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Evaluation of efficacy of impregnated curtains in experimental hen houses as a phlebotomine control tool in northeast Argentina

M. MANTECA ACOSTA[1,2], M. S. SANTINI[2,3,4], A. A. PÉREZ[5] and O. D. SALOMÓN[1,2,4]

[1]Instituto Nacional de Medicina Tropical, Ministerio de Salud de la Nación, Puerto Iguazú, Argentina, [2]Red de Investigación de Leishmaniasis en Argentina (REDILA), Argentina, [3]Administración Nacional de Laboratorios e Institutos de Salud, Centro Nacional de Diagnóstico e Investigación en Endemo-Epidemias, Buenos Aires, Argentina, [4]Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina and [5]Facultad de Ciencias Exactas y Naturales, Departamento de Ecología, Genética y Evolución, Universidad de Buenos Aires, Buenos Aires, Argentina

Abstract. The aim of this study was to evaluate the effectiveness of insecticide-impregnated curtains against the entry of phlebotomine (Diptera: Psychodidae) flies into experimental slatted hen houses in an area endemic for American cutaneous leishmaniasis (ACL). Three treatments in experimental dwellings containing three chickens each were applied using, respectively, an impregnated curtain (IC), a non-impregnated curtain (NIC) and no curtain (NC). A control site without chickens (WC) was included. The study used permethrin at 0.05 g/m^2. During each month for 1 year, each experimental hen house randomly received all treatments. Phlebotomine sandflies were captured using REDILA BL traps placed inside the hen house. Significant differences in abundances of phlebotomine flies/trap/night were observed between treatments ($\chi^2 = 17853.58$, d.f. = 3, $P < 0.0001$): 59.7% of phlebotomines were captured in the NC treatment, 26.3% in the NIC treatment, 8.0% in the IC treatment and 6.1% in the WC condition. *Nyssomyia whitmani* (Antunes & Coutinho) was the most abundant species in all collections (89.9%). These results showed a lower abundance of phlebotomines in the experimental hen house in the IC condition than in the hen house in the NC condition ($P < 0.05$) and that the presence of NIC represents an effective physical barrier against phlebotomines ($P < 0.05$). Therefore, the use of curtains may be an alternative eco-friendly method for the prevention of indoor ACL transmission in slatted dwellings, which represent a common house type in northeast Argentina.

Key words. chickens, leishmaniasis, mosquito nets, permethrin.

Introduction

American cutaneous leishmaniasis (ACL) is an endemic disease in the northern region of Argentina, where *Leishmania braziliensis* (Vianna) (Kinetoplastida: Trypanosomatidae) represents its main etiological agent. This disease is present across a region of 500 000 km^2 in 10 provinces of Argentina and although the incidence of ACL has increased since 1980, epidemic outbreaks in the province of Misiones, at the northeast international border, started in 1998 (Salomón *et al*., 2006). The latest recorded outbreak in the area around the city of Puerto Iguazú (Misiones) occurred in 2004 in a rural area known as 'Dos Mil Hectáreas', where the phlebotomine sandfly *Nyssomyia whitmani* was found to naturally harbour parasitic DNA (Salomón *et al*.,

Correspondence: Mariana Manteca Acosta, Instituto Nacional de Medicina Tropical, Ministerio de Salud de la Nación, Neuquén y Jujuy, Puerto Iguazú, Misiones 3370, Argentina. Tel.: + 54 3757 425001/425002; Fax: + 54 3757 425001; E-mail: mariana.manteca@gmail.com

2009). This species was also the most prevalent in the focus, particularly in animal shelters close to the ecotone of patches of recent deforestation (Salomón *et al*., 2009; Mastrángelo & Salomón, 2010; Fernández *et al*., 2012). Phlebotomines have been controlled by employing chemical insecticides on the walls of human dwellings, animal shelters and other domestic buildings (Alexander & Maroli, 2003). However, the use of large amounts of insecticide in natural environments poses a danger to non-target organisms and is unfeasible for achieving adequate coverage in forested areas. Therefore, in less anthropized landscapes, the use of insecticide-treated fabrics represents one of the most sustainable methods of reducing the intradomiciliary transmission of *Leishmania* spp. in communities surrounded by forest, where the diurnal resting sites of vectors are unknown or inaccessible (Alexander & Maroli, 2003).

