



RESEARCH ARTICLE

Evaluation of an ivermectin-based attractive targeted sugar bait (ATSB) against *Aedes aegypti* in Tanzania.

[version 1; peer review: 1 approved, 2 approved with reservations, 1 not approved]

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Abstract

Background

The control of vector borne arboviral diseases such as Dengue is mainly achieved by reducing human-vector contact and controlling the vectors through source reduction and environmental management. These measures are constrained by labour intensity, insecticide resistance and pro-active community participation. The current study intended to develop and test an ivermectin-based attractive-targeted sugar bait (ATSB) against *Aedes aegypti*.

Methods

The 48hour lethal concentration (LC90) of ivermectin against *Ae. aegypti* was determined through serial dilution experiment where five 30cm x 30cm x 30cm cages were set; into each, a 10% sugar solution treated with ivermectin were introduced. 40 *Ae. aegypti* were released into each cage and observed for mortality after 4, 8, 24 and 48 hours. The ivermectin-based ATSB was evaluated in a semi field system where ATSB and attractive sugar bait (ASB) were deployed into each compartment of the semi field and 100 female *Ae. aegypti* were released every day and recaptured the next day through human land catch and Bio-gent sentinel trap. The developed and semi-field tested ATSB was further tested in the field by deploying them in garages.

Results

The ivermectin 48hr LC90 of male and female *Ae. aegypti* was found to be 0.03% w/v. In the semi field system, the ATSB significantly reduced a free-flying population of *Ae. aegypti* within 24 hours (incidence rate ratio (IRR) = 0.62; [95% confidence interval (95%CI); 0.54-0.70] and p-

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value < 0.001). However, in the field, the ATSBs required the addition of yeast as a carbon dioxide source to efficiently attract *Ae. aegypti* mosquitoes to feed.

Conclusion

Ivermectin is an active ingredient that can be used in an ATSB for *Ae. aegypti* depopulation. However, further research is needed to improve the developed and tested ATSB to compete with natural sources of sugar in a natural environment.

Keywords

Attractive-targeted sugar bait, ivermectin, *Aedes aegypti*, vector control, Dengue fever, Tanzania

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Introduction

Dengue fever is a global public health concern estimated to threaten 100 to 200 million people per year worldwide, with 2.5 billion people worldwide approximated to be at risk (World Health Organization *et al.*, 2009). The disease is endemic in 100 countries, with Asian and South American countries being the most affected (Kyle & Harris, 2008). The disease is reported to have increased by 30-fold in the past half century (World Health Organization, 2012) and is projected to increase further in the coming years (World Health Organization [WHO], 2020a). Reports from Bhatt *et al.*, 2013 have shown that the disease is spreading globally and can no longer be viewed as a regional problem, with cases occurring in countries without past history of disease outbreaks (Bhatt *et al.*, 2013), including Tanzania (Ward *et al.*, 2017).

The primary and secondary vectors of the dengue fever are *Aedes aegypti* (L) and *Aedes albopictus* (Skuse), respectively (Barrett & Higgs, 2007). They are widely distributed all over the world as a result of the rapid expansion of poorly planned cities, lack of piped water systems, poor sewage systems, high urban population growth (Gubler, 2011; Guzman & Harris, 2015) and the ability of the vectors to oviposit in diverse environments (Gómez-Dantés & Willoquet, 2009; Hawley, 1988). Additionally, an increase in inter-continental travel involving trade and tourism (Messina *et al.*, 2019) has largely contributed to spread of the vectors as well as the disease.

For decades, there have been no effective recommended anti-viral drugs for dengue fever treatment. However, in 2015, WHO recommended the use of the CYD-TDV vaccine (tetravalent dengue vaccine) against dengue fever for travellers going to countries with a seroprevalence of 70% and above (Wilder-Smith *et al.*, 2019). In 2019, the United States Food and Drug administration (FDA) approved the vaccine for individuals residing in endemic countries (FDA Commissioner, 2019). Nonetheless, the vaccine protects only individuals who are seropositive (individuals who had a previous infection) and not seronegative ones (Sridhar *et al.*, 2018). Thus, disease prevention continues to heavily rely on vector control, mainly through source reduction (larviciding and environmental management), preventing human-vector contact (repellent and insecticide treated materials) and adult depopulation (insecticide space spraying, indoor space spraying) (Gómez-Dantés & Willoquet, 2009; Ranson *et al.*, 2010; Vontas *et al.*, 2012; World Health Organization, 2009). However, current control measures require untiring commitment from health authorities and community members, and is challenged by economic constraints in low- and middle-income countries (LMIC). In addition, insecticide-based tools are threatened by the emergence of insecticide resistance (Lima *et al.*, 2011; Ponlawat *et al.*, 2005; Rodríguez *et al.*, 2007; Vontas *et al.*, 2012). The development of a low-cost, peri-domestic intervention such as an attractive-targeted sugar bait (ATSB) against *Aedes* mosquitoes could have the potential to expand the intervention toolbox and improve disease control.

In recent years, ATSBs have surged as a novel vector control paradigm (Beier *et al.*, 2012; Müller *et al.*, 2008; Müller *et al.*, 2010a; Naranjo *et al.*, 2013; Qualls *et al.*, 2015b). The

intervention exploits mosquito sugar feeding behaviour, which is common to both male and female mosquitoes. Usually male mosquitoes exclusively feed on sugar for their whole life while females feed on sugar for survival, flight and fecundity enhancement (Foster, 1995) but require blood for egg-laying. Therefore, an intervention that targets this behaviour will concurrently target both male and female mosquitoes. ATSBs have been researched for controlling malaria vectors and have successfully reduced the vector populations (Müller *et al.*, 2008; Müller *et al.*, 2010b). Additionally, they have also been found to impact the density and fitness of *Aedes* mosquitoes (Ali *et al.*, 2006; Xue *et al.*, 2006) as well as other sugar-seeking vectors such as sand flies (Qualls *et al.*, 2015a; Saghaifipour *et al.*, 2017). Furthermore, ATSBs appear to reduce human landing rates (Junnala *et al.*, 2015; Xue *et al.*, 2006; Xue & Barnard, 2003), a parameter that highly influences disease transmission

Ivermectin was selected as the ATSB toxicant because of its holistic ability to reduce densities, fitness and virus replication in the mosquitoes. Ivermectin has been reported to reduce mosquito survival (Chaccour *et al.*, 2010), inhibits oviposition (Focks *et al.*, 1991) as well as reducing viral load of flaviviruses inside the mosquitoes including dengue viruses (Mastrangelo *et al.*, 2012; Wagstaff *et al.*, 2012; Xu *et al.*, 2018). In addition, ivermectin has a different mode of action: it targets the glutamate-gate chloride channel compared to other insecticides commonly used for vector control (Hemingway & Ranson, 2005), a property that may circumvent the existing resistance mechanisms in the mosquitoes (Foy *et al.*, 2011). Ivermectin also has a good safety record for use in humans and other mammals making it suitable for a bait solution in case of accidental ingestion. For more than three decades, it has been distributed in mass drug administration (MDA) campaigns against filariasis and onchocerciasis with over one billion doses donated by the Mectizan project (Crump & Omura, 2011). The safety of the endectocide is likely related to its target receptors in arthropods where upon being ingested, the ivermectin directly targets glutamate-gated chloride channels (GluCl), a receptor channel which exists in invertebrate nerve and muscle cells leading to paralysis and death of the insect (Fox, 2006; Sheriff *et al.*, 2005) but is non-existent in vertebrates. This study aimed at evaluating the efficacy of an ivermectin-based ATSB as a potential approach for controlling *Ae. aegypti* mosquitoes in urban Tanzania.

Methods

Mosquitoes

All experiments were conducted at Ifakara Health Institute Bagamoyo and Ifakara branches using disease free, insectary-reared *Aedes aegypti*. The mosquitoes were reared at 27±5°C and 40%-99% humidity at Ifakara Health Institute insectary in Bagamoyo and Ifakara, Tanzania. Larvae were fed daily with Tetramin® fish food while adults were maintained with 10% w/v sugar solution ad libitum with natural light regimen. For egg laying, they were fed cow blood through a membrane.

Laboratory experiment

Determination of ivermectin 48hr LC90 for *Ae. aegypti*. The dose of ivermectin sufficient to kill 90% of *Ae. aegypti* was determined in a laboratory using insectary-reared mosquitoes. Serial dilutions of ivermectin in 10% w/v sugar solution

were obtained starting with 0.01% of ivermectin as a starting point following a report by Tenywa *et al.* (Tenywa *et al.*, 2017) that found 0.01% of ivermectin in 10% w/v sugar solution was enough to kill more than 90% of malaria vector (*Anopheles arabiensis*) within 48 hours. To make a 0.01% ivermectin solution, 1ml of 1% injectable ivermectin (Ivomec®) was diluted in 100ml of 10% w/v sugar solution. The procedure was repeated to obtain other ivermectin concentrations: 0.001%, 0.0025%, 0.005%, 0.015%, 0.02%, 0.025%, 0.03%, 0.04%, 0.05% and 0.06%. The solutions were dyed with a food colouring dye (Carmoisin) at 0.5% v/v concentration for easy visualization of sugar fed mosquitoes.

