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Full Length Research Paper

Cross sectional study of bovine trypanosomosis and major clinical signs observed in Diga District, Western Ethiopia

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A cross sectional study was conducted on a total of 410 zebu cattle in selected Kebeles of Diga *Woreda*, Western Ethiopia. The purposes of the study were to determine the prevalence and species of trypanosomes infecting cattle using buffy coat darkground-phase contrast technique and thin blood smear and to assess the associations of common complaints observed by cattle owners with detected trypanosomosis. An overall prevalence of 5.85% was recorded and no statistical significant difference in the prevalence between the Kebeles involved in the present study. The species of trypanosomes identified were *Trypanosoma Congolese* (54.17%) followed by, *Trypanosoma Vivax* (37.5%) *and Trypanosoma Brucei* (8.33%). Trypanosome infection rate based on different age groups was not found statistically significant (P > 0.05). Sex-wise prevalence of 7.47 and 3.55% were recorded in female and male cattle, respectively. Upon case history assessment, the likelihood of cattle with poor body condition to be trypanosome positive was higher when compared to animals with good body condition (OR = 15.82, 95% CI = 5.9- 44.6). Besides cattle with anaemic status were 52.4 times (OR = 52.4, 95% CI = 8.2-216) more likely to have trypanosome infection than non anaemic animals. The mean PCV value of parasitemic animals (20.04%) was lower (P < 0.05) than that of aparasitemic cattle (26.85%). In conclusion, the result of this survey indicated that bovine trypanosomosis is potentially a major constraint to the livestock production and common clinical signs loss of weight and anemia could be considered as one option to keep the disease in check for cattle owners in the area.

Key words: Cattle, clinical signs, Diga *Woreda*, Ethiopia, prevalence, trypanosomosis.

INTRODUCTION

Ten million square kilometers of Africa are infested by the tsetse fly which transmits animal trypanosomosis. Animals lose condition and become progressively emaciated. Milk yield has been reported to decrease in dairy animals (CFSPH, 2009). Available data indicated that bovine trypanosomosis is one of the major impedements to livestock development and agricultural production in Ethiopia, contributing negatively to the overall development in general and to food self-reliance efforts of the nation in particular (Ministry of Agriculture and Rural Development (MoARD, 2004; Abebe, 2005). Anemia, generalized enlargement of the superficial lymph nodes, lethargy and progressive loss of condition are the major signs of bovine trypanosomosis (Radostitis et al., 2007, Blood et al., 1989).

The most prevalent trypanosome species in tsetse infested areas of Ethiopia are *Trypanosoma congolense* and *Trypanosoma vivax* (Abebe and Jobre, 1996; Abebe, 2005). The reported prevalence of bovine trypanosomosis

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varies from locality to locality depending on agro climatic conditions, seasons, and activities which were intended to control the impact of the disease. Abebe and Jobre (1996) indicated the prevalence of 58.5, 31.2 and 3.5% for *T. congolense*, *T. vivax*, and *Trypanosoma brucei*, respectively in southwest Ethiopia. A similar report was also recorded for Awi zone in northwestern part of the country (Kebede and Animuit, 2009), Bekele et al. (2010), recently reported prevalence of 70, 20 and 8% in cattle for *T. congolense*, *T. vivax*, and *T. brucei*, respectively, in southern rift valley of the country. Furthermore, an overall prevalence of 4.4% bovine trypanosome infection, with abundance of 36.36, 18.18 and 9.09% for *T. congolense, T. vivax* and *T. brucei*, respectively was also reported by Tadesse and Tsegaye (2010) from Bench Maji zone, South Western Ethiopia. Specific status regarding bovine trypanosomosis is not known for Diga Woreda of East Wollega zone, which is located very near to high risk area for tsetse transmitted trypanosomosis in the country. Therefore the objectives of the present study were to determine the prevalence of infection, to identify the trypanosomes species involved for the infection and to assess the association of cattle trypanosomosis with observed major clinical signs and complaints by cattle owners.

