Academic Journal of Animal Diseases 4(1): 01-09, 2015
ISSN 2079-200X

DOI: 10.5829/idosi.ajad.2015.4.1.86195

Epidemiology of Non Tsetse Transmitted Trypanosomosis in Cattle, in Amhara Regional State of Bahir Dar Zuria and Fogera District, Ethiopia

[1]Anwar Shifaw, [2]Mebratu Asaye and [3]Habtamu Biyazen

[1]College of Veterinary Medicine and Agriculture,
Addis Ababa University, P.O. Box: 34, Debre Zeit, Ethiopia
[2]Faculty of Veterinary Medicine,
University of Gondar, P.O. Box: 196, Gondar, Ethiopia
[3]College of Agro-Industry and Land Resource,
Haramaya University, P.O. Box: 226, Chiro, Ethiopia

Abstract: Trypanosomosis is a debilitating disease of man and domestic animals which is caused by haem of lagelate of the genus *Trypanosoma*, family Trypanosomatidae. The objectives of this study were: To determine the current status of bovine trypanosomosis in non-tsetse infested highland North Western part of Ethiopia, to assess an agreement between the parasitological and molecular tests and to assess the risk factors associated with prevalence of animal trypanosomosis in the area. A total of 357 cattle were examined for trypanosomes using microscopy (woo test) and molecular test (Internal Transcribed Spacer PCR). The study was conducted in two selected districts of North West of Amhara Regional state, namely Fogera and Bahir Dar Zuria. The local indigenous zebu cattle and Fogera breeds were considered for this study. To assess the status of bovine trypanosomosis, general information was obtained from the settler through structured questionnaire survey. About 800 µl of blood sample was collected by heparinized vacutainer tube from jugular vein for subsequent DNA extraction and PCR test. In the present study the overall prevalence of trypanosomosis was 1.4% and 43.3% recorded by woo test and PCR respectively. The ITS PCR result revealed that the common trypanosome species in the study area were *T. vivax, T. theileri* and *trypanozoa (T. evansi).* Among these *T.vivax* was the predominant species, which cover 81% of the total positive cases and followed by *T. theileri,* 10%. Significantly higher prevalence was obtained by using the ITS PCR than classical parasitological examination. Ths comparison shows that the better efficiency of molecular test, which will become in the future an efficient tool to estimate the prevalence of African trypanosomosis in affected area. The effect of host related risk factors (sex, age, body condition and treatment) on the prevalence of trypanosomosis was also studied in this research. However, significant difference ($p > 0.05$) was not observed. A prevalence difference obtained by the two techniques suggests molecular techniques are better for the diagnosis of the disease in the affected area.

Key words: Trypanosomes · PCR · Woo test · *Glossina* spp.

INTRODUCTION

Trypanosomosis is a debilitating disease of man and domestic animals. It is caused by haemoflagelate of the genus *Trypanosoma*, family Trypanosomatidae. It is transmitted by tsetse flies (*Glossina* spp) and biting flies [1] and the disease is characterized by parasitaemia, fever, anemia, loss of condition, reduced productivity and frequently high mortality which among the other factors limit the pace of rural development in tropical Africa [2-4]. Trypanosomosis complex is described by the World Health Organization [1] as serious diseases lacking effective control measure and all mammalian species are susceptible to the infection [5].

Corresponding Author: Mebratu Assaye, Faculity of Veterinary Medicine, University of Gondar, P.O. Box: 196, Gondar, Ethiopia. E-mail: assayemebratu@yahoo.com.

In Africa trypanosomosis is not only restricted to areas in which the vector tsetse fly (*Glossina* spp) can survive but also found outside the tsetse belt area as it is transmitted mechanically by flies of genus *Tabanus, Haematopota, Hippobusca stomoxys* and others. This type of transmission has caused the spread of *Trypanosoma evansi* and *T. vivax* outside the tsetse infested areas [6, 7].

In Ethiopia, there are five economically important animal trypanosome species which includes *T. congolense, T. vivax, T. brucei, T. evansi* [8] and *T. equiperdem* [9]. Among them the most prevalent species are *T. congolense* and *T. vivax*. Apart from the cyclical transmission of trypanosomosis by the *Glossina* spp, mechanical transmission is also a potential threat to livestock productivity in many parts of Ethiopia [7]. However, information on prevalence of the disease in animals and the vectors involved is scanty and sufficient data in compiled form is not available.

Accurate diagnosis of trypanosome infection in livestock is required for a proper understanding of the epidemiology of the disease in any geographical locality. However, high parasitaemia are usually evident only in early infections and in chronic phases of the disease. Parasitaemia are apparently absent from the blood for long intervals. This is due to the ability of the trypanosome to establish prolonged infections attributed to the phenomenon of antigenic variation [10]. Therefore, a diagnostic method with high degree of sensitivity and specificity is required like a molecular technique (PCR), besides direct (parasitological) and indirect (serological) diagnostic methods with varying degree of sensitivity and specificity [11, 12]. The polymerase chain reaction (PCR), using primers designed from satellite genomic DNA sequences specific for different taxonomic groups, has proven the most specific and sensitive experimental technique to detect trypanosomal DNA in either the vector or the host [13-17].