Experiments were done using two rounds of five cages (30cm × 30cm × 30cm) placed inside the insectary. Each ivermectin concentration was poured into a 30mL plastic container to 2/3 of its capacity. These containers are regularly used for delivering glucose to mosquitoes reared in the insectary. A Whatman paper (filter paper) was rolled up like a tube and then dipped into each container containing the sugar solution with ivermectin concentration. The sugar solutions were randomly placed into each of the 30cm × 30cm × 30cm cages without blinding the researchers. One cage with 10% w/v sugar solution without ivermectin served as a control for the experiment. The sugar solution progressed up the filter paper where the mosquitoes had access to it. 40, 3–6 days old, blood naïve and starved for 6–8 hours mosquitoes were introduced into each cage and allowed to feed on the soaked filter paper dipped into the container containing ivermectin in 10% sugar solution. For an entire experiment, a total of 2200 mosquitoes were used as per (Tenywa *et al.*, 2017). Mosquito mortality was recorded after 4-, 8-, 24- and 48-hours post-introduction of the treatments. For each ivermectin concentration, five experiments (replicates) were performed while changing the position of the cages after each experimental replicate to avoid bias due to cage positioning.

Similar experimental set up and procedures as described above were performed for male *Ae. aegypti* where eight ivermectin doses were tested: 0.005%, 0.01%, 0.015%, 0.02%, 0.025%, 0.03%, 0.04% and 0.05%. In this round of the experiment, 1800 mosquitoes were used.

Semi field experiment

Efficacy of ATSB on population density and survival of *Ae. aegypti* in a controlled environment. The efficacy of ATSB was determined in a semi field system at Ifakara Health Institute's mosquito city in Ifakara. The semi field system (biodome) is 19m × 29m long; sitting on a concrete slab and surrounded with a water moat which prevents mosquito predators from entering the biodome. Its floor is of mud and the walls are made of net which allows ambient conditions inside the biodome to be similar to the outside environment. The biodome has two compartments separated by a 4m × 29m corridor and is roofed with polyvinyl sheets (Ferguson *et al.*, 2008). A hut mimicking rural houses in terms of doors, windows, eaves, structure and size is installed in each of the two biodome compartments. The huts are made of red brick walls and grass roofs. To reduce the temperature inside the biodome that could lead to mosquito desiccation the biodome was modified by adding a thatch layer

beneath the polyvinyl roof. The entry doors were sealed with double nets and fixed with a zip in order to prevent mosquitoes from escaping the system when a person entered or exited the biodome.

The two compartments of the biodome were labelled as A and B. Five control-ATSB (ATSB without ivermectin) and ATSBs were made following Tenywa *et al.*, (Tenywa *et al.*, 2017) and deployed into each of the compartments. 100 *Ae. aegypti* mosquitoes aged 2 – 5 days, blood naïve and starved for 6–8 hours were released into each compartment at 6:00 am every day consecutively for 30 days, simulating every day emerging mosquitoes in the wild population. In compartment A, the five control-ATSBs were deployed in all 30 days of the experimentation while in compartment B, the 30 days of experimentation were done in three phases each of 10 days. During phase I (day 0 to 10 of experimentation) and phase III (day 21 to 30 experimentation) control-ATSB were placed in the compartment, while during phase II (day 11 to 20 of experimentation) the control-ATSB were replaced with ATSBs containing 0.03% ivermectin. To maintain quality of the control-ATSB and ATSB, both were replaced with a new one after every five days.

24 hours after every mosquito release, one Biogent (BG) sentinel trap was deployed into each compartment for three hours from 6:30am to 9:30am to collect mosquitoes for monitoring the *Ae. aegypti* population density. Mosquitoes collected from each compartment were killed with ethanol by spraying them, counted and recorded.

During phase II, human landing catches (HLC) were done in compartment A and B for 20 minutes from 6:00am to 6:20am before deployment of the BG sentinel trap at 6:30am to assess the impact of ATSB on *Ae. aegypti* survival. One volunteer wearing shorts, closed shoes, a long sleeve shirt and hat, sat inside each of the semi field compartment and conducted a human land catch to collect mosquitoes landing on his shins (Figure 1). To reduce bias due to differences in volunteer attraction to the mosquitoes, the volunteers were swapped every day between the two compartments. All collected mosquitoes from each compartment were kept in paper cups labelled with the collection date and compartment/treatment. The collected mosquitoes were transferred to the insectary and given 10% w/v sugar solution that is used for regular colony maintenance. Sugar was delivered by soaking a small ball of cotton wool into 10% w/v sugar solution and put on top of the netting covering the paper cup lid. Daily mortality was recorded for 30 days. After the 30 days, the mosquitoes that remained alive were sprayed with ethanol and discarded.

Field experiment

Preparation of attractive targeted sugar bait (ATSB) with and without a carbon dioxide source. ATSB were prepared following procedures described previously (Tenywa *et al.*, 2017) (Figure 2A). Three test bait stations were prepared as follows: 1) control bait consisted of plain water dyed with 0.5% v/v red food colouring dye; 2) test-ATSB consisted of 10% w/v of sugar solution and ivermectin (final concentration 0.03% w/v); and 3) test-ASTB+CO₂ consisted of the same bait as test-ASTB

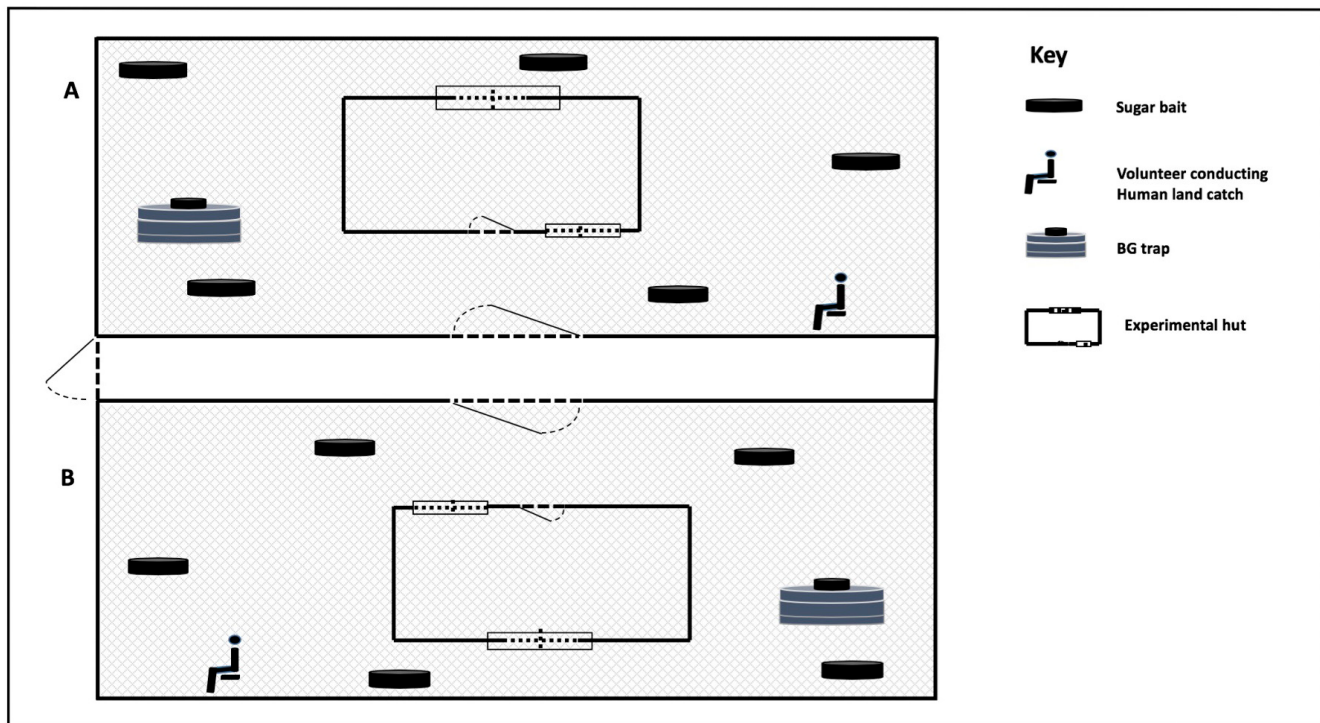


Figure 1. Illustration of attractive targeted sugar bait (test-ATSB or control-ATSB) stations deployed in a semi field. The control-ATSB and test-ATSB were deployed into either compartments A or B for 30 days with replacement of the control-ATSB by test-ATSB at day 10-20 in compartment B to determine its efficacy against *Ae. aegypti* population density in a controlled environment. One Bio-gent sentinel trap was deployed into each semi field compartment every day at 6:30am – 9:30am for 30 days. A volunteer conducted human land catch in each compartment from day 11 to 20 of the experimentation for 20 minutes from 6:00am to 6:20 am.



but with addition of a carbon dioxide (CO_2) source. The carbon dioxide was obtained from yeast. For that purpose, a total of 250grams of sugar (sucrose) and 17.5grams of yeast were dissolved into 2.5 litres of water in a 5 litre bottle. The bottle's lid was drilled to make a small hole where a small pipe was inserted into and connected to the test-ATSB+ CO_2 bait (Figure 2B). All the baits were covered with a wire mesh that was painted with rat glue (Tack-Tick Stronghold Glue®) to make a sticky trap (Figure 2C) trapping any mosquito attracted to land and feed on the bait.