Many studies have assessed the effectiveness of insecticide-impregnated mosquito nets as a method of controlling vectors of leishmaniasis (Alexander *et al*., 1995; Picado *et al*., 2010; Noazin *et al*., 2013), malaria (Alaii *et al*., 2003) and dengue (Vanlerberghe *et al*., 2011), and have agreed upon the effectiveness and efficiency of this method. Mosquito nets are cheap, sustainable and simple to use for the community itself in the form of insecticide-impregnated bednets, curtains or screens (Alexander & Maroli, 2003). Synthetic pyrethroids as insecticides combine the properties of low to moderate mammalian toxicity (Zaim *et al*., 2000), low volatility and high insecticidal activity (Alexander & Maroli, 2003).

The Dos Mil Hectáreas focus in the current study area presents a scenario of wild cycle/wild transmission of ACL (Salomón *et al*., 2008). The dwellings at risk are located close to protected natural areas and hence insecticide spraying is not an option as a method of vector control. In this context, the aim of this study was to evaluate the effectiveness of insecticide-impregnated curtains in experimental hen houses against the entry of vectors into slatted dwellings, which represent the usual house type in northeastern Argentina.

Materials and methods

Study area

This study was carried out in the city of Puerto Iguazú (province of Misiones, Argentina; 25°36′S, 54°35′W) near Iguazú National Park. The city is located in the phytogeographic region of the Paraná forest within the Amazon forest domain (Cabrera, 1971), which is shared by Argentina, Paraguay and Brazil. The Paraná and Iguazú rivers mark the borders between Argentina and its two neighbouring countries. The climate is subtropical without a dry season (Instituto Geográfico Militar, 1998); mean temperatures are around 25.7 °C in the warmest month (February) and 14.6 °C in the coldest month (July). This study was conducted on a local farm in the rural area known as Dos Mil Hectáreas (Fernández *et al*., 2012), adjacent to Peninsula Provincial Park (25°43′S, 54°35′W). In this rural zone, dwellings are built of wooden slats in a manner that leaves a space between each pair of adjacent slats. The farm has an area of 3 ha, occupied by a house, a pigsty, a tree near the house where chickens sleep and 1.5 ha of crop cultures, and is located on the edge of Peninsula Provincial Park.

Fig. 1. Experimental hen house built and installed on the farm adjacent to the border of the forest.

Experimental hen houses

Experimental hen houses consisted of four-sided rectangular enclosures (230 cm high, 150 cm wide) (Fig. 1). Two of the four walls and the floor of each hen house were made with slats of wood. The roof was constructed from a sheet of corrugated metal and the two remaining walls (200 cm high, 100 cm wide) from crimped wire mesh (mesh opening: 1.5 cm). Spaces between slats (5 cm) were left uncovered in order to simulate the usual design of houses in the area, which indicates that openness is critical to phlebotomine entry in experimental hen houses (Quinnell & Dye, 1994; Bray *et al*., 2010). Three of these dwellings were built on the farm area contiguous to the border of the forest of Peninsula Provincial Park (Fig. 1) and were spaced 50 m apart. One month before the beginning of the experiment, three chickens were placed in each experimental hen house and were given food and water *ad libitum*. A control site without chickens (WC) was selected on the border of the forest and 50 m from the nearest hen house.

Curtains

Curtains of black polyester net fabric (2 m^2; mesh size: 1 × 1 mm) were used. A survey carried out in the community of Dos Mil Hectáreas suggested that black is the colour of net fabric preferred by householders because it requires less frequent washing (L. Costa, unpublished observation, 2014). Curtains were impregnated with permethrin as insecticide (FLOP permethrin 10%; Chemotécnica SA, Ezeiza, Argentina) at 0.05 g/m^2 and dried before each sampling according to Rosendaal's Manual [World Health Organization (WHO), 1997]. Once dried, curtains were stored in airtight bags until use. Curtains were mounted only on the wire mesh walls of the hen houses, according to the assigned treatment.

Table 1. Example of random application of treatments over six consecutive nights in a given month.

	Hen house 1	Hen house 2	Hen house 3	Control site
2 nights	IC	NIC	NC	WC
2 nights	NC	IC	NIC	WC
2 nights	NIC	NC	IC	WC

NC, no curtain; NIC, non-impregnated curtain; IC, impregnated curtain; WC, without chickens (control).

