International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 3 (2014) pp. 378-388**
http://www.ijcmas.com

Original Research Article

Trypanosome infection rate of *Glossina morsitans* and trypanosomosis prevalence in cattle in upper Didessa valley western Ethiopia

Mulugeta Desta*

[1]Ministry of Agriculture National Tsetse and Trypanosomosis Investigation and Control center Bedelle -Ethiopia
[2]College of Natural and Computational Sciences Department of Biology, Haramaya University, P.o. Box, 138, Dire Dawa Ethiopia.
Corresponding author

K e y w o r d s

Cattle, apparent fly density, Glossina morsitans, Infection rate, Trypanosomosis, western Ethiopia.

A B S T R A C T

The survey was conducted in upper Didessa valley western Ethiopia in 2010. It was planned to determine the trypanosome infection rate, population density of *Glossina morsitans* and prevalence of trypanosomosis in cattle. A total of 200 flies were dissected. The overall infection rate of *G.morsitans* was 7.5%, among which 2 (6.25%) was male and 13 (7.73%) female flies. The prevalence was significantly higher (χ^2 = 99.82; P = 0.00) in female flies than male flies. In determination of tsetse flies population density, flies were trapped using baited stationary traps, and other biting flies were estimated in relation to altitude levels and vegetation types. Higher proportion of tsetse flies was caught in the riverine vegetation type followed by savanna and bush areas. Blood samples from 562 randomly selected cattle of both sex and different age groups were collected and examined with conventional haematological and parasitological techniques. Out of the total examined animals, 70 (13.24%) cattle were infected with trypanosomes. Most of the infections were due to *Trypanosoma congolense* (81.42%) followed by *Trypanosoma vivax* (12.85%), mixed infections of *T. congolense* and *T. vivax* (5.71%). There was no statistically significant difference (P > 0.05) in infection between male and female, and altitude levels. Mean packed cell volume (PCV) value of parasitaemic and aparasitaemic animals was significantly (P < 0.05) different. Diagnosis of trypanosomosis in tsetse or domestic livestock is a basic requirement for epidemiological studies as well as for planning and implementing control operations. Therefore, the results of this study should be used to define the strategy of disease control in places where tsetse and trypanosomosis challenge were reported.

Introduction

Tsetse are biological vectors of trypanosomes meaning that tsetse, in the process of feeding, acquire and then transmit small, single-celled organisms

called trypanosomes from infected vertebrate hosts to uninfected animals. Some tsetse transmitted trypanosome species cause trypanosomosis, an infectious disease. Trypanosomes are the parasite that causes trypanosomosis of humans and domestic animals (International Laboratory for Research on Animal Diseases (ILRAD), 1988; Connor, 1994). The most important species responsible for the disease complex commonly known as Nagana in livestock are *Trypanosoma brucei, Trypanosoma congolense* and *Trypanosoma vivax.*

In humans, tsetse transmitted trypanosomosis is called sleeping sickness. These trypanosomes are highly evolved and have developed a life cycle that requires periods in both the vertebrate and tsetse hosts. Tsetse transmits trypanosomes in two ways, *mechanical* and *biological* transmission. Mechanical transmission involves the direct transmission of the same individual trypanosomes taken from an infected host into an uninfected host. Biological transmission requires a period of incubation of the trypanosomes within the tsetse host. The term *biological* is used because trypanosomes must reproduce through several generations inside the tsetse host during the period of incubation, which requires extreme adaptation of the trypanosomes to their tsetse host. The entire process takes at least two or three weeks and the metacyclic trypanosomes are inoculated into the new host when the tsetse fly feed (Urquhart et al., 1995). In *T. vivax*, a similar process of cyclic development takes place except that it occurs entirely within the proboscis. Tsetse challenge is determined by the product of relative tsetse density, trypanosome prevalence in tsetse and the proportion of meals obtained by the tsetse from a defined host (Leak et al., 1988). The occurrence and impact of trypanosomosis on the other hand depends on tsetse challenge, host distribution, livestock breeds, farming practices and control practices (Rogers et al., 1996). Therefore, it is prudent to study the infection rate in the tsetse flies to obtain a reasonable indication about the risk of trypanosomosis to domestic livestock and consequently a useful parameter for prioritizing the strategy in the disease control techniques. Tsetse flies in Ethiopia are confined to the southern, western and southwestern regions between longitude 33° and 38° E and latitude 5° and 12°N. Tsetse infested areas lie in the low lands and also in the river valleys of Baro, Akobo, Didessa, Abay (Blue Nile), Ghibe and Omo (Langridge, 1976). Five species of tsetse flies are believed to be found in Ethiopia. These are *Glossina marsitans submorsitans, Glossina pallidipes, Glossina fuscipes fuscipes, Glossina tachinoides* and *Glossina longipennis* (Langridge, 1976; MOA, 1996; Ministry of Agriculture and Rural Development (MOARD), 2004). There are five economically important animal trypanosome species in Ethiopia. These are *T. congolense, T. vivax, Trypanosoma brucei brucei, Trypanosoma evansi* (Langridge, 1976) and *Trypanosoma equiperdum* (Dagnachew and Shafo, 1981).

