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Post Control Survey on Prevalence of Bovine Trypanosomosis and Vector Distribution in Ameya District, South West Shewa, Ethiopia

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Abstract- A cross sectional study was conducted from February to June, 2013 to determine prevalence of bovine trypanosomosis and population of tsetse and other biting flies, and to assess effects of integrated control strategy implemented in the last five years in Ameya district, South West Shewa, Ethiopia. Both primary and secondary data were used. Structured questionnaire survey was conducted by face to face discussion. Buffy coat technique was used for screening followed by thin smear technique for trypanosome species identification. Baited monopyramidail traps were deployed at a distance of 300m apart for 72 hours to catch flies. The district was identified as one of the areas affected by bovine trypanosomosis and infested by tsetse and other biting flies. Integrated control strategy has been implemented to reduce occurrence of the disease and its vectors. From 436 examined animals, 6 (1.4%) were positive. Two species of trypanosome, *Trypanosoma brucei* (3/6, 50%) and *Trypanosoma congolense* (2/6, 33.33%) were identified in a single infection while 16.67% (1/6) mixed infection of both species was obtained. There was no statistical significance difference ($p>0.05$) in prevalence of the disease among groups of peasant associations and age, and between sex groups. The mean packed cell volume of parasitemic and aparasitemic cattle was similar. A total of 6236 biting flies, including 6133 (98.35%) *Stomoxys*, 18 (0.29%) *Tabanus*, 14 (0.22%) *Chrysops* and 71 (1.14%) *Haematopota* were captured. However, tsetse fly was not captured. Statistical significance difference was observed in mean catch of *Stomoxys* and *Tabanus* ($p<0.05$) in different peasant associations. In conclusion, application of integrated control strategy undertaken in the district resulted in reduction of disease occurrence and tsetse flies. Thus, continuation of the ongoing integrated control strategy and dissemination of the strategy to neighboring districts to limit re-invasion with tsetse flies were recommended.

Keywords: Ameya district, biting flies, bovine trypanosomosis, integrated control strategy, tsetse flies.

1. INTRODUCTION

Livestock are of enormous importance socially and economically for nutritional and agricultural purposes in Africa (Uilenberg, 1990). In developing countries, diseases of livestock reduce agricultural output by up to 30% (FAO, 1990). Among animal diseases, presence of trypanosomosis, which is caused by trypanosome and transmitted cyclically by tsetse flies or

mechanically by biting flies (Maudlin *et al.*, 2004; Radostits *et al.*, 2007), is a major constraint on agricultural production and has a devastating effect on livestock and man. Tsetse flies are largely responsible for an uneven distribution of cattle in Africa, leading to overgrazing and severe environmental degradation in some areas and preventing the introduction of productive farming and livestock systems in other areas. Tsetse and trypanosomosis are problems that are closely linked with rural poverty, thus, tsetse fly is frequently referred to as the "poverty insect" (IAEA, 2003). It is also responsible for an annual loss of millions of dollars in livestock production as a result of the cost related to treatment, prevention and vector control efforts (Samuel *et al.*, 2001) and death of animals (Bett *et al.*, 2004).

Control of the disease can be based on control of the causal agent and its vector, and use of innate resistance of the host to the effects of the infection (Uilenberg, 1990). There are several methods that may be used to try to reduce trypanosomosis and its effects; a combination of these methods will be used in any campaign carried out on a continental scale (Pollock, 1982). In Ethiopia, several attempts have been made to control the disease with chemotherapy and chemoprophylaxis being the most widely applied methods. Vector targeted control practices have been implemented mainly through specifically designed joint projects of the Ministry of Agriculture and other non-governmental organizations (Tafese *et al.*, 2012).

As part of the Gibe river system, tsetse-transmitted animal trypanosomosis has been incriminated as the primary disease condition to highly curtail the production potential of its livestock sub-sector, particularly in the fertile lowland and midlands of Ameya district, South West Shewa zone, Ethiopia. The district was one of the highly affected areas by animal trypanosomosis and infested by different tsetse and biting flies (NTTICC, 2009; Denu *et al.*, 2012). Since intense studies in 2007/2008 in the district, integrated control strategy has been undertaken by support of governmental and non-governmental organizations. Thus, knowing the current status of the disease and its vectors is crucial to apply efforts towards combating the disease and reducing economic losses in the area. Therefore, the current study was conducted to determine the prevalence of the disease and its vectors, identification

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of vector flies and trypanosome species distributed in the area and to check the effectiveness of the undertaken control methods.