Understanding the epidemiology of the disease will facilitate the choice of suitable control method and it helps in planning for development strategies in the area. The present study was therefore carried out to determine the current status of bovine trypanosomosis in non-tsetse infested highland North Western part of Ethiopia, to assess the agreement between the parasitological and molecular tests and to assess the risk factors associated with prevalence of animal trypanosomosis in the area.

MATERIALS AND METHODS

Study Area: The study was conducted in two selected districts of North West of Amhara Regional state, namely Fogera and Bahir Dar zuria. Fogera is situated at 11°58' N latitude and 37°41' E longitude. The altitude ranges from 1774 to 2410 m.a.s.l. The mean annual rainfall is 1216.3mm and ranges from 1103 to 1336mm. The woreda is divided into 25 peasant association and 5 urban kebeles. Among 25 PAs; sampling was conducted in Shina, Kokit and Shaga with the respective elevation (altitude) of 1792 m.a.s.l, 1793 m.a.s.l and 1790 m.a.s.l. and location of 11° 53.402' N 037° 38.89' E, 11°58.780' N 037°42.397' E and 11°56.1' N 37°38.293' E respectively. The district is mainly dominated by one long rainy season. There are about one hundred fifty seven thousand cattle, seven point six thousand sheep, two hundred fort six point five thousand poultry, twenty one point eight thousand beehives, thirteen point two thousand donkeys and a very few horses and mules in the district [18].

Bahir Dar zuria district is located 565 Km North West of the capital, Addis Ababa. The elevation of the area is 1780 masl. The annual temperature and rainfall in the study area are about 9-34 °c and 900-1500 mm, respectively [19]. The wet season lasts from April to September where as the dry season lasts from October to March [20]. Among Bahirdar zuria's PAs, sampling was conducted in Meshenti with an altitude of 1700 m.a.s.l and located at 11°29' N 39°29' E and Zenzelima with an altitude of 1907m.a.s.l and located at 11°37.619' N 037°27.963' E [19].

Study Population: The local indigenous zebu cattle and Fogera breeds were considered for this study. They were kept under traditional extensive husbandry system with communal herding and mixed crop livestock production system. During the day, cattle were herded together and looked after by herd's men. Male cattle over three years of age were used as plauphing oxen. Animals work usually during the morning, particularly during wet season and graze the rest of the days.

Study Design and Methodology

Sampling Method and Sample Size Determination: The sampling method applied in the present study was simple random sampling including all body condition of the animals, age and sex. The desired sample size was calculated according to the formula given by Thrusfield

[21] based on the expected prevalence of 32% [22] and absolute desired precision of 5% at confidence level of 95%. As a result, a total of 357 cattle were sampled.

Questionnaire Survey: To assess the status of bovine trypanosomosis, general information was obtained from the settler through structured questionnaire survey. Information is sought on the history of settlement, socio-economic issues, farming system, livestock management and constraints and problems of bovine trypanosomosis in the settlement schemes. Information was acquired from the respondents on herd performance parameters, herd structure, cattle ownership and current disease management practice.

Sample Collection, Parasitological and Hematological Examinations: Whole blood sample was collected from jugular vein of each animal using heparinized vacutainer tube. Hematocrit capillary tube was filled with the blood and sealed with crystal clay. The capillary tube was centrifuged as soon as possible with a hematocrit centrifuge and PCV value was read by hematocrit reader for determination of anemia. Placed the hematocrit tube on viewing chamber and buffy coat layer at the center on the stage of compound microscope and examined at the level of buffy coat layer for the presence of trypanosome movement and the remaining blood sample send to molecular test for further diagnostic approach.

Molecular Test: About 800 µl of blood sample was collected by heparinized vacutainer tube from jugular vein and then transported (at room temperature) to Addis Ababa university, the Ethio-Belgium VLIR

Laboratory for subsequent DNA extraction and PCR test. The DNA extraction was carried out according to QIAGEN spin protocol [23].

Polymerase Chain Reaction (PCR): In this study Polymerase chain reaction which is based on detecting the ribosomal DNA Internal-transcribe spacer 1 (ITS-1) of the trypanosome was used. It is PCR in which all species of trypanosome can be detected in single reaction. The protocol of ITS-1 PCR was adopted from Claes *et al*. [24]. In general, 22.5 µl premix was made per reaction, containing: 14.65 µl H_2O, 1 µl $MgCl_2$. 1 µl forward primer, 1 µl reverse primer, 2 µl dNTPs, 2.5 µl buffer, 0.25 µl BSA and 0.1 µl hot start DNA polymerase (Table 1). Then 2.5 µl of sample was added to each PCR tubes containing reaction mix.

