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Trypanosome infection rate of *Glossina pallidipes* and trypanosomosis prevalence in cattle in Amaro Special District of Southern Ethiopia

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The survey was conducted in Amaro Special District, Southern Ethiopia in 2010. It was initiated to determine the trypanosome infection rate, population density of *Glossina* species and prevalence of trypanosomosis in cattle. *Glossina pallidipes* was the only species of tsetse found in the study area during the study period. A total of 202 flies were dissected. The overall infection rate of *G. pallidipes* was 6.93%, among which 1 (0.49%) was male and 13 (6.43%) 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 561 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, 74 (13.19%) cattle were infected with trypanosomes. Most of the infections were due to *Trypanosoma congolense* (78.37%) followed by *Trypanosoma vivax* (12.13%), mixed infections of *T. congolense* and *T. vivax* (8.1%) and the rest were *Trypanosoma brucei* (1.35%). 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.

Key words: Cattle, apparent fly density, Glossina pallidipes, Infection rate, Trypanosomosis, Southern Ethiopia.

INTRODUCTION

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.* They are usually transmitted by tsetse flies. Tsetse flies ingest trypanosomes present in the blood or lymph while feeding on an infected host.

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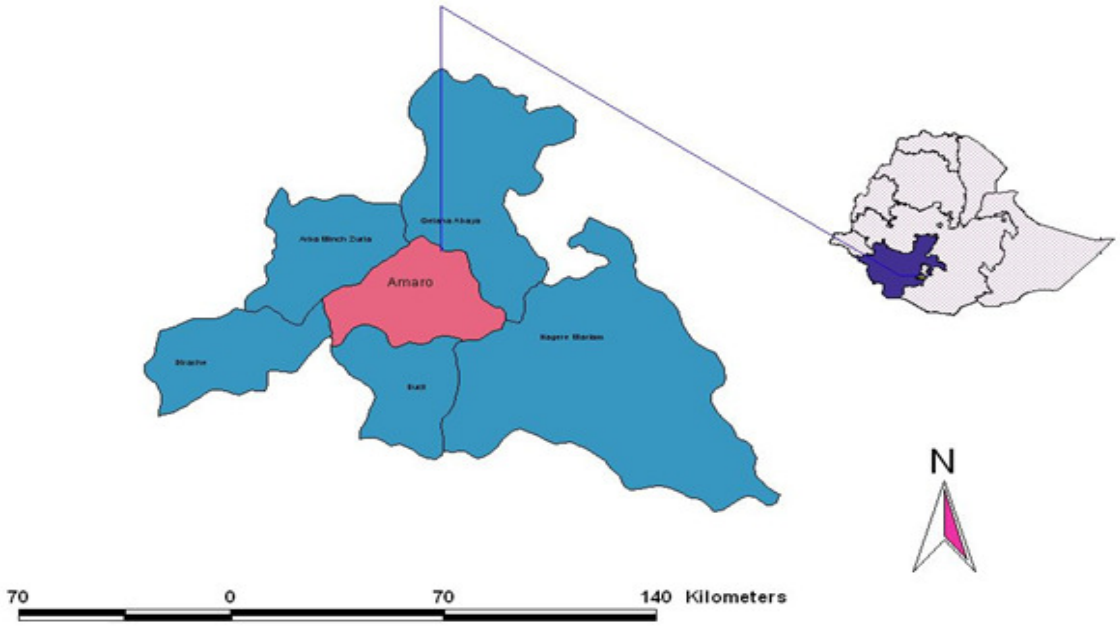

Figure 1. Location map of the study area.

There after the trypanosomes lose their glycoprotein surface coat and in the case of *T. brucei* and *T. congolense*, become elongated and multiply in the midgut before migrating forward to the salivary glands (*T. brucei*) and the proboscis (*T. congolense*). 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* species and prevalence of trypanosome in cattle in Amaro Special District of Southern Ethiopia.