II. MATERIALS AND METHODS

a) Study area

The study was conducted in Ameya district, South West Shewa zone, Ethiopia from February to March, 2013 (for prevalence of trypanosomosis survey) and from May to June, 2013 (for tsetse and biting flies survey). Gindo is the administrative town of the district and located at about 145 Km distant southwards of Addis Ababa, capital of Oromia region and Ethiopia. The district consists of midland and lowland situated in Gibe watershed, one of main rivers of Gibe-Omo River system. The district has 39 kebeles (peasant associations (PAs)), of which 19 (47%), 10 (36%) and 7 (17%) PAs are lowland, midland and highland, respectively. Five PAs having different sites, Gambela Ashute Talgo (Ashute site), Gombore Aliyi (Gombore, Eteya and Gombore Baticha sites), Mari Kereyu Sekela (Mari-Magari and Mari sites), Moko Ujuba Kota (Kota site) and Cha'a Kase (Atnafo site) were randomly selected for the study.

The topography of the areas is mainly plain and thoroughly cultivated. Mixed livestock and crop farming is the dominant form of production where rain-fed agriculture is the common production system. The vegetations are confined on the river sides, and the types of vegetations are dominated by thick and scattered thickets with short grasses on the upper reaches of the rivers. As inhabitants of the area are cutting down the trees for fire wood and charcoal, the vegetation is getting thinner and thinner. The wild life could not be found in a group due to lack of enough shelter as needed except some wild games.

The district has a total of 15,715 local and 37 cross breed cattle. In addition, there are 30,881, 34,815, 7,803, 3,150, 13,046 and 85,823 sheep, goats, horses, mules, donkeys and poultry, respectively (Socio-economic data, 2012).

b) Study protocol

Both primary and secondary data were used. The primary data were collected from field works. Secondary data were obtained from Livestock Development and Health Agency of Ameya district after provision of permission request letter, and published sources on works conducted in the district before five years. The obtained secondary data include prevalence of the disease, distribution and densities of tsetse and biting flies before five years, and control strategies applied in the last five years.

c) Questionnaire survey

A structured questionnaire survey was conducted to gather information on aspects of livestock management, socio-economics of the society, trypanosomosis and its vectors, and control strategies being

used to control the disease and its vectors. Fifty-one respondents were randomly selected from the households, who are residents of the district, and informed about purpose of the study. The questionnaire was administered by face to face discussion.

d) Study design and animals

A cross sectional study was conducted on 436 randomly selected local zebu breed cattle of both sexes. Age of animals was determined using owners' information. Accordingly, animals were categorized as young (<2 years), young adult ($\geq 2 < 5$ years), adult ($\geq 5 \leq 9$ years) and old (>9 years). Entomological survey was conducted in selected areas that seemed to be habitat for tsetse flies using monopyramidal traps.

e) Parasitological survey

A small quantity of paired blood samples were collected into heparinized haematocrit capillary tube (filled up to about $\frac{3}{4}$) from marginal ear vein by pricking with the tip of a sterile lancet after properly securing the animal and aseptically preparing the area. Buffy coat technique was used. The samples were centrifuged at 12000 rpm for 5 minutes. The capillaries were measured by haematocrit reader and a packed cell volume (PCV) value of each cattle was recorded.

The capillary tube was cut 1 mm below the buffy coat using diamond pencil. The content of the capillary tube was expressed onto a clean microscope slide and covered with a 22x22 mm cover slip. Then the slide was examined for trypanosomes based on the type of movement in the microscopic field with 40X objective lens magnification. Confirmation of trypanosome species of positive samples by morphological characteristics was performed by thin smear technique and examination by light microscopy of 100X objective lens magnification (Murray et al., 1977; Uilenberg, 1990).

f) Entomological survey

During entomological survey, 24 monopyramidal traps were deployed in selected sites (Gombore, Mari Magari, Mari and Kota sites) at a distance of about 300 m apart for 72 hours. Every trap was odour-baited with acetone (150 mg/hr release rate), 3-octen-1-ol (0.5 mg/hr release rate) and cow urine (1000 mg/hr release rate) (Terfa *et al.*, 2014). The underneath of each trap pole was smeared with grease in order to prevent the ants climbing up the pole towards the collecting cage that could damage the flies.

