Insecticide-impregnated netting as a potential surface treatment: an alternative to insecticide spraying for control of the leishmaniasis vector Lutzomyia longipalpis (Diptera: Psychodidae)?

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Research

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Abstract

Background: The sand fly, *Lutzomyia longipalpis,* is the main vector of *Leishmania infantum* in Brazil. A previous laboratory study showed that covering surfaces with insecticide-impregnated netting may provide an alternative method for killing sand flies. Synthetic male *Lu. longipalpis* sex/aggregation pheromone co-located with micro-encapsulated l-cyhalothrin demonstrated the potential of “lure-and-kill” to significantly reduce canine infection and sand fly densities. In this study we were interested to determine if insecticide impregnated netting could replace sprayed insecticide for *Lu. longipalpis* control.

Methods: We placed synthetic pheromone in experimental and real chicken sheds treated with a $1m^2$ surface of either sprayed insecticide or insecticide-impregnated netting. Two experiments in experimental chicken sheds were carried out to determine the effect of the insecticide treatments on *Lu. longipalpis* over 1-week and 16-week periods. We counted the number of *Lu. longipalpis* collected overnight and dead at 24 hours. Two longitudinal intervention studies were carried in real chicken sheds and compared the numbers of *Lu. longipalpis* (collected and dead at 24h) before adding the intervention (either the netting or sprayed insecticide treatments) with the numbers collected 24h after the intervention.

Results: In the first experiment all flies caught in the spray treated experimental chicken sheds were dead at 24 hours and in netting treated sheds 97% of females and 88% of males were dead at 24 hours (257 vs 225, Wilcoxon Signed Ranks Test *P*=0.043). The netting and spray treated traps were equally effective at killing both female and male *Lu. longipalpis* over the first 8-weeks however after 16-weeks both treatments killed a significantly lower proportion of females (64%vs 96%; *P*=0.000) and males 89%vs 100%; *P*=0.000) compared to the beginning. In the first of the longitudinal studies in real chicken sheds only the netting intervention significantly increased the proportion of females dead after 24h (60%vs81%; *P*=0.042). The subsequent study showed that both netting and spraying treatments had similarly significant impacts on the proportion of females dead after 24h (netting: 60%vs80%: P=0.0194 and spraying: 43%vs72%: *P*=0.0004).

Conclusions: The netting and spray insecticide interventions (with synthetic sex/aggregation pheromone) have similar impacts on the *Lu. longipalpis* population.

Background

Visceral leishmaniasis (VL) is an important neglected tropical disease around the world, with over 350 million people at risk of infection and an estimated 50,000 deaths per year[1]. Brazil is one of six countries that have 90% of all reported VL cases and the disease, caused by the Protist parasite *Leishmania (Leishmania) infantum* (Kinetoplastida: Trypanosomatidae), is transmitted by the sand fly *Lutzomyia longipalpis* (Diptera: Psychodidae) from infected domestic dogs, *Canis familiaris* (Carnivora: Canidae) the reservoir host, to humans[1, 2].

Despite the vector control strategies adopted by the Brazilian Ministry of Health (MoH) over the past 20 years, the geographical range of *Lu. longipalpis* is spreading [3, 4]. The MoH sand fly control programme

is reactive and on the diagnosis of a human case, the home of the infected person, and any other human or animal dwelling within a 200m radius, is sprayed with a residual insecticide[5]. In addition, the MoH proactively monitor dog infection and when an infected dog is identified, it is euthanised[6]. These vector and infection control strategies have not reduced the incidence of the disease in dogs or humans[7-11] and a recent analysis has shown that the burden of disease caused by VL more than doubled between 1990 and 2016[12].

Spraying insecticide for sand fly control is challenging for local health authorities because of the cost and effectiveness of the activity. In Brazil, the MoH recommend that after the initial insecticide treatment it must be repeated after three to four months[5]. In addition, to ensure effectiveness of insecticide application and to avoid the development of resistance in the vector, residual insecticide spraying requires trained people with appropriate infrastructure to ensure well-maintained spraying equipment and an effective application regime[13, 14].

In the peridomestic environment large aggregations of male and female *Lu. longipalpis* become established on or near host animals[15]. These heterogeneously distributed aggregations are where the females take a blood meal and mate. Although it is not clear why some aggregation sites are favoured over other potential sites, the aggregation behaviour is largely driven by the male produced sex-aggregation pheromone[16, 17]. The use of insecticide has a disruptive effect on *Lu. longipalpis* aggregation formation and those males that arrive first at an insecticide treated site are killed and therefore further recruitment of females and males is stopped[18]. A consequence of this disruption is that new sand fly aggregations may occur at sites that have not been treated with insecticide[18, 19]. In practice, this means that most of the insecticide used is wasted as a long-term *Lu. longipalpis* vector control tool[18]. The use of synthetic sex/aggregation pheromone in insecticide treated sites overcomes the disruptive effect of the insecticide by continuing to attract female and male sand flies[19, 20] and a controlled release formulation of the pheromone can attract *Lu. longipalpis* for up to 12 weeks[21] greatly extending the lethal effect of the insecticide[22, 23]. A trial of the synthetic sex/aggregation, (*S*)-9-methylgermacrene-B[24], formulated in a long-lasting controlled release device[20, 21] co-located with microencapsulated l-cyhalothrin in chicken roosting sites significantly reduced *Lu. longipalpis* densities, canine *Leishmania* parasite infection incidence, tissue loads and canine seroconversion incidence establishing the potential for this strategy to reduce disease incidence[25].

