SHORT COMMUNICATION

Evaluation and Modification of Off-Host Flea Collection Techniques Used in Northwest Uganda: Laboratory and Field Studies

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ABSTRACT Quantifying the abundance of host-seeking fleas is critical for assessing risk of human exposure to flea-borne disease agents, including *Yersinia pestis*, the etiological agent of plague. Yet, reliable measures of the efficacy of existing host-seeking flea collection methods are lacking. In this study, we compare the efficacy of passive and active methods for the collection of host-seeking fleas in both the laboratory and human habitations in a plague-endemic region of northwest Uganda. In the laboratory, lighted "Kilonzo" flea traps modified with either blinking lights, the creation of shadows or the generation of carbon dioxide were less efficient at collecting *Xenopsylla cheopis* Rothchild and *Ctenocephalides felis* Bouché fleas than an active collection method using white cotton socks or cotton flannel. Passive collection using Kilonzo light traps in the laboratory collected significantly more *X. cheopis* than *C. felis* and active collection, using white socks and flannel, collected significantly more *C. felis* than *X. cheopis*. In field studies conducted in Uganda, Kilonzo traps using a flashlight were similar in their collection efficacy to Kilonzo traps using kerosene lamps. However, in contrast to laboratory studies, Kilonzo flea traps using flashlights collected a greater number of fleas than swabbing. Within human habitations in Uganda, Kilonzo traps were especially useful for collecting *C. felis*, the dominant species found in human habitations in this area.

KEY WORDS flea trap, *Ctenocephalides felis*, *Xenopsylla cheopis*, flea, plague

During flea-borne disease surveillance and research activities, it is often necessary to collect information on the host-seeking (off-host) flea populations found in a given area. This information can be used along with other data to assess the risk of flea-borne disease transmission to humans residing in the same areas. For example, when rodents die from *Yersina pestis* infection, their fleas can be released into the surrounding environment and increase plague risk for humans (Gage 1998). Assessing the abundance of host-seeking fleas in human habitations is important because human infections in many of the world's plague foci are associated with domestic or peridomestic settings (Akiev 1982, Gratz 1999, Tikhomirov 1999). This is particularly imperative in Africa where >90% of the world's plague cases have occurred in the past two decades and where the ecology and epidemiology of plague are poorly understood (Kilonzo et al. 1992, Laudisoit et al. 2007, Eisen et al. 2008). Unfortunately, comparisons of the relative efficiencies of various techniques for sampling off-host flea populations in Africa have received little attention in the plague literature.

Host-seeking fleas found in traditional style mud huts in Africa have been collected previously using a light trap based on Kilonzo (1977). The original "Kilonzo" trap consisted of a kerosene hurricane lamp suspended above a pan of water (or other liquid) such that the light radiating from the lamp covers only the surface area of the pan. Fleas are presumably drawn to the trap because of heat and light emanating from the lamp's flame. This design was used for the collection of off-host fleas elsewhere in Africa (Kilonzo et al. 1992, Laudisoit et al. 2007, Sackal et al. 2008). A slightly modified Kilonzo trap that makes use of a flashlight (with either an incandescent or a light emitting diode [LED] bulb) instead of the kerosene lamp suspended over the pan of water also has been used recently in Uganda (Eisen et al. 2008; Centers for Disease Control and Prevention [CDC], unpublished data).

The purpose of this study was to evaluate the relative flea trapping efficacies of different modifications of the Kilonzo trap under laboratory conditions and compare this type of trap to active methods that relied on dragging pieces of cotton cloth over flea-infested surfaces or walking the area wearing white cotton socks. We also determined the relative efficacies of two types of Kilonzo traps and compared one Kilonzo

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Table 1. Numbers of *C. felis* and *X. cheopis* fleas captured in laboratory studies