In Ethiopia, few studies were conducted regarding trypanosome infection rate in tsetse fly while no studies were performed in the current study area. Therefore the objective of the present study is to determine the trypanosomes infection rate of *Glossina morsitans* and prevalence of trypanosome in cattle in upper Didessa valley western Ethiopia.

Materials and Methods

Study area

The study was carried out in upper Didessa valley western Ethiopia, from October to November. The study area was located between 7° 47` N, 37° 58`E, longitude, along the escarpment of Didessa river Dabo districts. The climate condition of the area is divided into: short rainy season (between Late March to May), long summer rainy season (from June to November) and dry season (from late December to April). The annual mean minimum and maximum temperature is 13.0 to 15.5 and 26.1 to 28.4°C, with an annual rainfall ranging from 735 to 1200 mm. The dominant floras were wood grass, acacia, *Ficas sycomorus* and other bushes. Major faunas in the study area were bush pig, antelopes, warthog, and others.

Study design

A cross-sectional study was conducted to determine the trypanosome infection rate and population density of *Glossina morsitans* and prevalence of trypanosomosis in cattle, in upper Didessa valley western Ethiopia.

Sample size and sampling method

The simple random sampling technique was used for the study of trypanosomes infection rates in *G. morsitans,* and stratified sampling method in cattle based on the herd common characteristics of the population using simple random sampling method and sample sizes were allocated using proportional allocation under which the sizes of samples from different strata were kept. The sample size was determined based on the expected prevalence rate of 50% and absolute desired precision of 5% at confidence level of 95%. As a result, a total of 384 animals were needed to be sampled (Thrusfield, 1995). However, in case of stratified sampling, the subjects are not independent and hence larger sample size has been required (Martin, 1978).

Tsetse flies survey

A total of 82 monoconical (Challier and Laveissiere, 1973) standard traps were deployed in the study area for tsetse fly trapping. All the traps were baited uniformly with octenol (1-oct-3-nel), acetone and three weeks old cow urine (Brightwell et al,). All odours were placed on the ground about 30 cm upwind of the trap. The poles of traps were greased to prevent fly predators, mainly ants. Traps were allowed to stay at the site of deployment for a period of 48 hr before collection. Trap deployment sites were selected to represent all vegetation type/habitat that could be related to fly multiplication, behavior, feeding, and other related aspects. After 48 h of deployment, the catchments of each trap was sorted by fly species and then counted, identified and analyzed. The apparent density of the tsetse fly was calculated as the number of tsetse catch/trap/day (Leak et al., 1987).

Sex determination

Tsetse flies were trapped using monopyramidal traps which were deployed along riverside and cattle roots. The flies were collected from the trap and before dissecting them the number of each sex and species of tsetse flies were recorded. Tsetse flies were identified as male or female by examining the posterior end of the abdomen. The male fly has a

lump on the ventral side of the abdomen (hypophgeum) at the posterior end but not in the female flies (Food and Agriculture Organization (FAO), 2000).

