Distribution Of Tsetse Flies And Significance To The Control Of African Trypanosomiasis In Busia County, Kenya

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Abstract

**Background:** Tsetse flies are the cyclical vectors of both human and animal diseases in Sub-Saharan Africa. Since 2012, Kenya has not recorded a case of human trypanosomiasis. However, African animal trypanosomiasis continues to be a major challenge to livestock production despite decades of control efforts. This study aimed to determine the prevalence of tsetse flies in Busia County post intervention and to build the capacity of local inhabitants in vector control activities.

**Methods:** This cross-sectional study was conducted between May 2018 and December 2018 in Teso South and Teso North sub-counties. Odour-baited biconical traps were deployed for 48 hours in each sampling area and captured tsetse flies were analysed for trypanosome infections. Additionally, training and field demonstrations were conducted as part of capacity building to enhance participation of local inhabitants in tsetse control activities.

**Results:** In Teso South sub-county, 62 tsetse flies were captured in Kwangamor, six in Obekai and 14 in Ngelechom sites. All the captured tsetse flies were classified as *G. fuscipes fuscipes*. In Teso North sub-county sites of Kapesur, three *G. pallidipes* were captured, while the Ikapolok sites yielded 12 tsetse flies all *G. fuscipes fuscipes*. The apparent density of tsetse flies was between 0.08 and 1.55 flies-per trap-per-day across the five study areas with *G. fuscipes fuscipes* being the dominant species. Microscopic examination of 72 *G. fuscipes fuscipes* identified three *T. vivax* and one *T. congolense* positive tsetse flies. No trypanosomes were observed in all the *G. pallidipes*. Overall infection rate of 1.39% and 4.17% was observed for *T. congolense* and *T. vivax* respectively. With regards to capacity building, a total of 26 community members were trained on tsetse fly control activities. Out of which five were selected as focal persons and were further trained on integrated vector management techniques and tsetse survey methods.

**Conclusions:** Tsetse flies in Teso South sub-county, albeit low density, harbour trypanosomes. This calls for an urgent attention to stop potential spread and transmission. Moreover, training of local inhabitants in tsetse control activities will help to strengthen and sustain efforts towards elimination of African trypanosomiasis in Busia County.

**Background**
Trypanosome parasites transmitted mainly through bites of infected tsetse flies (Glossina spp) are known causes of both humans and animals diseases affecting the poor and marginalised populations of Sub-Saharan Africa. In humans, two protozoan parasites: *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense* (1) cause Human African Trypanosomiasis (HAT) also called “sleeping sickness”. Infections with *T. b. gambiense* (gHAT) are usually chronic and mainly found in Western and Central Africa, while infections with *T. b. rhodesiense* (rHAT) are acute in nature and mainly found in East and South Africa (2). The role of animals in the maintenance of gHAT transmission is still unclear, while rHAT transmission, conversely, involves both wild animals and domestic animals as reservoirs (3–7). Furthermore, Game parks and animal conservation areas have become the main sources of exposure to travellers and tourists (8–11). Over the years, through the concerted efforts of the World Health Organization (WHO) and other stakeholders such as National governments, Non-governmental Organizations and pharmaceutical companies, have substantially reduced the number of HAT cases. This success has necessitated the current WHO target of eliminating gHAT in endemic foci by the year 2030 (7, 12–14). Kenya has no recent autochthonous case of HAT apart from two cases detected in non-endemic countries from tourists who had visited the Masai-Mara National Reserve in 2012 (10, 13).

On the contrary, most of the Sub-Sahara Africa tsetse endemic countries still suffer huge economic losses due to African Animal Trypanosomiasis (AAT) also called “nagana” that is caused by three *Trypanosoma* species: *Trypanosoma brucei brucei*, *Trypanosoma congolense* and *Trypanosoma vivax*. The disease continues to affect the economic welfare and livelihoods of many farmers and imposing major constraints to livestock health and productivity (15, 16). Furthermore, the surge in human population in many parts of Africa has led to increased land pressure and encroachment into tsetse infested areas for settlement and farming thereby increasing animal/human-fly contacts and potential for contracting trypanosomiasis (17).

