Efficiencies of stationary sampling tools for tsetse *Glossina fuscipes fuscipes* in western Kenya

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Highlights

- StS-target is more efficient in sampling *Glossina fuscipes fuscipes* than ES-target and biconical trap.
- Cost of catching a fly using the StS-target was 3.4 times lower compared to the biconical trap.
- Cost of catching a fly using StS-target was over 19 times lower compared to the ES-target.
- Tsetse fly collected from StS target are suitable for PCR base molecular tests.
- There is a presence of *Spiroplasma* in wild *G. fuscipes fuscipes* of western Kenya.

Abstract

Monitoring the effectiveness of tsetse control interventions that aim to reduce transmission of African trypanosomiasis requires highly efficient sampling tools that can catch flies at low densities. The sticky small target (SS-target) has previously been shown to be more effective in sampling *Glossina fuscipes fuscipes* compared to the biconical trap. However, its efficiency in terms of the proportion of flies it catches out of those that visit it has not been reported. Furthermore, there are no reports on whether tsetse samples caught using the SS-target can be used for subsequent processes such as molecular tests. In this study, we evaluated the efficiency of the biconical trap and targets for sampling *G. f. fuscipes*. All targets were tiny (0.25 m × 0.50 m) but varied in their capture system. We used targets with sticky surface (SS-targets) and those with an electrified surface (ES-targets). We also assessed the suitability of flies caught on the SS-target for molecular tests by amplifying DNA of bacterial communities. Randomized block design experiments were undertaken in Mbita area and Manga Island on Lake Victoria of western Kenya. Fly catches of each sampling tool were compared to those of the sampling tool flanked by electric (E) nets and analysed using a negative binomial regression. The detransformed mean catch for each sampling tool alone was divided by the detransformed mean catch of the sampling tool flanked by two E-nets to obtain its efficiency expressed as a percentage. A proportion of flies caught on the SS-target was preserved for molecular tests. Overall, the efficiencies of the biconical trap, ES-target and SS-target were 7.7%, 13.3% and 27.0% respectively. A higher proportion of females (69 to 79%) than males approached all the sampling tools, but the trap efficiency was greater for male *G. f. fuscipes* than females. Furthermore, sequencing the 16S rRNA gene from fly samples caught on the SS-target revealed the presence of *Spiroplasma*. Our results indicate that the a) SS-target is the most efficient trap to monitor *G. f. fuscipes*

population during interventions, with the biconical trap being the least efficient, and b) samples collected from SS-targets are suitable for molecular studies.

Key words: Biconical trap; ES-target; SS-target; Molecular tests; tsetse

1. Introduction

Tsetse control has been identified by the World Health Organization (WHO) as a key component in stopping transmission of human African trypanosomiasis (HAT) by 2030 (Mahamat et al., 2017). About 98% of HAT is caused by the protozoan parasite *Trypanosoma brucei gambiense* which are mainly transmitted by tsetse flies from the Palpalis group (Holmes, 2013; Tirados et al., 2015). In the last decade, significant research has been carried out to improve the cost effectiveness of targets as control tools for tsetse from the Palpalis group (Esterhuizen et al., 2011; Lindh et al., 2009; Mahamat et al., 2017; Tirados et al., 2015). This resulted in the development of "tiny targets" (comprising of panels made of black fine polyester netting treated with insecticide and blue material each measuring 0.25 m × 0.25 m) which are cost effective for control of *Glossina fuscipes fuscipes* (Shaw et al., 2015; Tirados et al., 2015). Furthermore, replacing the netting on the tiny target with black material and covering it with a transparent sticky film to make a sticky small target (SS-target) measuring 0.25 m × 0.50 m, was shown to be more effective for sampling *G. f. fuscipes* than the biconical trap commonly used for this species (Leak et al., 2008; Mbewe et al., 2018b). Electrocuting capture methods such as electric surfaces and nets have mainly been used to evaluate traps, odour baits and tsetse behaviour (Leak et al., 2008; Vale, 1974). Whereas electric surfaces capture flies that land on baits, electric (E) nets catch tsetse in flight (Vale, 1974).

All tsetse sampling tools and methods have biases (Vreysen et al., 2013). For instance, mobile trapping methods are biased towards catching more male than female and smaller than larger tsetse flies (Mweempwa et al., 2020). Stationary trapping methods such as the use of traps and targets generally tend to catch a higher proportion of females than males which is close to the natural tsetse population (Challier and Gouteux, 1980; Leak et al., 2008; Vreysen et al., 2013). The choice of the tool and method for sampling tsetse flies is influenced by its biases, efficiency and cost effectiveness (Abila et al., 2007). Notably, regardless of the method used, monitoring the effectiveness of a tsetse control intervention on tsetse populations require highly efficient sampling tools that can catch flies at low densities. The flies caught should be suitable for subsequent processing such as identification, counting, dissections and molecular tests. The biconical trap has been used to sample *G. f. fuscipes* for observational and experimental studies and monitoring effectiveness of control interventions (Mbewe et al., 2019, 2018a; Tirados et al., 2015). Further, the efficiency of the biconical trap has been studied (Lindh et al., 2009) and fly samples collected have been used in molecular tests (Lindh and Lehane, 2011). However, even though the SS-target has been shown to be more effective than the biconical trap in sampling *G. f. fuscipes*; it has not been evaluated for efficiency and it is not known whether the sticky substance on the SS-target could interfere with suitability of fly sample for molecular tests. Establishing its efficiency could not only widen the available choice of tsetse sampling tools for planning in tsetse control interventions that target *G. f. fuscipes* but also lead to better intervention outcomes. In this study, we evaluated the targets and biconical trap for their efficiency in catching *G. f. fuscipes*. All targets were tiny (0.25 m × 0.50) m using two capturing systems, either a sticky (SS-targets) or an electrified (ES-targets) surface. We also assessed whether *G. f. fuscipes* samples caught on the SS-target were suitable for molecular tests by amplifying DNA of the microbes associated with the insect.