Application of ATSB in a field setting. Field experiments were conducted between November and December 2019 in Tegeta, Kinondoni district in Dar es Salaam; the largest city and economic centre of Tanzania. The city is located at 6.48°S and 39.17°E along the Indian Ocean coast with 1100mm annual rainfall. It is a hub for international travel into and out of Tanzania and experiences frequent dengue outbreaks (Vairo *et al.*, 2016). A survey was conducted to identify *Ae. aegypti* breeding sites using BG sentinel traps deployed twice a day: 7:00am – 11:00am and 4:00pm – 7:00pm. Two vehicle garages with old used tires were identified as appropriate study sites and labelled as garage A and garage B. The garages were approximately 100 metres away from each other.

Three test-ATSB and test-ATSB+ CO_2 stations prepared as described above were deployed at either garage A or garage B. The stations of the same treatment were placed at 10 metres apart. To each treatment arm (test-ATSB and test-ATSB+ CO_2), three control baits: a bait that consisted of plain water dyed with 0.5% v/v red food colouring dye, were placed; each at three metres away from each treatment station. The treatment and control stations were left at the study sites for 24 hours. After 24 hours, the baits were checked for the presence of the mosquitoes that stuck on the bait station traps, removed and morphologically identified. In order to account for any variations due to difference in mosquito densities between the study sites, the

test-ATSBs and test-ATSBs+ CO_2 were swapped after every 24 hours between the sites. The experiments ran for a total of 12 days. And after every three days, the baits (test-ATSBs and test-ATSBs+ CO_2) and control were replaced with a new one so as to maintain the quality of the baits.

Data analysis

All data obtained were analysed using STATA package (Stata corp, College Station, TX).

Ivermectin LD90 against *Ae. aegypti*

A mean cumulative proportion mortality of mosquitoes for each ivermectin concentration was determined and compared to control (10% sugar solution) at 4, 8, 24 and 48 hours.

ATSB efficacy against *Ae. Aegypti* in the semi-field and field

Poisson regression model was employed to compare the number of mosquitoes caught in the semi-field compartments that had ATSBs vs control-ATSBs. Mosquito ages, compartments and day were considered as covariates. For the field test, a negative binomial regression model was performed to compare the number of mosquitoes that quested sugar from test-ASTB+ CO_2 and test-ASTB, and control bait consisted of plain water. The incidence rate ratio (IRR) and 95% confidence interval were obtained from the model.

Results

Ivermectin LD90 against *Ae. aegypti*

The results show that ivermectin is toxic to both male and female *Ae. aegypti* (Figure 3). In insectary conditions, approximately half of female and male *Ae. aegypti* mosquitoes provided with 0.02% (w/v) of ivermectin in 10% sugar solution died within 24 hours. Sugar solution containing ivermectin 0.03% (w/v) caused approximately 80% of mosquito mortality for both mosquito sexes within 24 hours and > 90% within 48 hours (Figure 3).

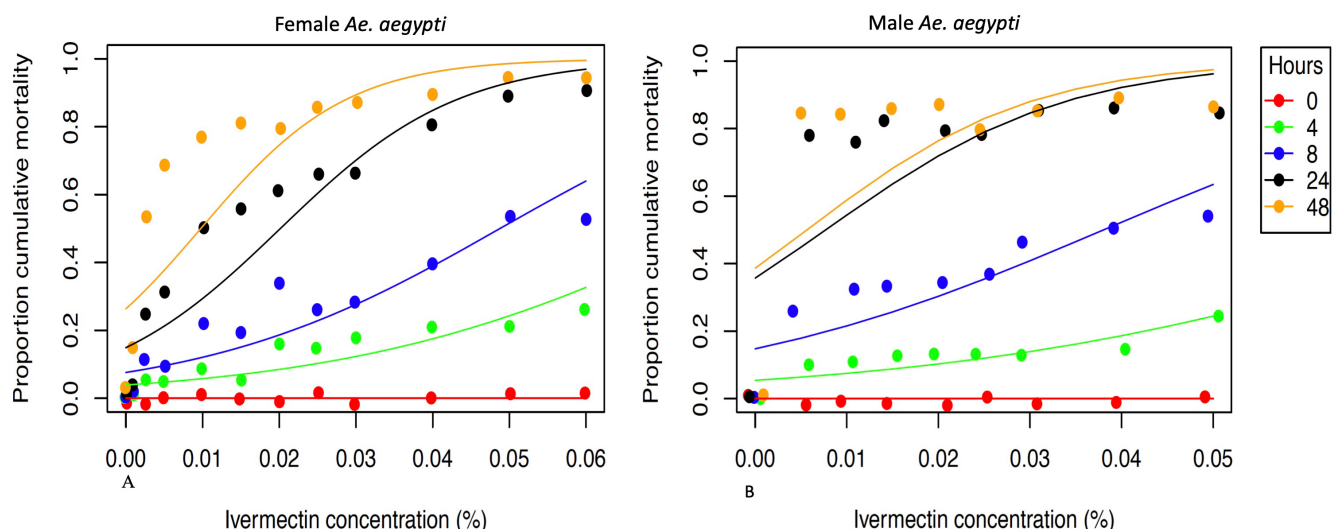


Figure 3. Mean cumulative proportional mortality of female (A) and male (B) *Ae. aegypti* post feeding on 10% sugar solution that contained different ivermectin concentrations.

ATSB efficacy against *Ae. aegypti* in a semi field environment

Mosquito recapture rate using BG-Sentinel traps in compartments A and B of the biodome were comparable before the introduction of ATSBs (incidence rate ratio (IRR) = 0.98; [95%confidence interval (CI): 0.87 – 1.12] and p- value ≤ 0.78) (compartment A, IRR=1). After introducing the ATSBs, mosquito recapture rate inside the compartment was reduced by 38% (IRR = 0.62; [95%CI: 0.54-0.70] and p-value < 0.001) (Table 1). Approximately 35% (95%CI: 32.0-38.0) and 56.8% (95%CI: 53.5-60.1) mosquitoes were recaptured in compartment with ATSB and control-ATSB respectively (Figure 4). ATSB reduced

Ae. aegypti survival time compared to control-ATSB (Figure 5). Within three days post recapture, 100% (n=253/253) and 7.5% (n= 23/306) of mosquitoes collected from a compartment with ATSB and a compartment with control-ASB respectively died (Figure 5).

ATSB efficacy against wild *Ae. aegypti*

Attractive targeted sugar baits (ATSBs) without carbon dioxide source optimized in a semi field system did not differ in attracting wild *Ae. aegypti* compared to a bait with plain water (IRR = 0.7; [95%CI: 0.36-1.17] and P-value ≤ 0.15) (Table 2). However, when a carbon dioxide source was added into the

Table 1. Cumulative number of recaptured mosquitoes (CR mosquitoes) and incidence rate ratio (IRR) of female *Aedes aegypti* recaptured in a semi field.