In Kenya agriculture accounts for over 24% of the country’s Gross Domestic Product (GDP); of which 12% is from the livestock sector. Therefore, AAT effects on agricultural production translates to a direct threat to the economy, food security and human welfare (18, 19). The Kenya tsetse and
trypanosomiasis eradication council (KENTTEC) recognises distinct diverse zones or “tsetse fly belts” in the country that are occupied by eight tsetse fly species. For instance, the Lake Victoria basin tsetse fly belt encompasses three counties in the western Kenya i.e. Busia, Bungoma and Siaya, and extends to parts of Uganda and Tanzania. This region is characterised by forested riverbanks, patches of dense vegetation and forested wetlands which are ideal habitats for tsetse flies (20, 21). It is a common practice for businessmen in this region to acquire livestock for beef from the cattle markets of Eastern Uganda, a practice that potentially could lead to the introduction of new trypanosomes and other animal diseases. Not long ago, cases of chemo-resistance to existing trypanocides have been reported in Uganda (22–24). Therefore, there is need for regular monitoring and surveillance of African animal trypanosomiasis and resistance to trypanocide drugs in the region.

Previous studies have shown that climate change coupled with human activities tend to modify environmental and biological conditions that affect the vector, host and parasites interactions (25–27). In addition, existing socio-economic and cultural factors also contribute to disease incidence and outcomes (28, 29). Over the past decade, Busia County has undergone rapid environmental changes, increased encroachment and use of wetlands and riverine for farming activities. Changes in land-use, deforestation, erosion and loss of biodiversity may affect the prevalence, distribution of tsetse flies, and disease transmission dynamics. Current data on tsetse distribution and population density given the new epidemiological settings in the county is lacking. To substantially achieve reduction in AAT and to maintain a downward trend in disease incidence, training of local inhabitants in vector control activities among other measures is paramount. This study set out to determine the current status with regards to tsetse distribution and to enhance local capacity in vector control activities.

Methods

Study area

The study was conducted in Busia County located in western Kenya. Based on the historical burden of African trypanosomiasis, Teso South and Teso North sub-counties were selected for the survey. As mentioned earlier, Busia is within the Lake Victoria basin tsetse fly belt that includes other counties
such as Bungoma to the north and Siaya to the southwest. It also borders Lake Victoria to the southeast and the Republic of Uganda to the west (Additional file 1). The tsetse surveys were carried out in five administrative villages namely: Kwangamor, Obekai and Ngelechom in Teso South sub-county; Kapesur and Ikapolok in Teso North sub-county. Subsistence farming and traditional rearing of livestock (cattle and small ruminants) are the main economic activities in the sampled areas.

Study Design
This cross-sectional survey was conducted as part of post intervention evaluation to determine the prevalence of tsetse flies in the five sites following past control activities by the Farming in Tsetse Controlled Areas (FITCA) project. In addition, capacity building of the local communities in tsetse control activities was done to enhance participation and for sustainability of tsetse control activities.

Community Sensitization And Capacity Building
Community sensitization was done through the local assistant chief’s forum or ‘baraza’ where the research staff together with the local administrators conducted awareness sessions and distributed the flyers. Information and educational materials for the community were developed and translated into local language (Ateso). Also, verbal consent and permission to access private lands for tsetse trapping was sought.

Training workshops were conducted in each village where local farmers were sensitized as part of establishing local capacity in tsetse control activities. A team of five focal persons was finally selected and offered further trained on basic tsetse biology and ecology, vector control techniques, baseline tsetse survey methods, trap deployment and servicing, interpretation of trap catches and data collection. Demonstrations and hands-on trainings in tsetse trapping and management of traps was done during the survey period.

Tsetse Surveys
The tsetse survey was carried out from May 2018 to December 2018. All potential tsetse fly trap locations were mapped and geo-referenced by Global Positioning System (GPS) prior to sampling. To ensure that all possible tsetse fly breeding sites were included, information on land use, different ecological niches based on vegetation type, drainage and human activities was considered during mapping. In addition, historical and anecdotal reports regarding tsetse fly availability in the sampling
sites was noted.

