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Comparative Analysis of the Effectiveness of Sand Fly Traps with Different Baits

Mong'are samuel[1], Ng'ang'a Zipporah[1], Ngumbi Philip[2], Johnstone Ingonga[2] and Ngure Peter[3]

1. *Jomo Kenyatta University of Agriculture and Technology, P.O Box 62000, Nairobi, Kenya*
2. *Kenya Medical Research Institute, KEMRI, P.O. Box 43640 – 00100 Nairobi, Kenya*
3. *Technical University of Kenya, P. O Box 52428-00200 Nairobi, Kenya*

Mong'are Samuel, Jomo Kenyatta university of Agriculture and Technology, Zoology Department P.O Box 62000-00200, Nairobi, Kenya

Abstract: *Sand flies are small haematophagous insects that transmit Leishmania parasites. Infection with Leishmania parasites results in diseases known as leishmaniases which can be grouped into three main forms: cutaneous, mucocutaneous and visceral leishmaniases. The CDC light trap is the standard surveillance technique used to determine sand fly densities in a given locality, although this technique has been hampered by several logistic issues. Therefore, this study sought to use host blood, urine and ripe fruits as baits in a modified trap to come up with a cheap device for sand fly surveillance. This study also aimed at identifying the sand fly species attracted to this new device. Defibrinated blood from cattle, goat, sheep and chicken; urine from cattle, sheep and goat and fruits: grapes, mangoes, bananas and apples were placed inside each trap to act as sand fly attractants. A total of 1302 sand flies were collected within 3 trapping nights. Blood baited trap yielded more sand flies (202.33 ± 2.85) while chicken baited trap trapped the least (65.00 ±1.53). Among the fruit baited traps, bananas attracted the highest number of sand flies (94.33 ± 4.63) followed by mango baited trap which caught (89.67 ± 2.33) although the difference between these two was not significant (P=0.682). 64.3% of the collected sand flies belonged to the genus Sergentomyia and 35.7% to Phlebotomus. The collected sand flies belonged to 7 species with P. martini (35.7%), S. schwetzi (34.1%) and S. antennata (15.7%) being the dominant species. Volatiles from plant and animal hosts may be used instead of light or carbon dioxide to improve the efficiency of traps for haematophagous insects. These volatiles can also be used to synergize each other and be incorporated in traps targeting sand flies.*

Key words: *Trap, sand flies, attractant, bait, leishmaniasis, fruit, blood, urine.*

I. Introduction

Leishmaniasis is a major vector-borne disease caused by obligate intramacrophage protozoa of the genus Leishmania infecting numerous mammalian species, including humans [1]. Leishmaniasis is endemic in areas of the tropics, subtropics, and southern Europe. Specifically, it is endemic in 88 countries and is the only tropical vector-borne disease that has been endemic to southern Europe for decades [2]. Currently, leishmaniasis has a wider geographical distribution pattern than understood before and it is considered to be a growing public health issue.

Leishmaniasis has been endemic in Kenya for a long time. The most prevalent forms are cutaneous leishmaniasis, visceral leishmaniasis and post kala-azar dermal leishmaniasis (PKDL) [3]. Visceral leishmaniasis is found predominantly in the arid, low-lying areas of the Rift Valley, Eastern and North Eastern provinces, whereas cutaneous leishmaniasis occurs over a wide range of environmental conditions, from semi-arid lowlands to high plateaus in the Eastern, Rift Valley, Central and Western provinces [4]. Visceral Leishmaniasis is endemic in Baringo, Koibatek, Turkana, West Pokot, Kitui, Meru, Keiyo, Marakwet, Mwingi, Tana River and Machakos districts [5].

