

Clinical Prevalence of Bovine Trypanosomosis in Onedistrict of Benishangul Gumuz Regional State Districts, in and Around Bambasi, North West-Ethiopia

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Abstract: The study was conducted from November 2009-March 2010 in and around Bambasi district indicates that the Trypanosomosis is one of the major economically important disease which affects the Agricultural activities, in reproduction and productivity of cattle. In this study cross –sectional study designed were used. A total of 385 cattle were examined and the diagnostic technique used includes PCV (Packed cell value), Hematocrit centrifugation technique (Buffy coat examination) and thin blood smear. The overall infection rate was 45.1% and the predominant species involved in the infection were *Trypanosomes congolense* accounted 19.74% and 11.68% in females and males, respectively. Followed by *trypanosome vivax* which are accounted 4.17% and 2.59% in females and males, respectively and mixed infection were also examined and scored; 1.82% in males and 4.41% infection rate of the total infection rate 45.1%. Statistical analysis of the results showed significant difference ($P<0.001$). The comparison of PCV values of parasitemia and aparasitemic animals in the study area also indicate statistically significant difference ($P<0.001$). And the PCV of 14% and 15% were minimal PCV values of infected cattle and PCV values of 16% and 17% were examined as the minimal PCV value of non-infected animals. The highest PCV was 35% of the non-infected cattle. Finally the results were discussed and recommended to alleviating the existing condition of Trypanosomosis in the study area.

Key words: Aparasitemic • PCV • Prevalence • Trypanosomosis

INTRODUCTION

Trypanosomosis is a widely spread unicellular flagellated protozoan disease which affects cattle, human and other wide range of hosts in sub-Saharan Africa [1]. The disease is frequently fatal and is a serious constraint to agricultural production in large parts of sub-Saharan Africa, exhibiting direct impacts on livestock productivity, livestock management and human settlement and indirect impacts on crop agriculture and human welfare [2]. Especially tsetse transmitted animal Trypanosomosis is an important constraint to livestock development in Africa. It occurs in around 10 million km² in 37 sub-Saharan countries [3] and constitutes a major threat to the survival and productivity of domestic livestock in sub-Saharan Africa [4].

African Trypanosomosis is one of the most important animal diseases encountered in all agro-ecological zones of the country and hinders the efforts made for food self-sufficiency [5] and the disease causes

about 3 million deaths every year in Africa and approximately 35 million doses of Trypanocidal drugs are being administered every year to enable livestock to survive in tsetse-infested areas [6] and it directly affects the milk and meat productivity of animals, reduces birth rates, increases abortion as well as mortality rates; all of these reduce the herd size and herd composition. The indirect impact of the disease mostly lies on crop production through the availability and cost of animals that provide traction power [2]. Trypanosomosis reduces work efficiency of oxen and discourages the introduction of drought animals in to crop farming [7].

The course of the disease may run from a chronic long lasting to an acute and rapidly fatal depending on the vector-parasite-host interactions. The disease is mainly characterized by intermittent fever, progressive anemia and loss of condition of susceptible hosts which if untreated leads to heavy mortalities [8]. The etiological agent of the disease is unicellular flagellated protozoan parasite of a genus *Trypanosoma*. Trypanosomes are

blood borne unicellular protozoan parasites dwelling in various body and tissue fluids. The parasite is known for more than a century, but still control of the disease remains elusive [9].

Several species of hematophagous tsetse flies of the genus *Glossina* are the vectors of African Trypanosomosis and are responsible for cyclical transmission of the parasitic protozoan between numerous vertebrate hosts. The vector is distributed over wide range of habitats covering about 10 million square kilometers of potential grazing lands in 37 countries which are rendered unsuitable for livestock breeding and farming across the African continent [10].

In Ethiopia, Trypanosomosis is one of the most important diseases that limit livestock productivity and agricultural development due to its high prevalence in the most arable and fertile land of southwest and northwest part of the country following the greater river basins of Abay, Omo, Ghibe and Baro [5]. Currently about 220,000 kilometer square areas of the above-mentioned regions are infested by five species of tsetse flies, namely, *Glossina pallidipes*, *G. morsitans*, *G. fuscipes*, *G. tachinoides* and *G. longipennis* [11]. Despite the considerable number of epidemiological studies carried out in Ethiopia on cattle and camels Trypanosomosis in parts of Southern Nations, Nationalities and Peoples' Region (SNNPR) and in Oromia and Amhara regions, information from Tigray and pastoral areas of Afar, belonging to the tsetse-free areas of Ethiopia, is scanty [12].