Experimental design

Sampling was conducted from February 2012 to January 2013, with the exception of the period from April to June, when phlebotomine abundances are low (Fernández *et al*., 2012). In January, logistical problems resulted in the absence of chickens in the experimental hen houses for 1 week, which did not coincide with sampling nights. The WC site was sampled from August 2012.

Three treatments were assigned to the hen houses in a sequential 3 × 3 Latin square design, using, respectively: (a) an impregnated curtain (IC); (b) a non-impregnated curtain (NIC), and (c) no curtain (NC). During each month, each of the three hen houses received each of the three treatments randomly in three consecutive 2-day periods. All hen houses had received all three treatments by the end of the 6 nights of sampling (Table 1).

Phlebotomines were captured with REDILA-BL traps (Red de Investigación de Leishmaniasis en Argentina, Argentina) (Fernández *et al*., 2015) which were set at 1.5 m above the ground from 17.00 to 09.00 hours inside every hen house and at the WC site. Maximum and minimum temperatures were recorded at all sites during all sampling nights. All phlebotomines captured were counted to calculate total abundances. The dependent variable 'phlebotomine abundance' was calculated as the average of the two samples (nights) of each period.

Proportions of N. whitmani among treatments

Samples of each combination treatment per month (30 samples) were randomly selected for determination to species level. The specimens were cleared with lacto-phenol (1 : 1) and identified under a microscope (400×), according to Galati (2003) and Shimabukuro *et al*. (2011).

Statistical analysis

A generalized linear mixed model with Poisson error distribution and log-link function was used to analyse the effects of treatments on phlebotomine abundance. The fixed factor in the model was 'treatment' and the random factors were 'hen house' and 'night'. Fisher's least significant difference (LSD) test was used to compare treatments. Proportions of *N. whitmani* in total sandfly captures by night were analysed using a generalized linear mixed model with binomial error distribution and logit link. The Shannon equitability index (E_H) was used as a measure of the evenness of species in each treatment. All statistical analyses were performed with InfoStat 2016 (Grupo InfoStat, FCA, 2016).

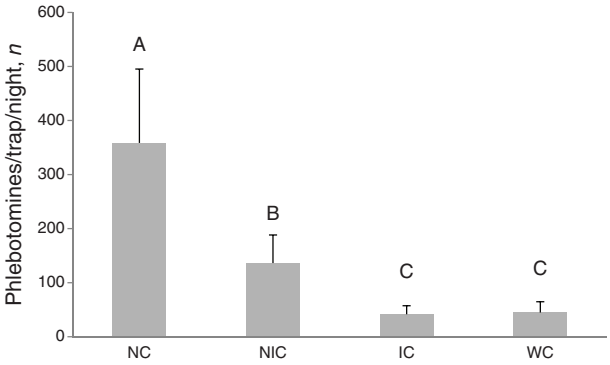

Fig. 2. Abundances of phlebotomines/trap/night (+SEM). Different letters above columns indicate significant differences. IC, impregnated curtain; NC, no curtain; NIC, non-impregnated curtain; WC, without chickens (control).

Results

Effectiveness of curtains

Of the overall phlebotomine capture, 59.7% of flies were found in the NC condition, 26.3% in the NIC condition, 8.0% in the IC condition and 6.1% in the WC condition. Significant differences in abundances of phlebotomines/trap/night were observed between treatments ($\chi^2 = 17853.58$, d.f. = 3, $P < 0.0001$) (Fig. 2). Average phlebotomine abundances in the IC [41.47, standard error of the mean (SEM) = 15.84] and NIC (136.22, SEM = 51.99) conditions were lower than in the NC (358.48, SEM = 136.78) treatment ($P < 0.05$). Significant differences emerged between the IC and NIC treatments ($P < 0.05$). In this trial, NIC reduced phlebotomine trapping abundance by 62.0% compared with NC, whereas IC caused a reduction of 88.4% in comparison with NC. In NC, the abundance of phlebotomines was 87.5% higher than in the WC condition ($P < 0.05$). No differences were found between the IC and WC (44.75, SEM = 20.03) treatments ($P > 0.05$).

Longitudinal study

Phlebotomine population dynamics showed variations in abundance according to month of sampling and treatment (Fig. 3). Treatments NC and NIC had similar patterns with higher levels of variation, and both showed the highest abundances of phlebotomines in November. By contrast, the IC and WC conditions showed patterns of lower variation during the sampling months and catches in IC did not exceed 190 phlebotomines/trap/night. The minimum temperatures recorded ranged between 13.06 °C (March) and 22.29 °C (December). The maximum temperatures recorded ranged between 26.13 °C (August) and 32.71 °C (December).