Age determination

In male tsetse, the age estimation was done according to the degree of wear or fraying observed on the hind margin of the wing. According to the degree of wear, flies were assigned to one or other of the six categories as described by Jackson (1946) and Challier (1965). After giving the wing fray category, the age was estimated using directions for estimating the mean age of a sample of tsetse flies, as mean wing fray was calculated as the sum of each category total divided by the sum of fly number for each category and finding the given value on the table as given in the FAO Training manual for tsetse control personnel (FAO, 2000). Female flies were age graded according to the contents of the uterus and the relative development of the four ovarioles. Ageing of the female tsetse flies using ovarian age determination was done by carrying out tsetse dissection and observing the contents of the uterus and the relative size of the follicles in each of the two ovarioles and in each of the two ovules that constitute each ovary. The sub-division of each of the age category was done as described by Saunders (1962) and followed as illustrated in the FAO Training manual for tsetse control personnel (FAO,1979).

Dissection of tsetse flies and Infection rate determination

Wings and the legs were removed from the flies. The dissection was carried out as described by the FAO Training manual for tsetse control personnel (FAO, 2000). Then, freshly killed tsetse flies were dissected under a dissecting microscope by using 0.9% normal saline. Trypanosome infections in the tsetse flies were identified using a compound microscope at a magnification of ×400, using the methods of Lloyd and Johnson (1924). Parasites found in the midgut, salivary glands and mouth parts were regarded as *Trypanozoon;* "*T. brucei*-type", those located in the mouth parts and midguts were *Nanomonas;* "*T. congolense*-type*"*, while those found in the mouth parts only was put in the group of *Duttonella*; *"T. vivax*-type infection", immature infections, when only the midgut was found infected. The Infection rate (IR) was calculated using the following formula:

$$\text{Infection rate (IR)} = \frac{\text{Number of tsetse flies infected}}{\text{Total Number of tsetse flies dissected over a given period}} \{100$$

Trypanosomes prevalence in cattle
Study animals

The study animals used were all age and sex group of *Bovine* species of local zebu breed of cattle. All of them were kept under extensive management system together with other livestock species. A total of 560 cattle were selected from study population by simple random sampling methods technique according to Thrusfield (2005) and Martin (1978), with 95% confidence interval, 5% desired absolute precision, and 50% expected prevalence.

Parasitological examinations

Blood samples were collected directly from the ear veins of the study animals into heparinized capillary tubes. The blood samples were examined by the capillary

microhaematocrit centrifugation method to estimate the packed cell volume (PCV) as an indicator of anemia. After determination of the PCV, the buffy coat (BC) was examined by dark ground/phase contrast microscope (Paris et al., 1982). For the purpose of species identification, a thin blood smear was prepared from the BC for those samples that were positive on BC examination and stained with giemsa stain and examined under a microscope using the oil immersion ×100 (Paris et al., 1982).

Data analysis

The data was entered into a Microsoft excel spread sheet to create a database and transferred to the statistical package for social sciences (SPSS) software programs of the computer before analysis. The SPSS version 16.0 software of the computer program were applied for the statistical analysis. The point prevalence was calculated for all data as the number of infected individuals divided by the number of individual sampled and multiplied by 100. The association between prevalence of trypanosome infection and the assumed risk factor was tested by chi-square, whereas student's *t* test was used to examine the differences in mean PCV value between parasitaemic and aparasitaemic animals.

Results and Discussion

From 82 traps deployed during the study period, a total of 4,702 flies were caught. Of these, 370 (7.86%) belong to *Glossina morsitans*, the remaining, 1840 (39.13%), were *Stomoxys*, 314 (6.67. %) *Tabanus* and 2178 (46.32%) were Heamatopota belonging to biting flies. The overall apparent fly density (tsetse) was 0.5 flies/trap/days (F/T/D). The difference in apparent fly density at PA level was 6.6 and 0.9 at cheleleki and alelitu, respectively. The number of fly counted was significantly different ($P < 0.05$) among PAs, and between tsetse and other biting flies (Table 1).