Evaluation	Type of trap	*C. felis*			*X. cheopis*		
		No. fleas captured (no. introduced)	% captured	Median (range)	No. fleas captured (no. introduced)	% captured	Median (range)
Dark pan trap (negative control)	Passive	2 (1,200)	0.2	0 (0–0.167)	11 (1,200)	0.9	0.167* (0.083–0.25)
Kilonzo (IC)	Passive	2 (1,200)	0.2	0 (0–0.083)	20 (1,176)	1.7	0.128* (0–0.765)
Kilonzo (IC)/shadows	Passive				3 (1200)	0.3	0
Kilonzo (LED)/blinking flashlight	Passive	5 (1,200)	0.4	0.083 (0–0.83)	17 (1,200)	1.4	0.083 (0–1.167)
Kilonzo with CO_2 attractant	Passive	3 (1,200)	0.3	0.042 (0–0.83)	3 (1,200)	0.3	0.042 (0–0.83)
White sock collection	Active	449 (1,200)	37.4	6.21* (2.75–9)	216 (1,178)	18.3	2.55 (2.04–4.50)
Dragging with flannel	Active	803 (1,200)	66.9	11.92* (7.92–13.67)	109 (1,200)	9.1	1.29 (0.833–3.08)

IC, incandescent; LED, light-emitting diode.

* Statistical analysis comparing trap type across species ($P < 0.05$).

design against the dragging method in traditionally constructed mud huts in northwestern Uganda.

Materials and Methods

Laboratory Studies. To create an arena environment for fleas in our laboratory, we purchased circular, galvanized steel livestock water tanks (Hutchison Inc. Grinnel, IA), which measured 1.6 m in diameter and 0.6 m in height and had a bottom surface area of 1.95 m^2. The interiors of the tanks were painted flat brown and were filled with ≈15 kg of sand (Medium Sand #1962, Quickrete, Atlanta, GA), creating a layer ≈0.6 cm in depth throughout the arena. For each evaluation, a trap was placed 10 cm from one side of the arena wall. For each of the six replicates within each of the experimental treatments and controls, fleas (mixed sex) were released 10 cm from the side directly across from the trap and ambient lights were turned off in the study area, leaving only the light from the flea trap for illumination (experimental treatments) or no illumination (negative controls). Fourteen hours after initiation, we removed the flea traps and counted the number of fleas captured by each trap. Six arenas were used for each replicate of five collection methods and the experiments were run for both laboratory bred *Xenopsylla cheopis* Rothschild (from the Centers for Disease Control and Prevention, Division of Vector-Borne Diseases breeding colony) and *Ctenocephalides felis* Bouché purchased from a commercial source (Heska Corp., Loveland, CO). For each method, ≈200 *X. cheopis* and 200 *C. felis* were tested within each of the six replicates for a total of ≈1,200 fleas used per method and species evaluated (Tables 1 and 2).

Passive flea trap methods included 1) the standard lighted Kilonzo flea trap design with a flashlight (GPR2AA2AA2D-B, Rayovac, Atlanta, GA) suspended over a pan of water to mimic the modified Kilonzo design described above; 2) the standard trap modified using a LED flashlight (SW75348, Schwin, Coral Gables, FL) with a blinking light; 3) the standard trap with the attachment of 12 curled, silver, reflective mylar strips (15.2 by 0.6 cm) to the incandescent flashlight such that when the flashlight was suspended upside down, the strips hung below the flashlight to create shadows on the bottom and sides of the arena (*X. cheopis* only); and 4) CO_2 attraction by using a previously described mixture of yeast and sugar placed in a small container in the center of the water pan (no light source) (Lorenzo et al. 1998). Each pan trap contained 2% saline with Tween 80, and petroleum jelly was applied to the rim of the pans to prevent fleas from escaping. One additional flea trap trial was run using a water pan not placed under a Kilonzo trap without the addition of any light source or modification (negative control).