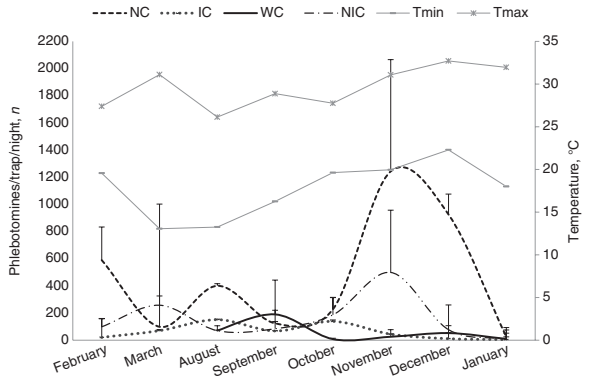

Fig. 3. Abundances of phlebotomines/trap/night by treatment and month of sampling (+SEM). IC, impregnated curtain; NC, no curtain; NIC, non-impregnated curtain; WC, without chickens (control); Tmin, minimum temperature; Tmax, maximum temperature.

Proportions of N. whitmani between treatments

The phlebotomine species identified included *N. whitmani* (89.9%), *Migonemyia migonei* (França) (5.0%), *Pintomyia pessoai* (Coutinho & Barretto) (2.7%), *Brumptomyia* sp. (1.7%), *Lutzomyia longipalpis* (Lutz & Neiva) (0.4%), and species of the *Evandromyia cortelezii* complex (Brèthes), *Psathyromyia* (*Psathytomyia*) *bigeniculata* (Floch & Abonnenc) and *Lutzomyia monticola* (Costa Lima) (< 0.1% each). Seven species were captured in each of the NIC and NC conditions, whereas four species were captured in each of the IC and WC conditions. The Shannon equitability index (E_H) for each treatment was 0.13 (IC), 0.04 (NIC), 0.11 (NC) and 0.1 (WC). *Nyssomyia whitmani* was the most abundant species in all treatments, accounting for over 78.0% of captures in all treatments. *Nyssomyia whitmani* and *M. migonei* were the only species found in all months and treatments. *Lutzomyia longipalpis* was found in IC and NIC in March and October. There were no differences in proportions of *N. whitmani* between the NC (0.91, SEM = 0.05), NIC (0.89, SEM = 0.05) and IC (0.93, SEM = 0.04) conditions.

Discussion

This study represents the first in Argentina to evaluate the effectiveness of impregnated curtains in reducing the community of phlebotomine sandflies in experimental hen houses that resemble intradomiciliary environments. The present results reinforce those achieved in other countries such as Colombia, Brazil, Venezuela, Italy, Iran, Syria, Sudan and Tanzania, where insecticide-treated fabrics were found to represent promising potential tools for protection against phlebotomines and other dipteran species (Alexander *et al.*, 1995; Yaghoobi-Ershadi *et al*., 2006). In addition, the current results show that the presence of impregnated curtains in slatted dwellings such as hen houses reduces the entry of phlebotomines, and that the non-impregnated curtain itself significantly reduces the capture of phlebotomines and thus represents an effective physical barrier against their entry. No differences in proportions of *N. whitmani* were detected between the NIC, IC and NC conditions, which suggests that the treatments produce no differential effect on this species, the main vector of ACL in the area.

The trial with experimental hen houses on the edge of the forest used a design adequate to test and evaluate different prevention and control tools, such as physical and chemical barriers, that have proven acceptability to the community. Furthermore, this design was able to quantify the effect of chicken presence on risk for ACL (using vector abundance as a surrogate for risk). Although *Leishmania* infection is unable to develop in some birds (Dantas-Torres & Brandão-Filho, 2006; Otranto *et al*., 2010), the chicken plays a key role in the epidemiology of leishmaniasis because it serves as an attractant for female sandflies (Alexander *et al*., 2002). The presence of hen houses was found to increase phlebotomine abundance by 87.5% in comparison with the control site (WC), which may indicate that hen houses provide artificial shelters as well as bloodmeal sources for phlebotomines, as proposed by Alexander *et al*. (2002).

