Efficacy of Light and Nonlighted Carbon Dioxide–Baited Traps for Adult Sand Fly (Diptera: Psychodidae) Surveillance in Three Counties of Mesrata, Libya

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Sand flies are important vectors of cutaneous leishmaniasis, especially along coastal towns of northwestern Libya where an estimated 20,000 cases have occurred from 2004 to 2009. Host-seeking traps are an important tool for sampling sand fly populations and surveying the incidence of Leishmania major and L. tropica within a given population. We evaluated the capture efficiency of CO2-baited BG-Sentinel, Centers for Disease Control and Prevention (CDC) light, CDC ultraviolet light, and nonbaited CO2 CDC light traps in 3 coastal townships during June, August, September, and November 2010. A total of 3,248 sand flies representing 8 species from 2 genera, were collected; most sand flies were identified as either Phlebotomus papatasi or P. longicuspis. Three of the traps captured significantly more sand flies compared to the BGSentinel baited with CO2 (P , 0.001). Three of 456 DNA pools extracted from sand flies were positive for Leishmania DNA, indicating a minimum estimated infection rate of 0.83% and 0.47% for P. papatasi and P. longicuspis, respectively.

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EFFICACY OF LIGHT AND NONLIGHTED CARBON DIOXIDE–BAITED TRAPS FOR ADULT SAND FLY (DIPTERA: PSYCHODIDAE) SURVEILLANCE IN THREE COUNTIES OF MESRATA, LIBYA

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ABSTRACT. Sand flies are important vectors of cutaneous leishmaniasis, especially along coastal towns of northwestern Libya where an estimated 20,000 cases have occurred from 2004 to 2009. Host-seeking traps are an important tool for sampling sand fly populations and surveying the incidence of *Leishmania major* and *L. tropica* within a given population. We evaluated the capture efficiency of CO_2-baited BG-Sentinel, Centers for Disease Control and Prevention (CDC) light, CDC ultraviolet light, and nonbaited CO_2 CDC light traps in 3 coastal townships during June, August, September, and November 2010. A total of 3,248 sand flies, representing 8 species from 2 genera, were collected; most sand flies were identified as either *Phlebotomus papatasi* or *P. longicuspis*. Three of the traps captured significantly more sand flies compared to the BG-Sentinel baited with CO_2 ($P < 0.001$). Three of 456 DNA pools extracted from sand flies were positive for *Leishmania* DNA, indicating a minimum estimated infection rate of 0.83% and 0.47% for *P. papatasi* and *P. longicuspis*, respectively.

KEY WORDS BG-Sentinel, Centers for Disease Control and Prevention light trap, *Phlebotomus papatasi*, *Phlebotomus longicuspis*, cutaneous leishmaniasis

INTRODUCTION

Female phlebotomine sand flies (Diptera: Psychodidae) are the primary vectors of *Leishmania*, a diverse group of protozoan parasites that infect an estimated 12 million people and result in 60,000 deaths annually in 88 countries (WHO 2011). Leishmaniasis can cause 4 distinct clinical manifestations known as localized, diffuse, mucosal, and visceral forms, of which the first three are classified as ''cutaneous'' leishmaniasis (CL) and are endemic in 70 countries worldwide (Reithinger et al. 2007). Of the 29 Old World sand fly species known to be or incriminated as vectors of *Leishmania*, *Phlebotomus papatasi* Scopoli and *P. longicuspis* Nitzulescu are reportedly the proven and primary vectors for leishmaniasis in North Africa, respectively (Killick-Kendrick 1987). Cutaneous and visceral leishmaniases are a considerable human health threat for much of coastal and interior Libya. Visceral leishmaniasis occurs sporadically in Tripoli and eastern parts of the country, with newly identified foci expanding south near the border with Chad (El-Buni et al. 1993).