Table 1: Reaction mixture used for PCR amplification (for one sample)

Reaction mixture	Concentration (µl)
H_2O	14.65
$MgCl_2$	1
buffer	2.5
dNTPs	2
ITS1-F	1
ITS1-R	1
BSA	0.25
Hot start	0.1
Sample DNA	2.5

The amplification of DNA in PCR was carried out in a 25 µl reaction mixture in which the thermal cycling was carried out in a Thermal Cycler using the following conditions: $94°C^{15:00}$ [$94\ °C^{0:30}$; $64°C^{0:30}$; $72\ °C^{0.30}$] 40; $72\ °C^{5:00}$ which means an initial denaturing step of 15 minutes at 94°C followed by 40 cycle of 94°C for 30 seconds, 64°C for 30 seconds and 72°C for 30 seconds and then final extension at 72°C for 5 minute. Typically, PCR consists of a series of 40 repeated temperature changes, called cycles (PCR cycle), with each cycle commonly consisting of 3 discrete temperature steps (denaturing at 94°C, annealing at 64°C, elongation at 72°C). The sequence of the primers and the expected amplification product for each species of the trypanosome was as indicated in Table 2 [25].

Gel Electrophoresis: In this study a total of total 15 µL of amplification product was loaded onto a 2% agarose gel and electrophoresis was conducted for 30 min at 100 V. The gel was stained with Ethidium bromide for 20 minutes and the samples were examined under UV light.

Table 2: Primer sequence, trypanosome species and the expected band sizes (Amplification product) on amplification with ITS1 R and ITS1 F primers.

Primer	Primer sequence	Trypanosome species	Amplification product
ITS1-F	5'TGTAGGTGAACCTGCAGCTGGATC-3'	*T.vivax*	150
ITS1-R	5' CAAGTCATCCATCGCGACACGTT-3'	*T. theileri*	350
		Trypanozoa	450
		T. congolense	650

Data Analysis: Row data on individual animals for parasitological examination, questionnaires and molecular test results were edited in the Microsoft excel spread sheet to create a data base using SPSS (statistical package for Social science) version 15 for data analysis. The prevalence of the disease was calculated by dividing the numbers of animals positive for the test by the total animals examined. The degree of the diagnostic test was assessed using KAPA stastics as described in Viera and Garret [26]. Furthermore chi-square test was applied to compare the prevalence of the trypanosomosis infection in different risk factors.

RESULTS

Parasitological Findings (Woo Test) Result: Out of 357 individuals considered in the study area for trypanosome infection, a prevalence of 1.4 % (5 positive cases) was detected. The prevalence rate in the study area of Bahir Dar zuria and Fogera was 0.8% and 0.6% respectively and there was no significance difference ($p > 0.05$) between the zones, peasant associations in the districts, sex, age, body condition and treatment by woo test (Table 3, 4, 5, 6).

ITS PCR Result: By ITS-1 PCR the overall prevalence of trypanosomes was 43.3%. The prevalence of trypanosomes was 19.5% and 23.8% in Bahir Dar zuria and Fogera district respectively. There was a significant difference ($p < 0.05$) observed between the zones and PAs (Table 7 and 8), however there was no significance difference ($p > 0.05$) between sex, age, treatment and body condition (Table 9 and 10).

ITS-1 PCR result revealed that the majorities of the detected infections were due to T. *vivax* (35%) followed by *T. theileri* (4.5%), mixed infection *(T. vivax and T. theileri* (2.5%), then by T*rypanozoon* (0.8%) and finally mixed infection of *T. theileri* and *Trypanzoon (*0.3%*)*. Among these *T. vivax* was the predominant species that covers 81% of the total positive cases (154 positive cases). There was statistically significant difference ($p < 0.05$) between the zones and peasant associations by ITS-1PCR. However there was no significant difference. ($p > 0.05$) observed between sex, age, body condition and treatment.

Comparison Between Parasitological and PCR Results: The two tests on the original data set showed a significant difference between the prevalence using PCR and the parasitological one ($p < 0.01$). Indeed it represented more than 30 times the prevalence detected parasitologically (43.3% vs. 1.4%). The main difference consisted in the number of positive cases of *T. vivax* and *T. theileri,* which dramatically increased using the molecular technique. Furthermore, PCR allowed the accurate diagnosis of mixed infections, which could not be detected by parasitological examination.

