Systematic Evaluation of Implemented Strategic Trypanosomosis and Vector control activities by the Application of Insecticides (Deltamethrin base) using Stationary attractive devices (targets), Ground sprays and Live bait techniques in upper Birbir valley, Ethiopia

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Abstract

Background

Trypanosomosis and vector control methods have evolved from game animal destruction and bush clearing, which mainly targeted on the removal of the source of food and shade, to insecticide spraying following the discovery of DDT. Baseline and control entomological and parasitological surveys or studies were carried out from September 2016 to February 2018, with the purpose intended to systematically evaluate the effectiveness of implemented strategic trypanosomosis and vector control activities.

Results

Baseline and control entomological surveys or studies were compared; and an overall apparent fly density of 2.61 flies/trap/day during the baseline survey was declined to 1.68 flies/trap/day with the rate of 64.37% after control with supporting evidence of a decrease in the daily mean catch of fly per single trap from $5.23 \pm 1.58$ to $3.35 \pm 1.58$, (Mean catch ± SD). Based on baseline and control survey or study results the overall prevalence of bovine trypanosomosis was compared and significantly declined from 7.91–4.81%, likelihood-ratio = 13.49, Pr = 0.000, with the rate of 60.81%. Furthermore, significantly better heard mean packed cell volume was observed during control survey or study 22.53 ± 4.48, 25.68 ± 4.04 (Mean PCV ± SD), when compared with baseline 21.87 ± 4.57, 25.41 ± 4.27 (Mean PCV ± SD), in parasite-infected and non-infected cattle respectively.

Conclusions

The study concluded that the use of stationary attractive devices (targets), Ground sprays, and Live bait techniques of Deltamethrin based insecticide had shown a significant decline in the density of tsetse fly (vector hosts) and trypanosomosis prevalence with a better heard mean PCV of infected and non-infected cattle. Therefore, the research recommended that such kind of control methods which are environmentally friendly or non-intrusive, cheap, and non-complicated for farmers and experts living at endemic tsetse belt areas.

Background

Tsetse fly (Glossina species) that are of major economic importance in Ethiopia are confined to the southern and western regions between longitude 33° and 38° E and latitude 5° and 12° N which covers about 220,000 km$^2$ fertile and arable agricultural land having great potential for livestock and crop production [1]. Five economically very important fly species, namely Glossina morsitans submorsitans, G. longipenis, G.pallidipes, G.tachinoides and G. fuscipes fuscipes exist that serving as cyclical transmitters of Trypanosoma congolense, Trypanosoma vivax, and Trypanosoma brucei. Trypanosomosis is a major priority disease in the country by causing direct and indirect losses to the livestock development resulting in the exclusion of animals from the infested potential areas, which consequentially affects agricultural crop production [2].

The disease African animal trypanosomosis (AAT) causes gradual health decline in infected animals reduces milk and meat production, increases risk of abortion, and animals eventually succumb to the disease [3]. This has an enormous impact on the livelihood of farmers who live in tsetse-belt (infested) areas, as infected animals cannot be used to plough the land, and animal husbandry is only possible when the animals are kept under regular
prophylactic treatment with trypanocidal drugs, often with consequential problems of drug resistance, counterfeited drugs, and suboptimal dosage. The presence of tsetse fly and trypanosomosis discourages the use of more-productive exotic and cross-bred cattle, affects the distribution of livestock populations, reduces the potential golden opportunities for livestock and crop production (mixed farming) and affects human settlements (people tend to avoid areas with tsetse flies) [4].

The course of the disease in animals is similar to the course of sleeping sickness in humans. Trypanosoma congoense and Trypanosoma vivax are the two most important species infecting cattle in sub-Saharan Africa. Other forms of animal trypanosomiasis are also known from other areas of the globe, caused by different species of trypanosomes and transmitted without the intervention of the tsetse fly [5]. Glossina insects are usually involved in the natural transmission of the parasite that the life cycle has two phases, one in the mammalian host and the other in the insect vector [6]. Tsetse flies ingest trypanosomes in the blood or lymph while feeding on an infected host, then undergo a cycle of development and multiplication in the digestive tract of the fly until the infective meta cyclic trypanosomes (meta trypanosomes) are produced [7]. There they undergo a transformation losing their typical trypanosome or trypomastigote and meta cyclic trypanosomes, which are infective, forms of the host [8].