2. Methodology

2.1. Study area

The field study was undertaken from February to April 2018 at the International Centre of Insect Physiology and Ecology (*icipe*) Thomas Odhiambo Campus (ITOC) (Latitude -0.429°, Longitude 34.204°) at Mbita point and Manga Island (Latitude -0.353°, Longitude 34.253°) on Lake Victoria in western Kenya (Mbewe et al., 2018; Tirados et al., 2015). Manga Island is inhabited by humans. The study site selection was based on documented history of the presence of *G. f. fuscipes* (Mohamed-Ahmed and Odulaja, 1997). Hippopotamus (*Hippopotamus amphibius*) and monitor lizards (*Varanus niloticus*) are potential pool of vertebrate host for *G. f. fuscipes* in the study area (Tirados et al., 2015). The natural lacustrine vegetation where tsetse flies find shelter has been reduced due to human activity (Mohamed-Ahmed and Odulaja, 1997). Even though the vector is present, Mbita area is known to be free of HAT (Simarro et al., 2012, 2010; Tirados et al., 2015).

2.2. Sampling tools

A biconical trap (Challier and Laveissiere, 1973), SS-target (Mbewe et al., 2018b) and ES-target and electric (E) net (Vale, 1974) were used to catch tsetse flies (Fig 1). The biconical trap had a diameter of 0.40 m at the widest point and a height of 1.30 m and sourced from Vestergaard-Frandsen (Lausanne, Switzerland). Flies collect in a non-return cage at the top of the biconical trap. The SS-target made by placing a plywood board of 0.25 × 0.50 m between and fastening it to two targets comprised blue and black cotton cloth panels each 0.25 × 0.25 m in size (Mbewe et al., 2018b). This was then covered with transparent sticky material (Luminos 4 adhesive rolls-ungridded: Rentokil Initial supplies, Liverpool, UK) which was not changed over the study period. The flies that land on the sticky surface were carefully removed using forceps and placed in storage containers. The ES-target comprised of blue and

black cloth panels each 0.25 × 0.25 m in size making it 0.25 × 0.50 m in dimension. The target was covered with an electric grid (Vale, 1974). The targets were made of similar looking blue and black cotton cloth (locally sourced and of unknown dye composition) to that used on the biconical trap. The electric (E) nets were made of fine black polyester netting (Swisstulle, Nottingham, UK) covered with an electric grid 0.5 m wide and 1.0 m height. The electric grids for the ES-target and E-net were supplied with pulses of high voltage using a High Voltage Spark Box (Early Warning Systems, Pietermaritzburg, South Africa) at 25 and 50 sparks/second, respectively. All electric grids were made of electrocuting copper wire 0.2 mm diameter and 8 mm apart (Vale, 1974). The electrocuting wires were blackened so that they could not be easily seen by tsetse flies (Packer and Brady, 1990; Vale, 1974). Consequently, the flies collided with the wires and were electrocuted. The electrocuted flies fell into a tray with soapy water below the grids (Lindh et al., 2009). The flies are then collected using forceps and placed in storage containers. Two of each sampling tool mentioned above were used throughout the study.

Fig 1: Sampling tools. Biconical trap (A), SS-target (B), ES-target (C) and E-net (D).

2.3. Study design and sample collection

The sampling tools were placed at sites with either open or dense vegetation with targets placed perpendicular to the Lake shore. Therefore, we used a series of randomized block

design (RBD) experiments (Torr et al., 2011) to compare treatments instead of the classical Latin Square design which requires that all trap sites should have similar vegetation as this could influence tsetse catches (FAO, 1992). In the randomised block experiments, trap sites were blocked, and treatments were randomly allocated to adjacent days at a site. The experiments were undertaken for four hours between 8:00 and 12:00 hours local time when *G. f. fuscipes* are most active (Mohamed-Ahmed and Odulaja, 1997; Omolo et al., 2009). Thereafter, all trapped flies were transported to the laboratory at ITOC in a locally sourced 50 L cool box for immediate sexing and counting. A proportion of the flies collected on the SS-target was preserved in 95% ethanol one month prior to molecular tests.

The first experiment with 12 replicates of RBD each comprising two adjacent days at a site, assessed tsetse catches of the biconical trap with and without two flanking E-nets. Similarly, the second experiment with 12 replicates of RBD assessed tsetse catches of the ES-target with and without two flanking E-nets. The third experiment, with 12 replicates of RBD assessed fly catches of the SS-target with and without two flanking E-nets. The fourth experiment compared catches of the biconical trap, SS-target and ES-target in nine RBD replicates comprising three adjacent days at a site. Each of the experiments, one to three, used two trapping sites and took 12 days while experiment four was done at three sites and took nine days. Experiments one to three were undertaken at ITOC while experiment four was undertaken on Manga Island.