DISCUSSION

In order to assess the current epidemiological situation of bovine trypanosomosis, a total of 357 cattle in two PAs of Bahirdar zuria and three PAs of Fogera districts were examined for the presence of trypanosome infection to determine the disease prevalence during the month April (at dry season). The overall prevalence of trypanosomosis in the current study detected by woo test was 1.4%. In this study the prevalence of trypanosomosis is much lower than the previous reports by Feyesa [27] which a study conducted on a district bordering Lake Tana, by Shimelis [22], a study conducted at Abay basin of north west Ethiopia, by Feyesa [27] at upper Didessa valley and by Yeshitila [28] at Sokoru district in Jimma zone. These all documented the overall prevalence of 6.1%, 14.68 %(31.65% *T. vivax*), 7.9% and 8.36% (36.55% *T. vivax*) respectively. Solano *et al.* [16] reported the overall prevalence of 5.2% by parasitological test in south west of Burkina Faso. These differences might be due to the failure to detect trypanosomes if the number of parasites is too low, as is the case with chronic infections [29], seasonal viration, microclimatic conditions which do not favor the devolvement and the multiplication of flies, management system and immunity of the herds, which all are considerable factors.

Due to seasonal differences, the prevalence of *T. vivax* infection is varied. In Nigeria a study conducted on zebu cattle has revealed a high infection rate during rainy season (9.5%) than in dry season (1.5%) by Kalu [30]. Tamasaukas *et al.* [31] has also indicated that bovine trypanosomosis was low during the dry season and this is due to major change in the environmental and agro-ecological conditions of the farms during dry season as compared to rainy season. Roulands *et al*. [32] found that the prevalence of cattle and sheep was 2-4 times lower in dry season than rainy season. In similar trend, Alekaw [27] reported that at dry season trypanosome infection decreased by the fact that the owners treat their animals. Hence, in concomitant with decrease in fly population during the dry season, self cure phenomenon of *T. vivax* infection also contributed the decreased prevalence [33].

Table 3: The prevalence of trypanosomosis between district by woo test

		Woo test negative	Positive	Total Number of animals examined
District	Bahir Dar Zuria	142(39.8)	3(0.8)	145(40.6)
	Fogera	210(58.8)	2(0.6)	212(59.4)
Total		352(98.4)	5(1.4)	357

There was no significant difference ($p > 0.05$) between the zones

Table 4: The prevalence of trypanosomosis among PAs by woo test

		Woo test negative	Positive	Total Number of animals examined
PA	Kokit	71(19.9)	1(0.3)	72(20.2)
	Meshenti	69(19.3)	3(0.8)	72(20.2)
	Shaga	72(20.2)	1(0.3)	73(20.4)
	Shina	67(18.8)	0(0)	67(18.8)
	Zenzelima	73(20.4)	0(0)	73(20.4)
Total		352(98.6)	5(1.4)	357

There was no significant difference ($p > 0.05$) between APs

Table 5: The prevalence of trypanosomosis between sex, age group and body condition by woo test

		Woo test (%)		
		Negative	Positive	Total Animals examined
Sex	F	208(58.3)	2(0.6)	210(58.8)
	M	144(40.3)	3(0.8)	147(41.2)
Age group	adult	225(63)	3(0.8)	228(63.9)
	old	59(16.5)	1(0.3)	60(16.8)
	young	68(19)	1(0.3)	69(19.3)
body condition	Good	162(45.4)	2(0.6)	164(45.9)
	Poor	19(53.2)	3(0.8)	193(54.1)
Total		352(98.6)	5(1.4)	357

There was no significant difference ($p > 0.05$) between between sexes, age group and body condition by woo test

Table 6: The prevalence of trypanosomosis between treatment by woo test

		Woo test negative	Positive	Total Animals examined
Treatment	Anthelmintic	120(33.6)	0(0)	120(33.6)
	Antitrypanosome	8(2.2)	0(0)	8(2.2)
	NO	222(62.2)	5(1.4)	227(63.6)
	Oxytetracyclin	2(0.6)	0(0)	2(0.6)
Total		352(98.6)	5(1.4)	357

There was no significant difference ($p > 0.05$) between treatments

Table 7: The prevalence of trypanosomosis between the two zones by ITS PCR

	PCR						
	N	T.v	T.v+Tt	Tt	Tt +Tz	Tz	Total Animals examined
S. Gonder	127 (35.6)	73 (20.4)	7 (2)	4(1.1)	0	1 (0.3)	212 (59.4)
W. Gojam	76 (21.3)	52 (14.6)	2 (0.6)	12 (3.4)	1 (0.3)	2 (0.6)	145 (40.6)
Total	203 (56.9)	125 (35)	9 (2.5)	16 (4.5)	1 (0.3)	3 (0.8)	357

N= Negative, T.v= *T.vivax*, Tt= *T. theileri*, Tz= *Trypanozoa*

No significant difference ($p > 0.05$) in prevalence between the zone by Woo test, but significance difference ($p < 0.05$) was observed by ITS PCR