In general, the diagnosis of trypanosome infection based on clinical signs alone is rather difficult, but haematological parameters like packed cell volume could be reliable indicators of the progress of the disease. Therefore, intermittent fever, anaemia and loss of body condition are important parameters used routinely for the tentative diagnosis of trypanosomosis in the areas where the disease is endemic and laboratory service is not available [9].

Trypanosomosis and vector control methods have evolved from game animal destruction and bush clearing, which mainly targeted on the removal of the source of food and shade, to insecticide spraying following the discovery of DDT. The insecticides were applied to the tsetse habitat either from the ground or from the air [10]. However, despite the success of the techniques in reducing tsetse population and disease transmission, they have been largely discarded for ecological and environmental reasons [11]. Currently, Stationary attractive devises or odour baited traps and targets, live bait techniques (insecticide sprayed cattle), ground spray, and Sequential Aerosol Technique (SAT) has been widely used throughout Africa, despite their specific limitations [12].

Therefore, the primary objectives of the study which was carried out from September 2016 to February 2018 intended to systematically evaluate implemented strategic trypanosomosis and vector control activities based on the study parameters of epidemiological association between bovine trypanosomosis, vector challenge and anemia in upper Birbir valley, additionally to investigate and analyses associated risk factors, determine the precise distribution and limits of a tsetse population in a given area, identify all the tsetse species and distribution present in the area, determine the relative abundance (apparent density) of each species present in time and space, and estimate the occurrence of trypanosome related anemia and analyses related risk factors.

Results

Baseline survey or study parameter results

Entomological baseline survey or study was conducted by 375 deployed functional monoconical standard traps baited with octenol (1-oct-3-nel), acetone (C₈H₁₆O); and 3 week old treated cattle urine in the main Bibir River and its principal tributaries sited in five different districts, namely Darimu, Seyo, Dale sadi, Bure, and Lalo kile. From a total
number of functional traps, 765(39.01%), 537(27.38%), 452(23.05%), and 207(10.56%) for *G.pallidipes*, *G.m.morsitans*, *G.f.fuscipes*, and *G.thachinoides* tsetse fly species were caught respectively. The predominantly distributed tsetse fly at the study area were savannah species including *G.pallidipes* and *G.m.morsitans* with an average mean catch of 2.04 ± 1.58 and 1.44 ± 1.58, (mean ± SD) per single trap when compared with other riverine species of 1.20 ± 1.58 and 0.56 ± 1.58, (mean ± SD) respectively for *G.f.fuscipes* and *G.thachinoides*. During the baseline survey in the Birbir river basin an overall apparent fly density of 2.61 flies/trap/day was recorded having the highest infestation record at Darimu district, (FTD = 6.39) and the lowest of all at Seyo district, (FTD = 0.79), in which both are located to the east and west water bank of the main river, Birbir. Regarding the findings of the sex proportion of flies, more female tsetse flies were caught, 70.27%, with a mean catch of 3.68 ± 1.58 flies per trap, than male 29.73% with a mean catch of 1.56 ± 1.58 flies per trap.