However, the results of the present study show differences in species richness between the treatments, although E_H was very low in all cases. Indeed, the treatments did not affect the evenness of species distribution and *N. whitmani* represented the dominant species in all cases. These results agree with the data recorded during the Dos Mil Hectáreas ACL outbreak and subsequent surveys (Fernández *et al*., 2012), and confirm the status of risk for *L. braziliensis* transmission.

This study facilitated recordings of the occurrence of *Lu. longipalpis*, the species incriminated as the main vector of *Leishmania infantum*, the causal agent of visceral leishmaniasis in Argentina. This species is abundant in areas with dense human populations and is highly adapted to peri-urban and urban settings (Harhay *et al*., 2011). In Puerto Iguazú, *Lu. longipalpis* was recorded for the first time in 2010 (Salomón *et al*., 2011) and had succeeded in colonizing a sector of the city by 2011 (Santini *et al*., 2013). However, there are no prior records in the outskirts of the city (Fernández *et al*., 2013), which shows that the presence of this species may indicate the ongoing urbanization of the area in recent years.

The monthly sampling profiles showing the dynamics of phlebotomine capture in each treatment can be divided into two patterns: (a) high-amplitude variation, and (b) low-amplitude variation. Treatments NC and NIC showed high levels of variation, although the variation in NIC was of a lower magnitude. The difference between the patterns observed in the NC and NIC conditions supports the suggestion that the non-impregnated curtain serves as a mechanical barrier that proportionally reduces vector entrance and represents adequate intradomiciliary protection in periods without extraordinarily high vector abundance events in stable endemic situations. By contrast, the hen houses in the IC and WC conditions showed low levels of variation. In the WC condition, this may be explained by the natural edge effect (Quintana *et al*., 2010) dynamics of phlebotomine species in the absence of a food source bait when only the light of the trap serves as an attractant. The difference between these patterns is more marked in the summer months, when minimum temperatures reach higher registers. This may be attributable to the association of temperature with increases in metabolism in adults and in flight activity in the search for food (Fernández *et al*., 2012). Finally, the low-amplitude variation in the IC treatment may be explained by insecticide repellency that

prevents vector entry into houses. Therefore, non-impregnated curtains may serve as a useful tool for protection during inter-epidemic periods and impregnated curtains may be useful during periods of high vector abundance or epidemics.

In order to develop effective control strategies that are socially acceptable, it is necessary to consider that *N. whitmani* in the studied area is a mainly sylvatic species with opportunistic feeding behaviour that includes domestic animals, and a high capacity for adaptation (Da Costa *et al*., 2007). Although a ban on chicken breeding might reduce sites of reproductive success or the attraction of vectors to sites close to houses, this measure is not feasible. Chickens are a source of food and income for the inhabitants of Dos Mil Hectáreas, as they are in other sites or countries where such poultry represents the type of livestock most commonly raised in low-income neighbourhoods (Alexander *et al*., 2002). In this context, given that the period when the vector is most active coincides with human resting time, locating hen houses far from human sleeping areas would serve as a preventive measure.

Although this study did not test its tools in inhabited houses, the curtains described herein may represent an easily accessible control tool for use by the whole community. Wilson *et al.* (2014) conducted a review to assess the efficacy of several insecticide-treated tools (bednets, curtains and screens) against eight vector-borne diseases prioritized by the WHO and found evidence of the protective efficacy of these tools against ACL. In the present study area, bednets are culturally acceptable only for children and adults claim that they cause discomfort in high temperatures (A. V. Mastrangelo, personal communication, 2012). However, the use of impregnated curtains on doors and windows may be very beneficial in dwellings at risk in Dos Mil Hectáreas and epidemiologically similar contexts. In addition, curtains could be used more intensively during warmer months as these represent the periods of highest vector abundance, although it is important to highlight that the efficacy of curtains depends on where domestic activities are carried out during the hours of vector activity (i.e. during twilight on verandas or in yards).

In conclusion, the present group assessed the effectiveness of curtains as an eco-friendly prevention tool to decrease the probability of phlebotomine entry to slatted dwellings located in endemic areas, mainly where houses are located close to protected natural areas such as in Puerto Iguazú. Subsequently, given that the feasibility and effectiveness of these tools have been established as proof of concept, the residual effects of curtains, their cost-effectiveness and the acceptability of the tool to the community will be assessed. Further, the hen house experimental design was shown to be a useful tool for the study of both anti-vector interventions and phlebotomine dynamics.

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