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# Comparative attractiveness of $CO_2$-baited CDC light traps and animal baits to *Phlebotomus duboscqi* sandflies

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## Abstract

*Background & objectives:* In order to understand sandfly bionomics, vector species identification, and to develop methods for sandfly control, there is a need to sample sandflies in any particular habitat. This survey was aimed at determining the best method of sampling *Phlebotomus (Phlebotomus) duboscqi* (Diptera: Psychodidae) in the field.

*Methods:* Different animal baits and $CO_2$-baited CDC light traps were used to attract sandflies released in an insect-proof screen-house located in the sandfly's natural habitat in Marigat, Baringo district of Kenya.

*Results*: Attraction of hungry *P. duboscqi* female sandflies by the goat (*Capra hircis*) was significantly higher than that of hamster (*Mesocricetus auretus*), Nile grass rat (*Arvicanthis niloticus*), gerbil (*Tatera robusta*) and chicken (*Gallus domestica)*. However, two rodent species, *A. niloticus* and *T. robusta* did not differ significantly. A linear regression analysis of weights of animal baits and number of sandflies attracted revealed an insignificant result. The fluorescent dyes used to distinguish sandflies of different day experiments seemed not to influence the sandfly numbers in relation to the studied sandfly behaviour.

*Interpretation & conclusion:* The similar attraction pattern of *P. duboscqi* in semi-field environment by $CO_2$-baited CDC light trap and the goat provides hope for solution to the problem of fast dissipating dry ice ($CO_2$ source) in the field. Goats can, therefore, also be utilized as deflectors of vectors of cutaneous leishmaniasis from humans in zooprophylaxis in *Leishmania major* endemic areas where the sandfly is found.

**Key words** Animal baits – $CO_2$-baited light traps – *Phlebotomus duboscqi*

## Introduction

Phlebotomine sandflies are vectors of leishmaniasis as well as other zoonotic and viral diseases. Female sandflies are also a biting nuisance even when they are not active vectors[1]. In Kenya, visceral leishmaniasis, caused by *Leishmania donovani* Laveran & Mesnil is transmitted by *Phlebotomus (Synphlebotomus) martini* Parrot. Cutaneous leishmaniasis is caused by *Leishmania major* Yakimoff & Schokhor, *L. tropica* Wright, and *L. aethiopica* Bray, Ashford and Bray. *L. major* is transmitted by *P. duboscqi* Neveu-Lemaire[2], one of the 16 sandfly species indigenous to Baringo district[3].

In order to understand sandfly bionomics, vector species identification, and to develop methods for sandfly control, there is need to sample sandflies in their habitat. Several sampling methods have been developed for this purpose. The most commonly used techniques for measuring sandfly abundance are CDC light traps and sticky traps[4,5]. While some traps are used either baited or unbaited, others are always used with baits. Odours from different sources, carbon dioxide and animals are common baits. For instance, sandfly catches can be increased by addition of a source of carbon dioxide such as pieces of dry ice[6] and traps baited with dry ice collect significantly more species and more mean totals than traps baited with carbon dioxide sachets[7].

*Phlebotomus duboscqi* breeds and rests mainly in animal burrows and to a lesser extent in tree holes and termite mounds[8,9]. The animal burrows are occupied by *Arvicanthis niloticus*, *Aethomys kaiseri*, *Tatera robusta* and *Taterillus emini*[10]. It is known to feed on rodents, ruminants, humans and carnivores[11]. It was earlier reported that *P. duboscqi* females are also attracted to chickens and mongoose (*Helogale* spp)[12]. However, baits were positioned strategically at known sandfly habitats.

In this study, attractiveness of various animal baits in sticky traps and dry ice baited CDC light traps toward *P. duboscqi* laboratory reared female sandflies was investigated within a greenhouse with insect proof net walls and roof.