Table 1.

| District      | Number of trap deployed | *G.pallidipes* | *G.m.morsitans* | *G.f.fuscipes* | *G.thachinoides* | Total | FTD   |
|---------------|-------------------------|----------------|-----------------|----------------|------------------|-------|-------|
| Darimu        | 75                      | 312            | 288             | 291            | 68               | 959   | 6.39  |
| Seyo          | 75                      | 47             | 43              | 24             | 5                | 119   | 0.79  |
| Dale sadi     | 75                      | 87             | 54              | 28             | 17               | 186   | 1.24  |
| Bure          | 75                      | 223            | 111             | 19             | 75               | 428   | 2.85  |
| Lalo kile     | 75                      | 96             | 41              | 90             | 42               | 269   | 1.79  |
| Total         | 375                     | 765            | 537             | 452            | 207              | 1961  | 2.61  |
| Percent (%)   | 39.01                   | 27.38          | 23.05           | 10.56          |                  |       |       |
| Mean fly catch/trap/day, SD | 2.04 ± 1.58 | 1.44 ± 1.58 | 1.20 ± 1.58 | 0.56 ± 1.58 | 5.23 ± 1.58 |       |       |

From total examined cattle for the parasite n = 1656; only n = 131 of them had found positive, with an overall prevalence rate of 7.91%. The highest trypanosomosis prevalence was recorded in Dale sadi and Darimu districts when compared with Bure which was the lowest of all with a statistically significant difference of prevalence between districts, pr = 0.000. Among considered risk factors the higher trypanosomosis prevalence was observed in poor body conditioned cattle 4.41% when compared with 2.84%, 0.66% for medium, and good body conditioned cattle respectively and the difference was found statistically significant, pr = 0.000, likelihood-ratio = 21.72.

Table 2.
Table 2

Prevalence of trypanosomosis and identified species in districts located to birbir valley during base line survey

| District   | T. congolense (%) | T. vivax (%) | Mixed infection (%) | Overall prevalence (%) | likelihood-ratio chi2, pr |
|------------|-------------------|--------------|---------------------|------------------------|-------------------------|
| Darimu     | 6.02              | 2.29         | 2.58                | 10.89                  |                         |
| Seyo       | 5.93              | 1.48         | 0.30                | 7.72                   |                         |
| Dale sadi  | 11.19             | 0.72         | 0.72                | 12.64                  |                         |
| Bure       | 2.06              | 0.29         | 0.00                | 2.35                   |                         |
| Lalo kile  | 4.82              | 1.13         | 0.85                | 6.80                   |                         |
| Total      | 5.80              | 1.21         | 0.91                | 7.91                   | 21.72, 0.000            |

Pr or p value < 0.005 considered as significant

The study demonstrated that trypanosomosis strongly causes anemia with mean PCV of infected cattle 21.87 ± 4.57 and non-infected cattle 25.41 ± 4.27 (Mean PCV ± SD) with 95% CI, Pr (T > t) = 0.0000 see Table 3.

Table 3

Statistical observation of anemia and parasite infection during base line survey

| Infection status | Observation | Mean PCV, SD   | CI (confidence interval) | Pr (T > t) |
|------------------|-------------|----------------|--------------------------|------------|
| Non infected     | 1525        | 25.41 ± 4.27   | 25.19418 25.62287        |            |
| Infected         | 131         | 21.87 ± 4.57   | 21.08091 22.65954        |            |
| Combined         | 1656        | 25.13 ± 4.39   | 24.91677 25.34048        | 0.0000     |

Pr (T > t) < 0.005 considered as significant

Table 3.

Control Survey Or Study Parameter Results

Similarly, the vector (tsetse fly) control survey was conducted by 375 deployed functional monoconical standard traps baited with octenol (1-oct-3-nel), acetone (C₈H₁₆O); and 3 week old treated cattle urine in the main Bibir River and its principal tributaries sited in five different districts, namely Darimu, Seyo, Dale sadi, Bure, and Lalo kile. From a total number of functional traps, 460(36.59%), 402(31.98%), 283(22.51%), and 112(8.91%) for G.pallidipes, G.m.morsitans, G.f.fuscipes, and G.thachinoides tsetse fly species were caught respectively. The predominantly distributed tsetse fly at the study area were savannah species including G.pallidipes and G.m.morsitans with average mean catch of 1.23 ± 1.58 and 1.07 ± 1.58, (mean ± SD) per single trap when compared with other riverine species of 0.75 ± 1.58 and 0.29 ± 1.58, (mean ± SD) respectively for G.f.fuscipes and G.thachinoides. During the control survey in the Birbir river basin an overall apparent fly density of 1.68 flies/trap/day was recorded having the highest infestation record again at Darimu district, (FTD = 3.84) and the lowest of all at Seyo district, (FTD = 0.17), in which both are located to the east and west water bank of the main river, Birbir. Regarding the findings of sex proportion of flies, more female tsetse flies were caught, 61.97%, with a mean catch of 2.08 ± 1.58 flies, than male 38.03% with a mean catch of 1.27 ± 1.58 flies per trap.
Table 4.