MATERIALS AND METHODS

Study area

The study was carried out in Amaro Special District of Southern Ethiopia, from August to September, 2010. The study area was located between 5° 47` N, 37° 58`E, longitude, along the escarpment of Amaro Special and Gelana Districts. The elevation ranges from 1100 to 3400 meters above sea level. The climate condition of the area is some what unique which divided into three seasons: short rainy season (between May and early June), long summer rainy season (from September 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. Amaro Special District is bordering Gelana District in the North and East, Burji Special District and Gamogofa Zone in the West (Figure 1).

Study design

A cross-sectional study was conducted to determine the trypanosome infection rate and population density of *G. pallidipes* and prevalence of trypanosomosis in cattle, in Amaro Special District of Southern Ethiopia.

Sample size and sampling method

The simple random sampling technique was used for the study of trypanosomes infection rates in *G. pallidipes,* 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 h 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:

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

Trypanosomes prevalence in cattle

Study animals

The study animals used were all age and sex group of *Bovine* species of local zebu and Boran breed of cattle. All of them were kept under extensive management system together with other livestock species. A total of 561 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. The study animals were selected from six Peasant Associations (PAs) of Amaro Special District of Southern Ethiopia.

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. Descriptive statistics was utilized to summarize data. 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

Tsetse fly survey

From 82 traps deployed during the study period, a total of 4,714 flies were caught. Of these, 370 (7.84%) belong to *Glossina* species, the remaining 1853 (39.30%) were *Stomoxys*, 309 (6.55%), *Tabanus* and 2182 (46.28%) were Heamatopota belonging to biting flies, while *G. pallidipes* was the only tsetse species found in the surveyed areas. The overall apparent fly density (tsetse) was 2.25 flies/trap/days (F/T/D). The difference in apparent fly density at PA level was 6.6 and 0.85 at Jelo and Dano, 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

A total of 202 tsetse flies were dissected during the study period. The overall trypanosome infection rate was 6.93%. More trypanosome infections were observed in female tsetse with an infection rate of 7.55% (Table 2). Overall 69.23% (or 9/13) of the trypanosome infections carried by the female tsetse were identified as belonging to the Duttonella group; these were classified as *T. vivax* and the 23.07% (3/13) were *Nanomonas;* "*T. congolense*-type" and the remaining 7.69% (1/13) were *Trypanozoon;* "*T. brucei*-type" infections. 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 ($P < 0.05$).

Prevalence of trypanosomes in cattle

Out of a total 561 cattle sampled, 74 (13.19%) were found to be infected with trypanosomes (Table 3). The prevalence of trypanosomosis at Dorbade and Kore Biko peasant association was 20.37 and 7.01%, 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,* and lastly by *T. brucei*. When the proportional frequency of trypanosomes was considered, *T. congolense* appeared 58 times while *T. vivax,* mixed infection of *T. congolense* and *T. vivax* and *T. brucei* were 9, 6 and 1 times, respectively. In other words, *T. congolense* being a major cause of infection exceeded the other trypanosome species by 78.37%. The overall trypanosome prevalence in the surveyed areas of the district comprised 13.19%, with a range of 8.77 to 20.37% while the overall mean PCV-value appeared to be 25.78% in a parasitaemic and 22.96% in parasitaemic animals. Of 292 males and 269 females examined, 39 (13.35%) and 35 (13.01%), 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 561 animals sampled, 95 (16.9%), 165 (290.4%) and 301 (53.6) were under age group 2 to 4 years, 5 to 7 years and > 7 years, respectively (Table 4). In each group, 12 (12.63%), 22 (13.33%) and 40 (13.28%) 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 (13.28%) and in animals from midland was 34 (13.07%). The difference between two ecological zones were 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.

DISCUSSION

The results on tsetse fly survey in this study revealed the presence of a single *Glossina* species, known as *G. pallidipes* and identified as the major vector of

Table 1. Summary of the results of entomological survey in Amaro Special District.