Infection rate

Infection rate was 7.5%. More trypanosome infections were observed in female tsetse with an infection rate of 7.73% (Table 2). Overall 73.33% (or 11/15) of the trypanosome infections carried by the female and male tsetse were identified as belonging to the Duttonella group; these were classified as *T. vivax* and the 26.66% (4/15) were *Nanomonas;* "*T. congolense*-type". There was significant difference in the proportion of tsetse infected with trypanosomes between male and female flies ($\chi 2 = 99.82$; $P = 0.00$) and also an age related effect in the number of trypanosome infections detected by microscopy with number of infected flies older than 31 days being significantly higher than those aged < 20 days ($P < 0.05$).

Out of a total 562 cattle sampled, 70 (12.45%) were found to be infected with trypanosomes (Table 3). The prevalence of trypanosomosis at kone and cheleleki sites were 18.51 and 10%, respectively. There was statistically significant ($P < 0.05$) difference in the prevalence of trypanosome infection between the two sites. According to the survey result obtained, *T. congolense* was the predominant species and found to be a major cause of infection in the study area followed by *T. vivax* and mixed infection of *T. congolense* and *T. vivax.* When the proportional frequency of trypanosomes was considered, *T. congolense* appeared

57 times while *T. vivax*, mixed infection of *T. congolense* and *T. vivax* and *T. brucei* were 9, 4, times respectively. In other words, *T. congolense* being a major cause of infection exceeded the other trypanosome species by 81.42%. The overall trypanosome prevalence in the surveyed areas of the district comprised 12.45%, with a range of 7.10 to 18.51% while the overall mean PCV-value appeared to be 25.78% in a parasitaemic and 22.96% in parasitaemic animals. Of 292 males and 270 females examined, 37 (12.67%) and 33 (12.22%), respectively were infected with trypanosome, but there was no significant difference ($P > 0.05$) between two sexes (Table 4). Age was categorized into three groups from randomly selected animals during blood sample collection. Out of 562 animals sampled, 95 (16.9%), 165 (290.4%) and 302 (53.7) were under age group 2 to 4 years, 5 to 7 years and > 7 years, respectively (Table 4). In each group, 10(10.52%), 20 (12.12%) and 40 (13.24%) were trypanosome positive and there was a significant difference ($P < 0.05$) among the age groups. Prevalence of trypanosome infection in cattle was analyzed according to the agro ecological zone: lowland (< 1500 masl) and midland (1500 to 1800 masl). Accordingly, the prevalence in animals sampled from lowland was 40 (13.24%) and in animals from midland were 30 (11.53%). The difference between two ecological zones was statistically not significant ($P > 0.05$).

Haematological findings

The mean PCV (%) values during the study period were 22.96 ± 2.61 in parasitaemic and 25.78 ± 4.06 in aparasitaemic animals. Statistical analysis was made using t-tests to compare mean PCV value of parasitaemic and aparasitaemic animals. When the results were compared, parasitaemic animals had lower mean PCV than aparasitaemic animals, and there is statistically significant difference ($P < 0.05$) between the two variables.

The results on tsetse fly survey in this study revealed the presence of *Glossina* morsitans in the savanna land of upper Didessa valley and identified as the major vector of trypanosomosis in the escarpment of Dabo district, western Ethiopia. As the survey concludes that *G.tachnoides* was one of the tsetse species found on the river side of Didessa River, it was not founded in the current study sites. The herds that were considered under this study were not under the risk of this species. Other biting flies including *Stomoxys*, haematopota, and *Tabanus* that transmit the parasites mechanically were also found in the study area. The overall apparent density of tsetse and other biting flies were 0.5 and 5.3 flies/trap/day (F/T/D), respectively. There was significant difference ($P < 0.05$) in tsetse flies density between surveyed peasant associations; cheleleki and alelitu sites ranging from 6.6 to 0.9. This might be attributed to the altitude and vegetation type and coverage of the two sites. The trypanosome infection rate in a population of tsetse may vary with sex, age and the sampling method (Jordan, 1974). Sex ratio and age composition of the flies were assessed in this study and higher numbers of female and adult flies were recorded. The presence of high number females might result in high population density which is indicative for future high infection rate. Similar results have been reported by Msangi (1999), Mohammed-Ahemed and Dairri (1987) and Leak (1999) which showed that in unbiased sample, female would comprise between 70 to 80% of the mean population.