Since 1971, CL cases have been reported from areas south and west of Tripoli and extending to the Tunisian border (Ashford et al. 1976). Over the last 2 decades reports of CL infections have increased in number and geographically within Libya, particularly along coastal regions. For example, Yafran, a mountainous town south of Tripoli with approximately 3,000 residents, reported 445 CL cases from February 1991 to December 1992 (El-Buni et al. 2000). Moreover, a clinical study conducted in 2006 and 2007 in the Al-Gadaheya and Al-Hisha villages of Sirte determined the annual incidence of CL among the village population was almost 1%, with the majority of cases (75%) affecting males from 12 to 40 years of age (Fathy et al. 2009).

Responding to a government mandate in 2007 to reduce the effects of epidemic levels of CL, the Libyan National Center of Diseases and Control (NCDC) initiated a rodent control campaign with the overarching goal of reducing CL infections by poisoning the natural rodent hosts of the *Leishmania* sand fly vectors. A labor- and time-intensive campaign was executed where aluminum phosphide (Phostoxin, Tripoli, Libya) pellets were placed inside rodent burrows to control the sand rat, *Psammomys obesus* Cretzschmar, the primary *Leishmania* spp. reservoir in Libya (Ashford et al. 1977). The intervention successfully reduced the rodent population by 85%, and the number of CL cases decreased from 7,180 in 2006 to 1,800 by 2008 (B. B. Annajar, unpublished data).

Unfortunately, for many Libyan regions affected by CL epidemics, the sand fly fauna and distribution remained unknown. Baseline adult sand fly surveillance data are an essential requirement for determining the population dynamics of anthropophilic sand flies responsible for human *Leishmania* spp. transmission (Alexander 2000). An active surveillance program also would provide valuable information to the

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NCDC on trap efficacy, and evaluate the effectiveness of controlling leishmaniasis transmission. Researchers from the NCDC and the US Naval Medical Research Unit No. 3 designed and implemented a surveillance study in 2010 to characterize the adult sand fly fauna in 3 *Leishmania*-endemic counties of Mesrata, Libya, and to determine the efficacy of different sand fly traps at these locations.

MATERIALS AND METHODS

Study area

A sand fly survey was conducted in the city of Taurgha, near the Libyan coast and east of Meserata City, Libya. This region has a typical Mediterranean climate (i.e., hot, dry summer and mild, wet winter), with annual precipitation of 20–30 cm and mean monthly temperatures from 14.8°C to 25.9°C (WorldClim 2005). An abundance of halophyte plants, namely *Zizyphus lotus* L. (Rhamnaceae) and *Halocnemum strobilaceum* (Pall.) M. Bieb. (Chenopodiaceae), were observed throughout the area. The areas where these plants are found are ideal sites for burrows of *Psammomys obesus* as well as the Libyan jird, *Meriones libycus* Lichtenstein, another important reservoir for CL (Ashford et al. 1977, Fathy et al. 2009).

We selected 3 counties (Al Sawadek, Al Kefah, and Al Fateh) in Taurgha in which to sample sand flies. These counties were the first to report a CL epidemic involving >7,000 cases (Fathy et al. 2009) and were therefore selected as ideal sites for our study. In each county, traps were placed at 2 sites separated at least 0.5 km and 50–100 m from residences, for a total of 6 trapping sites. Al Sawadek (32°01.368′N, 15°07.200′E to 32°01.279′N, 15°07.281′E), situated along a marsh, is characterized by numerous date palms (*Phoenix dactylifera* L.) and surrounded by dense vegetation of halophytes that harbor numerous rodent burrows. Al Kefah (31°58.773′N, 15°04.956′E to 31°58.695′N, 15°04.587′E) is a region of date palms mixed with almond trees, with many sheep and goat barns. Al Fateh (32°01.480′N, 15°02.327′E to 32°01.602′N, 15°02.201′E) is a region of cultivated onion and clover and scattered date palm trees interspersed with sheep barns and chicken coops.