Odour baited biconical traps developed by the International Centre of Insect Physiology and Ecology (ICIPE) were used for tsetse fly trapping. A total of 20 traps were deployed at a distance of 50-100 meters apart in each sampling site. All traps were baited with phenol sachets and acetone bottles placed at the base. After 48 hours, the traps were checked and emptied. The cages containing the flies were stored in cool boxes with moist cotton wool and shipped to the entomology laboratory for further analysis.

Vector Identification And Dissection
In the laboratory, flies were immobilised in a cool box containing cotton wool soaked in ethyl acetate for about five minutes. The flies were then counted, segregated and subjected to morphological identification. Biting flies of entomological importance to trypanosomiasis transmission were also recorded. To determine the presence of trypanosomes in the tsetse flies, all non teneral flies were selected for dissection following standard procedures. Briefly, using a clean pair of forceps, wings and legs of tsetse flies were removed. Under a dissecting microscope, the proboscis, salivary glands and midguts were teased out. The dissected parts were placed on a slide with a drop of normal saline and observed under a compound microscope at X100 and X400 Total Magnification.

Data analysis
Data collected was recorded and stored in computer spreadsheets and analysed using simple descriptive summaries such as percentages and presented in tables and figures. The apparent tsetse density for each study area was determined based on the average number of tsetse flies caught per trap per day. Tsetse fly infection rate was determined as the proportion of tsetse flies positive for trypanosomes.

Results
Local community participation and capacity building
A total of 26 community members (mostly representatives of farmers) were trained on integrated vector management and trypanosomiasis control approaches. In addition, a team of five focal persons was further trained on basic tsetse biology, vector control and survey methods.

Tsetse Fly Apparent Density
The tsetse fly survey activities were carried out in five study sites. Three in Teso South sub-county,
namely: Kwangamor, Obekai and Ngelechom; two in Teso North sub-county namely: Kapesur and Ikapolok (Fig. 1). A total of twenty trapping points were identified for each site where the odour-baited biconical tsetse traps were deployed for a period of 48 hours before harvesting the catches.

In Teso South sub-county, Kwangamor trapping site, a total of 62 tsetse flies and 23 biting flies of \textit{Stomoxys} \textit{spp} were captured. In the Obekai trapping site, six tsetse flies, two \textit{Tabanidae} and two \textit{Haematopota} \textit{spp} biting flies were captured. For the Ngelechom trapping site, 14 tsetse flies, two \textit{Tabanidae} and one \textit{Haematopota} \textit{spp} biting flies were captured. All the tsetse flies captured in these three trapping sites were \textit{G. fuscipes fuscipes}.

In Teso North sub-county, Kapesur trapping sites, three \textit{G. pallidipes} were captured. This tsetse species was caught only within the thicketed areas of Kapesur village. In the Ikapolok trapping site, a total of 12 tsetse flies and 30 \textit{Stomoxys} \textit{spp} of biting flies were captured. All the tsetse flies were \textit{G. fuscipes fuscipes}. Overall, 91 \textit{G. fuscipes fuscipes} and 3 \textit{G. pallidipes} were caught across the five trapping sites (Table 1). Kwangamor site had the highest apparent tsetse fly density of (1.55) while Kapesur site was the lowest (0.08). Details of the location of traps with tsetse flies is shown in Fig. 2.

The respective GPS coordinates for the traps is also provided (Additional file 2).

Table 1
Summary of tsetse fly catches after 48 hour deployment of odour-baited biconical traps in the five study sites.

| Study site (Village) | Traps with tsetse flies (%) | Total flies Captured | Tsetse Species Identification | Apparent Fly Densities (FTD)$^a$ |
|----------------------|----------------------------|----------------------|-------------------------------|----------------------------------|
|                      |                            |                      | Teneral Male | Non teneral Male | Teneral Female | Non teneral Female | FTD (%) |
| Kwangamor            | N = 20                     | 19 (95)              | 62            | \textit{G. fuscipes fuscipes} | 9               | 13               | 3       | 37       | 1.55 |
| Obekai               | 4 (20)                     | 6                    | \textit{G. fuscipes fuscipes} | 0               | 2               | 0                   | 4       | 8        | 0.15 |
| Ngelechom            | 5 (25)                     | 11                   | \textit{G. fuscipes fuscipes} | 3               | 2               | 2                   | 4       | 8        | 0.28 |
| Kapesur              | 3 (15)                     | 3                    | \textit{G. pallidipes}       | 0               | 1               | 0                   | 2       | 4        | 0.08 |
| Ikapolok             | 6 (30)                     | 12                   | \textit{G. fuscipes fuscipes} | 2               | 2               | 0                   | 8       | 4        | 0.30 |