Since anti-leishmanial vaccines are still being developed, the current control strategies for leishmaniasis rely on case management. However, case management is difficult to be conducted since it is restricted by factors like lack of access to affordable, active drugs, incorrect prescribing and poor compliance [1]. The best method of interrupting any vector-borne disease is to reduce man-vector contact. Sand fly control programmes in most visceral leishmaniasis foci have advanced slowly when compared to that of other haematophagus arthropods like mosquitoes, ticks and black flies. Measures employed include spraying houses with insecticides where sand flies are endophilic and using treated and untreated bed nets where sand flies are endophagic [6]. Personal protection using repellents and nets is an important aspect. In endemic areas, spraying with dichlorodiphenyltrichloroethane (DDT) and other residual insecticides is effective in sand fly control **[7].**

Insecticides pose adverse effects to the user and the environment. The effectiveness of these spraying programmes is not the only issue for concern but their side effects are also important on health and environment, and their potential for sustainability, which depends on the cost of the insecticides and their application. Sand flies have also developed resistance to the chemicals, mainly to DDT and in some cases to Malathion and pyrethroids [8].

The use of pheromones is another strategy of controlling insect vectors. This property of pheromones is yet to be exploited in the control of vectors of leishmaniasis. Research has shown that different animal baits and CO2-baited CDC light traps have great potential in trapping sand flies. Studies have also confirmed that natural products are potential sources of new and selective agents for the treatment of important tropical diseases caused by protozoans. There is need to explore these source and exploit them maximally because they are likely to lower the high cost of treatment, reduce resistance of drugs & insecticides and also reduce environmental pollution. Therefore, this study sought to modify an existing trap so that it can attract sand flies by using scent normally produced by host animals and plants. This study also aimed at identifying the sand fly species attracted to this new device. In studying this objective, we wanted to invent a trap which is light independent in trapping sand flies.

II. Materials And Methods

2.1Study site

The study was carried out during the dry season of July 2014 in rural environments around Marigat town. Trapping of sand flies was carried out in Rabai village, Marigat division**,** Baringo County. Identification of the captured sand flies was carried out at the Kenya Medical Research Institute, Centre for Biotechnology Research and Development (CBRD), Nairobi.

2.2 Study design

A comparative experimental design using several baits was used. Their effectiveness was based on the number of sand flies trapped. Trapping experiments were conducted using a Latin square design with 10 treatments rotated through 10 locations / sites over 10 nights of trapping. The traps were operated from late afternoon to the early morning of the following day (18:00 to 08:00) along different locations with a distance of approximately 150 m between any two trapping sites. The control experiment was set on the windward side and it involved a CDC light trap set at 100-150 meters away from the treatment area.

2.3 Design of the trap

The trap consisted of two main components; a 5-liter plastic bottle and a sticky paper trap mounted inside this plastic bottle. The trap was constructed as previously described [9] with slight modifications. Briefly, the sticky trap was constructed from 10 x10 cm white cardboard sheet (with 0.1 cm wide netting separated by 0.3 cm square holes) covered with castor oil as an adhesive. This trap was then mounted vertically inside a clear 5-liter plastic bottle. Three windows were cut in the sides of this plastic bottle. The windows were covered by the steel net to keep larger non-target insects out of the trap (plate 1). Below the windows is a reservoir for holding the bait.

Plate 1: Design of the trap

2.4 Baiting and setting the traps

The baits used include defibrinated blood from cattle, goat, sheep and chicken; urine from cattle, sheep and goat. The fruits used include mango (*Mangifera indica L.*), banana (*Musa sapientum*), apple (*Malus domestica*) and grapes. The selection of these baits was based on past research which had shown that goat and chicken blood attract a lot of sand flies in the field [10; 11] when compared to other mammals. Ripe fruits also have a great potential in attracting sand flies [12]. Blood was obtained from slaughter houses (abattoirs) within Marigat area while fruits were bought from local markets.

Blood was obtained fresh from a slaughter house and immediately defibrinated to prevent clotting. A sponge soaked in 0.5 liter of blood/urine (in case of fruits, 0.5kg of ripe or overripe fruits) was placed inside each trap. The baited trap was then hung on strong sticks fixed on the termite mound with the opening 50 cm above the ground (plate 2). To eliminate positional bias, traps were rotated clockwise each day. Insects drawn to the traps were collected promptly at 08:00 to prevent degradation. There were four controls. The first control trap was empty, the second trap had only water soaked sponge, the third trap had sponges soaked with fresh 10% sucrose solution and the fourth control was a CDC light trap.