Table 4
Tsetse flies’ distribution and their density in districts located to birbir valley during control survey

| District    | Number of trap deployed | G.pallidipes | G.m.morsitans | G.f.fuscipes | G.thachinoides | Total | FTD |
|-------------|-------------------------|--------------|---------------|--------------|----------------|-------|-----|
| Darimu      | 75                      | 192          | 218           | 152          | 14             | 576   | 3.84|
| Seyo        | 75                      | 11           | 8             | 6            | 1              | 26    | 0.17|
| Dale sadi   | 75                      | 69           | 52            | 39           | 21             | 181   | 1.21|
| Bure        | 75                      | 167          | 108           | 10           | 54             | 339   | 2.26|
| Lalo kile   | 75                      | 21           | 16            | 76           | 22             | 135   | 0.90|
| Total       | 375                     | 460          | 402           | 283          | 112            | 1257  | 1.68|
| Percent (%) |                         | 36.59        | 31.98         | 22.51        | 8.91           |       |     |
| Mean fly catch/trap/day, SD | 75                     | 1.23 ± 1.58  | 1.07 ± 1.58   | 0.75 ± 1.58  | 0.29 ± 1.58    | 3.35 ± 1.58 |

From total examined cattle for the parasite during control survey n = 1644; only n = 79 of them had found positive, with an overall prevalence rate of 4.81%. The highest trypanosomosis prevalence was recorded in Dale sadi and Seyo districts when compared with Bure which was the lowest of all with statistically significant difference of prevalence between districts, pr = 0.000. Among considered risk factors the higher trypanosomosis prevalence was observed in medium body conditioned cattle 2.62% when compared with 1.83%, 0.36% for poor and good body conditioned cattle respectively and the difference was found statistically not significant, pr = 0.064, likelihood-ratio = 5.49.

Table 5

Table 5
Prevalence of trypanosomosis and identified species in districts located to birbir valley during control survey

| District    | T. congolense (%) | T. vivax (%) | Mixed infection (%) | Overall prevalence (%) | likelihood-ratio chi2, pr |
|-------------|-------------------|--------------|---------------------|------------------------|--------------------------|
| Darimu      | 3.44              | 0.29         | 0.58                | 4.29                   |                           |
| Seyo        | 3.24              | 1.77         | 0.89                | 5.89                   |                           |
| Dale sadi   | 3.61              | 2.53         | 1.81                | 7.94                   |                           |
| Bure        | 1.16              | 0.29         | 0.29                | 1.73                   |                           |
| Lalo kile   | 3.00              | 1.20         | 0.60                | 4.80                   |                           |
| Total       | 2.86              | 1.16         | 0.79                | 4.81                   | 5.49, 0.064               |

Pr or p value < 0.005 considered as significant
The study was clearly demonstrated that trypanosomosis causes less severe anemia with mean PCV of infected cattle $22.53 \pm 4.48$ and non-infected cattle $25.68 \pm 4.04$ (Mean PCV ± SD) with 95% CI, $Pr(T > t) = 0.0000$ after control survey was conducted see Table 6.