Mosquitoes recaptured in semi field compartment with	N	n	CR Mosquitoes	IRR	95%CI	P-value
ASB	10	1000	569	1	-	-
ATSB	10	1000	350	0.62	0.54-0.70	<0.001

N number of replicates, n total number of released mosquitoes, CR-Mosquitoes cumulative recaptured mosquitoes, IRR incidence rate ratio and 95%CI confidence interval

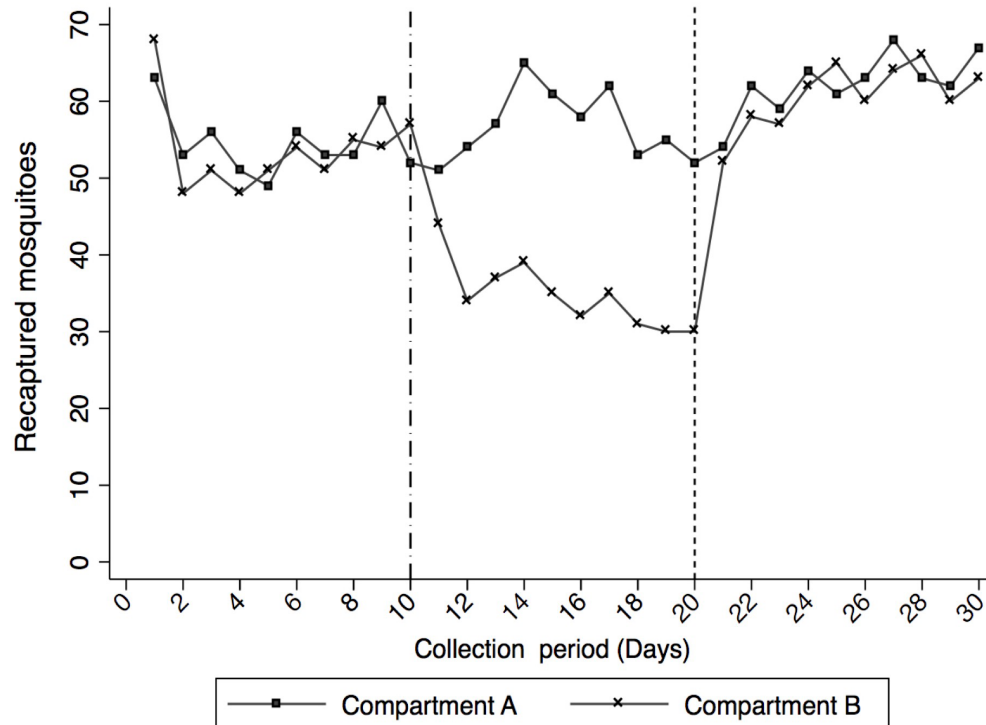


Figure 4. Mosquitoes recaptured in a semi field (controlled environment). **Compartment A** received attractive sugar bait without ivermectin (control-ASB) for 30 days of the experiments and **Compartment B** received ASB in phases I and III (day 0–10 and day 21–30) and in phase II (day 11–20) it received attractive targeted sugar bait with ivermectin (test-ATSB). A dash dot line shows when test-ATSB was introduced into the compartment B to replace control-ASB and a round dot line shows when the introduced test-ATSB was replaced with control-ASB.

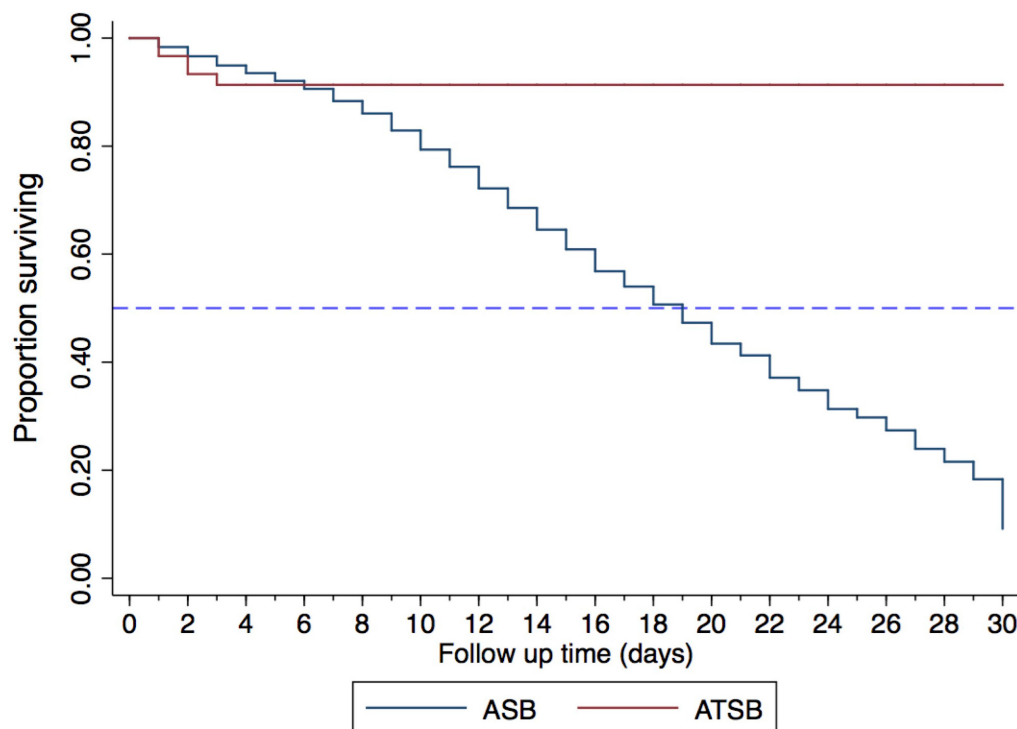


Figure 5. Effect of attractive targeted sugar bait on survival of female *Ae. aegypti* exposed to the baits in a semi-field system for 24 hours.

Table 2. Incidence rate ratio (IRR) of wild female *Ae. aegypti* attracted by ATSB.

ATSB sticky trap	Attracted mosquitoes	IRR	95%IRR CI	P-value
Bait with sugar and ivermectin	46	1	-	-
Bait with plain water (control)	40	0.7	0.36-1.17	0.15
Bait with sugar, ivermectin and CO ₂ source	172	4.4	2.52-7.83	<0.001

IRR captured mosquito incidence rate ratio, 95% IRR CI confidence interval.

ATSB, the incidence rate ratio of captured mosquitoes increased by approximately 4.4 folds (IRR = 6.8; [95%CI:4.11 – 11.30] and P – value < 0.001) in comparison to bait with sugar and ivermectin (IRR = 1) (Table 2).

Discussion

This study demonstrated that both female and male *Ae. aegypti* are equally sensitive to ivermectin when ingested in a sugar meal. It showed that mosquito mortality is a function of ivermectin concentration and time (ivermectin concentration α mosquito mortality) i.e. the higher the ivermectin concentration the faster

the mosquito is killed. Even though *Ae. aegypti* were sensitive to ivermectin, the mosquito species is not as sensitive as other mosquito species such as malaria vectors (Chaccour *et al.*, 2010; Sylla *et al.*, 2010) which are reported to be sensitive to ivermectin concentrations found in person's blood after receiving a therapeutic dose. In this study 0.03% of ivermectin killed more than 90% of both *Ae. aegypti* sexes within 48 hours; this dose is three times higher than that which killed the same proportion of female *An. arabiensis* in the same time period (Tenywa *et al.*, 2017), indicating that this mosquito species is less sensitive to ivermectin.

Moreover, it is known that even low ivermectin concentrations cause sub-lethal effects to mosquitoes including *Ae. aegypti* (Focks *et al.*, 1991; Tesh & Guzman, 1990) and *Ae. albopictus* (Tesh & Guzman, 1990) by reducing mosquito longevity, egg hatching rate and shortening the life of mosquito progenies. There may also be effects on the pathogen, as low therapeutic plasma concentration of ivermectin may have anti-dengue viral activity by reducing the ability of the dengue virus to infect mosquitoes (Xu *et al.*, 2018). Therefore, it may be possible to impact dengue vector populations using lower doses of ivermectin, that may still reach epidemiological significance by reducing vector survival, mosquito fitness and blocking viral transmission to the vectors. In this regard, reducing the ivermectin dose would also likely reduce the risk to non-target species when ivermectin is used in an ATSB whilst keeping its epidemiological impact. Further research is needed to investigate how sublethal doses of ivermectin delivered in sugar solution impact *Ae. aegypti* vectors, and how these effects would impact wild populations densities and transmission dynamics.

This study demonstrated that ATSB deployed inside a semi-field system remarkably reduced *Ae. aegypti* populations within twenty-four hours and had a suppressive effect on the free-flying population. The observed impact of the ATSB stations concurs with Müller *et al.*, 2008 and Xue *et al.*, 2008, which reported a similar impact of ATSB on *Ae. aegypti*, *Ae. caspius* population as well as *An. segentii* populations. Mosquito survival time for the mosquitoes collected from a compartment which had ATSBs was observed to be less than three days, contrasting with those from the control compartment where approximately half of them survived longer than nineteen days out of thirty days of holding period. The difference in survival time of the mosquitoes from the two groups suggests that the majority of the mosquitoes were attracted by the ATSBs and fed on it.