Table 8: The prevalence of trypanosomosis among PAs by ITS PCR

		PCR						
		N	T.v	T.v+Tt	Tt	Tt +Tz	Tz	Total Animals examined
PA	Kokit	46(12.9)	17(4.8)	5(1.4)	3(0.8	0(0)	1(0.3)	72(20.2)
	Meshenti	35(9.8)	27(7.6)	1(0.3)	6(1.7)	1(0.3)	2(6)	72(20.2)
	Shaga	49(13.7)	22(6.2)	1(0.3)	1(0.3)	0(0)	0(0)	73(20.4)
	Shina	32(9)	34(9.5)	1(0.3)	0(0)	0(0)	0(0)	67(18.8)
	Zenzelima	41(11.5)	25(7)	1(0.3)	6(1.7)	0(0)	0(00	73(20.4)
Total		203(56.9	125(35)	9(2.5)	16(4.5)	1(0.3)	3(0.8)	357

N= Negative, T.v= *T.vivax*, Tt= *T. theileri*, Tz= *Trypanozoa*

No significant difference ($p > 0.05$) in prevalence between the PAs by Woo test, but significance difference ($p < 0.05$) was observed by ITS-1 PCR.

Table 9: The prevalence of trypanosomosis between sexes, age and body condition by PCR

		ITS-1 PCR						
		N	T.v	Tt	Tz	T.v+Tt	Tt +Tz	Total Animals examined
Sex	Female	115(32.2)	10(2.8)	2(0.6)	79(22.1)	4(1.1)	0(0)	210(58.8)
	Male	88(24.6)	6(1.7)	1(0.3)	46(12.9)	5(1.4)	1(0.3)	147(41.2)
Age group	Adult	125(35)	8(2.2)	1(0.3)	88(24.6)	5(1.4)	1(0.3)	228(63.9)
	Old	41(11.5)	3(0.8)	1(0.3)	13(3.6)	2(0.6)	0(0)	60(16.8)
	Young	37(10.4)	5(1.4)	1(0.3)	24(6.7)	2(0.6)	0(0)	69(19.3)
Body condition	Good	94(26.3)	8(2.2)	0(0)	59(16.5)	3(0.8)	0(0)	164 (45.9)
	Poor	109(30.5)	8(2.2)	3(0.8)	66(18.5)	6(1.7)	1(0.3)	193 (54.1)
Total		203(56.9)	16(4.50	3(0.8)	125(35)	9(2.5)	1(0.3)	357

N= Negative, T.v= *T.vivax*, Tt= *T. theileri*, Tz= *Trypanozoa*

No significant difference ($p > 0.05$) in prevalence between the sex, age and body condition by Woo test and ITS1 PCR

Table 10: The prevalence of trypanosomosis between treatments by ITS1 PCR

	PCR						
Treatment	N	T.v	T.v+Tt	Tt	Tt +Tz	Tz	Total animals examined
Anthelmintic	75(21)	32(9)	5(1.4)	5(1.4)	1(0.3)	2(0.6)	120
Antitrypanosome	4(1.1)	3(0.8)	1(0.3)	0(0)	0(0)	0(0)	8
NO	123(34.5)	89(24.9)	3(0.8)	11(3.1)	0(0)	1(0.3)	227
Oxytetracyclin	1(0.3)	1(0.3)	0(0)	0(0)	0(0)	0(0)	2
Total	203(56.9)	125(35)	9(2.5)	16(4.5)	1(0.3)	3(0.8)	357

N= Negative, T.v= *T.vivax*, Tt= *T. theileri*, Tz= *Trypanozoa*

No significant difference ($p > 0.05$) in prevalence between treatment by Woo test and ITS1 PCR

Similar studies were conducted with this report (the current study) in a wide area of survey in Zambia by sinyangue *et al*. [34], which reported the prevalence in individual village and district and varied between 0 and 64% and this prevalence has varied not only between villages but also between visits. A prevalence variation that laid between 0 and 43% has been reported by Mwambo *et al*. [35] in Tanzania and also similar report was obtained in north west of Nigeria by Fajinmi *et al*. [4] with the overall prevalence of 1.8% (1.2% *T .vivax)* by standard trypanosome detection method.

The internal transcribed spacer (ITS) region of ribosomal DNA presents the advantages of being a multi copy locus (100-200 copies), having a small size (300-800bp) and varying from one taxon to another but highly conserved in size in a given taxon, making it a preferred diagnostic target for universal test [36, 37]. The PCR technique using primers designed on repetitive genomic DNA sequences of trypanosomes had already been used for epidemiological purposes. However, such investigations focused essentially on the identification of trypanosome species in tsetse flies [17, 38, 39, 46] and to a much lesser extent in cattle. The technique has been verified on blood samples of experimentally infected animals, confirming its higher sensitivity and specificity when compared to parasitological techniques [13, 40]. In the present work, we used the PCR technique as a diagnostic tool to assess the prevalence of animal trypanosomosis on samples collected in the field, North West of Ethiopia in Amhara region of Bahirdar zuria and Fogera district, at the geographic scale of an agro-pastoral zone.

