Comparison of Three Carbon Dioxide Sources on Phlebotomine Sand Fly Capture in Egypt

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ABSTRACT

Lighted Centers for Disease Control and Prevention (CDC) light traps were baited with carbon dioxide (CO2) produced from three different sources to compare the efficacy of each in collecting phlebotomine sand flies in Bahrif village, Aswan Governorate, Egypt. Treatments consisted of compressed CO2 gas released at a rate of 250 ml/min, 1.5 kg of dry ice (replaced daily) sublimating from an insulated plastic container, CO2 gas produced from a prototype FASTGAS (FG) CO2 generator system (APTIV Inc., Portland, OR), and a CDC light trap without a CO2 source. Carbon dioxide was released above each treatment trap’s catch opening. Traps were placed in a 4 × 4 Latin square designed study with three replications completed after four consecutive nights in August 2007. During the study, 1,842 phlebotomine sand flies were collected from two genera and five species. Traps collected 1,739 (94.4%) Phlebotomus papatasi (Scopoli), 19 (1.0%) Phlebotomus sergenti, 64 (3.5%) Sergentomyia schwetzi, 16 (0.9%) Sergentomyia palestinensis, and four (0.2%) Sergentomyia tiberiadis. Overall treatment results were dry ice (541) > FG (504) > compressed gas (454) > no CO2 (343). Total catches of P. papatasi were not significantly different between treatments, although CO2-baited traps collected 23–34% more sand flies than the unbaited (control) trap. Results indicate that the traps baited with a prototype CO2 generator were as attractive as traps supplied with CO2 sources traditionally used in sand fly surveillance efforts. Field-deployable CO2 generators are particularly advantageous in remote areas where dry ice or compressed gas is difficult to obtain.

KEY WORDS compressed CO2, Phlebotomus papatasi, CO2 generator system, surveillance, dry ice

The Old World phlebotomine sand fly Phlebotomus papatasi (Scopoli) is the primary vector of cutaneous leishmaniasis in North Africa, the Middle East, and Central Asia, including urban and rural areas of Afghanistan (Nadim and Rostami 1974, Faulde et al. 2006, Faulde et al. 2008) and Iraq (Mohsen 1983; Coleman et al. 2006, 2007).

Adult sand fly surveillance is of utmost importance for monitoring population trends and identifying pertinent vector species. Surveillance is conducted chiefly with CDC light traps (Alexander 2000, Faulde 2007). When traps are baited with CO2, sand fly capture can increase by an order of magnitude over those of nonbaited traps (Orshan et al. 2010). In addition, mammalian kairomones, such as lactic acid (Acree et al. 1968, Smith et al. 1970), octenol (French and Kline 1989), phenols (Kline et al. 1990), and acetone (Vale 1990, Mihok et al. 2007), have been blended with CO2 to increase trap catches of mosquitoes, tabanids, and tsetse flies. These same kairomones are being evaluated as synergists for CO2-baited sand fly traps (Dougherty et al. 1999, Beavers et al. 2004, Bernier et al. 2008).

In the United States, CO2 compressed in cylinders or tanks is sometimes used to bait traps for mosquito studies (Carestia and Savage 1967, Service 1993). Unfortunately, CO2 in this form is not readily available in North Africa, the Middle East, and the Central Asian Republics, and the size and weight of the cylinders make the transport and use of large numbers impractical. Another form of CO2 more commonly used in the United States is dry ice or frozen CO2 (Newhouse et al. 1966, Bordash et al. 1972). Dry ice can only be maintained for short periods and, like CO2 compressed in tanks, it is difficult to obtain in the Middle East and surrounding countries.

Public health and preventive medicine personnel working in the Middle East and surrounding countries need a source of CO2 that is easy to ship, will store for long periods, and will provide the quantities necessary to attract sand flies and other blood-feeding insects to surveillance traps. A prototype chemical CO2 gener-
ator has been developed to meet these requirements. The objective of our study was to evaluate this chemical CO₂ generator and determine its relative effectiveness for phlebotomine sand fly attraction compared with dry ice or compressed CO₂. The logistical advantages and disadvantages of using chemical CO₂ generators compared with dry ice or compressed CO₂ also are addressed.

Materials and Methods

The study was conducted in the small farming community of Bahrif, located on the east bank of the Nile River ~10 km north of Aswan in southern Egypt. Bahrif has been used by U.S. Naval Medical Research Unit No. 3 (NAMRU-3) personnel as an ideal study location for sand fly research over the past 30 yr owing to its abundance of sand flies and the absence of leishmaniasis in the surrounding area (Hoel et al. 2007). Hogsette et al. (2008) describes the agricultural produce and local flora and fauna in Bahrif, and Hanafi et al. (2001) provide a map of the village’s location in Egypt.

The four CDC light traps (model 512, John W Hock Co., Gainesville, FL) used in this study were equipped with CM-47 incandescent lamps. One trap without a CO₂ source (untreated control) was compared with the other three traps, each supplied with CO₂ from dry ice, compressed gas or the prototype chemical CO₂ generator.