2.4. Statistical analysis

Tsetse catches as counts from the sampling tools were analysed using a negative binomial regression with a log link and overdispersion parameter to determine the catch indices while taking into account the block and day of the experiment. These statistical analyses were performed in R. The detransformed mean catches of the trial arms in the negative binomial

model were obtained using the "*effects"* package in R (Fox, 2003) and used to calculate the catch index. The catch index is the detransformed mean catch of the treatment arm expressed as a proportion of the reference arm in the experiments. In experiments one to three, the flies caught in or on sampling tool alone represented the "actual" catch while the flies caught in or on the sampling tool flanked by two E-nets represented all the flies that visited the sampling tool. Therefore, the efficiency of the sampling tool was calculated as a percentage with the total fly catch of the sampling tool alone as the numerator and the total fly catch of the sampling tool flanked by two E-nets as the denominator (Vale et al., 1986). In the fourth experiment, the cost of catching a single fly was calculated by dividing the prevailing sampling tool cost divided by detransformed mean of each sampling tool. *P*-values less than 0.05 were considered statistically significant.

2.5. Suitability of flies for molecular tests

To assess the suitability of flies caught on the SS-target for molecular tests, each fly was removed from ethanol storage and rinsed three times using distilled water and air-dried overnight at room temperature. From the flies caught using the SS-target, 154 were randomly selected using random generated numbers from Excel (Microsoft Cooperation 2007). Then total genomic DNA was extracted from individual whole fly samples without the legs and wings using NucleoSpin® Tissue Kit (Macherey-Nagel GmbH & Co. KG, Duren, Germany) according to the manufacturer's instructions. Polymerase Chain Reactions (PCR) were carried out in 25 µl volumes using 2 µl of template DNA in a 5×PCR reaction buffer, 2.5 mM $MgCl_2$, 0.5 mM dNTPs, 10 pM of each primer and 0.1 µl of Super-Therm Taq DNA polymer (JMR Holdings, London, UK). The quality of template DNA was verified by amplification of insect-specific mtDNA using universal insect primers 12SAI-forward, 5'-AAACTAGGATTAGATACCCTATTAT-3'; 12SBI-reverse, 5'-

AAGAGCGACGGGCGATGTGT-3') (Simon et al., 1991) and endosymbiont gram positive bacterial 16S rRNA gene (Mateos et al., 2006). After each PCR run, 5 µl of the amplification were subjected to electrophoresis in TAE buffer (40 mM Tris, 20 mM acetic acid, 1 m MEDTA, pH 8.0) on 1.5 % agarose gel together with a 100 bp DNA ladder standard (Thermo Scientific, Whitehead Scientific, Cape Town, South Africa) and visualised using GelRed® (Biotium, Fermont, US) staining. The PCR conditions for the amplification of the insect specific mtDNA were as follows: denaturation at 94°C for 1 min 30 sec and the 35 cycles at 94 °C, 55 °C, 72 °C for 1 min each and 72 °C for 7 min. The PCR conditions for amplification of bacterial 16S rDNA were as follows: denaturation at 95 °C for 5 min and the 35 cycles at 94 °C for 30 sec, 59 °C for 30 sec, 72 °C for 1 min and 72°C for 10 min. Size selected bands of approximately 450 bp after amplification of the 16S rDNA gene for gram positive bacteria were cut from the agarose gel and purified using the Macherey-Nagel NucleoSpin® Gel and PCR Clean-up kit (Macherey-Nagel GmbH & Co. KG, Duren, Germany) according to the manufacturer's instructions. Two samples with three biological replicates each were then sequenced using 63F forward and TKSSsp reverse primers (Mateos et al., 2006). Chromatographs of the 16S rRNA gene sequences were manually corrected using CLC Main Workbench version 8.0.1 (QIAGEN, Aarhus A/S). The sequences were used as a query to run Basic Local Alignment Search Tool for nucleotides (BLASTn, National Center for Biotechnology Information, U.S. National Library of Medicine, Bethesda MD, USA) to identify homologous sequences from related species available in the GenBank using default settings. The "One Click" mode on the Phylogeny.fr platform (Dereeper et al., 2008) was used to reconstruct a phylogenetic tree through an automated process that successively performs multiple sequence alignment using MUSCLE (Edgar, 2004) with default settings to align these sequences (see Figure 2 for accession numbers); tree building using PhyML (Guindon and Gascuel, 2003) run with an approximate Likelihood Ratio Test

(Anisimova and Gascuel, 2006) for branch support; and tree drawing using TreeDyn (Chevenet et al., 2006). Sequences were deposited in Genebank under accession numbers MZ021341 and MZ021342.

