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The Dynamics of Trypanosomosis in and around Intensive Suppression Area in Southern TseTse Eradication Project Site, Ethiopia

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Abstract

This study was carried out in and around intensive suppression area of the Southern Tsetse Eradication Project in Gamo Goffa Zone, Southern Nations, Nationalities and people`s Region of Ethiopia. Assessment of the dynamics of the disease trypanosomosis was done by epidemiological surveys between December-Jan, 2008 (dry season) and April-May, 2009 (wet season). For epidemiological study bovine blood samples were collected from 700 animals and parasitological examination was carried out by dark ground/phase contrast buffy coat technique. Two species of trypanosomes namely **Trypanosomacongolense** *and* **Trypanosomavivax** *were identified. The proportions of them were 5.5% and 3.5% in areas of intensive suppression during the dry and wet seasons, and 2.7% and 14% in regular suppression area and uncontrolled area respectively. There was no significant ($p>0.05$) differences in means of PCV values during the dry and wet seasons in the intensive suppression areas. However, there was significant ($p<0.05$) differences in mean PCV values ($p<0.05$) between regular suppression and uncontrolled areas. In conclusion, results of seasonal and spatial dynamics of trypanosomosis in and around intensive suppression areas would be useful in planning and eradication program in the project area.*

Key words: *Cattle, Intensive Suppression Area, Prevalence, Trypanosomosis*.

Introduction

Trypanosomosis is the widespread protozoan disease complex affecting cattle and other wide range of hosts, including humans in the sub-Saharan Africa. The course of the disease may run from a chronic long lasting to an acute and rapidly fatal one depending on the vector-parasite-host interactions, characterized mainly by intermittent fever, progressive anaemia and loss of condition of susceptible hosts which if untreated leads to heavy mortalities (Bourn, 2001).

Control strategies in trypanosomsis concentrate on vector control, parasite control with chemotherapy and chemoprophylaxis and use of the inherent trypanotolerant trait in some breeds of animals (Holmes, 1997). Previous control techniques included

vegetation clearing, ground and aerial insecticide spraying and selective game destruction. These methods have been discouraged due to the high costs involved in addition to being environmentally un-friendly (FAO, 1992).

Therefore the objectives of this study were: to determine seasonal dynamics of trypanosomosis in intensive suppression area of Southern Tsetse Eradication Project area andto conduct a comparative evaluation of the prevalence of trypanosomosis in intensive suppression area, regular suppression area and uncontrolled area in Arba Minch field operation site of the project site.

Materials and Methods

Study area

The study was carried out in the Southern Tsetse Eradication Project (STEP) area within about 25,000 km^2 of the Southern Rift Valley of Ethiopia, located between 4^{0}45` and 7$^0$15` northern latitudes and 36^{0}40`and 38$^0$20` eastern longitudes in the country. The project is situated in the Southern Nations Nationalities and People's Regional State, Gamo Goffa Zone, Arba Minch Field operation site of the project area surrounded by highlands, Lakes and the Nechi Sar National Park. This area is divided in blocks. The first block comprises about 10,500 km^2 which is also divided into grids. Each grid covers about 100km^2. Specific study area is in Arba Minch Field Operation Site of the STEP and named as the intensive suppression area (ISA). Intensive suppression area (ISA) covers about 100km^2 areas. It is located between 6^0 05'and 6^{0}14` latitude and 37$^0$53` and 37^{0}62` longitude (STEP, 2007). The natural vegetation is predominantly wooded savannah grass land and large portion of the area is well irrigated cultivated land. The area is characterized by hot climatic condition where low and unevenly distributed rainfall patterns (wet season) and regularly high temperature (long dry season) are typical. Annual average rainfall ranges between 800 and 1000mm. The annual mean maximum temperature is about 34.3^oC and the annual mean minimum temperature is 12.5^oC (Krubel, 1985).

Study population

Study populations were Zebu cattle in and around intensive suppression area of the Southern Tsetse Eradication Project (STEP) site. Type of animal management practices in the study area is extensive where cattle are herded at the vicinity of lakes, rivers, within Nechi Sar National Park and hill side during the day.