A **B**


C **D**

Plate 2: Baiting and setting the traps: A shows experimental set up with traps around the termite mound, B shows defibrinated blood in petri dishes, C shows blood and urine in petri didshes ready for transfer into the traps, D shows a CDC light trap set at the entrance of a termite mound

2.5 Mounting and identification of trapped sand flies

The collected sand flies were transferred from the traps by using a camel hair brush wetted in distilled water. They were directly preserved in 70% alcohol for later processing and identification in the laboratory. Each trap was individually numbered and notes recorded on collection date, the total number of sand flies collected, phlebotomine species composition, sex ratios, number of blood-fed females, and the number of gravid females. In the laboratory, sand flies were washed in 2% detergent solution to remove hairs and other debris. Thereafter, the flies were rinsed in PBS then transferred to a microscope slide for dissection and mounting. Slides were made of head and genitalia for species confirmation. The heads were excised and mounted using gum chloral on slides upside-down so as to expose the cibarium and pharynx. The mounted 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].

2.6 Ethical and Biosafety considerations

Approval to conduct this research was granted by Kenya Medical Research Institute's independent ethical review committee, Scientific Steering Committee and Animal Care and Use Committee. Precautionary measures included putting on protective gear while in the laboratory and field to avoid sand fly bites.

2.7 Statistical analysis

Average yields of the different trap attractants were analyzed by using analysis of variance (ANOVA). Homogeneity of variance was assumed with Tukey post hoc analysis to reveal the extent of the difference among the groups in cases where ANOVA was significant. Chi square test was also performed to compare the yield of the individual traps. The probability level was set at $P = 0.05$.

III. Results

During this study, a total of 1302 sand flies were caught over 3 nights of trapping. Out of these, 576 (44.24%) were males while 726 (55.76%) were females. These difference in sex was significant ($t=-2.334$, $P=0.025$) with a male/female ration of less than 0.80. A significant difference was found among the different attractants used ($P=0.001$). Traps baited with blood were the most effective followed by fruit baited traps while urine baited traps were the least.

3.1 Blood baited traps

The trap baited with goat blood yielded more sand flies, 202.33 ± 2.85 (mean ± S. E) as compared to the rest (fig. 2). This was followed by sheep blood-baited trap which caught 142.67 ± 4.92 (Mean ± SE), cattle blood-baited trap 121.00 ±4.00 while chicken blood baited trap was the least 65.00 ±1.53 (mean± S. E). CDC light trap was significantly different from the experimental traps, 346.33 ±10.84 ($P<0.001$). There was no significant difference between the negative controls (water and sugar baited traps) which had 9.67 ± 1.20 and 13.00 ± 1.00 ($P= 0.999$) respectively. When averaged over the 3 nights, a significant difference was found among the four blood baited traps ($f=579$, $DF_{6,\ 14}$, $P=0.001$). However, the difference between sheep blood baited trap and cattle blood baited trap was not significant ($P=0.086$). 76% of the sand flies trapped by blood baited traps were females while 24% were males.

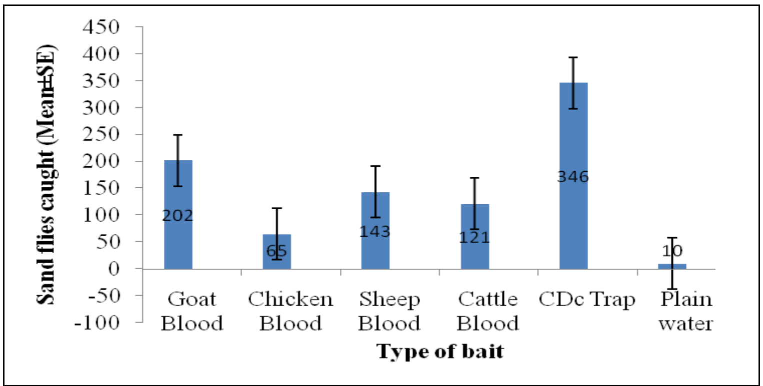

Figure 1: Mean Number of Sand Flies Caught By Blood Baited Traps Compared To Positive And Negative Controls