Active flea collection techniques evaluated in this study included 1) an individual who wore white, knee-high cotton socks and methodically traversed the arena for 40 s, taking care to cover the entire surface of the arena; and 2) an individual who dragged a 25- by 25-cm piece of white flannel across the floor of the arena for 40 s, taking care to cover the entire surface of the arena. To evaluate the above active methods, fleas were released into the center of the arena and allowed to move freely in darkness for 14 h before the use of the active collection technique. After collection, the pieces of flannel or the cotton socks were

Table 2. Number of field-collected fleas from Kilonzo-kerosene and Kilonzo-flashlight traps in human habitations in northwestern Uganda

Kilonzo type (trap-nights)	No. fleas	Flea identification				
		X. cheopis	*X. brasiliensis*	*C. felis*	*E. gallinacea*	*A. torvus*
Flashlight (n = 130)	77	7	2	67	0	1
Kerosene (n = 127)	128	3	0	114	11	0
Total (n = 257)	205	10	2	181	11	1

placed in a plastic bag and then transferred to a freezer to immobilize captured fleas. Once the fleas had been killed by freezing, the sock or flannel samplers were removed from the freezer and all fleas on each sampler were counted. After each trial, the flea arenas were cleaned and new sand was placed in each arena. Throughout the trials, the arenas were kept at ambient room temperature and humidity.

Field Studies. Flea collection studies were performed inside traditional housing in the plague-endemic districts of Arua and Nebbi located in northwest Uganda. Fleas were collected in the field using three methods: 1) a standard Kilonzo design trap consisting of a kerosene hurricane lamp suspended over a metal pan (25.4 cm in diameter); 2) a modified Kilonzo design identical to the standard design but equipped with a flashlight rather than a kerosene lamp (with incandescent bulb) suspended over a metal pan; and 3) swabbing the entire area of the floor with white flannel cloth (60 by 60 cm). Each pan trap contained 2% saline with Tween 80, and petroleum jelly was applied to the rim of the pans to prevent fleas from escaping. Village residents were requested to turn the flashlights on or light the kerosene hurricane lamps at night and leave these light sources lit throughout the night. To ensure that lights stayed lit throughout the trials, each night batteries were replaced in all flashlights and the kerosene lamps were filled to capacity with fuel.

We first sought to compare the standard Kilonzo design (kerosene lamp) with the modified Kilonzo design (incandescent flashlight). Using both types of pan trap (one each per hut), we collected fleas in 80–100 homes for 1–2 nights. Second, we compared the modified Kilonzo trap (with flashlight) with swabbing using three methods (136 huts each method): 1) swabbing at night (1a) followed by use of a Kilonzo trap (1b), followed by an additional swabbing in the morning (1c); 2) use of a Kilonzo trap in the evening (with no prior swabbing) (2a), followed by a swabbing in the morning (2b); and 3) swabbing in the morning with no previous swabbing or use of Kilonzo traps. As technicians proceeded through a village, they sequentially assigned homes to one of the three methods so that each method was evaluated under similar local conditions. After fleas were collected from the pans or swabs, they were stored in microcentrifuge tubes and later identified to species following published taxonomic keys (Hopkins 1947, Haselbarth 1966, Smit 1973).

Statistical Analysis. In both laboratory and field trials, we used nonparametric Kruskal–Wallis or Wilcoxon rank sums tests with χ^2 approximations to compare median numbers or proportions of fleas collected in traps. All statistical comparisons were run using JMP statistical software (SAS Institute, Cary, NC), and results were considered significant if $P < 0.05$.

Results

Laboratory Trials. In laboratory trials using *C. felis*, active collection with white socks or flannel collected a greater proportion of fleas than other methods (i.e., various modifications of the Kilonzo traps or the use of dark pan traps [negative control]) (Table 1). The proportion of *C. felis* captured by cotton socks, pieces of flannel or the Kilonzo trap methods differed significantly ($\chi^2 \geq 27.2332$, df = 5, $P < 0.0001$). Further pairwise comparisons revealed that the flannel pieces captured significantly more *C. felis* than did white socks ($\chi^2 \geq 5.0256$, df = 1, $P = 0.0250$), but both of these sampling methods were significantly higher than all other groups combined ($\chi^2 \geq 25.6889$, df = 2, $P < 0.0001$) after these other groups (modifications of the Kilonzo trap and dark pan trap controls) were determined to be similar ($\chi^2 \geq 4.1494$, df = 3, $P = 0.2458$).