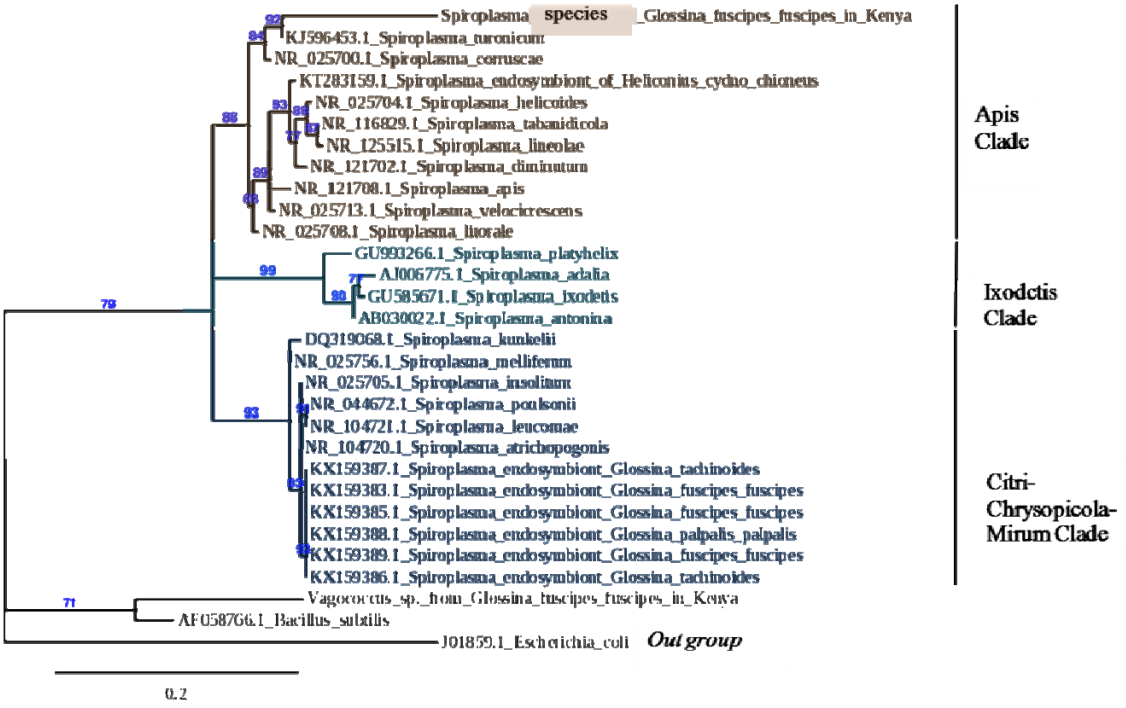

Fig 2: Approximate likelihood ratio test phylogenetic analysis of *Spiroplasma* and *Vagococcus* rRNA gene isolated from *G. f. fuscipes.* Bootstrap values are given at each node (only value < 50% are indicated). Genbank accession numbers are given on the left of each species.

2.6. Ethics statement

Entomological collections on the shores of Lake Victoria at ITOC were permitted by the owners (*icipe*). Other entomological collections were done on public land. The study was conducted in conformity with *icipe's* ethical rules for studies with animals.

3. Results

3.1. Trap efficiency of sampling tools

In experiments to determine the trap efficiency of the biconical trap, a total of 309 tsetse flies were caught with 213 (69%) females and 96 males. Out of these, 287 (92%) were collected from the biconical trap with E-net and 22 from the biconical trap alone. The overall catch index of the biconical trap compared to biconical trap with flanking E-nets was 0.68 (95% CI: 0.39 – 0.12%; Z= -9.743; P<0.0001). The catch index for males was higher than for females (Table 1). The overall efficiency of the biconical trap was 7.7% (95% CI: 4.9 – 11.4%). The biconical trap efficiencies for males (10.2%; 95% CI: 4.9 – 18.5%) and females (6.5%; 95% CI: 3.5 – 10.9%) were not significantly different (P=0.2764).

Experiments to determine the efficiency of the ES-target caught a total of 145 tsetse flies comprising of 105 (72%) female and 40 males. Of these, 128 (88%) were caught with the ES-target flanked by E-net and 17 with ES-target alone. The overall catch index of the ES-target compared to the ES-target flanked with E-nets was 0.13 (95% CI: 0.07 – 0.23; Z= -7.096; P<0.0001). The catch index for males was higher than for females (Table 1). The overall efficiency of the ES-target was 13.3% (95% CI: 7.9 – 20.4%). The efficiency was significantly higher (P=0.0002) for males (33.3%; 95% CI: 17.3 – 52.8%) than females (7.1%; 2.9 – 14.2%).

A total of 235 tsetse flies were caught in the experiment that determined the efficiency of the SS-target. Of these 165 (70%) were female and 70 were male. From these 185 (79%) were caught with the SS-target flanked by E-net and 50 with the SS-target alone. The overall catch index of the SS-target compared to the SS-target flanked with E-nets was 0.27 (95% CI: 0.19 – 0.37; Z= -7.706; P<0.0001). The catch index for males was higher than for females (Table 1). The SS-target had an overall efficiency of 27.0% (95% CI: 20.8 – 34.0%). The efficiency

was significantly higher (P=0.0037) for males (42.8%; 95% CI: 28.9 – 56.7%) than for females (21.3%; 95% CI: 14.4 – 28.2%).

A pairwise comparison showed that the overall efficiency of the SS-target significantly differed with the biconical trap (P<0.0001) and ES-target (P=0.0037). There was no significant difference between the overall efficiency of the ES-target and the biconical trap (P=0.0714).

Table 1: Detransformed means and indices of catches from experiments that investigated efficiency of biconical trap, ES-target and SS-target