Sand fly trapping

Adult sand flies were collected in June, August, September, and November 2010, using 4 traps at each collection site: 2 US Centers for Disease Control and Prevention (CDC) miniature light traps (model 2836; Bioquip Products, Rancho Dominguez, CA), a BG-Sentinel (BGS) trap (BioGents AG, Regensburg, Germany), and a miniature CDC ultraviolet light (CDCUV) trap (model 2848; Bioquip Products). With the exception of the BGS trap, all traps were placed along a transect 30 m from each other, mounted on a 2-m metal pole, and suspended 0.5 m above ground level. The CDC, CDCUV, and BGS traps were baited with 1 kg of dry ice (CO_2). The BGS trap does not use light as an attractant and was originally designed to capture diurnal biting *Stegomyia* mosquitoes (Hoel et al. 2010). The 2nd CDC trap served as a control (CDCC) and contained no CO_2. Traps were set at 1800 h and collected the following morning at 0700 h, constituting 1 trapping period. Traps were set for 5 consecutive nights each month (1 trial), randomizing their position to eliminate any within-site bias. Sand flies were euthanized by placing them in a dry-ice cooler for 1 h, enumerated, and dispensed into 1.5-ml plastic microcentrifuge tubes containing a 75% ethanol solution.

Sand fly identification and *Leishmania* testing

The head and terminal abdominal segments of all sand flies were slide-mounted, sexed, and identified to species using taxonomic keys of Lane (1986) and Lewis (1982). A subsample of female *Phlebotomus* spp. sand flies (8–20%) was retained for voucher specimens but not tested for *Leishmania*; the alimentary canal of remaining specimens was removed and preserved in 75% ethanol for polymerase chain reaction (PCR) testing. One to 5 females of the same species were pooled for *Leishmania* detection. The DNA was extracted from 456 such pools using the QIAGEN DNA Mini Kit (QIAGEN, Valencia, CA) and stored at −20°C. *Leishmania* DNA was analyzed using real-time PCR as previously described (Villinski et al. 2008). Each real-time PCR experiment included a positive control (DNA from a reference strain: *L. major* (Yakimoff and Schokhor), IPAP/EG/89/SI-177 and *L. tropica*, WR664) and a negative control (water). A subset (ca. 10%) of *Leishmania*-negative DNA pools were tested to verify DNA integrity by amplifying the sand fly 18Sr DNA gene (unpublished assay).

Statistical analysis

A randomized complete block design with sites as the blocking effect was used to determine capture rates between different trap types. Data were $\log_{10}(n + 1)$ transformed prior to statistical analysis, and effects of trap type, trap location (county), and trial (time of year) were evaluated by 3-way ANOVA using SPSS software v. 11.0.1 (SPSS 2001). Only untransformed data are presented in the text and tables. Statistical analyses were conducted using PROC GLM and multiple-means comparisons were made using Tukey's multiple range test ($\alpha = 0.05$). Data from malfunctioning traps were recorded and treated as missing values.

Table 1. Total sand flies captured and numbers of polymerase chain reaction pools submitted for *Leishmania* spp. testing from 5 female *Phlebotomus* species collected in 3 counties of Mesrata, Libya, during June, July, September, and November 2010.

Species	Total (%)	No. males	No. females	No. tested	No. pools[1]	No. pools positive for *Leishmania*
Phlebotomus longicuspis	1,773 (54.6)	1,051	722	671	242	1
P. papatasi	1,221 (37.6)	462	759	673	209	2
P. perniciosus	77 (2.4)	77	0			
P. langeroni	11 (0.3)	6	5	4	4	0
P. sergenti	6 (0.2)	5	1	1	1	0
Sergentomyia minuta	140 (4.3)	71	69			
S. antennata	16 (0.5)	16	0			
S. fallax	4 (0.1)	1	3			
Total	3,248	1,689	1,559	1,349	456	3

[1] Each pool contained 1 to 5 sand flies.