Table.1 Summary of the results of entomological survey

Site	Trap	Days	*G.species found*		*Other biting flies*				
			G.morsitans	***FTD***	***Sto***	***Tab***	***Hea***	***Total***	***F/T/D***
Chelelk	15	2	198	6.6	778	58	838	1674	55.8
kone	20	2	66	1.7	361	138	507	1006	25.15
kerka	20	2	45	1.1	305	55	362	722	18.05
mexi	20	2	48	1.2	360	30	410	800	20
Alelitu	7	2	13	0.9	36	33	61	130	9.29
Total	82		370	0.5	1840	314	2178	4332	5.3

F/T/D= flies/trap/days, Sto= stomyxs, Tab = tabanus, Hea= haematopota.

Table.2 The number of flies dissected and infection rate of *Glossina morsitans* based on sex and age.

Sex	**No. dissected**	**Age**	**No. of flies infected by trypanosome species (%)**			**Overall infection rate (%)**
			T. congolense	***T. vivax***	***T. brucei***	
Male	32	19	1(3.12)	1 (3.12)	0	2(6.25)
Female	168	31	3 (1.78)	10(5.95)	0	13 (7.73)
Total	200		4 (2)	11 (5.5)	0	15 (7.5)

Table.3 Prevalence of trypanosome infections and species of trypanosomes identified in cattle in the study area

Site	N	+ve	***Trypanosome species (%)***			**Overall infection (%)**
			T. congolense	***T. vivax***	**Mixed**	
Chelelk	100	10	9	1	0	10
kone	108	20	15	4	1	18.51
kerka	92	10	5	2	3	10.86
mexi	95	12	10	2	0	12. 63
Alelitu	110	14	14	0	0	12. 72
Bakagaba	57	4	4	0	0	7.01
Total	562	70	57	9	4	12.45

Table.4 Prevalence of trypanosome infection in relation to sex, age and altitude categories

Risk factor	N	+ve	*Trypanosome species (%)*			**Prevalence (%)**	**d.f**	χ2-value	***P-value***
			T. congolense	***T. vivax***	**Mixed**				
Sex									
Male	292	37	30	5	2	12.67	1	1.299	0.254
Female	270	33	27	4	2	12.22	-	-	-
Age									
2-4 years	95	10	10	0	0	10.52	2	117.348	0.000
5-7 years	165	20	16	4	0	12.12	-	-	-
>7 years	302	40	31	5	4	13.24	-	-	-
Altitude									
Mid-land	260	30	25	4	1	11.53	1	2.995	0. 083
Low-land	302	40	32	5	3	13.24	-	-	-
Total	562	70	57	9	4	12.45	-	-	-

A total of 200 *G.morsitans* were dissected, and an overall of 7.5% of *G. morsitans* in study site was harbors *T. vivax, and T. congolense. T. vivax* is the most prevalent species identified in the tsetse fly. According to Adams *et al*. (2010), *T. vivax* is considered to be one of the most important of the salivarian trypanosomes because of its pathogenicity to cattle and its relatively high infection rates in tsetse. More trypanosome infections were observed in female tsetse with an infection rate of 7.73% amongst the female flies while 6.25% infection rate was found in male flies. There was significant difference in the proportion of tsetse infected with trypanosomes between male and female flies ($\chi 2 = 2.01$; $P = 0.00$). The reason for a higher infection rate in females might be due to their better life expectancy as suggested by Jordan (1974). The lower infection rate found in male flies can be explained by the low average age of traped male flies (20 days or less). The overall trypanosome prevalence (13.24%) found in the present study is relatively high when compared with the apparent density of *G.morsitans* (0.5%) but it was well compromised with trypanosome infection rate of *G.morsitans* (7.5%). The relatively higher fly infection rate and trypanosome prevalence as compared to low tsetse challenge can be explained by the higher fly- animal contact. *T. congolense* in cattle was the most prevalent trypanosome species in the study area that accounts for the overall percentage of about 81.42% (57/70). Similar studies indicated that the most prevalent trypanosome species in tsetse-infested areas of Ethiopia are *T. congolense* and *T. vivax.* Rowlands *et al*. (1993) reported a prevalence of 37% for *T. congolense* in southwest Ethiopia. Abebe and Jobre (1996) reported an infection rate of 58.5% for *T. congolense*, 31.2% for *T. vivax* and 3.5% for *T. brucei* in southwest Ethiopia. In this research work, age was found to be a risk factor; higher infection rates were observed in adult animals in both altitude levels. This is logically associated to the fact that young animals are also naturally protected to some extent by maternal antibodies (Fimmen et al., 1982) but adult animals travel and cross-different vegetation types for grazing, watering, as well as for draught and harvesting crops to tsetse high challenged