MATERIALS AND METHODS

Study area

The present study was carried out in selected villages of Diga Woreda which is found in Oromia National Regional state, Western Ethiopia. The Woreda is located at 343 km away from Addis Ababa and has a total population of 58,826 and land area of about 40,788 ha. The study area has an altitude range of 1380 to 2300 m above sea level and receives an average annual rain fall of 1416 mm. The temperature range is 14.6 to 30.4°C and the annual average is 22.5°C. According to the Diga District agriculture and rural development office, agro-climatic classification of the *Woreda* is low land (58%), mid-altitude (42%) and no high land coverage (0%). The farming practice in the area is mixed where crop production and all classes of live stock are kept by farmers with the estimated population of cattle 57,586, sheep 11, 220, goats 6,091, horses 136, mules 46, donkeys 2,948 and poultry 31, 241(Central Statistic Authority (CSA), 2009)

Study animals

The study was conducted on local zebu cattle. These animals were raised in different villages of Diga district. The animals examined in this particular study were representing different Kebeles (the smallest administrative unit), sex, body conditions and age groups (calf, young and adult) and reared in extensive management system.

Study design

A cross sectional study was conducted from November 2010 to March 2011, by selecting 11 Kebeles out of 23 Kebeles found in the Woreda based on their accessibility and environmental variations.

A total of 410 animals were sampled, the number of animals sampled from each village was based on proportional weighting. At the Kebele level, animals were selected by simple random sampling using lottery method. The number of cattle sampled from a particular herd in a given Kebele depended on proportional weighting; but a minimum of 20 cattle per herd was fixed. The study animals were classified in different age groups. Animals less than one year (calf), 1 to 6 years and greater than 6 years. Prior to taking the blood sample, information about the history and the clinical signs for each sampled animals were discussed with the cattle owners and recorded and summarized. In addition, the body condition of each animal was also assessed by the amount of fat covering the rump, loin and degree of depression in tailhead area (Nicholson and Butterwarth, 1986).

Sample size

The sample size was calculated using expected prevalence of 50% and desired absolute precision of 0.05 as per the standard procedure described by Thursfield (1995). An estimated minimum sample size of 384 cattle was obtained, but we were able to involve 410 cattle for our study.

Sample collection

Cattle were properly restrained and following aseptic procedure, the marginal ear vein was pricked with the tip of sterile lancet to let blood in to capillary tube. Blood samples were collected in to two capillary tubes from each animal (Murray et al., 1977).

Parasitological examination

Buffy coat darkground-phase contrast technique (BCT)

Blood was collected in two heparinized microhematocrit capillary tubes filled up to 3/4th of their volume from each animal. One end of the tube was sealed with crystal sealant. The tube was placed in the microhematocrit centrifuge with sealant at the outer end. The blood in capillary tube was centrifuged at 10,000 rpm for 5 min. The tubes were taken from the hematocrit centrifuge and placed on the microhematocrit reader to determine the PCV. Animals with PCV value below 24% were considered to be anaemic (Murray et al, 1983). After reading PCV, the capillary tubes were cut 1 mm below the buffy coat including the top layer of the RBC and the contents were expressed onto slide and examined by dark ground-phase contrast microscope for detection of trypanosome (Murray et al., 1983)

Thin blood smear and Giemsa staining

For preparation of the thin smear, first the slide was polished with dry and clean cloth. The blood in microhematocrit capillary tube was expressed approximately 20 mm away from one end on the slide. The spreader (another slide) was placed on a head of the drop of the blood approximately at an angle of 45°. The spreader slide was drawn back to make contact with blood. Then, the blood was allowed to run to both ends of the spreader slide and spread the blood along the slide with steady motion. The slide was dried by waving it in the air and fixed for 5 min with methyl alcohol. The smear was flooded with Giemsa staining solution for 45 min. Excess stain was drained and washed off by using distilled water and allowed dry for examination. Then microscopic examination was made under oil emersion objective (Losos, 1986; Losos and kede, 1972).