Sample size

A total of 200 cattle were sampled from ISA during wet and dry season and 150 cattle in each of RSA and UCA by using systematic sampling technique. In ISA, four crush-points were selected and the area was densely populated with large numbers, of cattle herds. A large number of samples were studied due to the willingness of farmers and the availability of laboratory facility.

Study design

The study design was a cross-sectional one in which samples were collected from selected crush-point sites in the study area. Four crush-points from intensive suppression area (ISA), two crush-points from regular suppression area (RSA) and two crush-points from uncontrolled area (UCA). Intensive suppression area differs from the regular suppression area in that the number of targets deployed per km square in the former was larger than the later. About 100% of cattle populations in ISA were treated with pour-on insecticides every month, whereas only 20% of cattle populations in the RSA were treated with pour-on insecticides every three months. Uncontrolled area has no tsetse control measure is implemented.

Cross-sectional epidemiological survey was carried out in different crush-points by taking blood samples, cattle in intensive suppression area in the dry and wet seasons. Blood sampling was also carried out in regular suppression area and in uncontrolled area during the study period. Detection of trypanosome infection in animals was performed by Buffy coat technique (BCT) (Murray et al., 1977). Briefly, blood was collected from the ear vein by heparinized capillary tubes and processed for determination of packed cell volume (PCV) and examination of trypanosome species. Animals that had trypanosomes and those with PCV values of less than 25 were reported to respective owners.

Data management and analysis

Data for this study were obtained from the epidemiological and entomological surveys carried out in the study areas during the dry season between Dec-Jan, 2008, and wet season, between Apr-May, 2009. The database file was transferred to SPSS from Excel and GIS Arc view from access and then descriptive statistics and statistical analyses for associations and differences between dependent and independent variables performed. Data were analyzed by chi-square ($\chi 2$) test, independent sample t-test and one-way ANOVA. The $p=0.05$ was used as the level of significance.

Results and Discussion

The results of the parasitological examination carried out in intensive suppression area on 200 bovine blood samples during dry and wet seasons showed that 11(5.5%) and 7(3.5%) were positive for trypanosomes, respectively. The prevalence of trypanosomosis in each crush-point sites` in Abulo, Shara, Mille and Chalba were 5.7%, 8%, 6% and 2.1% in dry season and 4%, 0%, 12% and 2.7% in wet season, respectively. The highest prevalence was recorded in Mille (12%) and the lowest was in Shara (0%) in wet season. Most of the infections (6% in dry season and 3% in wet season) were due to *T. congolense* in ISA. This species accounted for 90% of the total infection rate in the area. The rest of 10% was due to *T. vivax* which was only diagnosed during wet season. The highest record of *T. congolense* (12%) was in Mille in wet season (Table 1).

Table 1. Distribution of trypanosome species (prevalence) during dry and wet seasons in intensive suppression areas

Crush Point	Season	Sample Size	*T. congolense* (%)	*T. vivax* (%)	Total (%)
Abulo	dry	53	5.7	0.00	6.0
	wet	50	2	2.0	4.0
Shara	dry	50	8	0.00	8.0
	wet	47	0.00	0.00	0.00
Mille	dry	50	6	0.00	6.0
	wet	52	12	0.00	12.0
Chalba	dry	25	2.1	0.00	2.1
	wet	73	2.7	0.00	2.7
Total	dry	200	6	0.00	6.0
	wet	200	3	1.0	4

There was no significant ($P > 0.05$) association between trypanosome infections and dry and wet seasons in ISA (Table 2). There was statistically significant ($p=0.001$) difference in means of PCV values in dry and wet seasons. Similar significant ($p=0.004$) differences were obtained at the Chalba crush-point (Table 3). Comparisons of means of PCV values in all crush-point sites by seasons in ISA illustrated by the following combination for significance of mean differences of PCV values in each season. One way ANOVA for PCV values among four crush-points in intensive suppression area showed significant ($P<0.05$) differences in means of PCV values among crush-points in ISA. All these combinations are shown in Table 4.