Trial arms	**Females**			**Males**			**Overall**		
	Detransformed mean catch (CI)	Catch index (CI)	*P* - value	Detransformed mean catch (CI)	Catch index (CI)	*P* - value	Detransformed mean catch (CI)	Catch index (CI)	*P* - value
Biconical trap + E-net	16.2 (12.6 – 21.0)	1	-	7.3 (5.4 – 9.7)	1	-	23.5 (18.3 – 30.3)	1	-
Biconical trap	0.9 (0.5 – 1.8)	0.06 (0.00 – 0.10)	<0.0001	0.7 (0.3 - 1.5)	0.10 (0.45 – 0.20)	<0.0001	1.6 (1.0 – 2.7)	0.07 (0.04 – 0.12)	<0.0001
ES-target + E-net	7.9 (6.0 – 10.5)	1	-	2.1 (1.2 – 3.1)	1	-	10.0 (7.8 – 12.9)	1	-
ES-target	0.6 (0.2 – 1.3)	0.07 (0.03 – 0.15)	<0.0001	0.7 (0.3 – 1.3)	0.33 (0.16 – 0.66)	<0.0001	1.3 (0.8 – 2.3)	0.13 (0.07 – 0.15)	<0.0001
SS-target + E-net	11.2 (8.9 – 13.9)	1	-	4.0 (3.0 – 5.4)	1	-	15.2 (12.7 – 18.1)	1	-
SS-target	2.4 (1.5 – 3.5)	0.21 (0.13 – 0.32)	<0.0001	1.7 (1.1 – 2.7)	0.43 (0.25 – 0.70)	<0.0001	4.1 (3.0 – 5.5)	0.27 (0.19 – 0.37)	<0.0001

3.2. Cost efficiency

In these experiments, a total of 371 tsetse flies were caught comprising of 208 (56%) females and 163 (44%) males. The SS-target, ES-target and biconical trap caught 234 (63%), 95 (26%) and 42 (11%) respectively. The detransformed mean catches of the biconical trap, electric target and SS-target were 4.6 (95%CI: 3.2 – 6.5), 10.4 (95% CI: 8.1 – 13.5) and 25.6 (21.0 – 31.2), respectively. This translated into the ES-target and SS-target significantly catching 2.3 (95% CI: 1.5 – 3.5; Z= $P < 0.0001$) and 5.6 (95% CI: 3.8 – 8.3; $P < 0.0001$) times more flies respectively than the biconical trap. For all the sampling tools except for the SS-target, the detransformed means for males were always higher than those for females (Table 2). The SS-target had the lowest cost of catching a single fly at USD 0.70 compared to USD 2.40 for the biconical trap and over USD 13.50 for the ES-target. However, the cost of the biconical trap was the lowest compared to the SS-target and ES-target (Table 3).

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Table 2: Detransformed means and indices of catches obtained from experiments that compared fly catches between the biconical trap, ES-target and SS-target

CI is 95% confidence interval

Trial arms	**Females**			**Males**			**Overall**		
	Detransformed mean catch (CI)	Catch index (CI)	*P* - value	Detransformed mean catch (CI)	Catch index (CI)	*P* - value	Detransformed mean catch (CI)	Catch index (CI)	*P* - value
Biconical trap (reference)	1.5 (0.8-2.9)	1	-	3.0 (2.0-4.6)	1	-	4.6 (3.2 - 6.5)	1	-
E- target	4.9 (3.2-7.6)	3.2 (1.6-6.7)	0.0019	5.5 (4.0-7.5)	1.8 (1.1-2.9)	0.0170	10.4 (8.1-13.5)	2.3 (1.5-3.5)	<0.0001
Sticky small target	16.5 (11.6-23.3)	10.6 (5.5-21.8)	<0.0001	9.3 (7.2-11.9)	3.0 (2.0-4.8)	<0.0001	25.6 (21.0-31.2)	5.6 (3.8-8.3)	<0.0001

Table 3: Approximate cost of catching a single fly using the biconical trap, ES-target and SS-target

Sampling tool	Approximate cost of sampling tool in USD	Flies caught per trapping device	Cost (USD)/ fly caught
Biconical trap	11.50	4.6	2.40
ES-target	>140*	10.4	<13.50
SS-target	18.30	25.6	0.70

USD is United States Dollar; *Does not include cost of spark box and charging of battery.

3.3. Suitability of flies for molecular tests

A total of 130/154 (86.7%; 95%CI: 80.2 – 91.7%) samples showed amplification of 16S rRNA gene of band size approximately between 400 and 500 base pairs. The BLASTn search showed that DNA sequences of amplified bacterial 16S rRNA gene from the two tsetse samples (SI 1) caught using the SS-target were related to *Spiroplasma turonicum* and *Vagococcus* spp with 90.7% and 99.3% identities, respectively. The DNA sequence related to 16S rRNA gene of *Spiroplasma turonicum* had a query cover of 87% and Expect (E) value of 9.0×10^{-133}. The DNA sequence related to 16S rRNA gene of *Vagococcus* spp showed a query cover of 99% and E-value of 0.0. Molecular phylogeny inferred from 16S rRNA gene of the *Spiroplasma* related sequence isolated from *G. f. fuscipes* caught on the shores of Lake Victoria in western Kenya showed that it belonged to the *Apis* Clade (Fig. 2). The DNA sequence related to *Vagococcus* spp had a different branch from *Spiroplasma* sp in the phylogenetic tree.

4. Discussion

Establishing the efficiency of sampling tools and prospects of using samples collected for downstream processes such as molecular tests is important to guide researchers in tsetse and African trypanosomiasis control to achieve better outcomes. In this study we report the

efficiencies of the biconical trap, ES-target and SS-targets to sample *G. f. fuscipes*. We also report molecular test results of the amplification of bacterial 16S rRNA gene from *G. f. fuscipes* collected using SS-targets.