3.2 Urine Baited traps

Urine from goat, cattle and sheep were used in baiting traps that were compared to CDC light trap and a negative control which was a trap without any bait (plain). A total of 96 sand flies were trapped over 3 nights of trapping. All the traps attracted more males than females (56% males, 44% females). There was no significant difference among the three baited traps. Sheep urine baited trap captured the highest number of sand flies, 37.67 ± 2.96, followed by goat blood baited trap 33.00 ± 1.53 and the least was cattle urine baited trap, 26.67 ± 1.45. Post hoc analysis of results using Tukey HSD revealed that there was no significant difference among these three traps ($P=0.879$). However, these catches were significantly different as compared to the positive control CDC light trap which captured 379.67 ± 10.11 ($P=0.011$). The negative control captured 11.67 ± 0.88 (mean ± SE) sand flies (fig.3).

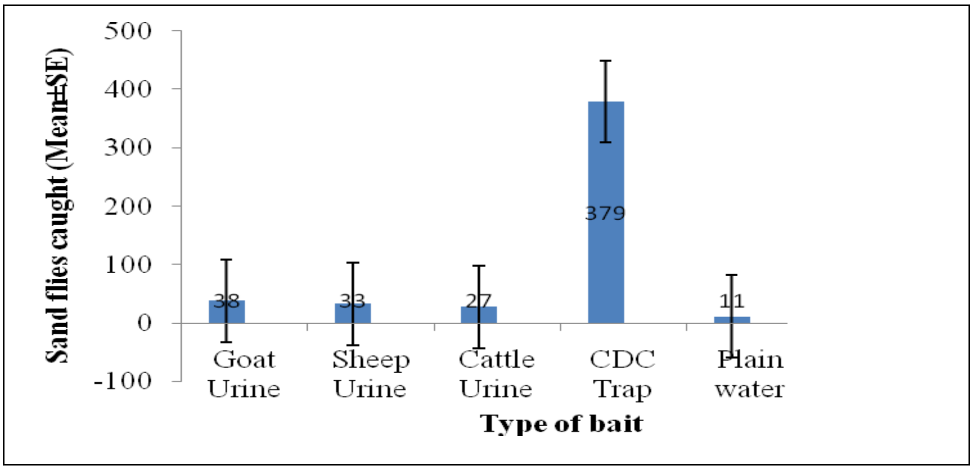

Figure 2 Mean Number Of Sand Flies Caught By Urine Baited Traps.

Comparing blood baited traps with urine baited traps revealed a significant difference in their catches (P<0.001) (fig. 4)