PA	Alt	Lat	Long	Traps	Days	*Glossina* spp found		Other biting flies caught				
						G.pallidipes	F/T/D	Sto	Tab	Hea	Total	F/T/D
Jelo	1300	5°47	37°58	15	2	200	6.6	783	58	841	1682	56.06
Dorbade	1324	5°51	37°55	20	2	64	1.6	365	138	503	1006	25.15
Shero	1153	5°45	37°54	20	2	46	1.15	307	54	361	722	18.05
Goble	1331	5°45	37°55	20	2	48	1.2	363	29	412	804	20.1
Dano	1100	5°44	37°45	7	2	12	0.85	35	30	65	130	9.28
Total	-	-	-	82	-	370	2.25	1853	309	2182	4344	26.48

PA = Peasant association, Alt = altitude, Long = longitude, F/T/D= flies/trap/days, Sto= stomyxs, Tab = tabanus, Hea= haematopota.

Table 2. The number of flies dissected and infection rate of *Glossina pallidipes* 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	30	19	0	1 (3.33)	0	1 (3.33)
Female	172	31	3 (1.74)	9 (5.23)	1 (0.58)	13 (7.55)
Total	202	-	3 (1.48)	10 (4.95)	1 (0.49)	14 (6.93)

$\chi^2 = 99.82$, $P = 0.00$.

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

PA	N	+ve	*Trypanosome species* (%)				Overall infection (%)
			T. congolense	*T. vivax*	*T. brucei*	Mixed	
Jelo	98	11	10	1	0	0	11.2
Dorbade	108	22	15	4	0	3	20.37
Shero	92	10	5	2	0	3	10.86
Goble	95	13	10	2	1	0	13. 68
Dano	111	14	14	0	0	0	12. 61
Kore biko	57	4	4	0	0	0	7.01
Total	561	74	58	9	1	6	13.19

$\chi^2 = 304$; $P = 0.00$.

trypanosomosis in Amaro special district, southern Ethiopia. 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 2.25 and 26.48 flies/trap/day (F/T/D), respectively. There was significant difference ($P < 0.05$) in tsetse flies density between surveyed peasant associations; Jelo and Dano of Amaro Special District ranging from 6.6 to 1.15. 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.

A total of 202 *G. pallidipes* were dissected, and an overall of 6.93% of *G. pallidipes* in Amaro Special District of Southern Ethiopia harbors *T. vivax, T. congolense and T. brucei. 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. Similar

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

Risk factor	N	+ve	Species detected				Prevalence (%)	d.f	χ^2-value	*P*-value
			T. congolense	*T. vivax*	*T. brucei*	Mixed				
Sex										
Male	292	39	31	5	0	3	13.35	1	1.299	0.254
Female	269	35	27	4	1	3	13.01	-	-	-
Age (years)										
2-4	95	12	10	1	0	1	12.63	2	117.348	0.000
5-7	165	22	17	3	0	2	13.33	-	-	-
>7	301	40	31	5	1	3	13.28	-	-	-
Altitude										
Mid-land	260	34	27	4	0	3	13.07	1	2.995	0.083
Low-land	301	40	32	5	0	3	13.28	-	-	-
Total	561	74	58	9	1	6	13.19	-	-	-

findings in other *Glossina* species were reported. An overall infection rate of 5·1% of *Glossina morsitans submorsitans* by the three species of trypanosomes was reported in Radom National Park of Bahr El Arab (Mohammed-Ahmed et al., 1989).

More trypanosome infections were observed in female tsetse with an infection rate of 6.43% amongst the female flies while 0.49% infection rate was found in male flies. There was significant difference in the proportion of tsetse infected with trypanosomes between male and female flies (χ^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 (13.19%) found in the present study is relatively high when compared with the apparent density of *G. pallidipes* (2.25%) but it was well compromised with trypanosome infection rate of *G. pallidipes* (6.93%). 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 78.37% (58/74). 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).

Conclusion

This study presents findings on the trypanosome infection rate of *G. pallidipes* and prevalence of cattle trypanosomes in Amaro Special District of southern Ethiopia. The study indicated that *G. pallidipes* was the only *Glossina* species with the apparent density of 2.25%. 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 mitigate the problem in the study area.

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