Comparative field study to evaluate the performance of three different traps for collecting sand flies in northeastern Italy

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ABSTRACT: Three standard methods for collecting sand flies (sticky trap, CDC light trap, and CO_2 trap) were compared in a field study conducted from June to October, 2012, at a site located in the center of a newly established autochthonous focus of canine leishmaniasis in northeastern Italy. Six traps (two sticky traps, two CDC light traps, and two CO_2 traps) were activated at the same time for a single night every two weeks during the season of sand fly activity. A total of 5,667 sand flies were collected and 2,213 identified, of which 82.1% were *Phlebotomus perniciosus*, 17.4% *P. neglectus*, 0.3% *Sergentomya minuta*, and 0.2% *P. mascitti*. The performances of all traps were influenced by their position inside the site, increasing with proximity to the animal shelters. CO_2 traps were more attractive for females of *P. perniciosus* and *P. neglectus*. CDC light traps showed an intermediate efficiency and were more attractive for *P. neglectus*, compared to other two traps. Results suggest that in northern Italy the CO_2 trap is a suitable sampling method for sand fly monitoring programs that include transmitted pathogen surveillance. ***Journal of Vector Ecology* 38 (2): 374-378. 2013.**

Keyword Index: *Phlebotomus mascitti*, *Phlebotomus neglectus*, *Phlebotomus perniciosus*, sampling methods, sex ratio, Italy.

INTRODUCTION

Over the last decades there has been a significant resurgence and northward spread of sand flies and sand fly-borne diseases in Europe (Dujardin et al. 2008, Maroli et al. 2013), where sand flies of the genus *Phlebotomus* are vectors of *Leishmania infantum*, the causative agents of canine leishmaniasis (CanL), cutaneous and visceral zoonotic human leishmaniasis, and viral diseases (Tesh et al. 1976, Maroli et al. 2013). The increase in sand flies and sand fly-borne diseases can be due mainly to ecological and climatic changes (Fisher et al. 2011) and to the increases in human migration, movement of infected dogs, and trends of globalization (Ferroglio et al. 2005, Aspock et al. 2008, Otranto et al. 2009, Maroli et al. 2013). In order to prevent the emerging risk of increasing sand fly distribution and density, and to develop methods for sand fly control, a correct approach to surveillance is necessary in Europe. A uniform method of collection is necessary to generate comparable entomological data, to understand vector ecology, and to obtain an improving knowledge of their bionomics and distribution. Several studies aiming to compare different sampling methods have been implemented for these goals (Kaul et al. 1994, Kasap et al. 2009, Kasili et al. 2009). The most commonly used methods to capture sand flies are sticky traps (non-attractant traps, consisting of paper impregnated with castor oil, useful to establish the density and seasonal trend of sand flies in an area), Centers for Disease Control (CDC) light traps, and CO_2 traps (suction traps that use a light source or dry ice as bait, respectively) (Alexander 2000). This paper describes a field study designed to test the different efficiency of these three traps, currently and routinely used in sand fly monitoring programs, and to evaluate which of these sampling devices should be used, according to the specific objectives of each entomological sampling program.

MATERIALS AND METHODS

Study area

The field study was conducted in one site (N 45.24942, E 11.67343) located in Calaone village (municipality of Baone), in the southern Colli Euganei, an isolated hilly area of the Province of Padova, in the central part of the Veneto Region (northeastern Italy). This site, at an altitude of 178 m above sea level (a.s.l.), was selected due to the evidence that Calaone village is located in the center of a newly established autochthonous focus of CanL in Colli Euganei area (Cassini et al. 2013) and that this specific site showed a high sand fly density.

Furthermore, environmental parameters (typical sub-Mediterranean climate) and the structure of the site area, such as the presence of different species of domesticated animals, animal shelters, soil rich in organic material, and dry walls with cracks and crevices, created the essential characteristics for sand fly resting and breeding sites (Killick-Kendrick 1999, Feliciangeli 2004).