Table 1. Prevalence of trypanosome infection in different villages of Diga Wereda, Western Ethiopia,

Kebeles	Numbers of animal examined	Numbers of animal positive (%)	Species of Trypanosame		
			Trypanosoma congolense (%)	*Trypanosoma vivax* (%)	*Trypanosoma brucei* (%)
Adugna	46	1(2.17)	-	1 (2.17)	-
Bikila	49	4(8.16)	2(4.1)	2(4.1)	-
Damaksa	26	1(2.17)	-	1(3.85)	-
Fromsa	51	3(5.88)	3(5.88)	-	-
Garuma	35	1(2.86)	1(2.86)	-	-
Gemechis	28	2(7.14)	1(3.57)	-	1(3.57)
Gudisa	66	4(6.06)	3(4.55)	1(1.5)	-
Ifa	60	4(6.66)	1(1.66)	3(5.0)	-
Jirata	49	4(8.16)	2(4.08)	1(2.04)	1(2.04)
Total	410	24(5.85)	13(3.17)	9(2.19)	2(0.49)

$\chi^2 = 3.34$; P = 0.99.

Table 2. Sex-wise prevalence of trypanosome infection.

Sex	Numbers of animal examined	Numbers infected and prevalence (%)	95% CI*	OR (95%CI)
Female	241	18(7.47)	4.15 - 10.79	
Male	169	6(3.55)	0.76 - 6.34	2.8(0.81-6.91)
Total	410	24(5.85)	3.58 - 8.12	

CI* = Confidence interval; $\chi^2 = 2.768$, P = 0.096.

Data management and analysis

Raw data generated for this study was stored in Microsoft Excel and the prevalence of bovine trypanosomosis in different age groups and sexes were analyzed by using STATA version 8.0; chi-square was used to compare the prevalence of trypanosome infection in different variables and to determine association between variables and the disease. Odds ratios (OR) were used to assess the association of trypanosomosis and observed clinical signs in sampled animals. The mean PCV values of parasitemic animals against that of parasitemic animals were compared using Paired t-test. In all cases differences between parameter were tested for significance at probability levels of 0.05.

RESULTS

Parasitological findings

The overall prevalence of bovine trypanosomosis was found to be 5.85% for Diga Woreda (95% CI: 3.58 to 8.12). The prevalences recorded for each Kebeles are summarized in Table 1. The highest (8.16%) prevalences were observed at Bikila and Jirata Kebeles and the lowest prevalence (2.17%) in Adugna Kebele. However, statistically there was no significant difference in the infection rate among the different Kebeles (P>0.05). In the present study, among 24 cattle detected positive13 (54.17%) were found to be infected by *T. congolese*, 9 (37.50%) by *T. vivax* and 2 (8.33%) cattle were infected by *T. brucei* Trypanosome infection prevalence between male and female animals showed no statistical difference (P > 0.05), even though there were slightly more infections in female animals (Table 2).

The age distribution of bovine trypanosomosis is presented in Table 3. Infections were observed in all the four age groups studied, with no statistically significant variations (P > 0.05)

The results showed that trypanosome infection prevalences among good and poor body condition animals showed statistically significant difference (P> 0.05). Higher prevalence was observed in poor body condition animals (Table 4).

The results of the monthly trypanosome infection showed higher and low prevalence rates in March and November respectively as it is illustrated in Table 5. There was no statistically significant difference in infection rate among the different months studied (P>0.05).

Hematological findings

The PCV value in the animals examined for trypanosome infection showed that the mean PCV value for the parasitemic cattle was 20.04 ± 0.46 while the mean PCV

Table 3. Trypanosome infection in different age groups.

Age group	Number of animals examined	Number infected and prevalence (%)	95% CI*
Calf (< 1 years)	27	1(3.70)	3.42 - 10.82
1-6 years	118	6(5.08)	1.12 - 9.04
>6 years	265	17(6.42)	3.47 - 9.37
Total	410	24(5.85)	3.58 - 8.12

CI* = Confidence interval; χ^2 = 0.504, P = 0.777.

Table 4. Trypanosome infection based on body condition.