In addition, due to limited logistic resources and poor diagnostic facilities, the exact burden and socioeconomic impact of African Animal Trypanosomosis is probably underestimated and information on prevailing trypanosome species and affected hosts remains inaccurate and fragmented [13]. Diagnosis of AAT is often based on clinical suspicion. Parasite detection is cumbersome in many cases where only low numbers of trypanosomes circulate in the host body fluids [14]. A more sensitive technique is the mini Anion Exchange Centrifugation Technique (MAECT) but the technique works best with *T. brucei* and *T. evansi* and has poor diagnostic potential for *T. congolense* and *T. vivax* [15].

According to Food and Agricultural organization (FAO), it is probably the only that profoundly affects the settlement and economic development of the major part of Africa [16]. This disease is also known as African Trypanosomes, because of its in African continent where the disease is most prevalence in this continent [17]. The African Animals Trypanosomosis (AAT) also called

Nagana and it is one of the major threats for the live-stock in Africa. Trypanosomosis (Nagana) which occurs in forty African countries with the exception of South Africa and Namibia is probably the most important disease of live-stock in the continent [18].

Trypanosomosis likely reduce the total production of live-stock by 10-50% [19]. The majority of farmers in sub-Saharan Africa still farming with hand, mainly because of animal diseases, which is mostly Trypanosomosis. Cross breed cattle cannot be introduced before tsetse is eradicated, because of the high risk of Trypanosomosis [20]. Trypanosomosis is a 'Greek' word, meaning (Trypano =borning, soma=body), species of Trypanosomes are parasites of a wide range of vertebrates, from fish to mammals [21]. Trypanosomes are unicellular organisms in which the Trypanosome is classified as flagellate protozoa from the genus Trypanosome of the family Trypanosomatidae which belongs to the order kinetoplastidae of the class zoomastigophara. The zoomastigophara is classified under the phylum Saricomastigophera [22].

The genus Trypanosome is further divided in to two sections: The selivaria; which contain subgenera, Duttonella, Nanomoanospynomanos and Trypanozoon. The salivarian Trypanosomes either undergo cycle development in insect before being transmitted mechanically [23]. The other section is the Sterocoraria which includes three subgenera Megatrypanum, Herpetsoma and Schizotrypanum. The distribution of pathogenic African Trypanosome species in livestock concludes with distribution of the insect vectors. The main pathogenic African livestock Trypanosomes are: *trypanosome congolense*, *trypanosome vivax*, *trypanosome brucei* are the principally transmitted by tsetse (*Glossina* spp), their cyclical vectors [23].

The non-cyclical transmissions occur through feeding of biting flies and insects, notably Tabanidae, Stomoxy and hypcidae. Trypanosomes do not multiply and die quickly, only can carry over a short distance, since it survives only for a limited time [22]. Mechanical transmission also occurs through needs during inoculation by syringe, surgical instrument and in carnivores by feeding on flesh infected carcass [24]. *Trypanosome theiri* and *trypanosome melophagium* transmitted by Tabanide flies and sheep kids, respectively.

All domestic livestock, but particularly important in cattle, also it is common in many wild animals. As with other vector borne disease, tsetse distribution and

density are most influencing factor, by spatial factors such as climate, vegetables and land utilization. The occurrence and impact of Trypanosomosis depend on tsetse challenge and on the number of other factors such as host distribution, livestock breed, farming practice and control practice [25]. The success of Trypanosomosis control program depends on the use of appropriate tsetse and Trypanosomosis control technologies suitable for the area. Therefore before starting any control program it mandatory assess the actual situation of the problem and any review the possible control options to be implemented in the area [26].

Before the establishment of the control of Trypanosomosis, it is important to study the area where the maximum benefits will be obtained. The most important criteria for the control of Trypanosomosis are the epidemiological status, economic cost and the efficacy of measured to control Trypanosomosis [25]. There are different methods of control system such as: chemotrapy, vector control, remove of vegetation, killing of wild animals, spraying of insecticides, trapping, sterile insect techniques (SIT), host protection: So among these technique chemical control of tsetse fly has been limited due to the non-specific enemies, while the sterile techniques (SIT) has high cost and requires significant external support [27].