| Infection status | Observation | Mean PCV, SD | CI (confidence interval) | Pr ($T > t$) |
|------------------|-------------|--------------|-------------------------|--------------|
| Non infected     | 1565        | 25.68 ± 4.04 | 25.48158 25.882       |              |
| Infected         | 79          | 22.53 ± 4.48 | 21.52876 23.53453     |              |
| Combined         | 1644        | 25.53 ± 4.11 | 25.33139 25.72944     | 0.0000       |

$Pr(T > t) < 0.005$ considered as significant

**Discussion**

According to Urquhart et al., 1992 [13], the epidemiology of African Animal Trypanosomosis in tsetse infested areas of Africa is determined by four biological factors, namely: trypanosomes, tsetse flies, reservoir hosts, and livestock. Baseline and control survey was conducted to evaluate the effectiveness of implemented strategic control activity using chemicals of Deltamethrin products as described in the methodologies based on the epidemiology of the disease which was determined by above mentioned biological factors.

Baseline and control entomological surveys or studies were compared, and an overall apparent fly density of 2.61 flies/trap/day during the baseline survey was declined to 1.68 flies/trap/day with the rate of 64.37% after control with supporting evidence of a decrease in the daily mean catch of fly per single trap from $5.23 \pm 1.58$ to $3.35 \pm 1.58$, (Mean catch ± SD) see Table 7 and Fig. 1. According to Vreysen et al., 2013 [14], insecticide-impregnated traps and targets can suppress tsetse fly’s population to low numbers within a few months of proper application with appropriate doses of odour attractants.
Table 7
Comparative tsetse flies’ distribution and their density in districts located to birbir valley during base line and control surveys

| District name | Number of trap deployed | Number of fly caught (base line) | Number of fly caught (control) |
|---------------|-------------------------|---------------------------------|-------------------------------|
|               |                         | Male | Female | Total | FTD | Male | Female | Total | FTD |
| Darimu        | 75                      | 297  | 662    | 959   | 6.39 | 234  | 342    | 576   | 3.84 |
| Seyo          | 75                      | 28   | 91     | 119   | 0.79 | 15   | 11     | 26    | 0.17 |
| Dale sadi     | 75                      | 61   | 125    | 186   | 1.24 | 68   | 113    | 181   | 1.21 |
| Bure          | 75                      | 102  | 326    | 428   | 2.85 | 122  | 217    | 339   | 2.26 |
| Lalo kile     | 75                      | 95   | 174    | 269   | 1.79 | 39   | 96     | 135   | 0.90 |
| Total         | 375                     | 583  | 1378   | 1961  | 2.61 | 478  | 779    | 1257  | 1.68 |
| Percent (%)   |                         | 29.73 | 70.27 |       |      | 38.03 | 61.97 |       |      |
| Mean fly catch/trap/day, SD |         | 1.56 ± 1.58 | 3.67 ± 1.58 | 5.23 ± 1.58 | 1.27 ± 1.58 | 2.08 ± 1.58 | 3.35 ± 1.58 |       |      |

Furthermore, Brightwell et al., 1991 [15] and Vale (1993) [16], explained that female tsetse flies are more attracted to a device like cloth or target that either kill the flies through tarsal contact with the insecticide applied to the target or caused starvation due to paralysis which exerted 2–3% additional daily mortality, which was in line with the result of this research. More female fly was caught during baseline survey 70.27% with 3.67 ± 1.58 (Mean catch ± SD) daily mean catch, however after control immediately declined to 61.97%, 2.08 ± 1.58 (Mean catch ± SD) with the rate of 88.1% see Table 7.

According to Leak et al., 1995 [17], live bait technique is based on insecticide treatment of livestock and exploits the blood-sucking behavior of both sexes of tsetse. Tsetse flies attempting to feed on cattle or other treated domestic livestock are killed by picking up a lethal deposit of insecticide on the ventral tarsal spines and on pre-tarsi whilst feeding which was effective in savannah fly species, although a significant decline in fly number and densities of G. pallidipes and G.m.morsitans was observed after control and approved by the current research entomological survey results, see Tables 1, 4 and 7.