Body condition	Number of animals examined	Number infected and prevalence (%)	95% CI*	OR(95%CI)
Good	364	8(2.20)	0.69 - 3.71	
Poor	46	16(34.78)	21.02 - 48.54	15.82(5.9-44.6)
Total	410	24(5.85)	3.58 - 8.12	

CI* = Confidence interval; χ^2= 78.682, P= 0.000.

value for the aparasitemic cattle was observed to be 26.86 ± 0.46 and the over all mean PCV value was 26.46 ± 0.23. There was statistically significant difference in the mean PCV value between the infected (20±0.22) and non infected animals (t = 7, P = 0.000).

In the present study, cattle owners were asked to describe the major clinical signs he or she observed on each sampled animals. Accordingly, the majority of them responded the occurrence of weight loss, milk reduction among adult cows and other health problems listed in Table 6. However the majority of complaints made by owners were not positively associated with trypanosome infection.

Cattle anaemic status (with low PCV) was positively associated with trypanosome infection (OR = 52.4, 95% CI = 8.2 - 216). However cattle with low PCV (anaemic) were 52.4 times more likely to be trypanosome positive than non anaemic cattle (Table 6).

DISCUSSION

The overall prevalence of 5.85% bovine trypanosomosis recorded in the present study is an indicator of the disease as a limiting factor to cattle production in the study area; in addition, this find is in agreement with a similar research conducted in Ethiopia NTTICC (2005) and Garoma (2009) found prevalence of 4.2 and 4.15% in Kenaf and Gari settlement areas of East Wellega , 4.2% was also recorded in South Achefer district in Amhara regional state by Denbarga et al. (2012). However our result is very low when compared to the reports of Afework et al., (2001) and Molalegen et al. (2011), from northwest of the country and 19% prevalence found by Abiy (2002) for Goro district of Southwest Ethiopia. The relatively low prevalence found in this study may be attributed to the frequent use of chemotherapeutic drugs, an increase in agricultural investment and decreased tsetse challenge in the area. In addition, the parasitological test has been reported to be less sensitive when compared with molecular techniques (Paris et al., 1982; Geysen et al., 2003).

In the present study, trypanosome infection rate observed in female was higher than male animals but not statistically significant. This observation coincides with the findings of Tefera (1994) in Arbaminch districts and Adane (1995) in and around Bahir Dar who reported no significant difference in prevalence between the two sexes. Similarly the infection rates of trypanosome in different age groups showed no statistically significant difference in the present study. This finding supports the result of the previous works by Sinshaw (2004), Tadess et al. (2011), Bitew et al. (2011) and Denbarega et al. (2012), who concluded that there is no significant difference in infection rate between different age groups. The possible explanation for the low prevalence of trypanosome infection observed in calves in the current study may be due to the fact that claves mostly remain confined around home and there is less exposure to tsetse fly whereas the older age groups might have faced the vector challenge during grazing in the field and at watering points.

In the present study, *Trypanosoma congolense* was the predominant species in the study area. The predominance of *T. congoloense* (54.17%) in the present study is in agreement with the previous works that documented 66.1% (Mereb et al., 1999), 60.9% (Afework et al., 2001) and 63.4% in southern part of Ethiopia (Terzu, 2006). In contrast, higher *T. congolense* proportions of 75% at Kone settlement (Tewelde et al., 2004), 85.2% at

Table 5. Prevalence of trypanosome infection in different months.

Months	Number of animals examined	Number infected and prevalence (%)	95% CI*
November	81	1(1.23)	0.03 - 6.60
December	123	9(7.32)	3.40 -13.10
January	112	10(8.93)	1.1 - 26.00
February	74	2(2.70)	0.3 - 9.20
March	20	2(10)	1.20 - 31.10
Total	410	24(5.85)	3.58 - 8.12

CI* = Confidence interval; $\chi^2 = 7.493$, P = 0.112.

Table 6. The relationship between trypanosomosis and some selected clinical signs observed or witness by the owners in cattle of Diga District , Western Ethiopia.