Likewise, laboratory trials using *X. cheopis* revealed that active collection with white socks or flannel collected a greater number of fleas than various Kilonzo trap modifications or dark pan traps (negative control) (Table 1). There was a significant difference in the proportion of *X. cheopis* captured by the various treatments ($\chi^2 \geq 28.8541$, df = 6, $P < 0.0001$). All Kilonzo modification groups were similar to each other and yielded significantly fewer fleas than flannel and white sock collection methods ($\chi^2 \geq 24.7780$, df = 1, $P < 0.0001$). Pairwise comparison revealed that similar proportions of *X. cheopis* were collected by the flannel and white sock collection methods ($\chi^2 \geq 3.6923$, df = 1, $P < 0.0547$) (Table 1).

Comparing the proportion of flea species captured per trap type, passive collection using pan traps (dark pan trap, Kilonzo light trap) collected significantly more *X. cheopis* than *C. felis* and active collection by using white socks and flannel collected significantly more *C. felis* than *X. cheopis* (Table 1).

Field Studies. Fleas collected from both field studies represented six species: *C. felis*, *X. cheopis*, *Xenopsylla brasiliensis* Baker, *Echidnophaga gallinacea* Westwood, *Afristivalus torvus* Rothschild (syn. *Stivalus torvus* in Hopkins 1947), and *Tunga penetrans* L. The majority of fleas collected were *C. felis.* Therefore, analyses were performed using *C. felis* data only.

In the first field study, which compared the Kilonzo-flashlight trap versus the Kilonzo-kerosene trap, the median number of *C. felis* captured in the two types of Kilonzo traps did not differ significantly ($\chi^2 \geq 3.3428$, df = 1, $P = 0.0675$). Traps collected at least one *C. felis* in 34.6% of the Kilonzo-kerosene traps and 24.6% of the Kilonzo-flashlight traps (Table 2).

In the Kilonzo flashlight traps versus swabbing trials, Mann–Whitney test for *C. felis* revealed significant differences among the six treatments (See Materials and Methods). A posthoc pairwise analysis revealed that methods 1b (Kilonzo after swab) and 2a (Kilonzo no prior swab) did not differ significantly from one another, indicating that swabbing did not remove a substantial proportion of the host-seeking *C. felis* population. Methods 1b and 2a did differ significantly from the other four methods (1a, swab before Kilonzo; 1c, swab next morning after Kilonzo; 2b, swab after Kilonzo-no prior swab; and 3, morning swab-no prior light trap or swab; $P < 0.0001$) (Table 3). Although the results for *E. gallinacea* were not significant, it suggests

Table 3. Number of field-collected fleas from Kilonzo-flashlight traps and swabbing in human habitations in northwestern Uganda

	Method 1a: swab before Kilonzo (evening) (n = 136 huts)	Method 1b: Kilonzo after swab (n = 136 huts)	Method 1c: swab next morning (n = 136 huts)	Method 2a: Kilonzo trap no prior swab (n = 136 huts)	Method 2b: Swab after Kilonzo trap no prior swab (n = 136 huts)	Method 3: Morning swab no prior Kilonzo trap or swab (n = 136 huts)
C. felis	2a	34b	2a	49b	2a	7a
X. cheopis	1	1	0	0	2	1
X. brasiliensis	2	0	0	1	1	0
T. pentetrans	0	1	0	1	0	0
E. gallinacea	6	0	2	4	0	5
A. torvus	0	0	0	0	1	0
Total	11	36	4	55	7	13
Median (range)	0 (0–3)	0 (0–5)	0 (0–2)	0 (0–6)	0 (0–2)	0 (0–4)

Proportions with the same lowercase letter were not significantly different ($P > 0.05$).

a trend toward collecting more of these fleas by swabbing than by use of the Kilonzo trap.