After a specified period of deployment, the flies caught in the collecting cage were sorted, identified, counted and the apparent fly density per trap per day was recorded. A hand lens was used for the identification flies based on the characteristic morphological structures at genus level.

g) Data analysis

All the collected data were coded and entered into the Microsoft Excel spreadsheet and analyzed using

Statistical Package for the Social Sciences (SPSS) 16.0 version. Frequency distribution and percentage were used to summarize data obtained from questionnaire survey and prevalence of the disease. Vector survey data were analyzed using student t-test and ANOVA to compare the mean catches in different study areas. In all cases, 95% confidence interval was employed.

III. RESULTS

a) Secondary data

Previous study was conducted by NTTICC (National Tsetse and Trypanosomosis Investigation and Control Center) in April, 2008 and reported in 2009 in two peasant associations (Mari Magari and Bareda Chilalu) before implementation of integrated control strategy. A total of 16 and 12 *Trypanosoma congolense* (*T. congolense*) and *Trypanosoma vivax* (*T. vivax*) were isolated from 192 examined cattle in the district with

11.45% and 17.7% prevalence rate in Mari Magari and Bareda Chilalu, respectively. Meanwhile, 40 monopyramidal traps were deployed and 28 *Glossina pallidipes*, 658 *Stomoxys* and 5 *Tabanus* flies were captured.

According to information from Livestock Development and Animal Health Agency of Ameya district, a total of 380 liters undiluted 18.75% deltamethrin were provided and poured on 25,350 cattle and 225 targets impregnated with 0.5% deltamethrin were deployed in the last adjacent five years (2007-2013) for 10 kebeles (PAs) in the district (Table 1). In addition, trypanocidal drugs and deltamethrin provided to the cattle owner by public and private veterinary clinics and pharmacies were also contributed for control activities and constitute the utmost proportion.

Table 1 : Trypanosomosis control mechanisms used in the last five years in Ameya district

Year	Poured on deltamethrin (liter)	Number of cattle sprayed	Target deployed
2007/08	100	5000	-
2008/09	170	7000	100
2009/10	50	4000	100
2011/12	70	4100	25
2012/13 (up to May, 2013)	90	5250	-
Total	380	25,350	225

b) Primary data

i. Questionnaire survey

The questionnaire survey result indicated that about 100% of the respondents practice mixed (livestock-crop production) farming system. Additionally, 9.8% (5/51) respondents participate in trade and feedlot activities. Cattle are reared as a source of milk, meat, manure, drought power, income generation, paying dowry, wealth banking, bleeding (sacrifice) and fattening.

Free grazing was the common practice (40/51 respondents (78.4%)) while free grazing combined with stall feeding during milking for lactating cows and drought oxen was also rarely used (11/51 (21.6%)). Crop residues like straws and residues of local beer called "atalaa" were identified as source of feed for stall feeding. Majority of the respondents (32/51 or 62.5%) were using bush and grassland areas to graze their animals, which are found either nearby the river bodies or not (Table 2).

The disease was first (49/51 respondents or 96%), second and fourth (1/51 respondent or 2% each) ranked among known animal diseases and resulted in death of 21 cattle owned by some of the 51 respondents in the last 12 months. From 51 respondents, 36 (70.6%) and 13 (25.5%) claimed decrease of milk production by

100% and 50%, respectively in lactating cows while all of them agreed on decreasing by half to complete cessation of drought power of oxen due to the disease. Effects of the disease like abortion, delay of first calving, long calving interval and birth of underweight calves were common in the area.

Majority of the respondents (37/51 (72.5%)) claimed as the disease was introduced in the district in 1970s while the rest (14/51 (27.5%)) said 20 years ago. About 90.2% (46/51) of the respondents responded as occurrence of the disease is decreased over last five years while the rest 9.8% (5/51) respondents claimed increased occurrence of the disease. However, respondents in both groups know and agreed on different control strategies performed in the district except 2 (3.9%) respondents (Table 2).