areas. *T. congolense* infection is a chronic disease that increase infection rates with age. *T. congolense* infection is usually higher in adult animals than younger ones (McDermott and Coelman, 1999).

In the present study, a relatively lower mean PCV values were observed in parasitaemic animals, but the difference is statically significant among aparasitaemic and parasitemic animals. The result of this study was in accordance with Rowlands et al. (2001) who observed in an increase in PCV value, the proportions of positivity decreases and hence mean PCV was a good indicator for the health status of animals in an endemic area. The lower mean PCV value in parasitaemic animals than the aparasitaemic animals is reported by several authors (Leak, 1987; Afewerk, 1998; Muturi, 1999; Tewelde, 2001). The development of anaemia is one of the most typical signs of trypanosomosis cased by *T. congolense* in the susceptible cattle breeds (Murray and Dexter, 1988). The level of anaemia or the PCV usually gives a reliable indication of the disease status and productive performance of an infected animal (Trail et al., 1991).

This study presents findings on the trypanosome infection rate of *G. morsitans* and prevalence of cattle trypanosomes in upper Didessa valley western Ethiopia. The study indicated that *G. morsitans* was the only *savanna* species with the apparent density of 0.5%. The trypanosome infection in vector and host animals were highly prevalent than tsetse population density in the study area. This result could be due to fly-cattle contact relationship which increases the prevalence of trypanosome in both vector and host animals. Therefore, vector controlling and treating infected cattle with prophylactic or chemotherapeutic measures should be given to overcome the problem in the study area.

References

Abebe G, Jobre Y 1996. Trypanosomosis: A threat to cattle production in Ethiopia. Rev. Med. Vet. 147:897-902.

Adams ER, Hamilton PB, Rodrigues AC, Malele II, Delespaux V, Teixera MMG, Gibson W 2010. New Trypanosoma Duttonella vivax genotypes from tsetse flies in East Africa. Parasitol. 137:641–650.

Afewerk Y 1998. Field investigations on the appearance of Drug Resistant Populations of Trypanosomes in Metekel District, North-West Ethiopia, MSc thesis, Addis Ababa University and Freie University of Berlin.

Brightwell R, Dransfield RD, Stevenson P, Williams B 1997. Changes over twelve years in population of *Glossina pallidipes* and *G.longpennis Diptera : Glossina* subject to varying trapping presure at. Nkurman, South-West Keniya. Bull. Entomol. Res.87:349-370.

Challier A 1965. Method for the determination of physiological age of *Glossina*. Insect Physiol. Biol. 6:241-248.

Challier A, Laveissiere C 1973. Un nouvean piège pour la capture des glossines description et essairs sur le terrain. Cah. Or stomser. Ent. Med. Parasitol. 11, 251–262.

Connor RJ 1994. Africa animal trypanosomosis. In: Coetzer JAW, Thomson GR, Tustin Rc Eds. Infectious disease of livestock with special reference to southern Africa, Oxford University press, Cape Town, pp. 203.

FAO 1979. Training manual for tsetse control personnel, Food and

Agriculture Organization of the United Nations, Rome, Italy.

FAO 2000. Food and agriculture Organization of the United Nations: Training manual for tsetse control personnel Vol.1.Rome, Italy.

Fimmen HO, Mehlitz D, Horchner F, Korb E 1982. Colstral antibodies and *Trypanosoma congolense* infection in calves. Trypanotolerance research and application. GTZ, No.116, Germany. pp173-187.