$^a$ Apparent tsetse density FTD = $\Sigma F/(T \times D)$ Where FTD means the average Fly count per Trap per Day, $\Sigma F$ is the total number of captured flies, $T$ is the total number of functional traps used and $D$ is the number of days (48 hours) for which the traps were functional.
Trypanosome Infection Rates
To determine tsetse fly infections with *Trypanosoma spp*, a total of 75 non teneral tsetse flies from the five study sites were dissected. Four (4) tsetse flies were positive for the *Trypanosoma spp* (Table 2). The Kwangamor site had two tsetse flies that were positive for *T. vivax* and one positive for *T. congolense*. The Obekai site had one tsetse fly positive for *T. vivax*. Trypanosomes were not observed in all the dissected tsetse flies from Kapesur and Ikapolok sites. Overall, *T. vivax* and *T. congolense* infection rate for Kwangamor site was 4.0% and 2.0% respectively, while the *T. vivax* infection rate for Obekai site was 0.17%. The *T. vivax* parasites were observed in the proboscis of the tsetse flies while the *T. congolense* parasites were additionally observed in the midgut and the salivary glands. The location of the four traps that yielded trypanosome-bearing tsetse flies in Kwangamor and Obekai sites is indicated in (Fig. 3).

Table 2

| Study site (Village) | Non Teneral Male | Non Teneral Female | Number Dissected | Number Positive | Infection Rate (%) |
|----------------------|------------------|--------------------|------------------|-----------------|-------------------|
| Kwangamor            | 13               | 37                 | 50               | 1               | 2.0<sup>a</sup>   |
|                      |                  |                    |                  | 2               | 4.0<sup>b</sup>   |
| Obekai               | 2                | 4                  | 6                | 1               | 0.17<sup>b</sup>  |
| Ngelechom            | 2                | 4                  | 6                | 0               | 0.0               |
| Kapesur              | 1                | 2                  | 3                | 0               | 0.0               |
| Ikapolok             | 2                | 8                  | 10               | 0               | 0.0               |

<sup>a</sup> *Trypanosoma congolense*; <sup>b</sup> *Trypanosoma vivax*

Discussion
Tsetse flies and trypanosomiasis mostly affect poor communities that are dependent on traditional methods of livestock keeping and small-scale crop production (24). Most farmers in Busia County have for a long time relied on chemotherapy and chemoprophylaxis as a means to maintain their livestock despite the constant threat from tsetse flies and other endemic diseases. Our findings showed that Busia County in general has a very low tsetse density and trypanosome infection rate. Two sampling areas i.e. Kwangamor and Obekai had trypanosome-infected flies while the other three sampling areas yielded non-infected tsetse flies. The area under study previously benefited under the African Union based Farming in Tsetse Controlled Areas (FITCA) project http://www.au-ibar.org/component/jdownloads/finish/24-fitca/665 that focused on tsetse control and treatment of animals. An approach that severely reduced tsetse densities by over 95% within the 1999–2004
program period. A number of follow up control initiatives by the Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC) maintained the success gained for a longer period of time (16, 30). This survey was conducted to assess if the tsetse populations had rebounded in this region. The most dominant tsetse fly in the study area was *G. fuscipes fuscipes* accounting for over 97% of the catches. The findings are consistent with the KENTTEC zoning of tsetse species in the country. Moreover, sampling sites consisted mainly of the riparian ecosystem which correlated well with the known ecologically aspects of this tsetse species (31–33). In landscapes that were ‘humanized’ or disrupted through cultivation, and clearance of bushes the tsetse were captured in traps that were set within crop fields. Human activities that disrupt the natural tsetse habitats may have forced *G. fuscipes fuscipes* to take refuge in cultivated fields. Such phenomena has been reported by previous research (17, 34, 35). The *G. pallidipes* tsetse flies were captured only in the hillocks of Kapesur dotted with thickets. No trypanosomes were observed in the *G. pallidipes* tsetse flies.