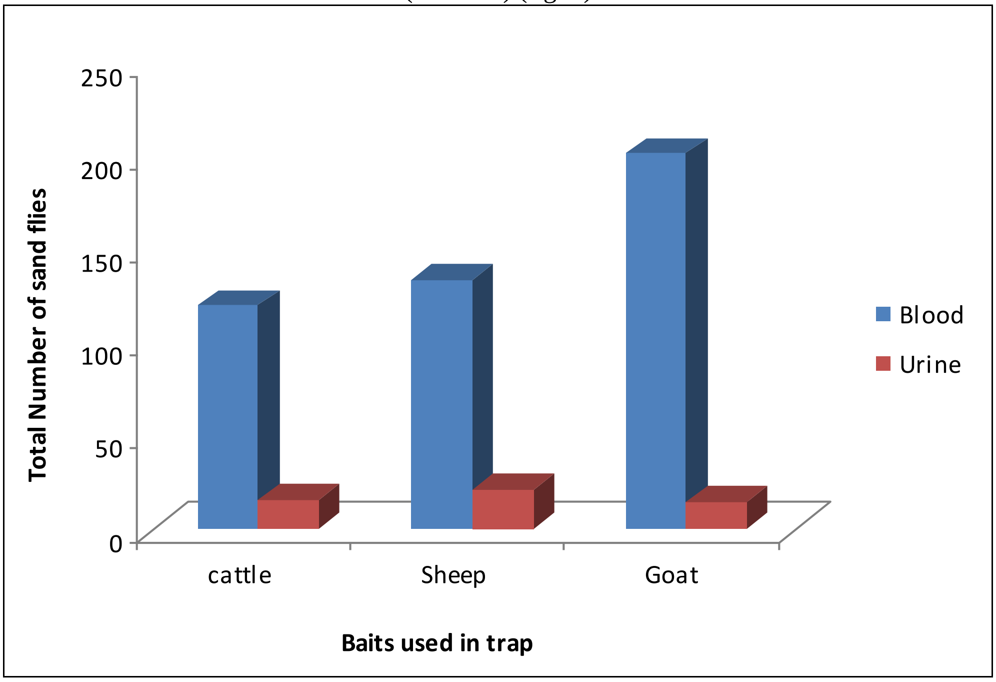

Figure 3: Comparing number of sand flies caught by blood baited traps and urine baited trap

Fruit baited traps

Fruits used as baits included bananas, apples, grapes and mangoes while CDC light trap and water were the controls. The average catch of the baited traps as compared to the controls was significantly different (f=259.364, DF 6, 14, P=0.001). Banana baited trap had caught the highest number of sand flies, 94.33 ± 4.63 followed by mango baited trap which caught 89.67 ± 2.33 (mean ± S. E). Apple baited and grapes baited traps caught 78.00 ± 4.62 and 57.00 ± 5.29 (mean ± S. E) sand flies respectively (fig. 5). However, the difference between banana baited trap and apple baited trap was not significant (P=0.682). The CDC light trap caught a significantly high number of sand flies (P=0.001) while the negative controls; water and sugar syrup baited traps caught 18.67 ± 6.17 and 17.00 ± 2.08 (mean ± S. E) respectively. Fruit baited traps attracted more males (66%) than females (34%)

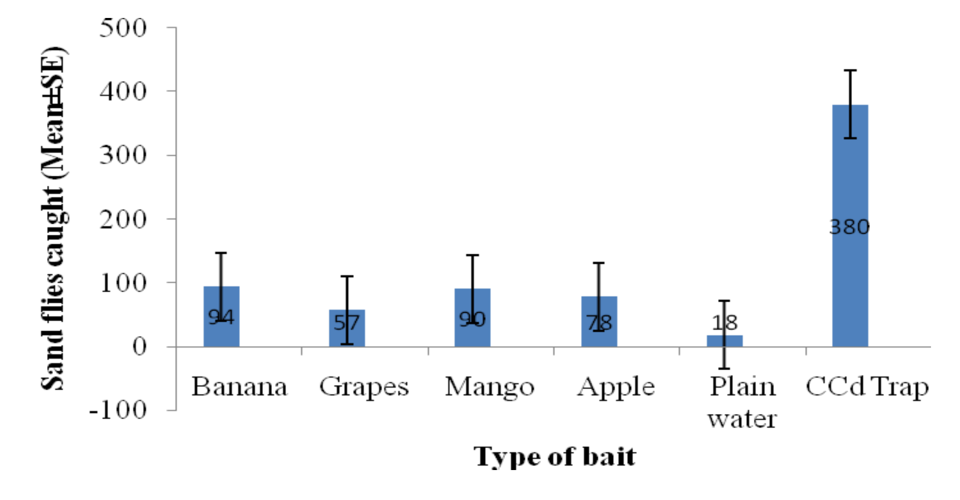

Figure 4: Comparison of number of sand flies caught by fruit baited traps

3.4 Identification of collected sand flies

A total of 1302 sand flies representing two genera and 7 species were collected using traps baited with three different types of baits: defibrinated blood, urine and fruits. 64.3% of the collected sand flies belonged to the genus Sergentomyia and 35.7% to Phlebotomus. In all the traps *Phlebotomus martini* dominated with a relative abundance of 35.7%. This was followed by *Sergentomyia schwetzi* (34.1%), *S. antennata* (15.7%), *S. clydei* (7.9%), *S. africana* 3.5%, *S. squamipleuris* (2.2%) while *S. dureni* (0.9%) was the least in relative abundance. Sand flies collected by blood baited traps were twice as numerous as those from fruit baited traps.