Table 2 : Summary of questionnaire survey on trypanosomosis and its vector in Ameya district

Variables	Total respondents	Frequency	Proportion (%)
Grazing area			
Grassland	51	11	21.6
Bush area	51	2	3.9
Bush and grassland	51	32	62.5
Bush area and bottom of valley	51	4	7.8
Grassland and top of valley	51	1	2
Bottom of valley	51	1	2
Season of occurrence of trypanosomosis utmost			
Wet season	51	9	17.6
Dry season	51	37	72.5
The same throughout a year	51	5	9.8
Know tsetse fly			
Yes	51	19	37.3
No	51	13	25.5
Know other vectors of trypanosomes	51	17	33.3
Know tsetse and other biting flies	51	2	3.9
Season of tsetse and other flies that transmit trypanosomosis abundance			
Wet season	51	38	74.5
Dry season	51	6	11.8
The same throughout a year	51	1	2.0
Don't know	51	6	11.8
Level of trypanosomosis in the last five years			
Increased	51	5	9.8
Decreased	51	46	90.2
Applied control strategies in the area			
Trypanocidal drugs	51	7	13.7
Trypanocidal drugs and trap/target	51	7	13.7
Trypanocidal drugs and pour on	51	12	23.5
Trypanocidal drugs, trap/target and pour on	51	21	41.5
Trypanocidal drugs, pour on and others (deforestation to cultivate the land, supplementary feeding)	51	2	3.9
Don't know any applied method	51	2	3.9

ii. *Prevalence of trypanosomosis*

In the current study, an overall 1.4% (6/436 cattle) prevalence of the disease was recorded. There was no statistical significance difference between sex ($p>0.05$), among PAs and age ($p>0.05$) groups (Table

3). Two species of trypanosomes, *T. congolense* (2/6 or 33.33%) and *Trypanosoma brucei* (*T. brucei*) (3/6 or 50%) were identified as a single infection and mixed infection of *T. congolense* and *T. brucei* (1/6 or 16.67%).

Table 3 : Prevalence of trypanosomosis in groups of different risk factors

Variable	Category	No. of examined	No. of positive (%)	Fishers' exact	p-value
PAs	Gambela Ashute Talgo	47	1(2.13)	2.801	0.532
	Gombore Aliyi	129	2(1.55)		
	Mari Kereyu Sekela	115	3(2.61)		
	Moko Ujuba Kota	73	0		
	Cha'a Kase	66	0		
Sex	Male	209	3 (1.44)	0.01	1.000
	Female	227	3 (1.32)		
Age (years)	<2	27	0	0.312	1.000
	≥2<5	150	2 (1.33)		
	≥5≤9	231	4 (1.73)		
	>9	28	0		

There was no observable difference in the mean PCV of the aparasitemic and parasitemic cattle. How -

ever, the mean PCV of parasitemic cattle are slightly higher. The mean PCV of aparasitemic cattle was 25.20

± 4.210 SD with a range of 11-38 while the mean PCV of parasitemic cattle was 25.50 ± 4.889 SD with a range of 19-31.

iii. Entomological survey

A total of 6,236 biting flies, of which 6,133 (98.35%) *Stomoxys*, 18 (0.29%) *Tabanus*, 14 (0.22%) *Chrysops* and 71 (1.14%) *Haematopota* were captured. However, no tsetse fly was captured. The apparent densities (fly/trap/day) of 85.18, 0.25, 0.19 and 0.97 were obtained for *Stomoxys*, *Tabanus*, *Chrysops* and *Haematopota*, respectively. The overall mean apparent density of the captured biting flies was 86.61 ± 65.72 SD.

Highest mean catch was recorded for *Stomoxys* (255.54 ± 196.648 SD) followed by *Haematopota* (2.96 ± 3.014) while the lowest mean catch was obtained for *Chrysops* (0.58 ± 1.018 SD) followed by *Tabanus* (0.75 ± 1.452 SD). Statistically, significance difference was obtained in the mean catch of *Stomoxys* ($F=4.777$, $p<0.05$) and *Tabanus* ($F=0.45$, $p<0.05$) among different PAs (Table 4). Further analysis revealed presence of statistical significance difference between Gombore and Mari Magari, and Gombore and Kota for *Stomoxys*, and between Gombore and Mari and Gombore and Kota for *Tabanus*.