In the field, the ivermectin-based sugar bait stations (test-ATSB) were inefficient at attracting wild *Ae. aegypti*. The shortcoming of the bait stations is probably attributed by the presence of other natural mosquito meals sources such as plants which were more attractive. The assumption made here corresponds to Xue *et al.*, 2008 which reported similar bait stations' outcome. Usually sugar baits work on basis of "attract and kill" principal, therefore, the existence of natural sugar sources (Beier *et al.*, 2012; Müller *et al.*, 2010b) has a critical influence on the bait's performance. In order for sugar baits to effectively reduce mosquito population in the field, they must release enough attractant volatiles to efficiently attract vectors and there with overcome competition from the natural sugar sources. Also, application strategy (bait stations or vegetation spraying) in the field impacts sugar bait efficiency. Vegetation spraying approach involves spraying the whole vegetations with toxic sugar solution, whereas the bait station approach presents the toxic sugar bait at one single point. In this context, bait station approach may hypothetically be regarded as less effective in attracting mosquitoes due to the fact that mosquito may take a longer time to allocate the solution. However, in spite of the hypothesised limitation, we think that bait stations minimise the possibility of targeting non-targeted organisms such as butterflies, bees, ants and others, making the strategy environmentally safer.

Furthermore, mosquitoes species use cues such as CO₂ and animal skin odorants to respond towards a host (Clark & Ray, 2016; Dekker & Cardé, 2011). Carbon dioxide is widely used as lure for mosquitoes trapping (Cilek *et al.*, 2011; Irish *et al.*, 2008; Meeraus *et al.*, 2008) as it mimicks human odour and receives a potential gain in vector surveillance and control platforms. This study demonstrated that adding carbon dioxide source into the developed ivermectin-based sugar baits increases the attractiveness of the bait against wild *Ae. aegypti*. This finding highlights that carbon dioxide can be a potential component of an ATSB bait to increase attractiveness to mosquitoes in the field.

Limitation and safety consideration

The ivermectin concentration used in the test-ATSBs in this study is relatively high; although it is unlikely that the bait concoction would be consumed by children, we recommend that further field research done on the baits should investigate these by either placing a protective grill over them or hanging them out of reach.

Conclusions

Ivermectin-based ATSBs successfully reduce laboratory *Ae. aegypti* populations by approximately 95% within three days in a controlled environment (semi-field) but failed to do so against wild *Ae. aegypti* in the field unless a carbon dioxide source was added. We recommend further research to improve bait station design, and attractant strategies such as plant-based volatiles.

Ethical approval

This study received an ethical approval from Ifakara Health Institute Review Board (IHI-IRB) No. IHI/IRB/No. 22-2017 and National Institute for Medical Research Review Board (NIMR-RB) No. NIMR/HQ/R.8a/Vol. IX/2813. This study was also granted permission to publish from Tanzania National Institute of Medical Research ref No: NIMR/HQ/P.12 VOL XXXIII/85. Furthermore, efforts were made to ameliorate any suffering of the mosquitoes used in the research, especially during euthanasia.

Data availability

Underlying data

Open Science Framework: Evaluation of an Ivermectin-based Attractive Targeted Sugar Bait (ATSB) against *Aedes aegypti* in Tanzania. <https://doi.org/10.17605/OSF.IO/3JESA>

This project contains the following files:

- ATSB attractiveness against wild *Aedes aegypti*.xlsx
- ATSB efficacy in a semi field system.xlsx
- Ivermectin dose response_female.xlsx
- Ivermectin dose response_male.xlsx
- Mosquito survival after exposure to ATSB.xlsx

Data are available under the terms of the [Creative Commons Zero "No rights reserved" data waiver](#) (CC0 1.0 Public domain dedication).

Authors' contributions

FCT, FOO and MFM designed the experiments. FCT, RKM, ABM and JJM conducted experiments. FCT and MFM analysed data. FCT, JKS and MFM wrote the paper and all other authors reviewed and contributed to the final draft. All authors read and approved the final manuscript draft.

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References

- Ali A, Xue RD, Barnard DR: **Effects of Sublethal Exposure to Boric Acid Sugar Bait on Adult Survival, Host-Seeking, Bloodfeeding Behavior, and Reproduction of *Stegomyia albopicta*.** *J Am Mosq Control Assoc.* 2006; **22**(3): 464–468.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Barrett ADT, Higgs S: **Yellow Fever: A Disease that Has Yet to be Conquered.** *Ann Rev Entomol.* 2007; **52**: 209–229.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Beier JC, Müller GC, Gu W, *et al.*: **Attractive toxic sugar bait (ATSB) methods decimate populations of *Anopheles* malaria vectors in arid environments regardless of the local availability of favoured sugar-source blossoms.** *Malar J.* 2012; **11**: 31.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Bhatt S, Gething PW, Brady OJ, *et al.*: **The global distribution and burden of dengue.** *Nature.* 2013; **496**(7446): 504–507.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Chaccour C, Lines J, Whitty CJM: **Effect of Ivermectin on *Anopheles gambiae* Mosquitoes Fed on Humans: The Potential of Oral Insecticides in Malaria Control.** *J Infect Dis.* 2010; **202**(1): 113–116.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Cilek JE, Hallmon CF, Johnson R: **Semi-Field Comparison of the Bg Lure, Nonanal, and 1-Octen-3-OL to Attract Adult Mosquitoes In Northwestern Florida.** *J Am Mosq Control Assoc.* 2011; **27**(4): 393–397.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Clark JT, Ray A: **Olfactory Mechanisms for Discovery of Odorants to Reduce Insect-Host Contact.** *J Chem Ecol.* 2016; **42**(9): 919–930.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Crump A, Ômura S: **Ivermectin, 'Wonder drug' from Japan: the human use perspective.** *Proc Jpn Acad Ser B Phys Biol Sci.* 2011; **87**(2): 13–28.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Dekker T, Cardé RT: **Moment-to-moment flight manoeuvres of the female yellow fever mosquito (*Aedes aegypti* L.) in response to plumes of carbon dioxide and human skin odour.** *J Exp Biol. The Company of Biologists,* 2011; **214**(Pt 20): 3480–94.
[PubMed Abstract](#) | [Publisher Full Text](#)
- FDA Commissioner: **First FDA-approved vaccine for the prevention of dengue disease in endemic regions.** FDA, 2019; (accessed 6.12.19).
[Reference Source](#)
- Ferguson HM, Ng'habi KR, Walder T, *et al.*: **Establishment of a large semi-field system for experimental study of African malaria vector ecology and control in Tanzania.** *Malar J.* 2008; **7**: 158.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Focks DA, McLaughlin RE, Linda SB: **Effects of ivermectin (MK-933) on the reproductive rate of *Aedes aegypti* (Diptera: Culicidae).** *J Med Entomol.* 1991; **28**(4): 501–505.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Foster WA: **Mosquito sugar feeding and reproductive energetics.** *Annu Rev Entomol.* 1995; **40**: 443–474.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Fox LM: **Ivermectin: uses and impact 20 years on.** *Curr Opin Infect Dis.* 2006; **19**(6): 588–93.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Foy BD, Kobylinski KC, da Silva IM, *et al.*: **Endectocides for malaria control.** *Trends Parasitol.* 2011; **27**(10): 423–428.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Gómez-Dantés H, Willoquet JR: **Dengue in the Americas: challenges for prevention and control.** *Cad Saude Publica.* 2009; **25** Suppl 1: S19–S31.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Gubler DJ: **Dengue, Urbanization and Globalization: The Unholy Trinity of the 21(st) Century.** *Trop Med Health.* 2011; **39**(4 Suppl): 3–11.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Guzman MG, Harris E: **Dengue.** *Lancet.* 2015; **385**(9966): 453–465.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Hawley WA: **Efficacy of attractive toxic sugar baits (ATSB) against *Aedes albopictus* with garlic oil encapsulated in beta-cyclodextrin as the active ingredient.** *J Am Mosq Control Assoc Suppl.* 1988; **1**: 1–39.
- Hemingway J, Ranson H: **Chemical control of vectors and mechanisms of resistance.** *Biology of disease vectors.* 2005; **2**.
- Irish SR, Chandre F, N'Guessan R: **Comparison of octenol- and BG Lure-Baited biogents sentinel traps and an encephalitis virus surveillance trap in Portland, OR.** *J Am Mosq Control Assoc.* 2008; **24**(3): 393–397.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Junnla A, Revay EE, Müller GC, *et al.*: **Efficacy of attractive toxic sugar baits (ATSB) against *Aedes albopictus* with garlic oil encapsulated in beta-cyclodextrin as the active ingredient.** *Acta Trop.* 2015; **152**: 195–200.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Kyle JL, Harris E: **Global Spread and Persistence of Dengue.** *Annu Rev Microbiol.* 2008; **62**: 71–92.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Lima EP, Paiva MHS, de Araújo AP, *et al.*: **Insecticide resistance in *Aedes aegypti* populations from Ceará, Brazil.** *Parasit Vectors.* 2011; **4**: 5.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Mastrangelo E, Pezzullo M, De Burghgraef T, *et al.*: **Ivermectin is a potent inhibitor of flavivirus replication specifically targeting NS3 helicase activity: new prospects for an old drug.** *J Antimicrob Chemother.* 2012; **67**(8): 1884–1894.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Meeraus WH, Armistead JS, Arias JR: **Field Comparison of Novel and Gold Standard Traps for Collecting *Aedes albopictus* in Northern Virginia.** *J Am Mosq Control Assoc.* 2008; **24**(2): 244–248.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Messina JP, Brady OJ, Golding N, *et al.*: **The current and future global distribution and population at risk of dengue.** *Nat Microbiol.* 2019; **4**(9): 1508–1515.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Müller GC, Beier JC, Traore SF, *et al.*: **Successful field trial of attractive toxic sugar bait (ATSB) methods in Mali advances the search for viable new tools to locally control and eliminate malaria in Africa.** *Malaria J.* 2010a; **9**: 210.
- Müller GC, Beier JC, Traore SF, *et al.*: **Successful field trial of attractive toxic sugar bait (ATSB) plant-spraying methods against malaria vectors in the *Anopheles gambiae* complex in Mali, West Africa.** *Malar J.* 2010b; **9**: 210.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Müller GC, Kravchenko VD, Schlein Y: **Decline Of *Anopheles sergentii* and *Aedes caspius* Populations Following Presentation Of Attractive Toxic (Spinosad) Sugar Bait Stations In An Oasis.** *J Am Mosq Control Assoc.* 2008; **24**(1): 147–149.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Naranjo DP, Qualls WA, Müller GC, *et al.*: **Evaluation of boric acid sugar baits against *Aedes albopictus* (Diptera: Culicidae) in tropical environments.** *Parasitol Res.* 2013; **112**(4): 1583–1587.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Ponlawat A, Scott JG, Harrington LC: **Insecticide susceptibility of *Aedes aegypti* and *Aedes albopictus* across Thailand.** *J Med Entomol.* 2005; **42**(5): 821–825.
[PubMed Abstract](#)
- Qualls WA, Müller GC, Khallaayoune K, *et al.*: **Control of sand flies with attractive toxic sugar baits (ATSB) and potential impact on non-target organisms in Morocco.** *Parasit Vectors.* 2015a; **8**: 87.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Qualls WA, Müller GC, Traore SF, *et al.*: **Indoor use of attractive toxic sugar bait (ATSB) to effectively control malaria vectors in Mali, West Africa.** *Malar J.* 2015b; **14**: 301.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Ranson H, Burhani J, Lumjuan N, *et al.*: **Insecticide resistance in dengue vectors.** *TropIKA.net.* 2010; **1**(1).
[Reference Source](#)