Table 7.

Based on baseline and control survey or study results the overall prevalence of bovine trypanosomosis was compared and significantly declined from 7.91–4.81%, likelihood-ratio = 13.49, Pr = 0.000, with the rate of 60.81%, which is due to direct intervention of conducted control activity see Fig. 1. According to recently published work of Meharen et al., 2020 [18], the abundance (dynamics) of tsetse distribution and density had a direct influence on the prevalence of the parasite when the fly density declines the prevalence of the parasite also lower.

Finally, significantly better heard mean PCV was observed during control survey or study when compared with base line in parasite infected and non-infected cattle see Table 8. According to Tasew and Duguma (2012) [19], heard mean PCV could be indicative of the status of infection of trypanosomosis in the tsetse (endemic) belt areas. Table 8.
Table 8
Comparative statistical observation of anemia and parasite infection during base line and control surveys

| Infection status                  | Observation | Mean PCV, SD  | CI(confidence interval) | Pr (T > t) |
|-----------------------------------|-------------|---------------|-------------------------|------------|
| During base line survey or study  |             |               |                         |            |
| Non infected                      | 1525        | 25.41 ± 4.27  | 25.19418 25.62287       |            |
| Infected                          | 131         | 21.87 ± 4.57  | 21.08091 22.65954       | 0.0000     |
| • With *T. congolense*            | 96          | 21.91 ± 4.57  | 20.98119 22.83131       |            |
| • With *T. vivax*                 | 20          | 22.6 ± 4.79   | 20.356 24.844           |            |
| • Mixed infection                 | 15          | 20.67 ± 4.32  | 18.27406 23.05928       |            |
| During control survey or study    |             |               |                         |            |
| Non infected                      | 1565        | 25.68 ± 4.04  | 25.48158 25.882         |            |
| Infected                          | 79          | 22.53± 4.48   | 21.52876 23.53453       | 0.0000     |
| • With *T. congolense*            | 47          | 21.98 ± 4.59  | 20.62977 23.32768       |            |
| • With *T. vivax*                 | 19          | 26.00± 2.85   | 24.62731 27.37269       |            |
| • Mixed infection                 | 13          | 19.46± 2.60   | 17.8893 21.03378        |            |

Pr (T > t) < 0.005 considered as significant

Conclusion
The study concluded that the use of stationary attractive devices (targets), Ground sprays, and Live bait techniques of Deltamethrin based insecticide had shown a significant decline in the density of tsetse fly (vector hosts) and trypanosomosis prevalence with a better heard mean PCV of infected and non-infected cattle. Therefore, the research recommended that such kind of control methods which are environmentally friendly or non-intrusive, cheap, and non-complicated for farmers and experts in the fight against trypanosomosis and tsetse (vectors) in endemic areas.

Methods
Description of the study area
The field study was conducted alongside the Birbir River and its principal tributaries with a purpose of promoting and developing strategic tsetse control and disease management in the study and its surrounding areas. There are about five randomly selected districts Bure, Darimu, Lalo kile, Dale sadi, and Seyo located alongside of the Birbir River basin which is lengthways of west wards drains into Abbay River (Blue Nile) which crossing the Ethiopian/Sudanese border finally drains into Egypt. The Birbir River of southwestern Ethiopia is a tributary of the Baro River, which it creates when it joins the Gebba at the latitude and longitude 8°14′28″N 34°57′39″E. It is politically important because its course defines part of the boundary between the West Welega and Illubabor Zones of the Oromia Region [20]. The study area has got sub-humid lowland climate to the southwest of the country that occurs between 1200 and 3020 meters above sea level at the escarpment of the valley with annual temperature
ranging from $16^\circ$C to $37^\circ$C. It receives high and reliable annual rainfall with an average of 1050 mm precipitation per annum. The main rainy season lasts from mid-June to late September and the shorter one from April to May. The predominant farming system of the area is characterized by mixed livestock and crop production, with livestock playing a vital role in agricultural activities, nutritional values and income generation. An East African Zebu type breed of cattle, which are also known as Abyssinian short-horned zebu, found in the study areas. This type of cattle breed is characterized by a pronounced susceptibility to trypanosomosis; as high prevalence rate, high drug resistance and a high number of treatments. Cattle in the study area kept are under traditional extensive husbandry system with communal herding, [21, and 22].