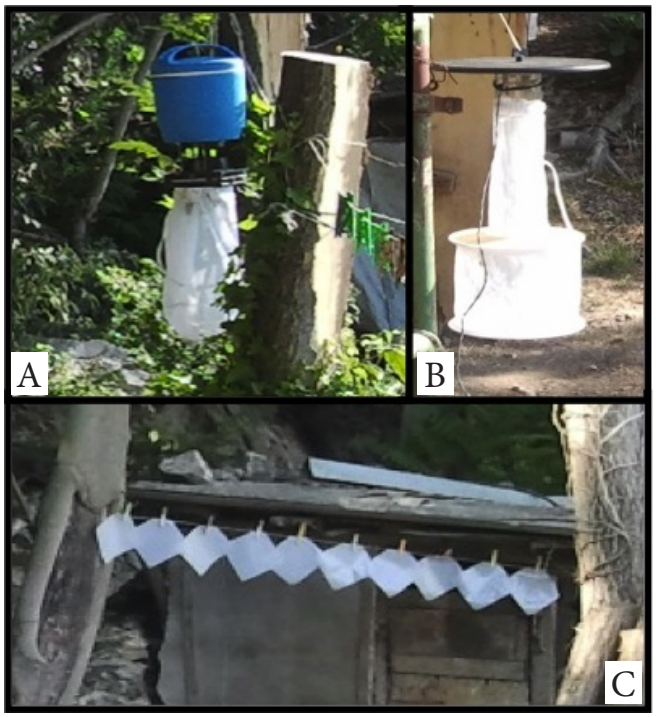

Figure 1. CO_2 gas trap (A), CDC light trap (B), and sticky trap (C: one trap is composed of ten papers) at collection points in the study site.

Trapping methods

Sand fly trapping was conducted during the active season (from 15th June to 3rd October, 2012), using six traps of three different types (two sticky traps, two CDC light traps, and two CO_2 traps): sticky traps (each one composed of ten papers 20x20 cm coated with castor oil), CDC light traps (Bioquip Products, Rancho Dominguez, CA, U.S.A.), and CDC-CO_2 traps (Byblos, Cantù, CO, Italy) filled with 1 kg dry ice (Figure 1). All traps were activated simultaneously, from 19:00 to 07:00, for a single night every two weeks. Captures were repeated for nine nights. Each trap was hung at a height of approximately 1.5 m above the ground. Figure 2 shows the position of the traps and animal shelters. Traps of the same type were located one in front of the other, near and far from the animal shelter (position 1 and 6), approximately 5 m distant. Therefore, for each capture, three traps were arranged near the animal shelters and three distant. The trap positions were changed every sampling day: the traps in positions 1 and 6 were moved toward positions 2 and 5; the traps located in places 2 and 5 moved toward positions 3 and 4; and the traps in places 3 and 4 moved toward positions 1 and 6. Collected specimens were identified and stored in 70% ethanol. At the end of the field trial, sand flies were mounted in Hoyer's medium and observed by microscopy for gender separation and identification up to species level, according to morphological features (Romi et al. 1994).

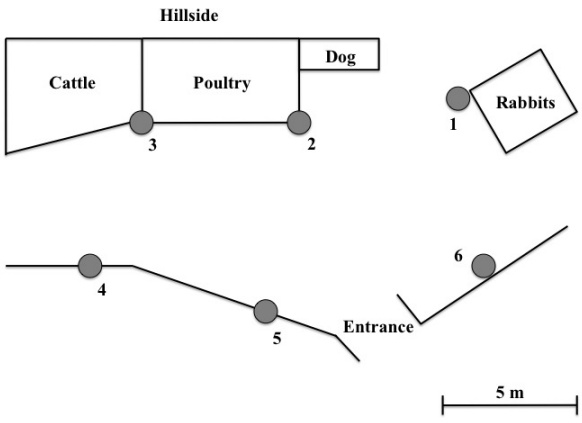

Figure 2. Design of the study site with the position of traps and animal shelters.