RESULTS

Thirty trap-nights, replicated 4 times, resulted in a total of 120 trap-nights (30 trap-nights/trial) and the capture of 3,248 sand flies, representing 8 species from 2 genera (Table 1). *Phlebotomus papatasi* and *P. longicuspis* represented 37% and 54% of the sand fly capture, respectively, and were subsequently analyzed for statistical differences among traps. Males comprised 52% of the total sand fly capture, with more male *P. longicuspis* (59%) collected compared to *P. papatasi* (38%). No female *P. perniciosus* Newstead or *Sergentomyia antennata* Newstead were collected from any traps (Table 1). Of the 3,248 sand flies captured, 1,349 were tested for *Leishmania*. Three pools were positive for *Leishmania* spp., indicating a minimum of 0.83% and 0.47% estimated infection rate for *P. papatasi* and *P. longicuspis*, respectively (Table 1). All positive pools were negative for *L. major*. Screening for other *Leishmania* spp. was not possible at the time due to logistical issues caused by civil unrest. However, given the published reports of *Leishmania* prevalence within Libya, it is likely, but not confirmed, that these sand flies were infected with *L. tropica*.

The statistical model tested for 3 effects: trap type, trap location (county), and trial (time of year). All 3 effects significantly contributed to the pattern of capture data observed in this study. Significantly more *P. longicuspis* were captured in the CDCUV (5.7 ± 0.8) and CDC (4.5 ± 0.3) traps compared to the CDCC (3.9 ± 0.6) and BGS traps (1.8 ± 0.2; Table 2) ($P < 0.001$). However, all 3 traps captured significantly more *P. papatasi* when compared to the BGS trap (Table 2). Based on data combined across collection dates and times, the CDC trap captured 8 sand fly species compared with 7, 7, and 5 for the CDCUV, CDCC, and BGS traps, respectively (Table 2). Trap location (county) had a significant effect in the model ($F = 23.95$, $df = 2,466$; $P < 0.001$) with more sand flies collected at Al Sawaedek (9.6 ± 1.5) compared to Al Kefah (5.9 ± 0.7) and Al Fateh (3.7 ± 0.5). This result is not unexpected as one would presume that basal population levels would vary among sites. Trial (time of year) also had a significant effect in the model. More sand flies were captured during August (3.2 ± 0.2) and September (3.7 ± 0.3) than in June (2.5 ± 0.2) and November (2.1 ± 0.3; $F = 123.6$; $df = 3,448$; $P < 0.001$). The greatest number of sand flies captured occurred during September ($n = 1,327$), whereas the fewest were collected during November ($n = 194$). This seasonal variation is a typical pattern for sand flies in this region and serves to underscore the times of year when risk of infection, and possibly sand fly control efforts, may be maximized (Ashford et al. 1977, El-Buni et al. 1993).

DISCUSSION

Our results demonstrate that CDCUV and CDC traps captured considerably more sand flies compared to BGS traps. Conversely, a previous study in Egypt demonstrated that BGS traps were more effective than CDC light traps (Hoel et al. 2010). Though they found no significant difference in capture rates among trap types, their study demonstrated that *P. papatasi* were collected 3-fold more often in the BGS trap compared to the CDC light trap. However, traps in their study were baited with twice as much dry ice (CO_2) compared to ours, which may influence the apparent inconsistencies between studies. When traps were collected in our study, no dry ice was observed in any of the containers. Therefore, a reduction of dry ice likely reduced the concentration and or the duration of CO_2. More sand flies were collected at Al Sawadek than at the other 2 sites. This area is characterized by abundant date palms, rodent burrows, and domestic livestock, all of which can contribute to high sand fly population density. Increased soil moisture from animal manure and bedding, which provides ideal sand fly oviposition and larval feeding sites, has been identified as an

Table 2. Numbers (mean ± SE) of 8 sand fly species collected in a trapping period from 4 trap types in 3 counties of Mesrata, Libya, during June, July, September, and November 2010.[1]

Species	Trap[2]				F	*P*
	CDCUV	CDC	CDCC	BGS		
Phlebotomus longicuspis	5.7 ± 0.8a	4.5 ± 0.3a	3.9 ± 0.6b	1.8 ± 0.2c	35.81	<0.0001
P. papatasi	3.2 ± 0.3a	2.9 ± 0.2a	2.6 ± 0.2a	1.8 ± 0.1b	15.70	<0.0001
P. perniciosus[3]	2.1 ± 0.6	2.3 ± 0.6	2.2 ± 0.5	1.0		
P. sergenti[3]	1.3 ± 0.3	1.0	0	1.0		
P. langeroni[3]	1.0	1.0	1.0	0.0		
Sergentomyia minuta[3]	1.6 ± 0.1	1.3 ± 0.1	1.2 ± 0.1	1.0		
S. antennata[3]	1.3 ± 0.3	1.0	1.0	0.0		
S. fallax[3]	0.0	1.0	1.5 ± 0.5	0.0		