Rodríguez MM, Bisset JA, Fernández D: **Levels of insecticide resistance and resistance mechanisms in *Aedes aegypti* from some Latin American countries.** *J Am Mosq Control Assoc.* 2007; **23**(4): 420–429.
[PubMed Abstract](#) | [Publisher Full Text](#)

Saghafipour A, Vatandoost H, Zahraei-Ramazani AR, *et al.*: **Control of zoonotic cutaneous leishmaniasis vector, *Phlebotomus papatasi*, using attractive toxic sugar baits (ATSB).** *PLoS One.* 2017; **12**(4): e0173558.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Sheriff JC, Kotze AC, Sangster NC, *et al.*: **Effect of ivermectin on feeding by *Haemonchus contortus* in vivo.** *Vet Parasitol.* 2005; **128**(3–4): 341–346.
[PubMed Abstract](#) | [Publisher Full Text](#)

Sridhar S, Luedtke A, Langevin E, *et al.*: **Effect of Dengue Serostatus on Dengue Vaccine Safety and Efficacy.** *N Engl J Med.* 2018; **379**(4): 327–340.
[PubMed Abstract](#) | [Publisher Full Text](#)

Sylla M, Kobylinski KC, Gray M, *et al.*: **Mass drug administration of ivermectin in south-eastern Senegal reduces the survivorship of wild-caught, blood fed malaria vectors.** *Malar J.* 2010; **9**: 365.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Tenywa FC, Kambagha A, Saddler A, *et al.*: **The development of an ivermectin-based attractive toxic sugar bait (ATSB) to target *Anopheles arabiensis*.** *Malar J.* 2017; **16**(1): 338.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Tesh RB, Guzman H: **Mortality and Infertility in Adult Mosquitoes After the Ingestion of Blood Containing Ivermectin.** *Am J Trop Med Hyg.* 1990; **43**(3): 229–233.
[PubMed Abstract](#) | [Publisher Full Text](#)

Vairo F, Mboera LEG, De Nardo P, *et al.*: **Clinical, Virologic, and Epidemiologic Characteristics of Dengue Outbreak, Dar es Salaam, Tanzania, 2014.** *Emerg Infect Dis.* 2016; **22**(5): 895–899.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Vontas J, Kioulos E, Pavlidi N, *et al.*: **Insecticide resistance in the major dengue vectors *Aedes albopictus* and *Aedes aegypti*.** *Pestic Biochem Physiol.* 2012; **104**(2): 126–131.
[Publisher Full Text](#)

Wagstaff KM, Sivakumaran H, Heaton SM, *et al.*: **Ivermectin is a specific inhibitor of importin α/β -mediated nuclear import able to inhibit**

replication of HIV-1 and dengue virus. *Biochem J.* 2012; **443**(3): 851–856.

[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Ward T, Samuel M, Maoz D, *et al.*: **Dengue data and surveillance in Tanzania: a systematic literature review.** *Trop Med Int Health.* 2017; **22**(8): 960–970.

[PubMed Abstract](#) | [Publisher Full Text](#)

WHO: **Dengue and severe dengue.** World Health Organisation, Geneva, Switzerland. 2020a; (accessed 8.11.21).

World Health Organization: **Dengue: Guidelines for Diagnosis, Treatment, Prevention and Control.** World Health Organization. 2009.

[PubMed Abstract](#)

World Health Organization: **Global strategy for dengue prevention and control 2012–2020.** 2012.

[Reference Source](#)

World Health Organization, Special Program For Research And Training In Tropical Diseases, TDR For Research On Diseases Of Poverty: **Dengue: guidelines for diagnosis, treatment, prevention and control.** World Health Organization. 2009.

[Reference Source](#)

Wilder-Smith A, Hombach J, Ferguson N, *et al.*: **Deliberations of the Strategic Advisory Group of Experts on Immunization on the use of CYD-TDV dengue vaccine.** *Lancet Infect Dis.* 2019; **19**(1): e31–e38.

[PubMed Abstract](#) | [Publisher Full Text](#)

Xu TL, Han Y, Liu W, *et al.*: **Antivirus effectiveness of ivermectin on dengue virus type 2 in *Aedes albopictus*.** *PLoS Negl Trop Dis.* 2018; **12**(11): e0006934.

[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Xue RD, Ali A, Kline DL, *et al.*: **Field evaluation of boric acid- and fipronil-based bait stations against adult mosquitoes.** *J Am Mosq Control Assoc.* 2008; **24**(3): 415–418.

[PubMed Abstract](#) | [Publisher Full Text](#)

Xue RD, Barnard DR: **Boric acid bait kills adult mosquitoes (Diptera: Culicidae).** *J Econ Entomol.* 2003; **96**(5): 1559–1562.

[PubMed Abstract](#) | [Publisher Full Text](#)

Xue RD, Kline DL, Ali A, *et al.*: **Application of Boric Acid Baits to Plant Foliage for Adult Mosquito Control.** *J Am Mosq Control Assoc.* 2006; **22**(3): 497–500.