Blood baited traps collected the highest number of sand flies with a relative abundance of 64.6% followed by fruit baited traps with 27.2% and urine baited traps trapped 8.2%. Blood baited traps collected sand flies representing 7 species. *P. martini* dominated with a relative abundance of 23% then *S. schwetzi* (19.5%), *S. antennata* (9.9%), *S. clydei* (5.8%), *S. africana* 2.9%, *S. squamipleuris* (2.1%) while *S. dureni* (0.9%) was the least. In the fruit baited traps *S. schwetzi* was the dominant species (11%), followed by *P. martini* (9.3%), *S. antennatus* (4.6%) while *S. clydei* was the least with 2.1%. *S. dureni, S. squamipleuris* and *S. afrikanus* were not collected in the fruit baited traps. Urine baited traps collected the least in terms of abundance and diversity. The collected sand flies represented four species: *S. schwetzi* (3.5%), *P. martini* (2.9%), *S. antennata* (1.2%) and *S. africana* (0.6%). *S.clydei*, *S. dureni* and *S. squamipleuris* were not represented.

There was a significant difference when *P. martini* (χ^2=17.964, P=0.0001) was compared with S. *schwetzi* sand flies (χ^2=6.366, P=0.012). These two species were different in the goat blood baited trap but in sheep blood they were not different (P>0.05). Among the blood, urine and fruit baited traps, the sand flies collected by blood baited trap was significantly different from the rest (χ^2=11.464, P=0.003). When comparing the two genera, there was a significant different among Sergentomyia and Phlebotomus species (χ^2=9.458, P=0.009). In terms of physiology, only 3 sand flies were gravid while 6 were blood fed. All were trapped by the blood baited traps. No blood fed or gravid sand fly was trapped by fruit baited and urine baited traps. S. schwetzi, S. antennata and P. martini species were attracted to all the three types of baits used (blood, urine and fruits).

IV. Discussion

4.1 Blood baited traps

Sticky traps, CDC light trap and CO_2 baited traps are mainly used for monitoring sand flies in order to understand their vector ecology so that effective traps for surveillance can be designed [14]. It is generally known that CDC light trap and CO_2 baited traps capture the highest number of sand flies in the field hence they are highly preferred for sand fly surveillance. However, these traps have shortcomings for example; they are expensive, need regular maintenance, are heavy and can stay in the field for only a limited amount of time [9]. Therefore, there is a need to come up with new, cheap and readily available traps for sand flies and other haematophagous insects. There is need for new devices which are dependent on host odours in trapping these vectors.

In this study, we used defibrinated blood, urine and ripe fruits to bait a modified sand fly trap. The findings that blood baited traps captured more sand flies than the rest of the baits shows that compounds released from animal hosts dominate over plant host. Significantly high numbers of female sand flies were attracted to the blood baited traps showing the significance of blood to the sand flies. Vertebrate blood is significant for reproduction and growth of haematophagous insects [15]. However, not any vertebrate animal can be a source of blood for sand flies as noticed in this study.