## Material & Methods

*The greenhouse design:* The model of greenhouse constructed was SAPPHIRE 960–4.25 m gutter height (Azrom Greenhouses, Israel), measuring

*Plate 1.* The greenhouse design

20 x 14.60 x 4 m. It had a cover roof and gable ending in insect proof net (225 x 770 μ) buried into the earth, 3 x 4 m entrance in the middle of one of the two longer sides with two swing doors (one to the entrance room and the other for the access to the greenhouse). The whole structure was supported by metallic posts and arches (Plate 1). On the outside perimeter of the greenhouse, shallow cement-corrugated trench was constructed and filled with water always to prevent ants from getting into the enclosure. A weather station (HOBO, Onset Computer Corporation, USA) set up nearby was used to regularly record outside temperature, wind speed and relative humidity. A data logger (HOBO, Onset Computer Corporation, USA), fitted at the centre on a metallic rod support at 1.5 m height was used to record temperature and relative humidity inside the greenhouse.

On completion of the construction, standard CDC traps were set inside for two consecutive nights to trap adult sandflies that may be therein. Standard CDC traps were again set inside for two nights to ascertain sandfly absence in the experimental area before beginning experiments.

*Experimental animals:* Hamsters were obtained from Kenya Medical Research Institute's (KEMRI) animal house. A young goat and chicken were bought from Marigat, Baringo district, the site where experiments were carried out. Two rodent species, *T. robusta* and *A. niloticus* were trapped from their natural habitats in Marigat. Approval for use of animals was granted by the Animal Care and Use Committee (ACUC) of KEMRI.

*Trapping of rodents:* Animal trapping was done using wire cage traps measuring 15.2 x 17.7 x 28 cm. Traps baited with maize flour meal mixed with peanut butter were left on site at night and during the day. They were set near rodent runs and burrow openings, inside and outside homesteads, along fences and around termite mounds. Traps were checked for catches in the morning at 0700 hrs and in the evening at 1800 hrs[10].

*Sandfly trapping, identification and colony establishment:* Sandflies for boosting the sandfly insectary established in Marigat were trapped within the Kenya Agricultural Research Institute (KARI) compound and Perkerra irrigation scheme using CDC light traps. Captured flies were aspirated into Perspex cage from the collection bags by the help of a mouth aspirator. Here, sandflies of the same species mated and were fed on blood from hamsters. Five days after feeding, these flies had oviposited. Those were then killed by freezing if alive and all (both dead and alive) kept at 4$^{o}$C in vials similarly labeled as chambers containing the laid eggs until processing. Heads of samples were excised and mounted using gum chloral on slides upside-down so as to expose the cibarium and pharynx. Slides were covered with cover slips and allowed to dry on the bench for 1–2 days. Species identification was performed thereafter by observing the cibarial armatures, spermatheca and the pharynx using identification keys[13]. Species names were matched with number on the individual oviposition chambers containing the eggs. From here on larvae hatching from eggs were of known identity. Meanwhile, larvae which hatched in 10 days after blood-feeding were regularly fed on larval food (mixture of rabbit chow and rabbit droppings) until they emerged to adults.

*Evaluation of attractiveness of $CO_2$-baited CDC light traps and animal baits:* These experiments were conducted in May 2008. A Latin square design of order six was set up in the greenhouse consisting of six baits. Different animal baits used in the experiments comprised of two common domestic animals (a goat and a chicken), laboratory reared rodent (Syrian golden hamster) and two rodents captured in the field (grass-rat and gerbil). These two rodents are among small mammals from which *L. major* has been isolated[10]. A five month old goat was selected in order to decrease biases due to large body sizes which reflect the amount of $CO_2$ released[14]. A CDC light trap baited with 1 kg of dry ice granules was included in all the six replicates as a control and this was compared with animal baits.

*Plate 2.* Baits arrangement in the greenhouse

During every experimental night, caged animal baits and a $CO_2$-baited light traps were set, 11 feet from each other in a circular pattern (Plate 2). The cages with animal baits were placed in top-side-open carton boxes lying on their wider lengths and measuring about 1 x 0.5 x 1 m. The boxes were lined on the inside with white paper sticky traps with a flap of white paper sticky trap over the open end (Plate 3). All open ends of boxes were placed such that they faced the centre of the arrangement 10.5 ft away. Plastic sheets were put on the ground in all the areas where the baited cages or light traps were hung. The sheets moved with the bait in subsequent replicates during their rotation in the greenhouse. Net traps hang about two feet from the ground over the baits in a portable sun shelter (1.5 m wide x 2 m long x 2 m tall) consisting of four galvanized aluminium poles covered with a roof made of water proof canvas.