[PubMed Abstract](#) | [Publisher Full Text](#)

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The abstract of this study by Tenywa *et al* states that this study 'intended to develop and test an ivermectin-based attractive-targeted sugar bait (ATSB) against *Aedes aegypti*, yet this does not reflect the results presented and it is important that the authors correct this. The results of the laboratory study demonstrate the killing effect of ivermectin fed in a sugar meal to male and female *Ae. aegypti*. The semi-field study suggests, despite issues with the experimental design which I discuss below, that *Ae. aegypti* released into a biodome in the absence (presumably, it is not mentioned) of competing sugar sources appeared to feed on sugar plus ivermectin in a prototype feeding station. The presence of devices with ivermectin over a 10-day period reduced the number (or percentage) of mosquitoes caught in daily collections relative to other time periods and the control compartment. Finally, the field experiment showed that free flying mosquitoes attempted to feed on stations inside garages and were caught on the sticky mesh lid of the device, and that a greater number of mosquitoes attempted to feed when a CO₂ source was attached to the stations. Thus, the study demonstrates toxicity of ivermectin and the attractancy of CO₂, using a prototype bait station which still has some serious development needs. Firstly, the stations seem to need to be replaced every 5 days, which is likely not feasible in full scale deployment of an intervention. There is also a safety issue and an environmental issue to non-target organisms to consider, with open containers of sugar water with a mesh grill kept at ground level with no means that I can see to protect children or other organisms from exposure.

Whilst this is an early proof of principle that ivermectin could be used in an ATSB deployment strategy, there is very little evidence of efficacy of this prototype and certainly it does not represent a final product which could be deployed. The results of the laboratory and semi-field experiments are confounded in the Conclusion, since a 95% reduction in population was certainly not demonstrated in the semi-field environment, and the comment that the stations failed to reduce populations in the field experiment is false since the experimental design was not set up to show efficacy. It is essential that the work presented is framed correctly, to correctly reflect the

results of the studies, and not overclaim the significance of the results – as it stands the conclusion is that an ATSB using ivermectin can only work with a CO₂ source, which is a claim potentially damaging to innovation in the development of ivermectin-based ATSBs. Critically, this study shows that CO₂ attracts mosquitoes to the stations, but not that it encourages feeding – stations may therefore mostly target blood feeding females who may not sugar feed, and without being confident that all of those females that are attracted feed and die you are potentially increasing the biting risk to people living nearby these stations. In reality, no attractant was included in the bait stations, and so it is inaccurate to label them ATSBs or expect them to compete with natural sugar sources and be effective in population control. On the subject of terminology, the Introduction refers to 'Attractive Targeted Sugar Baits', a name trademarked by Westham Co for their device. The generic term is 'Attractive Toxic Sugar Baits' which is more relevant here since there is nothing about the device that targets them to mosquitoes specifically.

Beyond the framing and accurate representation of the results, which I strongly believe must be corrected, I think there are issues with the experimental design of the semi-field and field experiments, as well as the analysis and visualisation of data which should also be addressed.

The major issue with the semi-field experiment is that the recapturing regime is problematic – 100 mosquitoes are released into the compartments at 6am, and at 6:30am a Bioagent trap is added to carry out collections and in phase 2 this included an additional HLC between 6am and 6:20am. There seems to be to be a high likelihood that a proportion of the captured mosquitoes would be the same ones which had just been released. It is thus confusing that 100% mortality was measured in the mosquitoes recaptured from the compartment with the toxic baits – unless the captured mosquitoes were screened for sugar feeding before mortality was scored, which should be mentioned in the Methods if so. In fact, a food dye was included in the experiments according to the Methods, but no data is presented on feeding rate for any experiment in the Results. Since there is no clear out of mosquitoes each day, with 50-60% of mosquitoes recaptured according to Figure 4 (if the Y axis does represent %, but in any case 100 mosquitoes were released every day), there would be 40-50% of mosquitoes remaining in the compartments from one day to the next, and so the populations would be expected to keep growing, unless there was 40-50% mortality in the compartments each day, which does not seem to match the data in Figure 4, or unless there was perfect recapture of all mosquitoes each morning which should be stated in the Methods if so.

I also have concerns about the field experiment, which is essentially a demonstration of the bait stations as a sticky trap, with and without an attractant (CO₂), and not a test of efficacy of the stations as seems to be claimed. There is no way to know which of the mosquitoes that were trapped would have fed on the sugar, nor any attempt to look at the effect on the population, particularly since the treatments were regularly swapped during the experiment. Given that, it's a minor point, but with the large number and high density of stations in small sites, the risk of trapping out needs to be addressed – there isn't a clear trend for reduced catches in the meta data but in many cases a large catch was followed by a lower catch the following day, particularly in garage 2. A major issue with this experiment is the inclusion of water controls and the way the data from them is handled. The captures in the water only controls were surely affected by the presence or absence of CO₂ in the nearby stations, since CO₂ will act as an attractant over an area of a few meters. Yet in the analysis the data from all water controls from both sites seems to have been combined, without accounting for the double trapping effort this constitutes. Doing some calculations from the meta data, when only compared against water on days when a competitor

station was tested then ATSB alone is significantly more attractive than water, though still not as attractive as an ATSB with CO₂. Thus it is not accurate to show, as in Table 2, that the bait without CO₂ attracted as many mosquitoes as water alone.

In several places the meta data does not match the results or methods presented in the paper – for example the text states that in the field experiment stations were swapped every 24 hours, but it seems from the meta data this was done every 3 days after day 1. The text states that the field experiment was conducted between November and December 2019, but the meta data suggests December 26th to January 6th. This needs to be carefully checked and misrepresentations corrected.

There has been a mistake made in the plotting of Figure 5, which does not match the text – the graph suggests only ~15% died in the ATSB compartment and 100% in the ASB compartment, whereas the text states 100% and 7.5% mortality, respectively. There is also an issue with the meta data presented for this experiment, where the sum values are incorrect in the summaries of the replicates. The numbers reported alive and dead in each replicate differs from the total numbers reported in the summary, so that the total number dead exceeds the number alive at the previous time point.

More minor issues to be resolved:

Methods:

- More information is needed about the mosquito colony used for the lab and semi-field experiments – origin, rearing details, quality control measures, insecticide resistance phenotype, any known resistance markers.
- In the first lab experiment and the semi-field experiment it is not clear whether male, female or both sexes were used.
- Why was a different range of concentrations used in the first and second lab experiments?
- It is not clear whether the stations tested in semi-field experiments included the sticky mesh lid, which is important to interpretation of the results and should be clarified.
- It would be interesting to compare the density of bait stations in the semi field experiment (5 stations in a 19x29m enclosure = 1 per 0.01 ha) and field experiment with the densities tested in published field trials of other ATSB products.
- Was there any vegetation or other natural sugar sources around the garages in the field experiment?
- In the field experiments, why were the treatment stations 10m apart, but the control stations at a distance of 3m?
- A lot more detail is needed in the data analysis section, and there are a few questions, for example, since compartment is a proxy for treatment in the semi-field experiment, why is it included as a fixed effect? Why is mosquito age included as a covariate when the same age of mosquito were used for all experiments?

Results:

- Figure 3 – without the inclusion of the 0% ivermectin data points it is not clear that there is a dose response in several of the groups. It is confusing the present the data by time point, and I would rather see time along the X axis and data plotted by concentration. As it is presented, the legend would be clearer if it was inverted to match the order of the lines on the graph.
- 'ATSB efficacy against *Ae. aegypti* in a semi field environment' – What do 35% and 56% recaptures represent, what's the denominator?
- Figure 4 – what does the Y axis show, % or #, mean or total, recaptures just from traps or using HLC as well?
- Figure 5 – how can you be sure the exposure is 24 hours, when there are daily releases and daily recaptures, but no way of knowing how long those you recapture have been in the compartment? Unless there was perfect recapture of all mosquitoes each morning which should be stated in the methods if so. I suggest being consistent in terminology to avoid confusion – ASB is used in the figure and control-ATSB in the text. What does the dotted line at 0.5 represent?

Discussion:

- I am not sure that the data from lab experiments supports the first sentence regarding males and females being equally sensitive to ivermectin, since more males than females were killed within 24 hours at the lower concentrations.
- The dose response shown in Figure 3 is also not clear, and so as shown does not seem to support the second sentence.
- How do the volumes of blood ingested by a female compare to the volumes of sugar ingested by males or females? It would be interesting to factor this in when comparing results from endectocide studies and this study with sugar.
- 'This study demonstrated that ATSB deployed inside a semi-field system remarkably reduced *Ae. aegypti* populations...' – I am not sure the data support such a strong claim. Certainly there was a reduction in recaptured mosquitoes in Phase 2, but without knowing whether a stable population had been established in the biodome which was then reduced by the deployment of stations the exact effect is not clear.
- 'The difference in survival time of the mosquitoes from the two groups suggests that the majority of the mosquitoes were attracted by the ATSBs and fed on it.' – to justify this claim we would need to understand the method more clearly, for example were the recaptured mosquitoes scored for sugar feeding and mortality only measured in those that had fed? What numbers of fed v unfed mosquitoes were captured?
- 'In the field, the ivermectin-based sugar bait stations were inefficient at attracting wild *Ae. aegypti*.' – as discussed above, if the analysis was repeated with the water only controls separated by treatment there is some evidence of attractancy, albeit less than with the inclusion of CO₂. Also as discussed above, there was no attractant included in the stations so I am not sure an attractive effect would have been expected. In this paragraph it would be useful to cite the recent paper about competition between ATSBs and natural sugar

sources by Muyaga *et al.*, (2023)¹.