Statistical analysis

Data on the total number of sand flies captured for each night and each trap were log-transformed to ensure normality (Shapiro-Wilk test), and Levene's test was implemented to check homogeneity of variances among groups. The analysis of variance was conducted with a two-way ANOVA, to assess the effect of type of trap (sticky, CDC light, CO_2 traps) and location (near or far from animal shelters) on the number of sand flies collected.

In order to estimate the number of different species and sex ratio of sand flies collected, a representative number of specimens (at least 100 sand flies) was identified for each sample (sample=total number of sand flies collected in one trap during one capture night). The total numbers of different species and the number of males and females for each species were estimated, applying the ratio among the identified specimens to the total number of sand flies collected for each sample (one trap/night). The effect of the type of trap on the species collected and sex within species captured was evaluated, comparing the relative abundance obtained from the sum of all the captures using the Pearson chi-square test.

RESULTS

A total of 5,667 sand flies was collected. Table 1 shows the total number of sand flies collected by the three traps, in relation to the position. Approximately, 66.7% of the phlebotomine fauna was collected by CO_2 traps, 24.8% using CDC light traps, and 8.4% with sticky traps. The two-way ANOVA highlighted a significant difference among the performances of the three different sampling methods ($F=4.214$; $DF=2$; $p=0.021$), and also the position (near or far from animal shelters) significantly influenced ($F=7.865$; $DF=1$; $p=0.007$) the number of captured sand flies. The interaction effect was not statistically significant ($F=0.772$; $DF=2$; $p=0.468$). Estimated marginal means are reported in Table 2.

Of the collected sand flies, a total of 2,213 specimens was identified. Among identified phlebotomine fauna, *Phlebotomus perniciosus* (Newstead 1911) was found to be the most abundant species (n=1,816; 82.1%), followed by *P. neglectus* (Tonnoir 1921) (n=386; 17.4%), *Sergentomya minuta* (Rondani 1843) (n=6; 0.3%), and *P. mascitti* (Grassi 1908) (n=5; 0.2%). Concerning these last two species, only

Table 1. Sand flies collected by sticky, CDC, and CO_2 traps, according to their position.

Traps	Distance to animal shelters	Captures (n)	Sand flies collected (n)	Median	Min	Max
Sticky	nearby	9	442	46	2	115
	far away	9	34	3	1	12
	Total	18	476	5.5		
CDC	nearby	8*	978	98.5	1	259
	far away	8*	431	26	1	160
	Total	16	1,409	60		
CO_2	nearby	8*	3,227	174	1	1,252
	far away	9	555	53	2	169
	Total	17	3,782	72		
Total		51	5,667	41		

*The trap failed to work on one night.

Table 2. Estimated Marginal Means of two-way ANOVA.

Position	Type of trap	n	Mean	Std. Error	95% C.I.
Nearby	Sticky	9	3.2940	0.5998	2.0859 to 4.5020
	CDC	8	3.8193	0.6362	2.5380 to 5.1006
	CO_2	8	4.4604	0.6362	3.1791 to 5.7417
Far away	Sticky	9	1.0319	0.5998	-0.1761 to 2.2400
	CDC	8	3.0333	0.6362	1.7520 to 4.3146
	CO_2	9	3.2614	0.5998	2.0534 to 4.4695

Table 3. Comparison between relative abundance of *P. perniciosus* and *P. neglectus*, according to the type of trap.

Species	Sticky N (%)	CDC N (%)	CO_2 N (%)	χ^2	p-value
P. perniciosus	414 (87.5%)	1,038 (73.9%)	3,342 (89.3%)	770.80	<0.001
P. neglectus	59 (12.5%)	366 (26.1%)	401 (10.7%)		
TOTAL	473	1,404	3,743		

Table 4. Comparison between relative abundance of male and female *P. perniciosus* and *P. neglectus*, according to the type of trap.