The overall prevalence of trypanosomosis in this study by ITS1 PCR was 43.3%. Among this *T .vivax* was the predominant species, which cover 81% of the total positive cases followed by *T. theileri* (10%) and the

others were mixed infection of *T. vivax* and *T. theileri* (2.5*%), T. theileri* and *Trypanozoon* (0.3%) and *Trypanozoa* (0.8%) had been recorded. *T. theileri*, which is non pathogenic haemoparasite [41]. This diagnostic test has high sensitivity and specificity, that investigates the new species which have not been reported in the previous studies by parasitological test within the same study area even in other part of Ethiopia since the technology applied for the first time for the identification of trypanosome species.

Using the PCR, a significantly higher prevalence was obtained than by classical parasitological examination (43.3% vs 1.4%). This was due to the higher sensitivity of the molecular technique which is able to detect less than one trypanosome per ml under experimental conditions [13]. Such prevalence differences as obtained by the two techniques would have a significant impact on the strategy selected for the control of the disease in affected area. Furthermore, PCR allowed the accurate diagnosis of mixed infections, which could not be detected by parasitological examination that reported with similar trends by Solano *et al.* [16] in Burkina Faso.

One animal presented with an infection (diagnosed by microscopy (woo test) as positive*)* was not recognized by the sets of primers used. This could reflect either a technical problem or the presence of trypanosomes which could not be recognized by the primers used, as has been reported previously [42] or sub detectable amount of DNA.

In the present study, the effect of risk factors like sex, age, body condition and treatment have no significant effect on the prevalence of trypanosomosis by both tests. Similar reports were conducted by Shimelis [22] and Tefera [43].

The infection rate in females was slightly higher than that of males but there was no significant difference on prevalence trypanosomosis in males and females. Similar results were reported by [32] which indicated that lactation stress results in higher prevalence of trypanosomosis. The possible suggestion in the present findings would be that of the physiological conditions of female animals like lactation, gestation and calving stress contributes a role in susceptibility to disease than the male ones.

Age was not found to be a risk factor in the present finding but slightly higher infection rate was observed in adult animals above 2 years of age. Similar results were reported by Shimelis [22] and Tefera [43]. This could be associated to the fact that animals which travel long distance for feed and drought as well as for harvesting crop are predisposed to stress. Roulands *et al.* [32] in ghibe valley indicated that suckling calve did not go out with their dams but graze at homesteads until weaned off. Young animals are also naturally protected to some extent by maternal antibodies [44], which result in low prevalence of trypanosome that observed in calves. The chronic state of trypanosome infection also contributes for higher prevalence in adult than young and old cattle. Roulands *et al.* [32] found that cows above 9 years of old had higher trypanocidal treatment than the corresponding value in 3 years old animals. This is the reflection of higher risk of trypanosomosis in adult than young.

Body condition and treatment have no significant risk factor in this study. This is due to as Desquesnes *et al.* [45] reported in sheep that depends on the management system, the clinical sign may vary from nil to significant loss of body condition if the management level of the animal is satisfactory, the clinical sign may be absent and infection inapparent even if the trypanosomosis circulating in the blood. Treatment is also not an important risk factor in this study. It might be associated with miss diagnosis, indiscriminate drug use, under dose, lower efficacy of the drug and drug resistance characteristic of the parasite.

CONCLUSION

Trypanosomosis in domestic livestock causes a significant negative impact in food production and economic growth in many part of the world, particularly in sub-Saharan Africa and it has greatly hampered people and animals settlement in considerable part of the world. Taking into account current views on sustainable control of African trypanosomosis, a need arises for tools that can assist in a rapid appraisal of the parasitological situation in agricultural and livestock areas and we concluded that the use of PCR to assess trypanosome prevalence in cattle seems a valuable tool for this purpose.

REFERENCES

1. WHO, 1998. Expert Committee on Control and Surveillance of African Trypanosomiasis. WHO, Technical Report Series No. 881, Geneva.
2. Abenga, J.N., F.N. Ewenzor, F.A. Lawani, C. Ezebuiro, J. Sule and K.M. David, 2002.Prevalence of trypanosomiasis in trade cattle at slaughter in Kaduna State, Nigeria. J. Parasitol., 23: 107-110.