The high number of sand flies captured by goat blood baited trap reveals that the goat is the preferred reservoir host for sand flies. These findings are consistent with previous studies which revealed that goat blood is the preferred blood meal for P. martini although other species prefer mixed feeding [16]. Therefore, it is likely that a goat has a strong cue which attracts sand flies for blood feeding [10]. It was also observed that chicken blood was the least in attracting sand flies in this study. However, other investigations have shown that chickens can be suitable hosts for Lutzomyia longipalpis population [11]. Chicken blood was favoured in Mauricio et al 2010 since he used dog, human, rabbit and chicken blood while in this study we used goat, cattle, sheep and chicken. Elsewhere, female sand flies which had fed on chicken blood had significantly delayed egg development compared to those fed on rabbits [17]. Therefore, chicken blood was not favoured because of its inhibitory effects on egg development and this may support our observation which showed that chicken blood attracted the least number of sand flies.

Sheep and cattle blood baited traps had a moderate attraction index for sand flies. This shows that cattle and sheep can be alternative hosts for sand flies in case the favoured sand flies are not available. Sand flies prefer blood feeding on mammalian hosts but some prefer reptilian hosts. Therefore, the attraction of sand flies to avians and bovids shows the role of these animals in the transmission of Leishmania parasites. Attraction of haematophagous insects to hosts may depend on the natural host preference or the availability and accessibility of the hosts within the local environment [18]. This explains why goat's blood was the preferred attractant in this study bearing in mind that almost every homestead in Rabai, Marigat division keeps goats more than any other domestic animal.

Contrary to blood, urine baited traps attracted very few sand flies showing that there are no attractants for sand flies in host urine. This may also be attributed to the fact that urine has no role in the physiology of sand flies. In the field haematophagous insects are attracted to host odour components and male pheromones which stimulate mating [19]. Little is known on the role of urine as a potent cue for biting insects. Very few sand flies were attracted to urine baited traps and this conquers with other findings which showed that feeding stimulants reported to attract mosquitoes and sand flies including host urine did not stimulate L. shannoni feeding [20] However, urine from host animals may attract or influence physiological conditions of other haematophagous insects including repelling or attracting them. Horse urine has been depicted as the best attractant during sampling of Glossina morsitans submorsitans [21]. Apart from morsitans flies, mosquitoes are also attracted to host urine which contains 4-methylphenol and 3-n-propylphenol as active substances [21].

Other studies have shown that addition of cow urine to habitats attracted oviposition by anopheline and culicine species [22]. The same might be expected in the case of sand flies since mosquitoes and sand flies have a closely related physiology. This finding shows that the presense of these animals in endemic areas might increase the risk of leishmaniasis or increase the breeding of sand flies in those areas. Therefore, incorporating host urine in traps might increase the efficiency of such traps hence reduction of sand flies from the locality.

4.2 Fruit baited traps

Sand flies generally feed on plant tissues like leaves, stems and flowers. They also feed on various sweet substances like honey dew and ripe fruits which contain a lot of sucrose, glucose, fructose and raffinose [23]. Once adults emerge, their first activity is sugar feeding which is the only source of energy for sand flies. Little research has been done on the attractiveness of sand flies to ripe fruits. However, the observation that banana and mango baited traps attracted more sand flies may be due to the speculation that bananas and mangoes contain high concentrations of sucrose and fructose for energy which the vectors use for host seeking, survival and reproduction. It is probable that as these fruits ripen and rot, they release volatile chemicals which attract sand flies and other insects. This can be supported by the observation that mosquitoes have been collected from peaches, apples, grapes and watermelons, particularly those damaged by rain and rot [24]. All the fruits used were attractive to the sand flies but the order of preference was banana, mango, apple and the least was grapes. The actual attractants are not known but sugar and CO2 emitted as part of metabolism can be some of the attractants since fermenting sugar yeast mixtures have been used as a source of carbon dioxide to attract and trap sand flies [25].

More males were attracted to the fruit baited traps because sugars are their main sources of food and energy. In the field, the main source of the sugar meal is from honey dew excreted by aphids and coccids and by feeding directly on plant tissues in the field [8]. Sugar/yeast mixture when allowed to ferment, it produces carbon dioxide which attracts sand flies in the same way as CO_2 baited traps [26]. There are also speculations that the attractants for sand flies in fruits are the volatile compounds affecting olfactory receptors or an insect's ability to detect CO_2 emissions [24].