	Sex	Sticky N (%)	CDC N (%)	CO_2 N (%)	χ^2	p-value
P. perniciosus	male	355 (85.7%)	863 (83.1%)	2,160 (64.6%)	171.23	<0.001
	female	59 (14.3%)	175 (16.9%)	1,182 (35.4%)		
	TOTAL	414	1,038	3,342		
P. neglectus	male	53 (89.8%)	290 (79.2%)	125 (31.2%)	208.18	<0.001
	female	6 (10.2%)	76 (20.8%)	276 (68.8%)		
	TOTAL	59	366	401		

females of *P. mascitti* were captured, three by CO_2 traps and two by CDC light traps, whereas *S. minuta* specimens (four males and two females) were caught by all types of trap. Of the identified flies, 1,573 (71%) were male and 640 (29%) female, for a sex ratio male/female of 2.46.

The trap efficiency for species and sex was compared only between *P. perniciosus* (estimated n=4,794) and *P. neglectus* (estimated n=826), because they represented the only two species with a significant abundance. Tables 3 and 4 show the relative abundance of these two species and inside each species for male and female with respect to the type of traps.

The CDC light trap was more efficient in capturing *P. neglectus* ($p<0.001$) compared to other traps, whereas the CO_2 trap was more attractive for females of both species ($p<0.001$).

DISCUSSION

The sticky trap, CDC light trap, and CO_2 trap are three standard methods for collecting adult sand flies during their periods of activity (Alexander 2000). Our study confirms that these traps differ in performance and that the CO_2 trap is more effective than the sticky trap in collecting sand flies and, at the same time, very similar in estimating the species composition (Veronesi et al. 2007, Kasap et al. 2009). The CDC light trap has an intermediate efficiency, which agrees with previous studies (Kasap et al. 2009). Besides, this trap is more attractive than the other two traps for *P. neglectus*, resulting in a different description of the species composition. This result may suggest a strong phototropism of this sand fly species.

Species with very low abundance, such as *P. mascitti*, may be difficult to detect using only non-attractive traps. In our case, this species was not captured by sticky traps. To our knowledge, this is the first report of *P. mascitti* in northeastern Italy. Furthermore, the study showed that all trap performances were affected by proximity to the animal shelters and, more generally, by the exact position chosen by the operator inside the site structures.

The overall percentage of male sand flies recorded (71%) during the study period is consistent with other published data (Reza and Mansour 2006, Kasap et al. 2009) and may be due to the 'lekking' behavior of the males (Killick-Kendrick 1999). However, this study showed a higher capacity of CO_2 traps to catch females of *P. perniciosus* and *P. neglectus* compared to the other two traps and agrees with previous studies (Maroli et al. 1997, Veronesi et al. 2007). It may represent an advantage for whenever sand fly monitoring associates the study of the ecological aspects with the detection of sand fly transmitted pathogens.

Although sticky traps constitute an inexpensive and simple method to randomly determine species composition and to provide more realistic estimation of sand flies densities than baited-traps (Alexander 2000), our study suggests that it may really be of low efficiency, mainly when the position chosen inside the site is not optimal. Out of a total of 5,667 sand flies captured by the six traps, only 34 were caught by the sticky trap located far away the animal shelters and therefore in the worse position. Besides, it is common knowledge (Maroli et al. 1997, Alexander 2000) that these traps are ineffective under particular climate conditions of high relative humidity, wind, and rain. CDC light traps are commonly used, associated with sticky traps, for sand fly monitoring activity in northern Italy, since the discover of the northward spread of CanL (Maroli et al. 2008). CO_2 traps are normally used for adult mosquito trapping during entomological surveillance activity in the study area (Mulatti et al. 2012), and their use in sand fly captures was only recently proposed in the country (Veronesi et al. 2007). According to our study, both types of traps seem to be more effective than sticky traps in terms of the total number of sand flies collected. However, CDC light traps could influence the correct assessment of species composition, preferentially attracting phototropic species, whereas the CO_2 trap showed higher performance in capturing sand fly females.

In conclusion, our results suggest that CO_2 traps are suitable for entomological surveys in northern Italy. In fact, their capacity to detect the presence of sand flies at low densities and their higher attractiveness for females (transmitted pathogens are found only in females) represent an advantage in areas characterized by emerging circulation of the phlebotomine fauna and increasing presence of pathogens transmitted by sand flies.

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