For the dry ice source, ~1.5 kg of dry ice were added nightly to a modified Igloo drink cooler (John W Hock Co.) with a 13-mm hole in the bottom center. A clip on the bottom of the cooler allowed a CDC light trap to be attached and separated from the cooler by ~6 cm. Thus, the cooler was suspended with the CDC trap affixed below.

For the compressed CO₂ source, CO₂ was released from 9-kg steel tanks metered at 250 ml/min by using a 5-psi single stage regulator with a 0.007 flow rate orifice microregulator (Clarke, Roselle, IL). The CO₂ flowed to the CDC trap through 6.4-mm Tygon tubing (Saint-Gobain Performance Plastic, Akron, OH) with the open end taped to the underside of the rain shield within 3 cm of the trap intake.

The FASTGAS (FG) system (APTIV Inc., Portland, OR) consisted of a mixture of two powdered food-grade reagents (900 g each) held in a 5-liter airtight plastic container with tubing connected to a gas outlet nozzle (Fig. 1). A gravity-fed water reservoir metered 1.5 liters of tap water through a restricted orifice (0.25 mm i.d. and 10 mm long) at the rate of 1.2–1.5 ml/min. The generator was ground mounted with its Tygon CO₂ exhaust tube taped to the underside of the rain shield within 3 cm of the trap intake. This low pressure system putatively produced from 200 to 300 ml of CO₂/min for ~12 h (P.K., personal observation).

All traps were activated within 1 h of sunset (1900–2000 hours) and trap contents collected within 1 h after sunrise (0600–0700 hours). Trap sites were ~30 m apart in a date palm (Phoenix dactylifera L.) grove between Bahrif village and the Nile River. Traps were hung on the base of the palms with the trap openings ~90 cm above the ground. The incandescent light produced by a trap at each particular trapping site was not visible from the other trapping sites. Trapping was performed in August 2007 to coincide with peak summer sand fly populations that occur in Bahrif (Hoel et al. 2007, Bernier et al. 2008). Approximately 95% of Bahrif’s sand fly populations has historically consisted of P. papatasi (H.A.H., personal observation), the primary vector of cutaneous leishmaniasis in Egypt. During the study, mean temperatures ranged from 25 to 43°C, relative humidity was usually ~20%, and wind speeds <10 km/h, with no precipitation.

Collected sand flies were anesthetized in large Igloo coolers (Igloo Products Corp., Katy, TX) stocked with dry ice, subsequently stored in 70% ethanol and transported to the NAMRU-3 laboratory for identification to species by using the taxonomic keys of Lane (1986).

Trap treatments were evaluated in a 4 × 4 Latin square design replicated three times for a total of 48 trap nights (12 trap nights for each treatment). Each trap treatment was set at one of four trapping sites for one night and rotated to the next trapping site on the following day. Sand fly trap data were normalized using log₁₀(n + 1) and subjected to two-way analysis of variance (ANOVA) to assess the effect of treatment (i.e., no CO₂, compressed CO₂, dry ice, and chemically generated CO₂ [FG system]) and trapping site on the
variance in the catch data. When ANOVA was significant, a post hoc analysis was performed using Tamhane’s t-test (equal variances not assumed). Statistical analysis was performed only for P. papatasi sand flies that represented the vast majority of the total catch. All analyses were performed using the SPSS statistical software package (SPSS Inc. 2005). Unless otherwise stated, α = 0.05.

Results and Discussion

In total, 1,842 sand flies in two genera and five species were captured over the 12-night trapping period. These species included P. papatasi (Σ = 1,739), Phlebotomus Sergenti (Parrot) (Σ = 19), Sergentomyia schwetzi Adler, Theodor & Parrot (Σ = 64), Sergentomyia palestinensis Adler & Theodor (Σ = 16), and Sergentomyia tiberiadiis Adler, Theodor & Lourie (Σ = 4), which represented 94.4, 1.0, 3.5, 0.9, and 0.2% of the catch, respectively (Table 1). Overall, females represented >50% of the catch for each species. The overall trap success for P. papatasi was dry ice (508) > FG (465) > compressed gas (432) > no CO2 (334) (Table 1).

The main effects model was not significant for treatment or trapping site (F = 1.29; df = 15, 47; P = 0.263), but, when separated by sex, catches of male P. papatasi differed significantly between treatments (F = 2.92; df = 3, 47; P = 0.044), although females did not (Fig. 2). However, with P. papatasi females there was a trend toward lower catches with the no CO2 treatment. Significantly more male P. papatasi were collected in traps baited with dry ice than with the no CO2 treatment; however, there were no significant differences between males captured by any of the treatments where CO2 was used (Fig. 2). Females of all species were collected in every treatment. The same was true for males, with the exception of males of P. sergenti and P. tiberiadiis, which were collected only in traps baited with the FG generator.