3. Swallow, B.M., 2000. Impacts of Trypanosomiasis on African Agriculture. Food and Agriculture Organization, Rome, Italy, pp: 52-57.
4. Fajinmi, A.O., O. Faleke, A.A. OMagaji, A.I. Daneji and M. Gweba, 2011. Presence of trypanosome species and determination of anaemia in trade cattle at Sokoto abattoir, Nigeria. Res. J. Parasitol., 6: 31-42.
5. Cattand, P., D. Phillipe, M. Guzman, J. Janin and A. Kroeger, 2005. Tropical diseases lacking adequate control, measures: dengue, leishmaniasis and African trypanosomiasis. Disease Control Priorities in Developing Countries, pp: 23-57.
6. Codjia, V., W. Mulatu, P.A.O. Majiwa and A.S. Pregrin, 1993. Epidemiology of bovine trypanosomosis in gibe valley, Southwest Ethiopia. Occurrence of population of T. congolense resistance to diminazine, isomethamidium and homidium. Acta Trop., 53: 151-163.
7. Abebe, G. and Y. Jobre, 1996. Trypanosomosis: A treat to cattle production in Ethiopia. Rev. Med. Vet., 147: 897-902.
8. Langridge, W.P., 1976. Tsetse and Trypanosomosis survey of Ethiopia. Ministry of overseas Department UK, pp: 1-40.
9. Dagnachew, Z. and K. Shafo, 1981.An investigation of dourine in Arsi administrative region. Eth. Vet. Bull., 4: 3-9.
10. Coetzer, J.A.W., G.R. Thomson and R.C. Tustin, 1994. Infectious diseases of livestock. Cape Town: Clyson press, Maitland, pp: 163-193.
11. Griener, M. and D. Bohning, 1994. Notes about determining the cut-off value in enzyme linked Immunosorbent assay (ELISA) reply. Prev. Vet. Me., 20: 307-310.
12. Jacobson, R.H., 1998. Validation of serological assays for diagnosis of infectious diseases. Rev. Sci. Tech. OIE., 17: 469-489.
13. Moser, D.R., G.A. Cook, D.E. Ochs, C.P. Bailey, M.R. McKane and J.E. Donelson, 1989 . Detection of Trypanosoma congolense and T. brucei subspecies by DNA amplification using the Polymerase Chain Reaction. Parasitol., 99: 57-66.
14. Masiga, D.K., A.J. Smyth, P. Hayes, T.J. Bromidge and W.C. Gibson, 1992. Sensitive detection of trypanosomes in tsetse flies by DNA amplification. Int. J. Parasitol., 22: 909-918.
15. Majiwa, P.A.O., R. Thatthi, S.K. Moloo, J.H.P. Nyeko, L.H. Otieno and S. Maloo, 1994. Detection of trypanosome infections in the saliva of tsetse flies and buffy-coat samples on antigenemic but aparasitaemic cattle. Parasitol., 108: 313-322.
16. Solano, P., J.F. Michel, T. Lefrançois, S. De La Rocque, I. Sidibé, A. Zoungrana and D. Cuisancea, 1999. Polymerase chain reaction as a diagnosis tool for detecting trypanosomes in naturally infected cattle in Burkina Faso. Veteri. Parasitol., 86: 95-103.
17. Reifenberg, J.M., P. Solano, G. Duvallet, D. Cuisance, J. Simporé and G.Cuny, 1999. Molecular characterization of trypanosomes isolates from naturally infected domestic animals in Burkina Faso. Vet. Parasitol., 71: 251-262.
18. FWARDO, 2003. Fogera woreda agricultural and rural development office annual progress report for the year 2003.
19. Census Central Statistics' Authority, 2009. Ethiopia Agricultural Enumeration Result for Amhara. Region. 29-55.
20. Ministry of agriculture, 1998. Ethiopian livestock development strategy. Ministry of agriculture (MOA), Addis Ababa, Ethiopia. N0. 12.
21. Thrusfield, M.V., 1995. Veterinary epidemiology 2nd Ed. Blackwell Science, Oxford, pp: 185-199.
22. Shimelis, D., 2004. Epidemiology of bovine trypanosomosis in Abay basin areas of North West of Ethiopia. MSC Thesis, FVM, AAU. Debre Zeit, Ethiopia.
23. QIAamp, 2010. DNA investigator hand book for purification of DNA. QIAGEN. USA, pp: 1-60.
24. Claes, F., E.C. Agbo, M. Radwanska, M.F.W.Tepas, T. Baltz, D.T. De Waal, B.M. Goddeeris, E. Classen and P. Buscher, 2003. How does Trypanosoma equiperdum fit in Trypanozoon group? A cluster analysis by RAPD and multiplex-endonuclease genotyping approach. Parasitol., 26: 425-431.
25. Njiru, Z.K., C.C. Constantine, S. Guya, J. Crowther, J.M. Kiragu, R.C. Thompson and A.M. Davila, 2005. The use of ITS1 rDNA PCR in detecting pathogenic African trypanosomes. Parasitol. Res., 95: 186-192.
26. Viera, J. and M. Garrett, 2005. Understanding inter-observer agreement. Kappa statistic. Fam. Med., 37: 360-363.
27. Feyesa, R., 2004. Current epidemiological situation of bovine trypanosomosis in Limushay tsetse control area of upper Didesa valley. MSC Thesis, FVM, AAU. Debre Zeit, Ethiopia.
28. Yeshitila, A., 2006. Prevalence of bovine trypanosomosis in sokuru woreda, Jimma zone, Oromia region, South West of Ethiopia. MSC Thesis, FVM, AAU. Debre Zeit, Ethiopia.
29. Nantulya, V.M., 1990.Trypanosomiasis in domestic animals: the problems of diagnosis. Rev. Sci. Tech. Off. Int. Epiz., 9: 357-367.