Table 4 : Significance of mean catch of biting flies using ANOVA test

Variable	F-test	p-value
<i>Stomoxys</i>	4.777	0.011
<i>Tabanus</i>	3.204	0.045
<i>Chrysops</i>	0.976	0.424
<i>Haematopota</i>	0.405	0.751

IV. DISCUSSION

a) Questionnaire survey

Dependence of the community on mixed farming system is consistent with previous work of Denu *et al.* (2012) in the same area. Livestock are reared for different purposes, which are integral part of agricultural activity and used as a source of milk, meat, manure, drought power, income generation, wealth accumulation (as a bank), for dowry payment and worshipping by scarifying or bleeding the animal. However, livestock production has been hindered by many devastating animal diseases, of which trypanosomosis is the first ranked disease by majority (49/51, 96%) of the respondents. The current result agrees with the previous result in the area (Denu *et al.*, 2012) and work of Terfa (2008) in Gawo-Dalle district, Kellem Wollega zone, Ethiopia, in which all the respondents ranked trypanosomosis as first. About 72.5% (37/51), who are elders, agreed as the disease was first introduced and recognized in the area in 1970s while the rest, younger and immigrants, estimated 20 years ago. Similarly, 100% indigenous habitants of in Gawo-Dalle district, Ethiopia claimed as the disease was introduced in 1960s (Terfa, 2008).

Twenty one cattle owned by some of the respondents were died due to the disease in the last 12 months (from date of the interview). Abortion, delay of first calving, long calving interval and birth of under-weight calves, reduction in milk production and drought power of oxen were also the important in the area, which led to struggle with subsistence livelihood of the community and consistent with Maudlin *et al.* (2004). According to Vreysen (2006), the disease is among well known livestock production constraints in Africa, mainly in rural poor community and considered as a root cause

of poverty. The overall economic loss caused by the disease was estimated to be US \$1408-1540 million per annum (NTTICC, 2006).

About 72.5% (37/51) of the respondents said that the disease occurs mostly in dry season. This disagrees with the finding of Denu *et al.* (2012) who reported higher prevalence of the disease in wet season than dry season in the area. The difference may be due to observable effects of the disease during dry season in combination with feed shortage and concurrent diseases.

Majority of the respondents (37.3% or 19/51) know tsetse flies, 33.3% (17/51) knows other biting flies that can transmit the disease while 3.9% (2/51) knows both biting and tsetse flies. About 74.5% (38/51) said both tsetse and biting flies are abundant during wet season. According to the respondents, these flies are responsible to transmit the disease during watering and grazing in forest, grassland, bush area, bottom and top of valleys of different vegetation types. The current findings are consistent with work of Denu *et al.* (2012).

b) Prevalence

In the current study, prevalence of the disease was 1.4%. However, in 2007/2008 higher prevalence rate of 33.5% and 17.83% in the late rainy and dry seasons, respectively was recorded in three districts of South West Shewa (Denu *et al.*, 2012) from which two were included in the current study. In addition, higher prevalence of 11.45% and 17.7% was obtained in Mari Magari and Bareda Chilalu sites of Ameya district in 2008 (NTTICC, 2009). This indicated the decreased occurrence of the disease over the last five years and is consistent with response of the majority of the respondents (46/51, 90.2%). Similarly, study conducted in two

areas (controlled and non-controlled area) indicated lower prevalence of the disease in the controlled (9.1%) than in non-controlled area (15.1%) in Hawa Gelan district of Kellem Wollega zone, Ethiopia (Fantahun *et al.*, 2012).

The difference between the current and previous studies in the same area is due to application of integrated control strategy and professional intervention recommended from the previous works. These control methods include treatment with trypanocidal drugs, deploying of impregnated targets, applying pour on of deltamethrin and increased clearance of the tsetse fly habitat for cultivation by the community. In addition, frequent treatment of the infected cattle and season of the study, dry season, might be reasons for lower result of the current study.

Two species, *T. congolense* and *T. brucei*, were isolated in the current study. *Trypanosoma brucei* was the dominant species, 50% (3/6) as single infection and 16.67% (1/6) as a mixed infection with *T. congolense*. However, *T. congolense* and *T. vivax* were the only reported species in the previous two studies of the same area before five years with *T. congolense* as the dominant species (NTTICC, 2009; Denu *et al.*, 2012). In contrast to the current result, *T. brucei* was the least prevalent species isolated in some parts of Ethiopia while *T. congolense* and *T. vivax* were the dominant (Abebe and Jobre, 1996; Abebe, 2005; Mulaw *et al.*, 2011; Tafese *et al.*, 2012). The difference might be attributed to sensitivity of tests and delayed examination of buffy coat smear (Uilenberg, 1990).