We observed that the efficiency of the SS-target (27.0%) was about 19 percent points more than that of the biconical (7.7%) and about 13 percent points more than the ES-target (13.3%). This is consistent with an earlier study that showed that the SS-target is a more effective tool for sampling *G. f. fuscipes* than the biconical trap (Mbewe et al., 2018b). The efficiency of the biconical trap in our study of 6.5% for females and 10.2% for male is lower than 21% and 40% for females and males respectively observed by Lindh et. al (2009). This variance could be due to differences in methods used among other factors which may include differences in environmental factors during the time the studies were undertaken (FAO, 1992). Whereas we used two E-nets to flank the biconical trap, Lindh et al. (2009) used one E-net to flank the biconical trap. Furthermore, the fly density was higher in the study by Lindh et al. (2009). This could have also contributed to the observed differences in efficiencies of the biconical trap as trap efficiency positively correlates with fly density (Vreysen et al., 2013). Nevertheless, in both studies, the efficiency for trapping females was lower than that for males despite a higher proportion of females visiting the trap. This observation was consistent across all the sampling tools studied and with literature (Leak et al., 2008); and could be an indication of sex based behavioural differences in landing on and entering visually attractive stationary sampling tools. In nature, there is a higher proportion of female tsetse flies than males (Leak et al., 2008). From our results, the SS-target caught a higher proportion of females than males compared to the biconical and ES-target. Therefore, the sex ratio of tsetse flies caught on the SS-target was more representative of the natural sex ratio than the other traps.

We also observed that the cost of catching a fly using the SS-target was 3.4 and over 19 times lower compared to the biconical trap and the ES-target, respectively. Nevertheless, a more rigorous study to determine the cost effectiveness of deploying SS-target for monitoring tsetse populations during an intervention is required. Furthermore, regarding catch indices, the SS-target caught more tsetse flies than the biconical trap and the electric target. Elsewhere, Oloo et al. (2014) showed that larger targets (1.0 × 1.5 m) covered with sticky material caught 4 to 6 times more *G. f. fuscipes* than the biconical trap. Whereas it has already been established that the SS-target is more effective for sampling *G. f. fuscipes* than the biconical trap and can be used for field experiments (Mbewe et al., 2019, 2018b), it had not been compared to the electric target. It would be expected that two target-based sampling tools could show similar catch indices. Surprisingly, the SS-target seems to catch more *G. f. fuscipes* than the electric target. This warrants further investigation to establish the possible explanations for the observation. Such explanations could be used to further optimise and make the sampling tools more cost effective for use in tsetse control interventions.

Phthalogen blue is known to be among the most attractive to tsetse. However, it has been difficult to find local sources of phthalogen blue cotton material since the mid 1990's (Lindh et al., 2012). In our study, all targets were made of blue and black cotton material locally sourced and of unknown dye composition and reflectance spectra. Therefore, we recommend for further investigations into the reflectance spectra of the blue and black cotton materials used to make the targets. A study by Lindh et al. (2012) showed that the reflectance of the blue colour was responsible to the level of attractiveness of the targets to tsetse.

Usually, tsetse flies caught during monitoring of the effectiveness of a control intervention are not only used to establish the fly densities but also other processes such as dissection and molecular tests to establish fly population characteristics. Population characteristics such as

ovarian age and genetics can be used to monitor and evaluate the progression of tsetse control interventions; whether or not there is reinvasion of tsetse flies from neighbouring un-treated areas (Leak et al., 2008). Therefore, tsetse flies caught using sampling tools should suitable for subsequent processes such as dissections and molecular based tests. In the current study we showed that tsetse flies caught on the SS-target were suitable for total genomic DNA extraction. Whereas insect DNA can easily be detected due to the large quantities in a whole fly, bacteria are small, and this could diminish the amount of DNA for detection using molecular tests. Interestingly, amplification of bacterial DNA from two flies and subsequent sequencing revealed the presence of *Spiroplasma* and *Vagococcus* genera. More than 23 bacteria have been isolated from tsetse and *Spiroplasma* is documented as a novel endosymbiont in *G. f. fuscipes* and *G. tachinoides* (Demirbas-Uzel et al., 2018). However, its role in the fly is unknown (Schneider et al., 2019). Molecular phylogeny from the 16S rRNA gene DNA sequence showed that *Spiroplasma* isolated from a single fly of *G. f. fuscipes* in the current study belonged to the *Apis* clade. Bacteria from the *Vagococcus* genera have been isolated from the digestive tract of *G. pallidipes* (Malele et al., 2018). Therefore, it is likely that *Vagococcus* isolated from *G. f. fuscipes* inhabits the digestive tract of the fly. However, only studies that will isolate the bacteria from the digestive tract of the fly can ascertain this claim. Documentation of bacterial communities in insect vectors is of interest for prospects of symbiont mediated approaches to control vector borne diseases (Chandel et al., 2015; De Vooght et al., 2014; Mbewe et al., 2015).

5. Conclusion

This study has shown that SS-target is the most efficient trap to monitor *G. f. fuscipes* declines during control interventions. It has also shown that samples collected from SS-targets are suitable for molecular studies and revealed the presence of *Spiroplasma* in wild *G.*

f. fuscipes. Therefore, we recommend future studies to explore the role of *Spiroplasma* in the biology of tsetse and Africa trypanosomiasis control.

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Data availability statement

All datasets used and/or analysed during the current study are available upon reasonable request from the corresponding author.