**Sampling Method And Sample Size Determination**

Since there was an insufficient human or material resource to survey the whole area in detail and the area was heterogeneous in all respects (vegetation, climate, altitude, land use, distribution of human habitation, etc.) to isolate certain homogeneous, or similar, subareas, and to obtain a representative sample area a stratified sampling method was applied. The target populations were all local breed cattle of all age groups and sex found in each randomly selected study site. The sample size was determined by using Thrusfield formula (1995) with the expected prevalence of the disease in respective of the area was 50%, since there was no officially reported prevalence previously minimum of 384 heads of cattle. However, the conducted study was longitudinal and involves the maximum number of sample size [23].

**Study Design And Methodologies**

A longitudinal study was carried out from September 2016 to February 2018 with the primary objective of systematic evaluation of implemented strategic trypanosomosis and vector control activities based on the study parameters of the epidemiological association between Bovine trypanosomosis, vector challenge and anemia in upper Birbir valley.

Initially, during the late September 2016 baseline data were collected by conducting entomological, parasitological, and hematological surveys to estimate the epidemiological association between Bovine Trypanosomosis, vector challenge and anemia in upper Birbir valley which was considered as baseline study parameters before the strategic trypanosomosis and vector control activities were started.

Implemented strategic trypanosomosis and vector control activities were based on the use of Stationary attractive devices (targets), Ground sprays and Live bait techniques [14] which composes deployment of about 6,400 Deltamethrin 20% based chemical impregnated cloth targets baited with 3-week old cow urine between 150-meter distance along sides with Birbir river and tributaries, estimated area coverage of more than 1500 km$^2$ Deltamethrin 20% based diluted to 0.02% ground spry along sides with Birbir river and tributaries, and impregnation of more than 85% of cattle population with Deltamethrin 1% pour-on application using T-bar spryer, with in all selected districts of Birbir valley for continuative three years between two periodic intervals per annum.

Finally, to evaluate the success of implemented control activities again entomological, parasitological and hematological surveys were conducted during early February 2018 and the epidemiological association between Bovine Trypanosomosis, vector challenge and anemia in upper Birbir valley were compared with baseline study parameters.
Entomological Survey Or Study

Before commencement of the study, a total of 375 monoconical standard traps baited with octenol (1-oct-3-nel), acetone \((C_8H_{16}O)\); and 3 weeks old treated cattle urine were deployed in the main Birbir River and its principal tributaries sited in five randomly selected districts of Bure, Darimu, Lalo kile, Dale sadi and Seyo located alongside the Birbir River basin which is lengthways of westwards drains into Abbay River (Blue Nile). Traps were placed approximately 45 cm from the ground baited with the aforementioned odours placed in three different bait dispensers. The poles of traps were greased to prevent the possible fly predators, mainly ants. The traps were positioned for two consecutive days with a mean interval between traps of 250 meters, in most likely areas for finding tsetse, based on the presence of gallery forests, and the location of rivers and streams after clearing up to 2–3 meters’ radius of the trap site to enhance the visibility of the traps and to prevent from the possible fire damage. The traps were deployed in georeferenced locations to map and easily display the distribution of tsetse flies on the GIS map. The apparent density (arithmetic mean catches per trap per day) of flies was calculated by dividing the total number of tsetse flies captured \((\Sigma F)\) by the product of the number of functioning traps used to catch them \((T)\) and the number of days for which the traps were operational \((D)\); \(FTD = \frac{\Sigma F}{T \times D}\). The identity of tsetse species present in the area was done using conventional identification keys of, Buxton1955 [24], Mulligan 1970 [25], FAO 1982a [26], Meharennet et al., 2020[18]. Sex of all collected flies was identified by observing the posterior end of the ventral aspect of the abdomen by hand lens in which male flies were identified by enlarged hypopygium in the posterior ventral part of the abdomen which is absent in female flies.