Table 2. Chi-Square (χ^2) test results for association of prevalence with dry and wet seasons in each crush-point site in ISA.

	Season						
	Dry			Wet			
Crush-Points	Sample Size	No. Positive	Prevalence (%)	Sample Size	No. positive	Prevalence (%)	P-value
Abulo	53	3	5.7	50	2	4	1.000
Shara	50	4	8	52	0	0	0.054
Mille	50	3	6	25	3	12	0.394
Chalba	47	1	2.1	73	2	2.7	1.000
Total	200	11	5.5	200	7	3.5	0.470

Table 3.t-test for equality of means of PCV values of crush-points in ISA by seasons

Test Variable	Crush-Points	Season	Mean	SD	SD ±	P-Value	95 % CI LB	UB
	Abulo	dry(n=53)	24.49	4.218	0.579	.000*	1.503	5.078
PCV		Wet(n=50)	21.20	4.916	0.695			
	Shara	dry (n=50)	23.36	3.415	0.483	0.832	-2.043	1.648
		wet(n=52)	23.56	5.662	0.785			
	Mille	dry (n=50)	22.84	4.483	0.634	0.394	-1.167	2.927
		wet(n=25)	21.96	3.529	0.706			
	Chalba	dry (n=47)	25.23	4.156	0.606	0.004*	-3.705	-0.703
		wet(n=73)	27.44	3.986	0.467			
	Total	Dry(n=200)	23.89	4.20	.297	0.589	-1.206	0.686
		wet(n=200)	24.15	5.35	.378			

In the three study areas, the means of PCV values and average prevalence were inversely related Thus, when crush-points in ISA were arranged in an increasing order of PCV values, the prevalence decreased in the same order of the crush-points(Figure1 and 2). The results of the parasitological examination carried out in regular suppression areas on 75 bovine blood samples from Fura and 75 from Faragosa showed that 4 (5.3%) were positive for two species of trypanosomes. There was no significance (P>0.05) association between trypanosome infections in the two crush-points of RSA (Table 5). Mean PCV value was higher in Faragosa than Fura. The converse was true for prevalence. This was also in agreement with trend of trypanosome infections and but PCV values were inversely related. Statistically there was no significant (P>0.05) difference in means of PCV values between the two crush-points in RSA (Table 6).