References

Abila, P.P., Okello-Onen, J., Okoth, J.O., Matete, G.O., Wamwiri, F., Politzar, H., 2007. Evaluation of the cost-effectiveness of pyramidal, modified pyramidal and monoscreen traps for the control of the tsetse fly, *Glossina fuscipes fuscipes*, in Uganda. J. Insect Sci. 7, 1–7. doi:10.1673/031.007.4701

Anbutsu, H., Fukatsu, T., 2003. Population Dynamics of Male-Killing and Non-Male-Killing Spiroplasmas in *Drosophila melanogaster*. Appl. Environ. Microbiol. 69, 1428–1434. doi:10.1128/AEM.69.3.1428

Anisimova, M., Gascuel, O., 2006. Approximate Likelihood-Ratio Test for Branches: A Fast, Accurate, and Powerful Alternative. Syst. Biol. 55, 539–552. doi:10.1080/10635150600755453

Challier, A., Gouteux, J.P., 1980. Ecology and epidemiological importance of _*Glossina Palpalis*_ in the Ivory Coast forest zone. Insect Sci. Its Appl. 1, 77–83.

Challier, A., Laveissiere, C., 1973. A new trap for catching flies (Glossina : Diptera , Muscidae) : description and field trials. Ser. notebooks ORSTOM Med. Entomol. Parasitol. 11, 251–262.

Chandel, K., Parikh, R., Mendki, M., Shouche, Y., Veer, V., 2015. Isolation and characterization of Vagococcus sp from midgut of *Culex quinquefasciatus* (Say) mosquito. J Vector Borne Dis 52, 52–57.

Chevenet, F., Brun, C., Bañuls, A.-L., Jacq, B., Christen, R., 2006. TreeDyn: towards dynamic graphics and annotations for analyses of trees. BMC Bioinformatics 7, 439. doi:10.1186/1471-2105-7-439

De Vooght, L., Caljon, G., De Ridder, K., Van Den Abbeele, J., 2014. Delivery of a functional anti-trypanosome Nanobody in different tsetse fly tissues via a bacterial symbiont, *Sodalis glossinidius*. Microb. Cell Fact. 13, 156. doi:10.1186/s12934-014-0156-6

Demirbas-Uzel, G., De Vooght, L., Parker, A.G. et al. Combining paratransgenesis with SIT: impact of ionizing radiation on the DNA copy number of Sodalis glossinidius in tsetse flies. BMC Microbiol 18, 160 (2018). https://doi.org/10.1186/s12866-018-1283-8

Dereeper, A., Guignon, V., Blanc, G., Audic, S., Buffet, S., Chevenet, F., Dufayard, J.-F., Guindon, S., Lefort, V., Lescot, M., Claverie, J.-M., Gascuel, O., 2008. Phylogeny.fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Res. 36, W465–W469. doi:10.1093/nar/gkn180

Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32, 1792–1797. doi:10.1093/nar/gkh340

Esterhuizen, J., Rayaisse, J.B., Tirados, I., Mpiana, S., Solano, P., Vale, G. a., Lehane, M.J., Torr, S.J., 2011. Improving the cost-effectiveness of visual devices for the control of riverine tsetse flies, the major vectors of human African trypanosomiasis. PLoS Negl. Trop. Dis. 5, 1–8. doi:10.1371/journal.pntd.0001257

FAO, 1992. Training Manual for TSETSE CONTROL PERSONNEL Volume 4. FAO, Rome.

Fox, J., 2003. Effect Displays in R for Generalised Linear Models. J. Stat. Softw. 8, 1–27.

Guindon, S., Gascuel, O., 2003. A Simple, Fast, and Accurate Algorithm to Estimate Large Phylogenies by Maximum Likelihood. Syst. Biol. 52, 696–704. doi:10.1080/10635150390235520

Holmes, P., 2013. Tsetse-transmitted trypanosomes - Their biology, disease impact and control. J. Invertebr. Pathol. 112 Suppl, S11-4. doi:10.1016/j.jip.2012.07.014

Leak, S., Ejigu, D., Vreysen, M., 2008. Collection of Entomological Baseline Data for Tsetse Area-Wide Integrated Pest Management Programmes, FAO/IAEA. FAO, Rome.

Lindh, J.M., Lehane, M.J., 2011. The tsetse fly *Glossina fuscipes fuscipes* (Diptera: Glossina) harbours a surprising diversity of bacteria other than symbionts. Antonie Van Leeuwenhoek 99, 711–720. doi:10.1007/s10482-010-9546-x

Lindh, J.M., Torr, S.J., Vale, G.A., Lehane, M.J., 2009. Improving the cost-effectiveness of artificial visual baits for controlling the tsetse fly *Glossina fuscipes fuscipes*. PLoS Negl. Trop. Dis. 3, e474. doi:10.1371/journal.pntd.0000474

Mahamat, M.H., Peka, M., Rayaisse, J.B., Rock, K.S., Toko, M.A., Darnas, J., Brahim, G.M., Alkatib, A.B., Yoni, W., Tirados, I., Courtin, F., Brand, S.P.C., Nersy, C., Alfaroukh, I.O., Torr, S.J., Lehane, M.J., Solano, P., 2017. Adding tsetse control to medical activities contributes to decreasing transmission of sleeping sickness in the Mandoul focus (Chad). PLoS Negl. Trop. Dis. 11, 1–16. doi:10.1371/journal.pntd.0005792

Malele, I., Nyingilili, H., Lyaruu, E., Tauzin, M., Bernard Ollivier, B., Cayol, J.L., Fardeau, M.L., Geiger, A., 2018. Bacterial diversity obtained by culturable approaches in the gut of Glossina pallidipes population from a non sleeping sickness focus in Tanzania:

Preliminary results. BMC Microbiol. 18. doi:10.1186/s12866-018-1288-3

Mateos, M., Castrezana, S.J., Nankivell, B.J., Estes, A.M., Markow, T.A., Moran, N.A., 2006. Heritable endosymbionts of *Drosophila*. Genetics 174, 363–376. doi:10.1534/genetics.106.058818