30. Kalu, A.U., 1996. Current status of tsetse fly and animal trypanosomosis in the Jos Plateau, Nigeria. Pev. Vet. Med., 27: 107-113.
31. Tamasaukas, R., H. Ruiz, H. Roa, A. Baldizan, A. Gonzalez and A. Guirra, 1996. Epidemiological diagnosis of trypanosome of cattle in Venezuela. In: Proceedings of the first symposium on new world trypanosome. Georgetown, Guiyan, November, 20-22, 1996 Inter American institute for cooperation on agriculture (IICA). Technical cooperation Agency in Barbados Eds. Sandravokary and mark Desquesnes, pp: 45-55.
32. Roulands, G.J., W. Mulatu, S..M. Nagda, R.B. Dolan and G.D.M. d'Ieteren, 1995. Genetic variation in packed red cell volume and frequency of parasaetemia in East Africa zebu cattle exposed to drug resistance trypanosomes. Livestock Produ Scien., 43: 75-84.
33. Gardiner, R.P., 1998. Recent studies of the biology of Trypanosoma vivax. Advance Parasitol., 28: 229-317.
34. Sinyangue, L., N. Machila, J. Mubanga, V. Delespoux, J. Brandt, S. Geert, P.H.A. Holmes and M.C. Eister, 2001. Trypanocidal drug resistance in Easter province of Zambia .In: Internation Scientific Council for trypanosomosis research and control (ISCTRC), 25th meeting Mombasa, Kenya, 1999. Pub. OAU/STRC, pp: 165-167.
35. Mwambo, H., J.M. Ndung'u, G.A. Murilla, L. Munga, L. Sanyangue, N. Machina, P.H. Holmes and C.M. Eislet, 2002. Trypanocidal drug resistance in Tanzania. In; International scientific council for trypanosomosis research and control (ITCTRC), 25th Meeting, Mombasa Kenya, 1999.Pub. OAU/STRC, pp: 168-174.
36. Desquesnes, M. and A.M. Davila, 2002. Applications of PCR-based tools for detection and identification of animal trypanosomes: a review and perspectives. Vet Parasitol., 109: 213-231.
37. Dlugosz, E. and M. Wisniewski, 2006. Molecular diagnostic of parasites using rRNA gene sequence. Wiad Parazytol., 52: 263-269.
38. McNamara, J.J., C. Laveissière and D.K. Masiga, 1995. Multiple trypanosome infections in wild tsetse in Côted'Ivoire detected by PCR analysis and DNA probes. Acta Trop., 59: 85-92.
39. Solano, P., J.M. Reifenberg, S. Amsler-Delafosse, I. Kaboré, D.Cuisance and G. Duvallet, 1996. Trypanosome characterization by PCR in Glossina palpalis gambiensis and G. tachinoides from Burkina Faso. Med. Vet. Entomol., 10: 354-358.
40. Clausen, P.H., A. Wiemann, R. Patzelt, D. Kakaire, C. Poetzsch, A. Peregrine and D. Mehlitz, 1998. Use of a PCR assay for the specific and sensitive detection of Trypanosoma Spp. in naturally infected dairy cattle in peri-urban Kampala, Uganda. Ann N Y Acad Sci., 1: 849: 21-31.
41. Villa, A., C. Gutterez, E. Gracta, B. Moreno, G. Characon, P.V. Sanz, P. Buscher and L. Taurafter, 2008. Presence of trypanosoma theileri in Spanish cattle. Annals of the Newark Academy of Sci., 1149: 352-357.
42. Katakura, K., C. Lubinga, H. Chitambo and Y. Tada, 1997. Detection of Trypanosoma congolense and *T. brucei* subspecies in cattle in Zambia by Polymerase Chain Reaction from blood collected on a filter paper. Parasitol. Res., 83: 241-245.
43. Tefera, S., 1994. Prevalence of bovine trypanosomiasis in Arbaminch district.DVM thesis: Addis Ababa University, Faculty of Veterinary Medicine. Debre Zeit, Ethiopia.
44. Fimmen, H.O., D. Mehlitz, F. Horchner and E. Karabe, 1982. Colostral antibodies and trypanosoma congolense infection in calves .trypanotolerance Recherché and Applications.GTZ, NO. 116, Germany, pp: 173-184.
45. Desquesnes, M., 1996. Evaluation of three antigen detection tests (monoclonal trapping ELISA) for African trypanosomes with an isolate of Trypanosoma vivax from French Guyana. Ann. N. Y. Acad. Sci., 791: 172-184..
46. Masiga, D.K., J.J. McNamara, C. Laveissière, P. Truc and W.C. Gibson, 1996. A high prevalence of mixed trypanosome infections in tsetse flies in Sinfra, Côte d'Ivoire, detected by DNA amplification. Parasitol., 112: 75-80.