The severity of the disease depends on the species and strains of Trypanosomes involved. The ability of Trypanosomosis to change their surface coat continuously leads to the exhaustion of the anti-body production by the host, that cause immunosuppressant of the host [1]. Sound knowledge of the basic features of Trypanosomes enables the identification of each species and so the exact cause of disease, once the basic features possessed, all Trypanosomes are appreciated, the diagnostic difference can be recognized and the species identified [28].

So my present study was conducted in BenishangulGumuz Regional state which is one of the nine regions of Ethiopia that is found northern west of the country, a low and one of the places where tsetse flies are reproduced and Trypanosomosis is endemic in occurrence. This Regions contains twenty districts, among these Bambasi district is the largest having large number of human population and cattle population. So the objectives of the study are:

- To established the present prevalence rate of Trypanosomosis in Bambasi
- To identify the species of Trypanosomes present there.

MATERIALS AND METHODS

Study Area: The present study was conducted on the prevalence of bovine Trypanosomosis in BenishangulGumuz Regional state in one of region districts Bambasi district. The region is consists of three administrative zones, namely Assosa zone, Kemashi zone and Metekel zone and twenty districts. The study area is located in low land area of BenishangulGumuz. The altitude of the area ranges from 1100-1450 m.a.l. bordering the Dabus river system to east direction. Bambasi district is situated in north east part of the Assosa town on 45kms. Bambasi district located 9°45', N and 34°45' east. The area covers about 76,140 hectares of the land with the total populations with the total population of 40,985. Topography of the area is marked by hill, sleep slopes and flat surface of the land.

The area has a sub-humid climate with a moderate hot temperature with less variation in average temperature between day time and night. It receives high and reliable annual rain fall. The rain fall in the area is bimodal. The mean annual rain fall is recorded over 1375 mm year period at MOA Bambasi station was 1350 – 1400 mm. The long dry season lasts from December – May. The area experiences a mean annual temperature of about 32°C. The highest average monthly temperature occurs in May (29-32°C) month, where the mean maximum temperature is 35°C. The coldest month is August when the average monthly minimum temperature 21°C.

The vegetation denominated by wooded bamboo trees and savannah grasses. Bamboo is the most common woody vegetation in the district. The livestock management system is mixed farming system. The livestock in the species in the area are: Bovines, Caprine, Ovine and Equidae (Donkey). The livestock population in the district as recorded from Bambasi district Agricultural and rural development (BWARD), the animal population of Bambasi is estimated as cattle -30,783, sheep- 5670, Goats – 9322, Donkey – 2907 and Chickens – 20390. The local human population is principally engaged in the livestock crop production activities. The major crop growing in the area are Maize, Losses and Fruits like Mango, Banana, Lemon, Orange and others. The most known disease in the area Trypanosomosis which is transmitted by tsetse flies in the low land, along the Dabus river basin. The major livestock in the region are: Trypanosomosis, Pastuerellosis and internal and external parasites are the known one in the area.

Study Design: The study type was cross sectional survey conducted to determine prevalence of Bovine Trypanosomosis from November 2009 – March 2010. Blood sample were collected from the ear vein of the clinically infected cattle by using lancet needle and heparinized micro-hematocrit capillary tube to its 3/4th volume. The study animals were zebu cattle under extensive traditional husbandry system. The animals graze the communally owned pasture land throughout the year. The area managed under the same agro-ecology without ant supplementary feeding.

A total of 385 blood samples were collected from cattle comes to Bambasi veterinary clinic, from those from Bambasi town and around the town. The sample size was determined by using 95% level of confidence interval and the expected prevalence of 50% of Trypanosomosis with the desired absolute precision of 5% and simple sample random sampling method was used [29]. The formula used is shown below:

$$n = \frac{1.96^2 p_{exp}(1-p)}{d^2}$$

where:

p_{exp} = expected prevalence

P = prevalence

d = desired absolute precision

n = required sample size

Parasitological Examination: After blood samples are collected using the above mentioned parasitological technic the laboratory diagnostics procedure followed step by step to identify the species of the parasite in sample collected. The capillary tube were loaded on micro – hematocrit centrifuge systematically and centrifuged at 1200 rpm for five minutes [30].