There was a significant difference between *C. felis* collected in the Kilonzo trap (flashlight) with no prior swab (method 2a) and swabbing only (method 1a + method 3) ($\chi^2 = 40.5725$, df = 1, $P < 0.0001$), indicating that the Kilonzo trap is a more efficient method of collecting *C. felis* than swabbing. There was no significant difference between the total number of *C. felis* collected per hut by a combination of swabbing and Kilonzo trap (methods 1a + 1b + 1c) and the number of fleas collected per hut by Kilonzo trap alone (method 2a) ($\chi^2 = 0.0648$, df = 1, $P = 0.7990$).

Discussion

Our laboratory trials indicated that the active collection (flagging and recovery from socks) of *C. felis* and *X. cheopis* was significantly more effective than passive collections by Kilonzo flea traps. Light and dark Kilonzo traps collected more *X. cheopis* than *C. felis*, but active collections using flannel and white socks collected more *C. felis* than *X. cheopis* in laboratory trials. In contrast, our field studies indicated that Kilonzo flashlight traps collected more *C .felis* than *X. cheopis.* We believe this difference between laboratory and field results may be caused by the lack of movement and stimulation of fleas in the arena studies. Other studies have shown that fleas are stimulated by movement and air currents (Osbrink and Rust 1985). The field studies were performed in huts inhabited by homeowners and other potential hosts (usually dogs, chickens, pigs, or a combination) who were frequently moving in their homes in the course of normal activities which might stimulate fleas to seek a host. Fleas in the arena studies may not have been adequately stimulated to move toward the Kilonzo traps. Also, low numbers of fleas collected using the swabbing method in the field may have been due to the difficulty of swabbing the entire floor of each hut. Huts often contained furniture and other items placed on the floor, which may have impeded complete sampling of the floor area.

The differences in the number of fleas collected in the field per species may have been due to the number of *C. felis* being higher than the number of other fleas in the huts. Under laboratory conditions, Kilonzo traps collected more *X. cheopis* than *C. felis*, indicating that differences seen in the field were probably due to *C. felis* being more abundant than *X. cheopis* in the huts and not due to the trap being more efficient at capturing *C. felis.* In the field, we would have expected to collect more *X. cheopis* if populations of these fleas were roughly equal in abundance to *C. felis,* but because the numbers of off-host *C. felis* in Ugandan huts are probably many times higher than those of *X. cheopis*, the former flea can be expected to be captured in much higher numbers during interepizootic periods. However, it should be noted that the numbers of off-host *X. cheopis* would be expected to increase suddenly whenever a plague-related die-off causes sudden high mortality among local rat populations. Our findings are consistent with other studies in Uganda which found that *C. felis* was the most commonly encountered flea in Kilonzo type traps (Eisen et al. 2008; CDC, unpublished data), although the numbers of fleas captured in our study were considerably lower than the total numbers captured in other studies done elsewhere in Africa (Kilonzo et al. 1992, 2006; Laudisoit et al. 2007). The reasons for these differences are not entirely clear but might reflect actual differences in the densities of fleas living within the huts in other African nations.

We selected modifications to the Kilonzo design that were relatively simple and inexpensive so that, if effective, these modifications could be used under field conditions in Africa. Although previous studies have indicated that fleas can be successfully collected using light traps (Rust and Dryden 1997, Creswell and Stratman 2007), mimicking shadows (Osbrink and Rust 1985), by using an intermittent light source (Dryden and Broce 1993), and by CO_2 generation, (Benton and Lee 1965, Miles 1968), our modifications of the Kilonzo design proved ineffective at improving trap efficiency in the laboratory. Consistent with our laboratory findings, other studies have reported success using white socks (Osbrink et al. 1986) and flannel drags (Barnes et al. 1972, Cully et al. 1997, Karhu and Anderson 2000) to collect fleas.

Although the true efficiency is unknown, Kilonzo traps using a flashlight remain a useful method for the collection of fleas, particularly *C. felis* fleas in tradi-

tional homes in Africa. More research is needed to determine the efficacy of these traps during plague epizootics when the numbers and assemblages of fleas are likely to change.

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