemiology and control of trypanosomiasis. Tydskr S Afr vet Ver 70: 172-176.
10. Abebe G, Jobre Y (1996) Trypanosomosis: A threat to cattle production in Ethiopia. Rev Med Vet 147: 897-902.

11. ARDODW (2011) Agricultural and Rural Development Office of Dangur District Annual Report. pp: 25-28.
12. Thrusfield M (2005) *Veterinary Epidemiology*. 3rd edn. Blackwell Science, Oxford, p: 233.
13. Pasquini C, Spurgeon T, Pasquini S (2003) *Anatomy of domestic animals: Systemic and regional approach*. 10th edn. Sudz Publishing, USA, p: 255.
14. Nicholson MG, Butterworth MH (1986) A guide to condition scoring of Zebu cattle. International Livestock Center for Africa (ILCA), Addis Ababa, Ethiopia.
15. Paris J, Murray M, McOdimba F (1982) A comparative evaluation of the parasitological techniques currently available for the diagnosis of African trypanosomiasis in cattle. *Acta Trop* 39: 307-316.
16. Murray M, Murray PK, McIntyre WI (1977) An improved parasitological technique for the diagnosis of African trypanosomiasis. *Trans R Soc Trop Med Hyg* 71: 325-326.
17. Bekele J (2004) Evaluation of Deltamethrin applications in the control of tsetse and trypanosomosis in the southern rift valley areas of Ethiopia. *Vet Parasitol* 168: 177-184.
18. Food and Agriculture Organization (2009) Key for the identification of adults of the species of *Glossina*. Collection of entomological baseline data for tsetse area-wide integrated pest management programmes. Chapter 5, Food and Agriculture Organization of The United Nations, Rome.
19. Leak SGA, Woume KA, 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.
20. Tafese W, Melaku A, Fentahun T (2012) Prevalence of bovine trypanosomosis and its vectors in two districts of East Wollega Zone, Ethiopia. *Onderstepoort J Vet Res* 79: E1-4.
21. Bekele M, Nasir M (2011) Prevalence and host related risk factors of bovine trypanosomosis in Hawa-gelan district, West Wellega zone, Western Ethiopia. *Afr J Agri Res* 6: 5055-5060.
22. Dagnachew S, Shibeshi S (2011) Prevalence and vector distributions of bovine trypanosomosis in control (*Sibu Sire*) and noncontrol (*Guto Gida*) districts bordering Upper Anger valley of East Wollega zone, Western Ethiopia. *Ethiop Vet J* 15: 77-86.
23. Mihret A, Mamo G (2007) Bovine trypanosomosis in three districts of East Gojjam Zone bordering the Blue Nile River in Ethiopia. *J Infect Dev Ctries* 1: 321-325.
24. Cherenet T, Sani RA, Panandam JM, Nadzr S, Speybroeck N, et al. (2004) Seasonal prevalence of bovine trypanosomosis in a tsetse-infested zone and a tsetse-free zone of the Amhara Region, north-west Ethiopia. *Onderstepoort J Vet Res* 71: 307-312.
25. Dagnachew S, Girma H, Abebe G (2011) A cross-sectional study on bovine trypanosomosis in Jawi district of Amhara Region, Northwest Ethiopia. *Ethiop Vet J* 15: 69-78.
26. Teka W, Terefe D, Wondimu A (2012) Prevalence study of bovine trypanosomosis and tsetse density in selected villages of Arbaminch, Ethiopia. *J Vet Med Anim Health* 4: 36-41.
27. Tadesse A, Tsegaye B (2010) Bovine trypanosomosis and its vectors in two districts of Bench Maji zone, South Western Ethiopia. *Trop Anim Health Prod* 42: 1757-1762.
28. Ali D, Bitew M (2011) Epidemiological study on bovine trypanosomiasis in Maokomo special district Benishangul-Gumuz regional State, Western Ethiopia. *Glob Vet* 6: 402-408.
29. Zeleke G (2011) Preliminary survey on tsetse flies and trypanosomosis at grazing fields and villages in and around the Nech Sar National Park, Southern Ethiopia. *Ethiop Vet J* 15: 59-67.
30. Mulaw S, Addis M, Fromsa A (2011) Study on the prevalence of major trypanosomes affecting bovine in tsetse infested Asosa district of Benishangul Gumuz Regional State, Western Ethiopia. *Glob Vet* 7: 330-336.
31. Rowlands GJ, Mulatu W, Authié E, d'Ieteren GD, Leak SG (1993) Epidemiology of bovine trypanosomiasis in the Ghibe valley, southwest Ethiopia. 2. Factors associated with variations in trypanosome prevalence, incidence of new infections and prevalence of recurrent infections. *Acta Trop* 53: 135-150.
32. Kahn CM, Line S (2005) *The Merck Veterinary Manual*. 9th edition. Wiley Publishers, USA, pp: 18-35.
33. Pereckiene A, Kaziūnaite V, Vysniauskas A, Petkevicius S, Malakauskas A, et al. (2007) A comparison of modifications of the McMaster method for the enumeration of *Ascaris suum* eggs in pig faecal samples. *Vet Parasitol* 149: 111-116.
34. Afework Y (1998) Field investigation on the appearance of drug resistant population of trypanosomes in Metekel district, Northwest Ethiopia. MSc Thesis, Addis Ababa University and Freie Universität Berlin, Faculty of Veterinary Medicine, Ethiopia.
35. Tewelde N (2001) Study on the occurrence of drug resistant trypanosomes in cattle in the Farming in Tsetse Control Areas (FITCA) project in Western Ethiopia. MSc Thesis, Addis Ababa University, Faculty of Veterinary Medicine, Debre-Zeit, Ethiopia.
36. Rowlands GJ, Leak SG, Peregrine AS, Nagda SM, Mulatu W, et al. (2001) The incidence of new and the prevalence and persistence of recurrent trypanosome infections in cattle in southwest Ethiopia exposed to a high challenge with drug-resistant parasites. *Acta Trop* 79: 149-163.
37. Office of International Epizootics (2012) *Trypanosomosis (tsetse-transmitted) Manual of diagnostic tests and vaccines for terrestrial animals*. Paris, France.
38. Marcotty T, Simukoko H, Berkvens D, Vercauteren J, Praet N, et al. (2008) Evaluating the use of packed cell volume as an indicator of trypanosomal infections in cattle in eastern Zambia. *Prev Vet Med* 87: 288-300.
39. Regassa F, Abebe G (2009) Current epidemiological situation of bovine trypanosomosis in Limu Shay tsetse controlled area of upper Didessa valley. *Ethiop Vet J* 13: 19-33.
40. Tilahun G, Balcha F, Kassa T, Birrie H, Gemechu T (1999) Tsetse and trypanosomiasis plot trial at Pawe settlement area, Tana Bales valley, Metekel zone, North-Western Ethiopia. Proceedings of the 13th Annual Conference of the Ethiopian Veterinary Association, Addis Ababa, Ethiopia.
41. Jordan AM (1986) *Trypanosomiasis control and African rural development*. Longman, London, UK.