[1] $n = 120$ trap-nights per trap. Means followed by the same letter on the same line are not significantly different ($P > 0.05$), Tukey's mean separation applied to $\log(n + 1)$-transformed data.
[2] Trap types: BGS, BG-Sentinel trap; CDC, Centers for Disease Control and Prevention miniature light trap; CDCC, nonbaited control CDC trap; CDCUV, miniature CDC ultraviolet light trap. With the exception of the CDCC trap, all traps were baited with 1.0 kg of dry ice.
[3] No statistical analyses conducted as collections were nominal.

environmental risk factor for *L. major* infection among farm workers in Libya (Fathy et al. 2009). Previously in Egypt, Hoel et al. (2010) showed that more sand flies were collected in sites having a greater number of date palms and concluded that date palms may serve as important sugar sources and resting sites for adult sand flies.

Light source and bait type are known to affect trap efficacy for sand flies and other insects. Burkett et al. (2007) determined that a similar style of ultraviolet CDC trap was effective at collecting large numbers of sand flies in Iraq compared to the common incandescent CDC light trap. However, their study compared nonbaited CO_2 light traps only. Carbon dioxide is a known host-seeking cue for many female phlebotomine sand flies and is often used to improve sand fly trap yields, especially among anthropophilic sand fly species (Alexander 2000). Traps baited with CO_2 alone (without light) are known to attract significantly more *P. papatasi* compared with nonbaited CO_2 traps (Beavers et al. 2004). However, in our study all light traps, including the nonbaited CO_2, captured significantly more *P. papatasi* and *P. longicuspis* compared to the BGS trap (Table 2). Significantly more *P. longicuspis* were captured in the CO_2-baited light traps compared to light alone (Table 2), suggesting light may increase sand fly captures in the presence of CO_2. The apparent preference of these 2 species for light traps compared with CO_2 alone necessitates further evaluation. Additional sand fly surveillance is warranted for this region of Libya to assess the effectiveness of Libyan NCDC leishmaniasis control programs. While light traps are typically used to gauge the success of control measures by the number of sand flies captured, interpretations should be guarded as few sand flies may actually come in contact with a control area (Alexander 2000). Unfortunately, severe civil unrest ensued in March 2011 in this region of Libya, preventing any further evaluation of sand fly surveillance and control strategies.

Our sand fly fauna results are similar to previous studies conducted by Ashford et al. (1977) and El-Buni et al. (1993), demonstrating that *P. longicuspis* and *P. papatasi* composed the majority of sand flies captured in Libya. We determined that DNA pools extracted from *P. papatasi* contained *Leishmania* DNA. Although we excluded *L. major* as the causative agent, we were unable to confirm the parasite species.

Relatively few *Leishmania*-infected sand flies were collected during this study; minimum infection levels were estimated between 0.47 and 0.83. Given the numerous rodent burrows found throughout the area, and cost of dry ice and trap maintenance, it may prove useful to supplement sand fly surveillance with other collection methods for this region. For example, sticky traps placed at the entrance of rodent burrows are effective at collecting adult sand flies (Obenauer et al. 2011) and may provide a better assessment of sand flies infected with *Leishmania* spp. To develop a comprehensive sand fly surveillance and control strategy for Libya, investigations into environmental factors affecting the vector distribution and parasite burden among the indigenous population are warranted. The increased incidences of CL have been attributed to transmission cycle changes from sylvatic to peridomestic environments and are usually caused by a displacement of susceptible individuals into a *Leishmania*-endemic area and are often triggered by natural disasters or armed conflicts (Reithinger et al. 2007). The current civil unrest in Libya may contribute to further CL transmission, and additional resources will likely be required to reduce the number of CL cases to levels reported before the unrest.

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