Wet Blood Films, procedure small drop of blood is placed on to a clean glass slide covered with a cover slip to spread the blood as a monolayer of the cell. This examined by light microscope (x40). The microscope objective is detecting any motile Trypanosomes. But it will not enough to identify the species of Trypanosomes properly.

Stained Thin Smear: A drop of blood is placed from one end of a clean microscope slide and a thin film is drawn out in the usual way. The film is dried in air briefly, fixed in methyl alcohol for two minutes and allowed to dry.

The smears are then stained by Giemsa and the stained slides must slides for 30 minutes. This techniques permits detailed morphological studies and identification of the Trypanosomes species. The same technique used with the lymph nodes biopsies.

Data Analysis: During the study periods the owner's name, address, animal sex, age [25] recorded using the animal's blood sample collection (Health) format. Hematological and parasitological data were handled similarly. Data on individual animals and parasitological examination results were inserted into MS-excel Microsoft wares. Then the entered data was transferred to spss software program as described in the protocol of Thrust field [29]. Descriptive statistics for explain prevalence, students t-tests for comparison and p-value for decision making were used.

RESULTS

Parasitological Findings: In this study area from November, 209 to March, 2010 a total of 385 heads of cattle were examined, out of which 173 animals were infected with various species of Trypanosomes. The overall prevalence of Trypanosomosis disease in was estimated to be 45.1% (173 cases out of 385). Out of the total animals examined 121 cases (31.5%) were due to Trypanosomes Congolese, 28 cases (7.3%) due Trypanosomes vivax and mixed infections were recorded for 24 cases (6.2%). Trypanosome congolense was estimated to be the most infective species of Trypanosomes in the study area followed by Trypanosome vivax indicated in table one below.

Totally 385 samples were collected and from the total 212 cattle were found aparasitaemic and 173(45.1%) examined to be the carriers of the disease. The above table indicated that the Trypanosome congolense 121 cases (31.4%) were shown more common occurrences in the study area than Trypanosomevivax 28(7.3%). Mixed infection was recorded in 24cases (6.2%) among the total of 173 cases. The prevalence of Trypanosomosis is varying in both sexes, in which females are more affected more frequently than male. From the results obtained during examination were 77 (49.7%) and 48(21%) in female and male respectively, which is statistically significant ($p < 0.05$), but the result indicates that there is no significant difference between the occurrences of Trypanosomevivax and mixed infection of both Trypanosomes species.

Table 1: Prevalence of Trypanosomes infection in Bambasi district among cattle presented to the veterinary clinic.

District	Total	Positive cases	Prevalence (%) within species		
Bambasi	385	173(45.1%)	<i>T. congolense</i> 121(31.4%)	<i>T. vivax</i> 28(7.3%)	Mixed infection 24(6.2%)

Table 2: the prevalence of Bovine Trypanosomes across different age and sex groups

variables	category	Animas sampled	Infection prevalence (%)	F value	P value
Sex	male	230	21	35.05	P<0.001
	females	155	49.7		
Age	<2 years	2	0	14.94	P<0.001
	2-6 years	331	29		
	>6years	52	52		

Table 3: Effect of parasitaemic on packed cell volume

Animal status		Animal sample(N)	Mean PCV(%0	F value	P value
parasitaemic	<i>T. congolense</i>	121	17.13	295.32	<0.001
	<i>T. vivax</i>	28	16.36	44.05	0.002
	mixed infection	24	16.42	35.78	0.001
	Total positive	173	49.91		
Total sampled		385			

Statistically significant difference (p<0.05) was seen between PCV values of parasitaemic and aparasitaemic cattle.

DISCUSSION

The study was revealed that the Trypanosomosis caused by different species of Trypanosomes were common in and around Bambasi town in domesticated livestock specially cattle. The overall disease prevalence was 45.1%. From the total prevalence obtained *trypanosome congolense* accounts for about 31.4% of the total positive sampled cattle. These relative high frequencies related with nutritional deficiencies and suitability of the area for reproduction of tsetse fly. The prevailing poor veterinary services, improper application of drug administration by non-professional could also be responsible for the application of the disease in the area as an endemic situation.