Case history		No. Examined	No. positive (%)	OR	95% CI
Weight loss	Yes	163	3(1.84)	4.71	1.45 - 15.11
	No	247	21(8.5)*		
Decrease in milk yield	Yes	160	8 (4.96)	3.8	1.42 - 10.12
	No	54	9 (18.6)*		
Loss of weight and appetite	Yes	128	3(2.34)	3.3	1.03 - 10.56
	No	282	21(7.4)*		
Loss of weight and coughing	Yes	115	8(6.9)*	1.3	0.55 - 3.06
	No	295	16(5.4)		
Diarrhoea and weight loss	Yes	16	2(12.5)*	2.4	0.0 - 10.5
	No	394	22(5.6)		
Dry feces and coughing	Yes	85	6(7.1)*	1.28	0.51 - 3.25
	No	325	18 (5.5)		
Anemia status (based on PCV)	Anaemic*	134	23 (17.91)*	52.4	8.2 - 216
	Non anaemic	276	1 (0.36)		

*The reference group for comparison.

Arbaminch Zuria districts (Woldeyes and Aboset, 1997) and 84% in Ghibe Valley (Rowlands et al., 1993) were documented. These high ratios of *T. congolense* suggest that the major cyclical vectors or Glossina species are more efficient transmitters of *T. congolense* than *T. vivax* in east Africa (Langridge, 1976).

According to Abebe (2005), *T. congolese* and *T. vivax* are the most prevalent trypanosomes that infect cattle in tsetse infested and tsetse free areas of the Ethiopia, respectively. In the tsetse infested areas of the country, though the prevalence of *T. congolense* was found to be high; a considerable number of examined animals were also harboring *T. vivax* infection which supports the result of the present study.

An assessment of the difference between mean PCV value of parasitemic and aparasitemic cattle was found to be 20.04 and 26.86, respectively and there was statistically significant difference between both groups ($P < 0.05$). This result agrees with the report of Haile (1996) who reported that the mean PCV value of parasitemic animals were significantly ($P < 0.05$) lower than that of aparasitemic animals.

Analysis of trypanosome infection prevalence on the basis of body condition revealed that cattle in poor body condition has 15.8 times more association with positivity to trypanosome when compared with cattle having good body condition. This result is in agreement with previous work by Mussa (2002) who also documented significant difference in infection rate between poor and good body condition cattle.

In the current study, anaemia was more frequently (OR: 52.4, 95% CI: 8.2 - 216) observed among trypanosome positive animals than trypanosome negative cattle. The positive relationship between anemia and trypanosomosis is of course a well established fact (Monga et al., 2004; Radostitis et al., 2007). So clinical manifestations particularly anaemia, could be taken into consideration with ecological conditions, might provide sufficient grounds for a putative diagnosis for trypanosomosis and its impact. In relation with the anemia one should also consider other factors such as the presence of nutritional deficiency and other diseases which are common in

Ethiopia (Mukasa et al., 1989; Berhanu, 2002) could also contribute for anaemia and poor body conditions we observed in this study.

The reported weight loss condition and reduction in milk by the animal owners in the examined animals were found to have no statistical positive associations with animals' which were infected with trypanosome. However a Delphi technique study done on expert opinion on key signs for clinical diagnosis of bovine trypanosomosis in some other East African countries considered weight loss as one of the important clinical sign for bovine trypanosomosis (Magona et al., 2004). Further, more the absence of positive association between trypanosome infection and loss of weight, a reduction in milk yield and other reported clinical signs (Table 6) could also depend on many factors such as stages of the disease, seasons and the type of study design (crossectional) we followed. In addition, it is difficult to attribute some common clinical signs to a single disease, which can be observed as a result of infection of many other diseases, which is very common in extensive cattle keeping system in country like Ethiopia. However previous reports (Kristjanson et al., 1999; FAO, 2000; Radostitis et al., 2007), indicated that clinical signs especially weight loss and milk reduction are common observations in trypanosomosis.

In conclusion, bovine trypanosomosis is potentially a constraint to the live stock production and the disease was commonly associated with loss of body condition and anemia in the study area. These signs could be used as an important input in creating awareness in the area of treatment, control and prevention of bovine trypanosomosis.

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