Mbewe, N.J., Mweempwa, C., Guya, S., Wamwiri, F.N., 2015. Microbiome frequency and their association with trypanosome infection in male *Glossina morsitans centralis* of Western Zambia. Vet. Parasitol. 211, 93–98. doi:10.1016/j.vetpar.2015.04.027

Mbewe, N.J., Saini, R.K., Irungu, J., Yusuf, A.A., Pirk, C.W.W., Torto, B., 2019. Responses of *Glossina fuscipes fuscipes* to visually attractive stationary devices baited with 4-methylguaiacol and certain repellent compounds in waterbuck odour. PLoS Negl. Trop. Dis. 13, e0007510. doi:10.1371/journal.pntd.0007510

Mbewe, N.J., Saini, R.K., Torto, B., Irungu, J., Yusuf, A.A., Pirk, C., 2018a. Effects of vector control on the population structure of tsetse (Glossina fuscipes fuscipes) in western Kenya. Acta Trop. 179, 1–9. doi:10.1016/j.actatropica.2017.12.015

Mbewe, N.J., Saini, R.K., Torto, B., Irungu, J., Yusuf, A.A., Pirk, C.W.W., 2018b. Sticky small target: an effective sampling tool for tsetse fly *Glossina fuscipes fuscipes* Newstead, 1910. Parasit. Vectors 11, 268. doi:10.1186/s13071-018-2840-6

Mohamed-Ahmed, M., Odulaja, A., 1997. Diel activity patterns and host preferences of *Glossina fuscipes fuscipes* (Diptera: Glossinidae) along the shores of Lake Victoria, Kenya. Bull. Entomol. Res. 87, 179–186.

Mweempwa, C., Mbewe, N.J., De Deken, R., 2020. Wing length of tsetse caught by stationary and mobile sampling methods. Acta Trop. 204, 105333.

doi:10.1016/j.actatropica.2020.105333

Omolo, M.O., Hassanali, A., Mpiana, S., Esterhuizen, J., Lindh, J., Lehane, M.J., Solano, P., Rayaisse, J.B., Vale, G.A., Torr, S.J., Tirados, I., 2009. Prospects for developing odour baits to control *Glossina fuscipes* spp., the major vector of human African trypanosomiasis. PLoS Negl. Trop. Dis. 3, e435. doi:10.1371/journal.pntd.0000435

Oloo, F., Sciarretta, A., Mohamed-Ahmed, M.M., Kröber, T., McMullin, A., Mihok, S., Guerin, P.M., 2014. Standardizing Visual Control Devices for Tsetse Flies: East African Species Glossina fuscipes fuscipes and Glossina tachinoides. PLoS Negl. Trop. Dis. 8. doi:10.1371/journal.pntd.0003334

Packer, M.J., Brady, J., 1990. Efficiency of electric nets as sampling devices for tsetse flies (Diptera: Glossinidae). Bull. Entomol. Res. 80, 43–47. doi:10.1017/s0007485300045909

Rayaisse, J.B., Esterhuizen, J., Tirados, I., Kaba, D., Salou, E., Vale, G.A., Lehane, M.J., Torr, S.J., Solano, P., 2011. Towards an Optimal Design of Target for Tsetse Control : Comparisons of Novel Targets for the Control of Palpalis Group Tsetse in West Africa 5, 1–8. doi:10.1371/journal.pntd.0001332

Schneider, D.I., Saarman, N., Onyango, M.G., Hyseni, C., Opiro, R., Echodu, R., O'Neill, M., Bloch, D., Vigneron, A., Johnson, T.J., Dion, K., Weis, B.L., Opiyo, E., Caccone, A. & Aksoy, S. (2019). Spatio-temporal distribution of Spiroplasma infections in the tsetse fly (Glossina fuscipes fuscipes) in the northern Uganda. PLoS Neglected Tropical Diseases, 13(8), e0007340.

Shaw, A.P.M., Tirados, I., Mangwiro, C.T.N., Esterhuizen, J., Lehane, M.J., Torr, S.J., Kovacic, V., 2015. Costs Of Using “Tiny Targets” to Control *Glossina fuscipes fuscipes*, a Vector of Gambiense Sleeping Sickness in Arua District of Uganda. PLoS Negl. Trop.

Dis. 9. doi:10.1371/journal.pntd.0003624

Simon, C., Franke, A., Martin, A., 1991. Polymerase chain reaction: DNA extraction and amplification., in: Hewitt, G.M., Johnson, A.W., Young, J.P.. (Eds.), Molecular Techniques in Taxonomy. Springer, Berlin, pp. 329–355.

Tirados, I., Esterhuizen, J., Kovacic, V., Mangwiro, T.N.C., Vale, G.A., Hastings, I., Solano, P., Lehane, M.J., Torr, S.J., 2015. Tsetse Control and Gambian Sleeping Sickness; Implications for Control Strategy. PLoS Negl. Trop. Dis. 9, e0003822. doi:10.1371/journal.pntd.0003822

Vale, G.A., 1974. New field methods for studying the responses of tsetse flies to hosts. Bull. Entomol. Res. 199–208.

Vreysen, M.J., Seck, M.T., Sall, B., Bouyer, J., 2013. Tsetse flies: Their biology and control using area-wide integrated pest management approaches. J. Invertebr. Pathol. 112, S15–S25. doi:10.1016/j.jip.2012.07.026