Furthermore, animals greater than two years old were more prone to the Trypanosomosis (P>0.05), which may due chronic nature of the disease in the cattle. A significant association of a disease with sex was observed where the prevalence in the females were higher than in males (p<0.001). In contrast, local and cross breed which were managed under the same environmental condition were seed not equally affected by Trypanosomes, suggesting that the local breeds are developing resistance of the disease. The prevalence bovine Trypanosomes were not only influenced by sex, age and breed, but most of the time it was affected by presence absence of tsetse fly vectors and agro-ecological situation of the area.

The prevalence (45.1%) of Trypanosomosis observed in the study area was in the line with the previous findings (39.8%), NTTICC [11] that was reported in the neighboring and present association of the present study area. The results suggested that the presence of Trypanosomosis infection in Bambasi district was slightly higher than the prevalence of other tsetse infested area of the country that observed by Murray [31] who report the prevalence of 31% in kindo-koysa district of southern Ethiopia.

The presence of many drug venders and drug administration is largely performed by unprofessional in the presence of trypanosomes *congolense*; in the Bambasi district may be indicative for high prevalence of Trypanosomosis. The higher proportion of trypanosome *congolense* (31.1%) in Bambasi district shows similar prevalence with trypanosome species from other tsetse infested regions of the country. Abebe and Jobre [5] who reported a prevalence of 34.5% for Trypanosome *congolense* in gibe district. Different researchers reported relatively lower prevalence of Trypanosome *congolense* in different regions of the country [2, 32, 33].

They reported a prevalence of 17.2%, 21%, 23% and 17.5% of Trypanosome *Congolese* in metekel district, southern rift valley and upper Didessa valley of tsetse infested region respectively. The development of anemia was the most reliable indicator of the progress of Trypanosomes infection [34]. The disease was assumed that the numerous concurrent diseases and nutritional

factors interfere with the anemia development and profoundly PCV is reliable indicator of anemia [3]. Thus, significant difference in PCV of the cattle due to Trypanosomosis in the ruminants was obtained in various studies done so far and that of Trypanosomes infections [5, 35, 36].

During PCV determination a value of 24-46% [35] was considered to be normal range. In the study area trypanosomes infections resulted in a significant decline in PCV, similar results reported by Mihiret [36] in and around Bahir Dar. A higher infection rate was observed in adult animals and animals above two years of age in the stud area, but sucking calves are at low risk of the disease because of they do not go out with their dam and grazing at home lands until they are weaned off [37]. Young animals are also naturally protected to some extent by maternal antibodies [38]. This could results in low prevalence of the Trypanosomes that was observed in the calves also as obtained from the study.

The difference between mean PCV value of parasitaemic and aparasitaemic indicates that Trypanosomosis is involved adversely by lowering PCV value of infected animals to the level of 14% [30] or may due to compound effects of poor nutrition and haematophagus helminthes infection [32]. A field report made by Alelign in 2003 for Thesis fulfillment in Benishangul regional state shows that the prevalence of Trypanosomosis in one of Assosa district kebeles, Urahkebele was 8.3% and Astostokebele was 23%, similarly in this study the prevalence of Trypanosomosis in Assosa district Abramo site was 16.6%, Afasim is 9.4% Amba (Village-7) is 32%. Likely the survey made by IIRAD, Tewelde [34] showed that the prevalence of Trypanosomosis in the two sites of Assosa district was 33.3% in the sites of Megelle -36 kebele and 27.8% in village-9. Studies done in Ghibe south west of Ethiopia indicate the prevalence of 41% in adult cattle and reduced to 16% during the year of tsetse control [37].

CONCLUSIONS

The study recorded high prevalence (45%) of Trypanosomosis. From result obtained of the prevalence in the study area displayed that Trypanosomosis is the major disease of animals in the study area, that potentially threat and effect the health, production, reproduction and productivity of cattle in Bambasi district. Infections with Trypanosomosis were found to be negatively affect the PCV value and body condition of affected animals.

Thereby it denoted that Trypanosomes infection of cattle in the study area, in which mostly calves' loss weight and decrease in growth rate, particularly where it was showed that Trypanosome congolense with the prevalence of 31.4% and Trypanosomes vivax with the prevalence of 7.3% profound dominated in the study area in Bambasi district. Trypanosomes congolense and Trypanosome vivax were exhibited that they are mainly transmitted by tsetse flies. And based on the above conclusions the following recommendations were forwarded:

- The prevalence of Trypanosomosis founded in the study area was seems to be high, therefore, attention should be given to the implementation of Trypanosomes control strategies to minimize the risk of the disease in the study area.
- Major concerns should be concentrated on the identified species of trypanosomes with the highest prevalence and control measures should be targeted accordingly.
- Research works conducted on the biology of Trypanotolerant, utilizations and propagations of Trypanotolerant breeds should be reinforced.