*Plate 3.* Design of carton box (sticky trap) used for housing animal baits

In all, 400 starved, 2–3 days old *P. duboscqi* reared in a nearby Marigat sandfly insectary as has been done previously[15], were released at the centre of the design at 1830 hrs. Traps were inspected the following day at 0630 hrs. CDC trap collection bags were labeled according to collection date. Using a camel hair brush, samples from sticky traps were recovered and counted. Before leaving, animal baits were provided with maize meal for food and water. Sandfly numbers in each of the traps were compared. Different fluorescent dyes were used to mark sandflies used for each experimental day.

Temperature and relative humidity conditions outside and inside the greenhouse during the experimental nights were as follows: temperature outside ranged from 18.71–32.34°C with a mean of 24.01°C, and inside ranged from 15.62–31.52°C with a mean of 21.97°C; and relative humidity outside ranged from 20.5–90% with a mean of 61.41%, and inside ranged from 20.9–98% with a mean of 70.84%. Wind speed and direction outside were 1.23 mph ± 136.32 S.D. respectively. There was no rain during the baits comparison study.

*Data management:* Data were entered in MS Excel sheet and thereafter imported into STATA 9.2, (STATACORP, TX, USA) for analysis. Results of the differences in bait performance were analysed statistically using Kruskal-Wallis test. Comparisons of pairs of given baits were done by Mann Whitny tests. Associations of log transformed sandfly numbers and bait weights were tested by linear regression. Tables of summary descriptive statistics and informative graphical displays were also constructed. Summaries of some data were done using pivot tables.

## Results

Sandfly numbers for all the six baits were compared and were significantly different among the baits ($\chi^2$= 17.788, $p$ = 0.0012). $CO_2$-baited light traps and the goat elicited the highest attraction of hungry sandflies though there was no statistically significant difference between the two baits (Z = 0.08, $p$ = 0.9361) (Fig. 1). *Arvicanthis niloticus* and *T. robusta*, rodents known to be reservoirs of *L. major* transmitted by *P. duboscqi,* did not show any difference in terms of sandfly attraction (Z = 0.484, $p$ = 0.6285). Two domestic animals known to attract *P. duboscqi*, goat and chicken, exhibited significant differences (Z= –2.807, $p$ = 0.0050). There was no difference in sandfly numbers due to change in positions during rotation of various baits in greenhouse ($\chi^2$ = 7.540, $p$ = 0.1819). All the flies were trapped on the sticky surface before they fed and no flies were captured in the net trap.

Linear regression detected an association between the weight of the bait and the log transformed number of sandflies attracted (F = 35.78, $p$ = 0.0000) (Fig. 2). When the bait that was preferred (goat) was left out in regression model, there was no association between the weight of the bait and the number of sandflies attracted (F = 1.44, $p$ = 0.2435) (Fig. 3).

## Discussion

That *P. duboscqi* sandflies blood-feed on ruminants[11] and domestic animals[8] are observations well-supported by the results of the current study. The goat, used among other baits such as hamster, *A. niloticus*, *T. robusta* and chicken, was most preferred. This is, however, contrary to other findings from Baringo district[12] in which chicken was superior to the goat

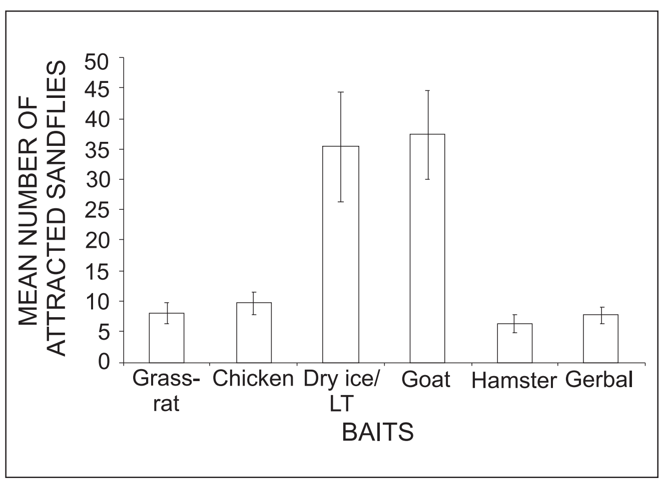


*Fig. 1:* Mean (± SE) number of *P. duboscqi* sandflies attracted to different baits