Water and sugar syrup baited traps which were the negative controls caught the lowest number of sand flies. This shows that there were no attractants in the negative controls except moisture which is also present in the ripe fruits. Apples and grapes attracted significantly more sand flies as compared to the negative controls, an observation in agreement with previous studies where these fruits attracted more sand flies than the controls [24]. This shows that ripe and rotting fruits release compounds which can attract biting insects. Therefore, CO_2 and other volatiles emitted by fruits can be used by flies to locate their vertebrate hosts or plants for sugar feeding. Some of these volatile compounds may probably mimic insect hormones used during mating. It is likely that as overripe fruits are fermenting, they emit chemicals which are similar to host seeking cues that stimulate either blood feeding or mating behavior in haematophagous insects and biting insects are believed to be attracted to ripe fruits based on attractive volatile compounds released by plants [24]. This shows that emanations from fermenting sugars can be used as a source of CO_2 to attract and trap sand flies [25]. Carbon dioxide can enhance trap collections [27]; hence if traps can be baited with odours from host animals together with carbon dioxide, the attractive efficiency of these traps will be greatly improved. Such traps will be easy and less costly to use as compared to CDC light traps.

4.3 Identification of sand flies

Human landing collections, the CDC light trap and the sticky paper traps are the standard surveillance techniques used to determine sand fly densities in a given locality [28]. Phlebotomine sand flies have a wide distribution and diversity in Marigat Division. Females and males depend on sugar as a source of energy but females also need blood meals for egg development [29]. Effective vector sampling is necessary for predicting disease outbreaks in a given area.

In this study, lures from different vertebrates and fruits were used in place of light and CO_2 which attracted sand flies from two genera. These traps collected sand flies from seven species within Rabai, Marigat division with *P. martini, S. schwetzi* and *S. antennata* being the dominant species. This study concurs with studies using CDC light traps in Marigat which trapped 11 species of sand flies with *P. martini and S. schwetzi* being the dominant species [30].

The high number and dominance of *P. martini, S. schwetzi* and *S. antennata* in all the baited traps suggest that these three species of sand flies have a wide range of blood meal and source of sugars hence they may show mixed feeding in nature. This shows that host blood and ripe fruits can mimic human hosts in the field hence these traps can yield a true representative of sand fly species in a given locality. *P. martini* is the main vector for visceral leishmaniasis [31] in Kenya although others may be there. The high number of trapped *P. martini* concurs with the high prevalence of visceral leishmaniasis in Marigat division. There is little literature showing the relationship between host urine and sand flies although cow, goat and sheep attracted 4 species of sand flies (S. schwetzi, P. martini, S. antennata and S. africana).

The result showed that very few blood fed females were collected despite a very high population density of sand flies that were caught by the traps. This shows that majority of the sand flies in Marigat division blood feed on wild animals or they find their way to human habitation for blood feeding. This observation is in agreement with previous studies which suggested that the majority of sand flies either migrates to villages for feeding on their preferred hosts or those feeding outdoors on wild animals are probably as widely dispersed as their wild hosts are, thus, becoming rare in the trap collections [32].

Conclusion and recommendation

Volatiles from plant and animal hosts may be used instead of light or carbon dioxide to improve the efficiency of traps for haematophagous insects. These volatiles can also be used to synergize each other and be incorporated in traps targeting sand flies. These compounds released by animals and plants are wastes which can be used as lures for vectors for surveillance purposes. In this study, sand flies showed highest preference to goat blood and ripe banana and mango fruits. Urine attracted sand flies by chance. Studies such as these are important in preventing sand flies from human contact by the presence of animals which constitute a natural protective measure against sand fly bites. Therefore, the number of sand flies that access humans is greatly reduced if there are a lot of animals in the local environment. They are also important for determining sand fly host preferences disease agents that they transmit. Further research should focus on identifying the insect attractants in host blood and ripe/rotting fruits then use them in improving the attractive efficiency of these traps instead of light. Such traps will be easy and less costly to use as compared to CDC light traps.

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