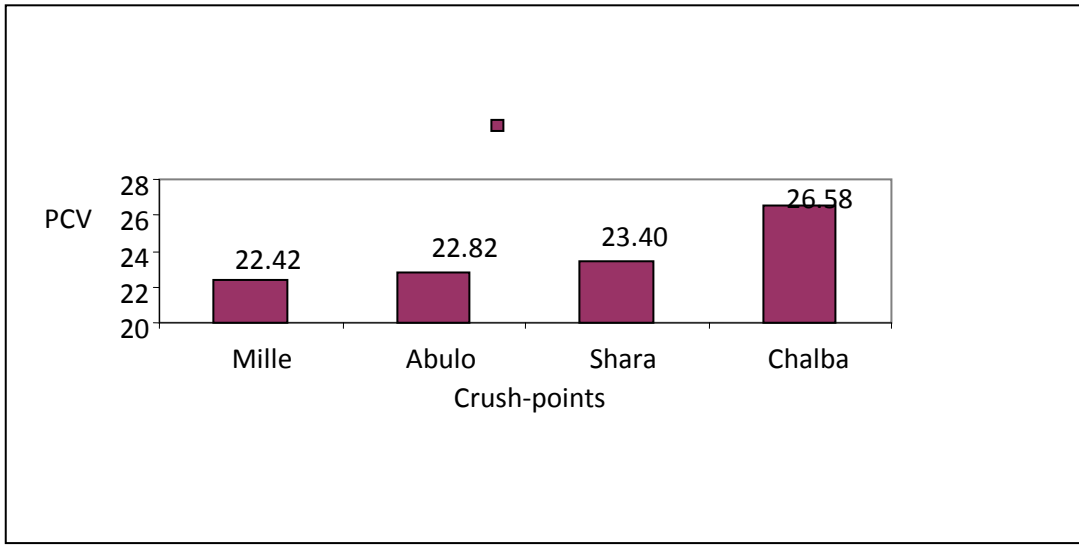

Figure 1.Mean of PCV values in each crush-point in the ISA

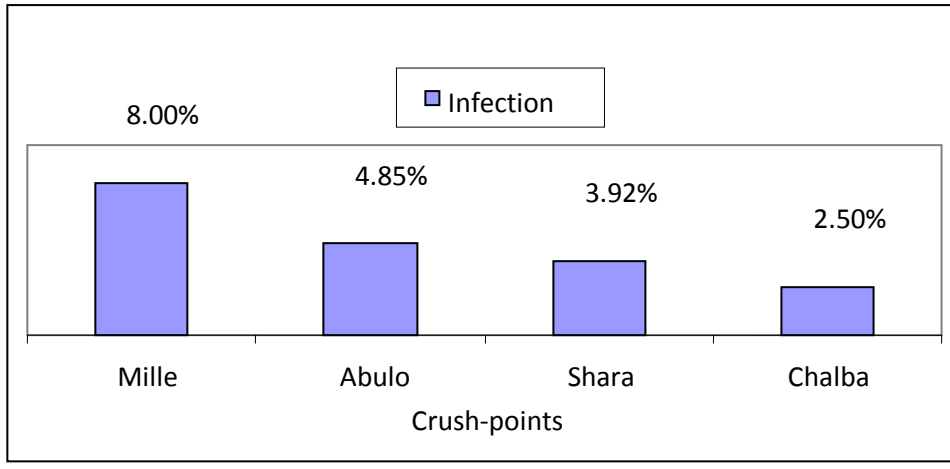

Figure 2.Prevalence of infection by crush-point in the ISA

The results of the parasitological examination carried out in uncontrolled area(UCA) by collecting blood samples from 70 cattle from Shecha and 80 from Kanchama found that 11(15.7%) and 10 (12.5%) were positive for trypanosomes respectively. Chi-square test described that there was no significant (P>0.05) statistical association between trypanosome infections detected in the two crush-point sites (Table 5). Most of the infections in this site were due to *T. congolense* which was about 81% of total infection and the remained 19% is due to *T. vivax*. Prevalence of *T. congolense* was 11.3% and

that of *T. vivax* was 2.7%. In this area out of total infection with *T. congolense* the prevalence in Shecha crush-point was 41.2% and in Kanchama it was 58.8% (Table 7). In contrast with the previous results in this study, higher mean PCV value was recorded in site where higher prevalence was observed, in other words prevalence and PCV values were directly associated (Table 6 & 7).

Table 4. One way ANOVA test for multiple comparisons of means of PCV values by using Tukey HSD in dry and wet seasons

Crushpoint	Crushpoint	Mean Difference	Std. Error	p-value	95% Confidence Interval	
Abulo dry	Shara dry	1.13057	0.86451	0.895	-1.50393	3.76506
Abulo dry	Mille dry	1.65057	0.86451	0.545	-0.98393	4.28506
Abulo dry	Chalba dry	-0.74348	0.87859	0.990	-3.42089	1.93394
Shara dry	Mille dry	0.52000	0.87701	0.998	-2.15259	3.19259
Shara dry	Chalba dry	-1.87404	0.89090	0.414	-4.58894	0.84086
Mille dry	Chalba dry	-2.39404	0.89090	0.129	-5.10894	0.32086
Abulo wet	Shara wet	-2.35769	0.86854	0.121	-5.00446	0.28907
Abulo wet	Mille wet	-0.76000	1.07412	0.996	-4.03324	2.51324
Abulo wet	chalba wet	-6.23836	0.80497	0.000*	-8.69142	-3.78529
Shara wet	Mille wet	1.59769	1.06721	0.808	-1.65450	4.84988
Shara wet	chalba wet	-3.88066	0.79573	0.000*	-6.30557	-1.45576
Mille wet	chalba wet	-5.47836	1.01615	0.000*	-8.57495	-2.38177

* The mean difference is significant at the .05 level.