Young adult ($\geq 2 < 5$ years) and adult ($\geq 5 \leq 9$ years) were more susceptible to the disease than young (< 2 years) and old (> 9 years) cattle. However, there was no statistical significance difference among different age groups. The higher prevalence was observed in adult (1.73%) followed by young adult (1.33%). The current result is slightly in line of agreement with work of Denu *et al.* (2012) in which 16%, 22% and 24% infection rate was reported in calves (< 1 year old), adult (1-4 years old) and older animals (> 4 years old), respectively in dry season with absence of statistical significance difference among age groups. This could be associated to the fact that adult cattle travel long distance for grazing and draught as well as harvesting crops in areas of high tsetse challenge (Denu *et al.*, 2012). According to Rowlands *et al.* (1995), sucking calves do not go out with their dams but graze at homesteads until they are weaned off, which is also true for this study area. Furthermore, protection of young animals by natural maternal antibodies to some extent (Fimmen *et al.*, 1992) could be considered as one factor for low prevalence in young cattle.

There was no statistical significance difference between sex groups even though the prevalence of the disease was slightly higher in males than females. This is consistent with work of Tafese *et al.* (2012). However,

Zecharias and Zeryehun (2012) reported slight higher prevalence in female cattle with absence of statistical significance difference in Arba Minch, Ethiopia. Absence of significance difference between sex groups may be due to an equal chance of exposure to the parasite (Tafese *et al.*, 2012).

The mean PCV of parasitemic and aparasitemic cattle of the current study was almost similar. However, the PCV of cattle was significantly influenced by trypanosome infection in the previous study in the area (Denu *et al.*, 2012). The lower PCV of apparently trypanosome free cattle could be due to various concurrent diseases and nutritional interference with development of anemia, conversely many cattle having high PCV also show to be infected in which it may be occurred due to early infection (Fantahun *et al.*, 2012).

c) Entomological survey

During the current survey, tsetse flies were not captured. However, four genera of biting flies were captured. Previously, the current study area was infested with *Glossina pallidipes*, *Stomoxys* and *Tabanus* (NTTICC, 2009; Denu *et al.*, 2012), *Glossina morsitans submorsitans*, *Glossina fuscipes fuscipes*, *Haematopota* and *Chrysops* (Denu *et al.*, 2012). A total of 6236 biting flies were captured. From these, about 6133 (98.35%) were *Stomoxys* while the rest was tabanids which slightly agrees with previous report of 6.48% and 4.51% tabanids and 81.02% and 88.06% *Stomoxys* (muscid) during the late rainy and dry seasons, respectively (Denu *et al.*, 2012). Similarly, higher number both tsetse and biting flies were captured in non-controlled area than controlled area in Hawa Gelan district (Fantahun *et al.*, 2012). The difference between the current and previous situation of tsetse flies might be attributed to implementation of tsetse flies control strategy undertaken in the district.

The mean apparent density of the captured biting flies of the current study was 86.61. The highest was recorded for *Stomoxys* (85.18 fly/trap/day) followed by *Haematopota* (0.97 fly/trap/day) while the lowest was recorded for *Chrysops* (0.19 fly/trap/day) followed by *Tabanus* (0.25 fly/trap/day). Denu *et al.* (2012) reported lower apparent density of muscids (*Stomoxys*) of 18.66 and 15.04 fly/trap/day in late rainy season and dry season, respectively; however, the apparent densities of tabanids (*Tabanus*, *Chrysops* and *Haematopota*) are comparable (1.49 and 0.77 fly/trap/day in late rainy and dry season, respectively).

In conclusion, the prevalence of the disease and its vectors, especially tsetse flies, were reduced when compared with situation of the area before five years. Implementation of integrated control strategy of the causal agent and its vectors were successfully resulted in reducing the occurrence of the disease. Thus, the integrated control strategy on application should be continued and disseminated to the

neighboring districts to avoid re-invasion of the area with tsetse flies.

V. ACKNOWLEDGEMENT

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