REFERANCES

1. Brown, C.G.D. and A.G. Lukins, 1990. Disease caused by protozoa. Hand book on animal diseases in Tropics (Swell and Brocklesby), 4th. ed. Bailiere. Tindall, London.
2. Swallow, B., 2000. Impact of Trypanosomosis in Africa, Agricultural. PAAT Technical and scientific series. N0. 2. Rome. Italy.
3. OUA/STRC, 2001. Trypanosomosis, Tsetse and Africa, the year 2001, report.
4. Oluwafemi, R.A., A.A. Ilemobade and E.A.O. Laseinde, 2007. The impact of African animal trypanosomosis and tsetse on the livelihood and wellbeing of cattle and their owners in the BICOT study area of Nigeria. *Sci Res Essay*, 9(9): 380-383.
5. Abebe, G. and Y. Jobre, 1996. Trypanosomes threats to the cattle productions in Ethiopia. *Revue mod, vet*, 147: 872-897.
6. Mattioli, R.C. and J. Slingenbergh, 2013. Programme Against African Trypanosomiasis (PAAT) Information System. <http://www.fao.org/ag/AGInfo/programmes/en/paat/disease.html>.
7. Omotainse, S.O., J.O. Kalejaiye, P. Dede and A.J. Dada, 2004. The current status of tsetse and animal Trypanosomosis in Nigeria. *J. Vet Sci.*, 1: 1-9.