There was significant (p=0.000) difference in mean PCV values among different study areas (Table 8). Chi-Square test showed that there was significant (p=0.000) association between study areas and trypanosome infection (Table 9).

One way ANOVA test was used for multiple comparisons of dependent variable PCV in each study areas. The results showed there was significance of mean differences of PCV values (Table 10). In case the mean difference was statistically significant ($p<0.05$).

Total prevalence of *T. congolense* in the study area was 5.29% and *T. vivax* was 0.86%. Out of the total infection rate 86.05% was due to *T. congolense* and 13.95% was due to *T. vivax.*

Results of Chi-square test showed that there was significant (p=0.000) association between infection and study areas (Table 11).

There was also a significant ($p<0.05$) associations of trypanosome species within each study area (Tables 12 and 13). Distribution of trypanosome species varied by areas.

Prevalence of infection and PCV values were inversely related among study areas. There was a significant ($p<0.05$) difference in mean PCV values and a significant ($p<0.05$) association among infection and all study areas (Tables 9 and 10).

Table 5. Results of Chi-square test for associations of prevalence and crush-points.

Crush-points	Sample Size	No. positive	Prevalence (%)	p-value
Abulo	50	2	4	
Shara	52	0	0	0.060
Mille	25	3	12	
Chalba	73	2	2.7	
Faragosa	75	0	0	0.120
Fura	75	4	5.3	
Kanchama	80	10	12.5	0.641
Shecha	70	11	15.7	

Table 6. Independent Samples t-Test for mean difference in PCV values in crush-points of RSA and UCA.

Test variable	Crush-points	Sample size	Mean	SD	SD Error of mean	p-value	95 % CI for mean LB	UB
PCV	Faragosa	75	25.81	4.33	0.500	0.795	-1.3153	1.7153
	Fura	75	25.61	5.04	0.581			
	Kanchama	80	21.21	3.54	0.395	0.036	-2.8491	-0.0973
	Shecha	70	22.69	4.95	0.592			

Table 7. Distribution of Trypanosome species in two crush-point sites in UCA

Area	Prevalence of Trypanosome species in Uncontrolled Area		
	T. congolense	*T. vivax*	Total
Shecha (n=80)	7	4	11
Kanchama (n=70)	10	0	10
Total (N=150)	17(11.33%)	4 (2.7%)	21

The present study attempted to identify the prevalence of trypanosomosis in intensive suppression area (ISA) which comprises randomly selected four crush-points (Abulo, Shara, Mille and Chalba) and apparent densities of tsetse flies in dry and wet seasons. Previous study in 15 sites suggests that the apparent density of *G. pallidipes*in those localized control sites may have been reduced by 92 percent while, the prevalence of trypanosomes in livestock in those areas decreased by 58 percent (Alemu *et al*., 2007). In the current study, infection was reduced from 5.5% in dry season to 3.5% in wet season and similarly average apparent densities of tsetse fly were reduced from 1.6 FTD in dry season to 0 FTD in wet season. A combination of chemoprophylaxis against the disease and insecticidal application on cattle against the vector might have greatly improved the trypanosomosis situation in the area. There was however, no significant association between trypanosome infections and the two seasons in the ISA. Previously, in some parts of the project areas, parasitological data were collected in 61 randomly selected points in 1st and 2nd rounds, from where 7285 and 8234 blood samples were collected

and examined which showed 558 (7.66%) and 99(1.20%) cattle were positive for trypanosomosis respectively (STEP, 2000). But, P. Van den Bossche (2001) showed that there is a high association ($P < 0.05$) between the vector and occurrence of bovine trypanosomosis.

Table 8. Results of ANOVA to test for significance of mean differences in three study areas

								95 % CI for mean	
	Area	Sample size	Mean	SD	SD Error	F-test	p-value	LB	UB
PCV	ISA	200	24.15	5.35	0.378	23.42	0.000	23.40	24.89
	RSA	150	25.71	4.68	0.382			24.96	26.47
	UCA	150	21.90	4.30	0.351			21.21	22.59
	Total	500	23.94	5.07	0.227			23.50	24.40

Table 9. Results of Chi-square test for significance association between infection and study areas

Study Areas	Sample Size	No. Positive	Prevalence (%)	p-value
ISA	200	7	3.5	
RSA	150	4	2.7	0.000
UCA	150	21	14	
Total	500	32	6.4	

Table 10.Multiple comparisons of dependent variable PCV in all study areas.