- 'mosquito may take a longer time to allocate the solution' – this is not clear, do the authors mean to say 'locate the station'?

Conclusion:

- Discussed above, this conclusion is not very clear as written, and I don't believe it accurately represents the results from the study performed or their implications.

References

1. Muyaga LL, Meza FC, Kahamba NF, Njalambaha RM, et al.: Effects of vegetation densities on the performance of attractive targeted sugar baits (ATSBs) for malaria vector control: a semi-field study. *Malar J.* 2023; **22** (1): 190 [PubMed Abstract](#) | [Publisher Full Text](#)

Is the work clearly and accurately presented and does it cite the current literature?

No

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

No

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Rosemary Lees is a medical entomologist, with expertise in development and evaluation of vector control tools and mosquito control interventions, and development and validation of bioassay methodologies for the evaluation of insecticide-based products and for monitoring insecticide resistance. Daniel McDermott is a medical entomologist and statistician with an expertise in study design and evaluation of vector control interventions in semi field and community based trials.

We confirm that we have read this submission and believe that we have an appropriate level of expertise to state that we do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Reviewer Report 03 July 2023

<https://doi.org/10.21956/wellcomeopenres.19287.r58211>

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Fernando Ariel Genta 

Laboratory of Insect Physiology and Biochemistry, Oswaldo Cruz Institute - Oswaldo Cruz Foundation (IOC-FIOCRUZ), Rio de Janeiro, Brazil

The manuscript by Tenywa *et al.* brings a straightforward description of the application of ivermectin-based ATSB against *Aedes aegypti* mosquitoes. It is well-designed, and the results are very interesting, pointing out this strategy's potential and limitations. I have only very minor comments or requests.

Introduction:

- "...as well as other sugar-seeking vectors such as sand flies..." - Please include the new references on ATSB against sand flies provided, Ferreira *et al.* 2018¹ and 2022²).
- "...a parameter that highly influences disease transmission" - Missing final dot.
- "...fitness and virus replication in the mosquitoes." - Remove "the".

Methods:

- "...they were fed cow blood through a membrane." - Please provide which anticoagulant was used and membrane.
- "... 100 *Ae. aegypti* mosquitoes aged 2 – 5 days, blood naïve and starved for 6–8 hours were released into each compartment" - Females/males only? Or mixed? Please clarify.

Results.

- "Sugar solution containing ivermectin 0.03% (w/v) caused approximately 80% of mosquito mortality for both mosquito sexes within 24 hours and > 90% within 48 hours (Figure 3)." - Data in Figure 3A does not fully support this sentence, the female 24h mortality is around 60% not 80%.
- Figure 3. Mean cumulative proportional mortality of female (A) and male (B) *Ae. aegypti* post feeding on 10% sugar solution that contained different ivermectin concentrations. - Please describe what is the meaning of the curves in the graph, in the figure legend. I see poor fitting in the 24 and 48h data. Is it possible to calculate a LD50 or LD90 using a probit-logit fitting tools?
- Figure 5. Effect of attractive targeted sugar bait on survival of female *Ae. aegypti* exposed to the baits in a semi-field system for 24 hours. - Please give the number of mosquitoes used for each curve. These are females or males?

References

1. Ferreira TN, Brazil RP, McDowell MA, Cunha-Júnior EF, et al.: Effects of anti-Leishmania compounds in the behavior of the sand fly vector *Lutzomyia longipalpis*. *Pest Manag Sci.* 2022; **78**

(7): 2792-2805 [PubMed Abstract](#) | [Publisher Full Text](#)

2. Ferreira TN, Pita-Pereira D, Costa SG, Brazil RP, et al.: Transmission blocking sugar baits for the control of Leishmania development inside sand flies using environmentally friendly beta-glycosides and their aglycones. *Parasit Vectors*. 2018; **11** (1): 614 [PubMed Abstract](#) | [Publisher Full Text](#)

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Insect Biochemistry and Physiology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 15 June 2023

<https://doi.org/10.21956/wellcomeopenres.19287.r58210>

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Tainá Neves Ferreira

Laboratório de Biologia de Insetos (LABI), Universidade Federal Fluminense, Niterói, State of Rio de Janeiro, Brazil

Abstract

- "Ae. aegypti were released into each cage and observed for mortality after 4, 8, 24, and 48 hours." - Why do you check only until 48 hours? Please explain better why not evaluate more

time.

Methods

Mosquitoes

- *"The mosquitoes were reared at 27±5°C and 40%-99% humidity at Ifakara Health Institute insectary in Bagamoyo and Ifakara, Tanzania"* - Very high variation of temperature and humidity. Was this range just for lab tests or were they combined with data from semi-field tests as well? Try to describe it in more detail.
- *"Similar experimental setup and procedures as described above were performed for males (...)"* - Was the procedure described done with females? Please clarify in the text, as in other insects (kissing bugs, for example) the males also feed on blood.

Semi field experiment

- *"To maintain the quality of the control-ATSB and ATSB, both were replaced with a new one after every five days."* - Did you do any previous tests evaluating the stability of ivermectin mixed with sugar? How did you establish the exchange every 5 days? It is important to describe these data to ensure that the ivermectin is not being degraded or that there is loss through evaporation, since the temperature can reach 32°C and the humidity is below 50%.

Field experiment

- *"Preparation of attractive targeted sugar bait (ATSB) with and without a carbon dioxide source. Three test bait stations were prepared as follows: 1) control bait consisted of plain water dyed with 0.5% v/v red food coloring dye; 2) test-ATSB consisted of 10% w/v of sugar solution and ivermectin (final concentration 0.03% w/v);"* - Why was there no dye in the other baits? How to know if the mosquitoes were feeding on the test baits if the solution is colorless? Have you thought of using another dye in a different color?

Data analysis

- Explain a little more how these analyzes were carried out. It is not clear how the analyzes were done for users of other software.

Discussion

- *"The difference in survival time of the mosquitoes from the two groups suggests that most of the mosquitoes were attracted by the ATSBs and fed on them."* - With the use of another dye, you would be sure of the food.
- *"The vegetation spraying approach involves spraying the whole vegetation with a toxic sugar solution, whereas the bait station approach presents the toxic sugar bait at one single point. In this context, the bait station approach may hypothetically be regarded as less effective in attracting mosquitoes because mosquitoes may take a longer time to allocate the solution."* - This part could be enhanced with the works made by Günter C Müller and Yosef Schlein *et al.* There seems to be a missing reference in this paragraph.
- Did you manage to do any preference tests, even in the laboratory, to assess the preference for control bait (ASB) and toxic bait (ATSB) in the same cage or room? It would be important data to assess the preference of mosquitoes for the bait compared to other sources of natural sugars, especially in the absence of CO₂.

Conclusions

- *"We recommend further research to improve bait station design and attractant strategies such as plant-based volatiles."* - In addition, to plant volatile compounds, you could think of other more specific attractant compounds for mosquitoes or even for *Aedes aegypti* as

pheromones. This would reduce limitations with non-target species.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Entomology, vector borne, diseases, parasite-vector interaction (invertebrate), vector control, parasitology (trypanosomatids).

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 20 January 2022

<https://doi.org/10.21956/wellcomeopenres.19287.r47841>

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Gaurav Kumar

ICMR-National Institute of Malaria Research, New Delhi, India

The manuscript evaluates ivermectin based sugar baits both under laboratory and field settings. But before consideration of manuscript for indexing, some clarifications are required from authors.

- In semi field conditions, approximately 35% and 56.8% mosquitoes were recaptured in compartment with ATSB and control-ATSB respectively. Which shows less attractiveness of ivermectin based sugar bait. Did authors test for the repellent effect of ivermectin, if any?

- After the introduction of Ivermectin-based sugar bait, there was merely a 38% decline in *Aedes* recapture rate. Authors should discuss it in comparison to other ATSBs with different toxicants like boric acid. The reduction rate does not seem as exciting as under natural field conditions, it may be lowered.
- Authors used term ATSB in which T stands for targeted while the common full form of ATSB is somewhat different. Authors should explain why they have done so.
- Moreover, no attractant was used in lab and field conditions therefore it should be toxic sugar baits (TSB) rather than ATSB.
- Authors should also explain the effect of Ivermectin sugar bait on male and female mosquitoes under field settings. As Males may be targeted more by the baits.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Vector biology and control

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.