8. Bourn, D.M., R.S. Reid, D.J. Rogers, W.F. Shnow and G.R.W. Wint, 2001. Environmental Change and the Autonomous Control of Tsetse and Trypanosomosis in Sub-Saharan Africa: Case Histories from Ethiopia, Gambia, Kenya, Nigeria and Zimbabwe. Oxford, UK: Environmental Research Group Oxford Limited.
9. Baral, T.N., 2010. Immunobiology of African trypanosomes: need of alternative interventions. *Journal of Biomedicine and Biotechnology*, 2010:24.
10. Kuzoe, F.A.S., 1993. Current situation of African trypanosomiasis. *Acta Tropica*; 54(3-4):153-162. doi: 10.1016/0001-706X(93)90089-T. [PubMed] [Cross Ref]
11. NTTICC, 2004. Annual report for the period of 7th June, 2003 to 6th July 2004. Bedelle, 1.
12. Sinshaw, A., G. Abebe, M. Desquesnes and W. Yoni, 2006. Biting flies and Trypanosomavivax infection in three highland districts bordering Lake Tana, Ethiopia. *Vet Parasitol.*;142:35-46. doi: 10.1016/j.vetpar.2006.06.032. [PubMed] [Cross Ref]
13. Fikru, R., B.M. Goddeeris, V. Delespaulx, Y. Moti, A. Tadesse, M. Bekana, 2012. Widespread occurrence of Trypanosomavivax in bovines of tsetse- as well as non-tsetse-infested regions of Ethiopia: a reason for concern? *Vet. Parasitol*, 190:355-61. doi: 10.1016/j.vetpar.2012.07.010. [PubMed] [Cross Ref]
14. Büscher, P., 2001. Diagnosis of human and animal African trypanosomiasis. In: Black SJ, Seed JR, editors. *The African Trypanosomes*. Boston: Kluwer Academic Publishers, pp: 5163.
15. Büscher, P., N.D. Mumba, J. Kabore, V. Lejon, J. Robays and V. Jamonneau, 2009. Improved models of mini Anion Exchange Centrifugation Technique (mAECT) and Modified Single Centrifugation (MSC) for sleeping sickness diagnosis and staging. *PLoS Negl Trop Dis.*;3:e471. doi: 10.1371/journal.pntd.0000471. [PMC free article] [PubMed] [Cross Ref]
16. FAO, 1998. A field guide for diagnosis, treatment and prevention of African Trypanosomes.
17. Wright, P.F., E. Nilsson, E.M.A. Van Rooij and M. Lelenta, 1995. Action of enzyme Linked Immunosorbent assay 77(49.73%) techniques for the detection of antibody infections disease diagnosis. *Rev. Scic. Tech. offin. Epiz*, 12: 435-450.
18. ILIRI, 1996. Newsletters International Livestock Research Institute (ILIRI), Livestock for development 1, January, 1996, Addis Ababa, Ethiopia.
19. ISCTRC, 1997. Estimating the cost of Animals Trypanosomes in Africa. International Laboratory for research on Animal Diseases, Nairobi, Kenya, pp: 1-4.
20. PAAT, 1990. Comments on SIT draft position paper by discussion group members of PAAT.
21. Kettle, D.S., 1996. Medical and Veterinary Entomology UK, CAB. International, walling ford, pp: 503-557.
22. Urquhart, G.M., I.L. Dumcan, A.M. Dunn and F.A. Jennings, 2003. *Veterinary Parasitology*. 2th. ed. Black Well Publishing, pp: 212-217.
23. Hoare, C.A., 1996. Trypanosomes of mammals. Black well scientific publications, Edinburgh, U.K., pp: 749.
24. Seifert, H.S.H., 1996. *Tropical Animal Health*, Kluwer Academic Publisher Boston, London, pp: 230.
25. Snow, W.F. and P. Rawlings, 1999. Methods for the rapid appraisal of African animal Trypanosomosis in the Gambia. *Prev. Vet. Med.*, 42(2): 67-86.
26. Dermott, J.M.C., 1996. Requirements for diagnostic tests to improve field investigations into the epidemiology and control of Trypanosomosis antigen ELISA for trypanosomes, Evaluations of performance proceeding of the work shop at ILRI Nairobi, Kenya, 9-11: 3-4.
27. Awoke, K., 2002. Study of Trypanosomosis and its vectors in Humba and Merabworedas, of Eastern Ethiopia: *Journal of Ethiopian veterinary association*, IV: 81-83.
28. ESTC, 1997. Ethiopian science and Technology Commission. Integrating the sterile insect technique to eradicate testes from southern rift valley, project proposed.
29. Uilenberg, G., 1997. A field guide for diagnosis, treatments and prevention of African Animals Trypanosomosis, adapted from the original edition by Boyt, W.P., FAO. Rome.
30. Thrustfield, M., 2005. *Veterinary Epidemiology*. 3rd. London, UK: Black Well Science.
31. Murray, M., 1997. Parasitological techniques for the diagnosis of African Trypanosomosis, R. imber, C.D., Evans, D.A. and Doig, S.J., Trypanosome brucei miniature anion- exchange centrifugation techniques for detection of low parasitoids. Adaptation for field use. *Trans. R. Soc. Trop. Med. Hyg*, 73: 312-317.
32. Ademe, M. and G. Abebe, 2001. Field study on drug resistant Trypanosomes of cattle (*Bosindicus*) in Kindo-koysa district, southern Ethiopia. *Bull. Anim. Hlth. prod. Afri*, 48: 131-132.

33. Afework, Y., 1998. Field investigation on the appearance of drug resistant Trypanosomosis in Metekel district, Northwest of Ethiopia; MSC thesis, Addis Ababa with universitat, Berlin.
34. Tewelde, N., 2001. Study on occurrence of drug resistant Trypanosomosis in cattle in the farming in the tsetse control areas (FIFACA) project in the western Ethiopia; MSC, Thesis, Addis Ababa University and frieUnversitat, Berlin.
35. ILRAD, 1994. International Laboratory for Research in animal's disease. Animal Report, Nairobi, Kenya.
36. Blood, D.C. and O.M. Radostits, 2007. Veterinary Medicine: A Text Book of Diseases of Cattle, Sheep, Pigs, Goats and Horses. 10th. BailliereTindall.
37. Getinet, Y., 1994. Prevalence of bovine Trypanosomosis in DebireMarkos district of eastern Gojjam Administrative Zone. Faculty of veterinary medicine, AddisAbaba, DVM, Thesis.
38. Rawlands, G.H., S.G.H. Leak, W. Mulatu, S.M. Nega, A. Wilson and G.D.M. d'Ieter, 2001. Use of deltamethrin sprays on insecticides for the control of cattle Trypanosomosis in the presence of high tsetse fly invasions. Med. Vet. Entomol, 15: 87-96.