The tsetse flies in Teso South sub-county were mainly *G. fuscipes fuscipes* and a small proportion (4.0%) were infected with *T. vivax*. The infections with *T. congolense* were disproportionately lower (2.0%) in the sampled areas. Previous research has shown that *G. fuscipes fuscipes* is a better vector for *T. vivax* leading higher infection rates among animals in this region (16, 30, 33, 36). The numerous tsetse and trypanosomiasis control activities implemented in Busia County over the past decade have immensely contributed to lowering tsetse densities and infection rates. For this success to be sustained, continuous monitoring and identification of residual tsetse breeding pockets and early deployment of control measures is crucial. This calls for the involvement of the local inhabitants in tsetse and trypanosomiasis control operations for sustainability.

**Conclusion**

Our findings revealed the existence of trypanosome-infected tsetse flies which could potentially serve as a source of seeding to the neighbouring regions. Training of local inhabitants in tsetse and trypanosomiasis control activities should be supported and integrated in the county animal health and veterinary services. Given the low tsetse densities and infection rates, elimination of trypanosomiasis
in Busia County is feasible.

Study Limitations

The trypanosome infections were determined by microscopy technique which is a less sensitive method. There is a possibility that some infected tsetse flies may have been missed. Though not the goal of the study, parasitological analysis on animal would have enriched the findings.

List Of Abbreviations

AAT: African animal trypanosomiasis
FITCA: Farming in tsetse controlled areas
FTD: Fly count per trap per day
GPS: Global positioning system
HAT: Human African trypanosomiasis
ICIBE: International centre for insect physiology and ecology
IVM: Integrated vector management
KENTTEC: Kenya tsetse and trypanosomiasis eradication council
PATTEC: Pan-African tsetse and trypanosomiasis eradication campaign

Declarations

Acknowledgement

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Availability of data and materials

All relevant information has been provided in this current manuscript.
**Ethical approval and consent to participate**

The Kenya National Council for Science Technology and Innovation provided a research permit (Ref: NACOSTI/P/18/76628/18964) for the study to be conducted in Busia County.

**Consent to publish**

Not applicable

**Authors’ contributions**

FA designed the study and wrote the draft manuscript. FA, TM, and OM data collection and review of manuscript. FA performed the laboratory experiments and analysed the data. MM review of manuscript, project administration and supervision. All authors read and approved the final manuscript.

**Competing interests**

The authors declare no competing interests including any financial, personal or other relationships with other people or organization that could inappropriately influence, or be perceived to influence the work.

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Additional File Legends

Additional file 1:
File format pdf extension .pdf
Title of data: Location of Teso South and Teso North in Busia County, Kenya.
Description of data: This figure is a map showing the location of the study sites where sampling was conducted.

Additional file 2:
File format pdf extension .pdf
Title of data: Global positioning system coordinates for the odour-baited biconical traps with tsetse
flies in the five study sites after 48 hours deployment.

Description of data: GPS coordinates indicating individual location of traps that yielded tsetse flies in the sampled sites.

Figures

Figure 1

Surveyed areas and the distribution of all the deployed tsetse fly traps in the five study sites. The shaded areas under the map indicate surveyed sites while the dots indicate location of odour-baited traps.
Figure 2

Distribution of odour-baited biconical tsetse traps in the five study sites and fly yields after 48 hours deployment. Dots represent location of individual traps, shaded dots show tsetse fly catches while the size of the shaded dots is relative to the number of tsetse flies captured. Unshaded dots represent traps with no tsetse flies.
Location of traps with tsetse flies harbouring Trypanosoma parasites in Kwangamor and Obekai sites. Unshaded dots represent traps with tsetse flies that did not have trypanosomes, while shaded dots represent presence of trypanosomes in the captured tsetse flies. For Kwangamor site, a total of three tsetse flies were positive with trypanosomes. Trap Kwm3 (T. congolense), Trap Kwm11 (T. vivax) and Trap Kwm13 (T. vivax), while the Obekai site had one positive tsetse fly in Trap Ob18 (T. vivax). Kwangamor and Obekai sites are located in Teso South sub-county.

Supplementary Files
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