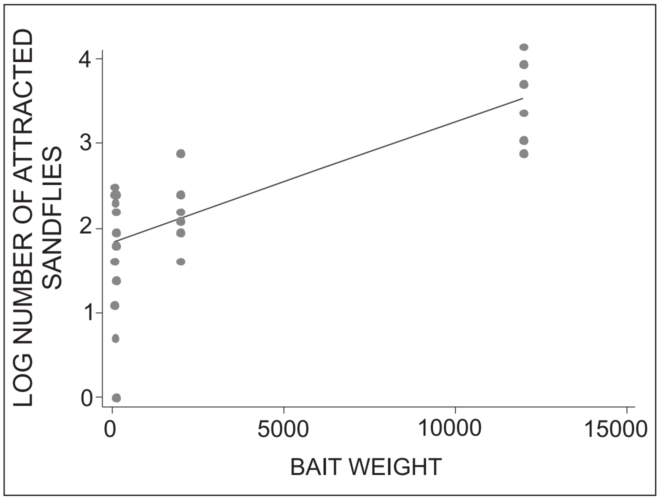


*Fig. 2:* Regression fit: an association between weights of animal baits and log transformed attracted number of sandflies

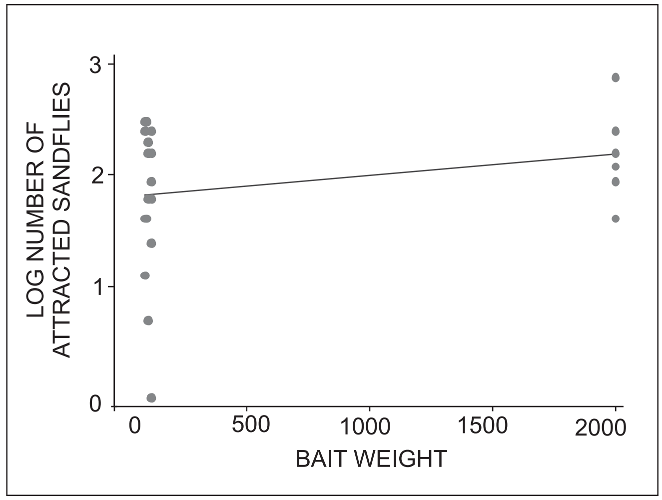


*Fig. 3:* Regression fit: an association between weights of all animal baits without goat and log transformed attracted number of sandflies

in terms of attraction. Host preference in the enclosed greenhouse provided equal chances for attraction of *P. duboscqi* by various baits hence yielding more reliable results. In the former study, baits were stationed in different areas with unknown number of sandflies and conditions in them may not have necessarily been the same. In this study, attraction by the goat was significantly higher than that of chicken and there existed no differences among the six bait positions in which baits were rotated in the greenhouse. The fluorescent dyes used to distinguish sandflies of different day experiments also seemed not to influence any of the studied sandfly behaviour. This is a fact that has been demonstrated in *Lutzomyia* spp sandflies[16].

Two rodent species, *A. niloticus* and *T. robusta*, known to host *L. major*[10] were also less attractive compared to the goat. Sandflies blood-feeding on these rodents could actually be owed to the fact that burrows they live in are also habitats for *P. duboscqi*.

To test the findings that bigger animals elicit more attraction due to higher amount of carbon dioxide emitted[14], a linear regression analysis without the goat but having largely weight varying baits was done. There was no significant difference. The high attraction by the goat could, therefore, be due to other factors that can be detected by sandflies.

## Conclusion

A similar attraction pattern of *P. duboscqi* in semi-field environment by dry ice-baited CDC light traps and the goat provides hope for solution to the problem of fast dissipating $CO_2$ source in the field especially when high atmospheric temperatures are prevailing. Since the traps baited with dry ice significantly attract sandflies[7] are as good a goat bait, a design using a light trap in combination with goat(s) as bait(s) is a feasible venture for vector sampling during disease surveillance and other exercises. Goats can therefore be utilized as deflectors of vectors of cutaneous leishmaniasis from humans in zooprophylaxis as has been suggested before[8]. Repellents, used as means of personal protection can also be used to supplement zooprophylaxis. These control methods could be effective because *P. duboscqi* is purely exophagous.

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