Hematological Survey Or Study

Paired blood samples were collected from the auricular vein (marginal ear vein) of each animal using two haematocrit capillary tubes. The tubes were filled with blood \(\frac{3}{4}\) of its height and sealed with crystal sealant. Packed cell volume (PCV) value for each collected blood sample was estimated, recorded and analyzed for the determination of anemia and comparison of infected animals with non-infected animals and only cattle with PCV \(\leq 24\%\) were considered as anemic [27].

Parasitological Survey Or Study

After the PCV value of each collected blood sample was determined the capillary tubes were cut 1 mm below the Buffy coat to include the top layer of RBCs. The content of the capillary tube was expressed on to a clean microscopic slid, mixed and covered with a coverslip. Then the slides were examined for trypanosomes based on the type of movement in the microscopic field. Confirmation of trypanosome species by morphological characteristics was done after staining with Giemsa and examination with oil immersion microscopy under \(\times 100\) power of magnification [28].

Finally, during sample collection; age, sex, Peasant Association, altitude and body condition of each animal were recorded. On subjective basis body condition of examined animals were evaluated during sample collection. They were classified as poor, medium and good relative to the average body condition of local animals (zebu) [29].

Data analysis
Data on entomological studies, collected from each deployed trap, were coded into appropriate variables and inserted into MS Excel Spreadsheet Program, 2010 (Microsoft Corp.) to create a database and transferred to STATA-12 software of the computer program. The data processing was carried out based on both qualitative and quantitative data analyzing methods. For the qualitative test statistical analytic methods, table, frequency distribution, mean, standard deviation, were some of the methods employed. On the other hand, for the quantitative test statistical analytic methods, the prevalence rate including point prevalence, the difference in mean tsetse density based on their respective sex ratio was analyzed using independent t-test; Chi-square (c2) test statistics was employed whether the results obtained were arose due to chance or not for the entomological data. The test statistics were used to test the overall disease prevalence with an absolute desired precession of 5% at the level of 95% confidence interval.

Abbreviations

CI: Confidence Interval; PCV: packed cell volume; SD: Standard Deviation; FTD: fly/ trap/day, DDT: Dichlorodiphenyltrichloroethane

Declarations

Ethics approval and consent to participate

The National Institute for the Control and Eradication of Tsetse fly and Trypanosomosis and Ministry of Agriculture, Ethiopia authorized the fieldwork. The purpose of the study was clearly explained to the cattle owners’ and veterinary officers and informal consent were obtained through verbal consent from Institute (National Institute for the Control and Eradication of Tsetse fly and Trypanosomosis) technical team and cattle owners because since the field work (research) was authorized by the Institute and Ministry of Agriculture no need of written consent requested only verbal consent was enough to proceed. Participants’ involvement in the study was on voluntary basis; participants who were unwilling to participate in the study and those who wish to quite their participation was informed to do so without any restriction. The study doesn’t involve any human and animal trial or experiment.

Consent for Publication

Not applicable, all participant authors had read and approved the submission

Availability of data and materials

The datasets supporting the conclusion of this research article are available upon request to the corresponding author.

Competing of interest

The authors have declared that no competing interest exists

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Author contributions
MB: Conceptualization, Methodology and Writing-original draft, Data curation, Formal analysis and Writing-review and editing

TD: Data collection and curation, Writing-review and editing

SD: Data curation and Formal analysis

TM: Writing-review and editing

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Figures

Figure 1

Spatial tsetse flies’ distribution, density and trypanosomosis prevalence during base line and control surveys. Map which was made by using Environmental Systems Research Institute (ESRI) software, 2015. Arcgis version 10.4.1. Redlands, California, USA.