Area	Area	Mean Difference	Std. Error	p-value	95% CI	
ISA	RSA	-1.5658(*)	.5246	.008	-2.799	-0.333
RSA	UCA	3.8133(*)	.5608	.000	2.495	5.132
UCA	ISA	-2.2475(*)	.5246	.000	-3.481	-1.014

* The mean difference is significant at the .05 level.

Table 1. Association of infection and study areas

Study Areas	Sample Size	No. Positive	Prevalence (%)	p-value
ISA	200	7	3.5	0.0001
RSA	150	4	2.7	
UCA	150	21	14	
Total	500	32	6.4	

Table 22. The association of the prevalence of T. congolense with each study area

Areas	Sample Size	No. positive	Prevalence (%)	p-value
ISA	200	6	3	0.0001
RSA	150	3	2	0.0001
UCA	150	17	11.3	0.0001

Table 33. The association of the prevalence of T. vivax with each study area

Areas	Sample Size	No. positive	Prevalence (%)	p-value
ISA	200	1	0.5	0.035
RSA	150	1	0.7	0.027
UCA	150	4	2.7	0.000

Even though there is causal relationship between prevalence of trypanosomosis and apparent densities of tsetse flies, 3.5% prevalence in the absence of tsetse fly in the ISA in wet season might be caused by the inaccessible tsetse flies in the pocket areas by conventional tsetse control methods or uncontrolled movement of large number of animals in the area from uncontrolled area. This dynamics of trypanosomosis and tsetse fly in dry and wet season should be considered prior to implementation of any control measure. Introduction of huge number of animals in the area may also haphazard the main monitoring activities in the area. Previous study in other selected parts of the project area showed an overall disease prevalence rates during the two rounds parasitological surveys of 7.66% and 1.20%, respectively. Preliminary analysis of the result of two round parasitological survey and four cycles of entomological survey showed that there was a very good overlap between the vector and the parasite distribution in the project area (STEP, 2000).

Animals in Mille are herded in wooded grass land near shores of Lake Abaya which offers favorable conditions for the vector tsetse flies to thrive. This calls for another attention to revise control and monitoring schedules of the project so that the relationship between vegetation coverage and disease prevalence should be considered during different control activities. The study underscores the usefulness of cross-sectional studies as a

precursor to tsetse and trypanosomosis control interventions. The results of the survey, besides identifying herds and villages where trypanosomosis constituted a major problem, as far as animal health is concerned, also provided information on the prevalence of trypanosome species and efficacy of monitoring program in the intensive suppression area.

Alemu*et al*. (2007) indicated that the community-based tsetse suppression does not cover all of the tsetse-infested areas in the STEP block-1 and therefore, some cattle herds remain with disease in areas that are not adequately covered by the community fly control measures. The operational programme should include the introduction of a set of implementation rules and regulations conducive to the special needs of an operational AW-IPM campaign, i.e. an efficient management structure and the provision of adequate financial flexibility.

The lowest prevalence (2.5%) record was in Chalba. The area has large proportion of cultivated land covered with riparian forest which prohibits the presence of the vector. Social motivation is excellent in farmers to bring animals for pour-on insecticide treatment. In Shara crush-point area, zero prevalence was registered in wet season which is probably due to the fact that animals were herded along hill sides of high altitude areas where tsetse flies could not survive.