Ford J, Makin MJ, Grimble RJ 1976. Trypanosomosis Control Program for Ethiopia. Ministry of Overseas Development of Great Britain. Pp.1-30 not sited

ILRAD 1988. AnimalReport of International laboratory for Research on Animal Diseases Nairobi, Kenya.

Jackson CHN 1946. An artificially isolated generation of tsetse flies Diptera. Bull Ent.Res.37**:**291-299. Jordan AM 1974. Bulletin of Entomological Research. 63:361-399.

Langridge WP 1976. A Tsetse and Trypanosomosis survey of Ethiopia. UK Ministry of Overseas Development, London :1- 118

Leak SGA 1988. Determination of tsetse challenge and its relationship with trypanosomosis prevalence. In: Livestock production in tsetse infested areas of Africa, Nairobi, Kenya. ATLN:43-52.

Leak SGA, Woume KA, Colardelle C, Duffera W, Feron A, Mulingo M, Tikubet G, Toure M, Yangari G 1987. Determination of tsetse challenge and its relationship with trypanosomosis prevalence. In: Livestock production in tsetse infested areas of Africa, Nairobi, Kenya. ATLN :43-52.

Lloyd L, Johnson WB 1924. The trypanosome infection of tsetse flies in Northern Nigeria. Bull. Entomol. Res. 14:265.

McDermott JJ, Coleman PG 1999. Modeling; Research into trypanosomosis epidemiology- the essential contributions of theory, model, diagnostics and field studies.

Mohammed-Ahemed MM, Dairri MF 1987.Trypanosome infection rate of *G. pallidipes* during wet and dry season in Somalia. Trop. Anim. Health Prod. 19:11-20.

Mohammed-Ahmed MM, Ahmed AI, Ishag A 1989. Trypanosome infection rate of Glosina Moristans submoristans in Bahr El Arab, South Darfur Province, Sudan. Trop. Anim. Health Prod. 11:239 – 244.

Msangi S 1999. Distribution, density and infection rates of tsetse in selected sites of Southern Rift valley of Ethiopia. MSc Thesis, Faculties of Veterinary Medicine, Addis Ababa University Ethiopia.

Murray M, Dexter TM 1988. Anemia in bovine African trypanosomosis. Acta Tropica, 45:389-432.

Murray M, Trail JCM, Turner DA, Wissocq Y 1983. Livestock productivity and Trypanotolerance. Network Train. Manual. ILCA. 4-10. not sited

Muturi KS 1999. Epidemiology of bovine trypanosomosis in selected sites of the Southern Rift valley of Ethiopia. MSc Thesis, Faculties of Veterinary Medicine, Addis Ababa University Ethiopia.

Rowlands GJ, Mulatu W, Authie E, Leak SGA, Peregrine AS 1993. Epidemiology of bovine trypanosomosis in the Ghibe valley, southwest Ethiopia. Acta Trop. 53:135-150.

Rogers DJ, Hay SI, Packer MJ 1996. Predicting the distribution of tsetse

flies in West Africa using temporal Fourier processed meteorological satellite data. Anm.Trop.Med.Parasitol. 96 3:225-241.

Rowlands GJ, Leak SGA, Peregrine AS, Nagda SM, Mulatu W, D'Ieteren GDM 2001. The incidence of new and the prevalence of recurrent trypanosome infection in cattle in south west Ethiopia exposed to a high challenge with drug –resistant parasite. Acta. Trop.79:149-163.

Saunders DS 1962.Age determination for female tsetse and age composition of samples of *G. pallidipes*, *G.fuscipes* and *G.brevipalpis*, Bull. Ent, Res. 53:579-595.

Swallow BM 2000. Impacts of Trypanosomosis on African Agriculture. PAAT Technical and Scientific Series 2, Food and Agriculture Organization FAO, Rome, pp 52. Not sited

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, Ethiopia

Trail JCM, D'Ieteren GDM, Maile JC, Yangari G 1991. Genetic aspects of control of anaemia development in trypanotolerant N'Dama cattle. Acta Trop. 48:285-291.

Urquhart GM, Armour J, Duncan JL, Dunn AM, Jennings FW 1995. Veterinary Parasitology. The University of Glasgow, Elbs .pp.203-212.