A common problem encountered in the field by personnel tasked with making vector-borne disease threat assessments is a lack of readily available CO2 for use with trapping systems (Coleman et al. 2006, 2007, 2009). As mentioned, dry ice can be difficult to obtain, and there is a rapid loss rate if not used quickly. Compressed gas cylinders offer the most consistent flow rates of any CO2 delivery system; however, regulators are expensive and the tanks are heavy, bulky and hard to conceal, making them easy targets for theft.

The prototype FG CO2 generator system seems to be a good alternative to the dry ice and compressed gas methods. The FG generator system compared well in our studies against dry ice and compressed CO2 delivery methods (Table 1; Fig. 2). It captured males of two species that were not captured by the other two methods (Table 1).

The FG system is simple to deploy in the field and requires nothing more than water to activate. It has no moving parts, is easy to assemble and is only slightly bulkier and heavier (3 kg) than modified Igloo coolers (John W Hock Co.) used in our studies to hold the dry ice. The use of commercial off-the-shelf components, food grade chemicals, drinking water, and nonhazard-

Table 1. Number of males and females and percentage of total females (±95% CI) of P. papatasi, P. sergenti, S. schwetzi, S. palestinensis, and S. tiberiadiis sand flies captured in CDC light traps baited with three sources of CO2 (or without CO2) and operated with incandescent light over 12 nights in Bahrif, Egypt, during August 2007.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>P. papatasi</th>
<th>P. sergenti</th>
<th>S. schwetzi</th>
<th>S. palestinensis</th>
<th>S. tiberiadiis</th>
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| aTreatment 1, dry ice; treatment 2, FG generator; treatment 3, compressed gas; treatment 4, no CO2.
| b% Σ %, percentage of total females.
| c% Σ %, percentage of total females.
| dΣ, grand sum.

Fig. 2. Mean ± SE catches of male and female P. papatasi sand flies captured in CO2-baited or unbaited CDC light traps operated with incandescent light. CO2 sources consisted of dry ice (1), an FG CO2 prototype generator system (2), or compressed CO2 (3). Catches were performed over 12 nights in Aswan, Egypt, during August 2007. Asterisk (*) indicates significantly fewer males trapped compared with dry ice treatment.
ous waste makes the FG system inherently safe, and it requires no special training or protective gear to use. In addition, reagents can be stored indefinitely at ambient temperatures until needed (as long as they are kept dry).

There are some minor disadvantages of using this system. One is a delay in full capacity CO$_2$ generation (200–300 ml/min) upon activation, but this is only by 5–10 min. Another is the reagents, which must be properly mixed to achieve maximum CO$_2$ generation, or the water orifice, which can become clogged. If this occurs, CO$_2$ production is diminished or stops completely. We experienced neither of these malfunctions during our study.

Although CO$_2$ flow rates were not quantified hourly during this study, all three systems were still producing CO$_2$ during morning trap catch collections. Igloo coolers still contained small amounts of dry ice, and compressed gas cylinders and FG containers showed positive pressure from their exhaust tube ports. A manufacturer (APTIV Inc.) study indicated that the FG system produced CO$_2$ at $\approx$260 ml/min for 14 h at 21°C and 360–460 ml/min for 10 h at 35°C by using identical water delivery rates and reagent volumes. At steady temperatures, CO$_2$ production tends to increase during the first 3–4 h from 200 to $>$400 ml/min and then stabilize for another 5–7 h, followed by a fairly rapid drop below 200 ml/min. In a second independent laboratory study, hourly CO$_2$ production averaged $\approx$300 ml/min over a 12-h period, with production dropping rapidly after 12 h and ceasing at 16 h (P.K., personal observation).

Like dry ice, CO$_2$ production levels from the FG system are positively correlated with ambient air temperatures. However, given the high ambient nighttime temperatures common to North Africa and Middle Eastern regions during the summer sand fly season (25–27°C mean low temperatures), it is reasonable to expect that this generator system would produce a minimum of 200 ml/min of CO$_2$ for at least 8 h. Thus, optimal CO$_2$ levels would be produced during hours of peak sand fly activity, which is mainly from dusk to several hours past midnight (El Said et al. 1986; Morsy et al. 1993).

In summary, CO$_2$ gas produced by a prototype gas generator, the FG CO$_2$ generator system, performed as well as the standard CO$_2$ sources (e.g., dry ice, compressed gas) used with CDC light traps for sand fly surveillance. This particular generator system uses nonhazardous food grade chemicals and water to generate CO$_2$ throughout the night at rates comparable with dry ice and compressed gas systems. The cost of this model has not yet been given nor has production begun; however, with respect to safety, effectiveness, ease of use, and in terms of logistical burden, we found the FG generator system competitive with dry ice and compressed gas for use with CDC light traps. Development of this prototype and other similar commercial CO$_2$ generation systems will enable entomologists and public health personnel to enhance sand fly surveillance efforts in remote settings where dry ice or compressed gas is difficult, if not impossible, to obtain.

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