In Africa, the primary vector for *T. congolense, T. vivax,* and *T. b. brucei* is the tsetse fly. These trypanosomes replicate in the tsetse fly and are transmitted through tsetse fly saliva when the fly feeds on an animal. Trypanosomosis is also mechanically transmitted by tsetse and other biting flies through the transfer of blood from one animal to another. The most important mechanical vectors are flies of the genus Tabanus, but Haematopota, Liperosia, Stomoxys, and Chrysops flies have also been implicated. In Africa, both *T. vivax* and T. b. brucei have spread beyond the "tsetse fly belts", where transmission is principally by tabanid and hippoboscid flies (Roder, 1984). In the current study the dominant trypanosome species were *T. congolense* with total prevalence of 5.3% and *T. vivax* was 0.9%. This shows that the main vector of the parasite for disease transmission in the study area is tsetse fly.

To assess the impact of trypanosomosis and compare the pathogenicity of different species and strains should be studied and documented to determine priority areas in control and monitoring programs.

Low community awareness and participation were observed in the study area during study time in some sites of intensive suppression area. Possible reasons would be reduction in disease challenge due the previous control measures. Time of application of insecticides doesn't match with work time of farmers and most of the animals move to the grazing areas before technical persons arrives at spraying crush-points. This requires the integration between local administration focal persons and technical officers.

In the RSA, regular treatment of animals was being carried out with frequency less than that of intensive suppression area. The crush-points were in the northern part of intensive suppression area. Prevalence of 2.7% and 0% were recorded in crush-points Fura and Faragosa, respectively. The higher prevalence in Fura might be due to the ecology of the area which is wooded grass land which is favorable climate for the vector. Though the season in the area is the wet one, Faragosa crush-point site suffers from shortage of rain which makes it difficult for the vector to exist. Most of the animals in Fura crush-point area were introduced from the other side of the Lake Abaya by boat for traction purpose. In this area, there are also very large movements of animals to the markets. These movements might predispose to the disease since there is no tsetse control measures practiced on the other side of the Lake Abaya. Society of this regular suppression area was well aware of the control measures and willing to treat animals with pour-on insecticides to keep targets on the deployed site. The prevalence of trypanosomosis 1.33% obtained in this study could be reduced by additional input of control measures.

In the UCA, there are no tsetse control measures implemented by the project. Kanchama with semi-sedentary farming system keeps cattle at the same grazing area in bush land with cattle from Shecha Town. Prevalence of trypanosomosis was 12.5% in Kanchama and 15.7% in Shecha. Over all, a prevalence of 14% in uncontrolled area was recorded. This is in agreement with the findings of Bergenie (2005) in Arba Minch field operation site where the prevalence of trypanosomosis during pre-intervention, was higher (23%) than post intervention (11%). Aniamals in Kanchama had been on the other side of the NNP in dry season and were moved to the present study area in wet season for grazing. Excess forage was available in the area and animals in Kanchama feed on it the whole day without tracking a long distance for feeding. The prevalence difference may be due to differences in management practices. Cattle in the town were kept by herdsmen in the grazing time and tethered by laborers at home. Usually there was no regular follow up by owners of animals in the urban area on animal health conditions.

The changes in the distribution of the different species of tsetse flies, would suggest that there had been considerable ecological instability attributable to man. Continuous annual burning of bushes and clearing of land for cultivation, which removed certain kinds of trees, and savannah land essential to tsetse species as well as hunting for food by pastoralists of wild animals (natural hosts) which are the main feed sources of tsetse had decreased the abundance and spread the. On the other hand suitable vegetation and the presence of suitable hosts favored the distribution and spread of tsetse flies to new areas (SRVL, 1998).

In some nearby sites of the study area, where no tsetse control was implemented had high tsetse population density. But gradual destruction of the vegetation in the area for extensive agricultural practices for crop production and for fire wood farmers experience low burden of the tsetse and trypanosomosis challenge (STEP, 2007). Since land use policies can determine tsetse and trypanosomosis dynamics, it should be set

and revised according to the population expansion, settlement programs and long term land use strategies. The unrelenting human pressure on land associated with the population growth in the study area may eventually get rid of most of the problem of Savannah tsetse species, but at the cost of uncontrolled land degradation.In conclusion, results of seasonal and spatial dynamics of trypanosomosis in and around intensive suppression areas would be useful in planning and eradication program in the project area. Integrated disease eradication approach should be followed and it needs integration among all stakeholders.

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