A previous laboratory study found that Blue Olyset netting (Sumitomo Chemical Co. Ltd., Tokyo, Japan), impregnated with 2% permethrin, was an effective replacement for sprayed microencapsulated l-cyhalothrin (20 mg a.i.m^{-2}) (Demand CSW; BASF PLC, Cheshire, UK)[26]. That study proposed that as insecticide impregnated netting can remain active for several years and is widely available as the main intervention against malaria transmission and thus readily available, its use in *Lu. longipalpis* control could overcome some of the challenges of residual insecticide spraying (staff training, dose control, cost, efficacy, incidental environmental contamination and support infrastructure)[27].

The aim of these experiments was to determine if application of insecticide impregnated netting under field conditions could be an effective replacement for residual insecticide spraying for *Lu. longipalpis* control. Thus, we compared the lethal effect of a-cypermethrin impregnated netting (Interceptor^{Ò}, BASF Chemical Co.) with l-cyhalothrin residual spray in experimental chicken sheds and permethrin (2%) + piperonyl butoxide (1%) impregnated netting (Olyset Plus^{Ò}, Sumitomo Chemical UK PLC) with a-cypermethrin residual insecticide spray in real chicken sheds.

Both residual spay insecticides are currently recommended by the Brazilian MoH for *Leishmania* vector control, Interceptor^{Ò} netting is currently used for malaria vector control in endemic parts of Brazil and Olyset Plus^{Ò} is currently used for malaria vector control in endemic parts of Africa.

The netting and spray treatments used in the experimental chicken sheds were different to those used in the real chicken shed experiments because we were only able to use those insecticide treatments (spray and net) that were available to the project at the time. The objective of the experiments was to compare the effect of spray insecticide treatments with netting insecticide treatments and not to compare the efficacy of the different insecticides.

Methods

Study site

The study took place in Governador Valadares (GV), a municipality of approximately 280,000 people in Minas Gerais State, Brazil (18°51' W; 41º57'S, altitude 170m, Fig. 1) 320 km northeast of Belo Horizonte, the state capital. This area is a focus of intense VL transmission and is also endemic for cutaneous leishmaniasis where the sand fly vector, *Lu. longipalpis* is abundant[28, 29]. GV is situated in the Rio Doce basin within the Atlantic Forest region and local topography consists of valleys and hills. The climate is the Aw type (tropical sub-warm and sub-dry) according to the Köppen–Geiger classification[30]. GV has an average temperature of 24.2°C, ranging from 15.2 to 33°C, and the average annual rainfall is 1109 mm concentrated between October and March[31].

Fig. 1 Location of trapping sites for the experiments in the municipality of Governador Valadares, Brazil.

Insert Figure 1

Experiments were carried out in the private gardens and yards of volunteer householders. The dates of trapping and location of the sites are summarised in Additional file 1. Typically, the householder's gardens consisted of a walled-in area at the front or back of the property which contained fruit trees, other types of shrubs and mature trees and animal shelters. Experiments 1 and 2 were carried out in experimental chicken sheds (2015) in the Vila Parque Ibituruna neighbourhood and experiments 3 and 4 were carried out in the householder's own chicken sheds (2019) in the Vila Isa and Vilage da Serra, neighbourhoods. These neighbourhoods are typical peri-urban areas, with homes built near the Área de Preservação Ambiental (APA) Ibituruna forest reserve.

The inclusion criteria for all experiments were that the households had a yard containing a chicken shed with chickens and that *Lu. longipalpis* were present. Presence of *Lu. longipalpis* was confirmed through preliminary sampling in the householder's chicken shed. Solvent (hexane) extracts of individual sand flies collected in GV both prior to and during field experiments were examined by coupled gas chromatography-mass spectrometry (GC/MS)[32] to confirm that they produced (*S*)-9-methylgermacrene-B sex/aggregation pheromone.

Taxonomic identification of sand fly species and their sex (male or female) for all experiments was by microscope (Nikon SMZ 445) examination of morphological characteristics. Male *Lu. longipalpis* were initially identified by the presence of a pale spot on abdominal tergite IV and then confirmed by the morphological characteristics of the genitalia[33, 34]. Females were dissected and the cibarium and spermathecae examined to confirm species identification[34].

Chicken sheds

Experimental chicken sheds used in experiments 1 and 2, were constructed from 4 plywood panels each measuring 105 cm high x 55 cm wide arranged in a square plan (55 cm x 55 cm). The panels were held together by plastic cable-ties passed through holes (10mm diameter) in the top and bottom corners of each panel. Sand flies were collected in miniature suction traps manufactured in Brazil (Hoover Pugedo (HP))[35]. The light bulb was removed from the trap and instead a pheromone lure, containing 10mg of synthetic sex pheromone ((±)-9-methylgermacrene-B), was attached to the underside of the lid of each trap[21]. The trap was suspended inside the experimental chicken shed from a wooden dowel (20 mm diameter) placed across the top of the shed. Sand flies were collected in a nylon Barraud cage (22cm x 22cm x 22cm) suspended below the HP trap. A chicken, supplied with food and water, from the household flock was placed on the ground inside the experimental chicken shed overnight.

The chicken sheds used in experiments 3 and 4 belonged to the householders and were constructed primarily out of locally available recycled wood but also included corrugated metal, asbestos sheet and plastic. Their primary function was to shelter the chickens from nocturnal predators and thus they have walls, a roof and a door. Once closed after dusk the chickens remained in the shed throughout the night until they were released by the householder in the morning. The sheds used in the study were selected on the basis of their size (range 1 to 10m^2) and the number of chickens that they contained (5 to 30).

Insecticide treatment

For experiments 1 and 2 a single layer of netting (Interceptor®, BASF S.A., São Paulo, Brazil) impregnated with a-cypermethrin (6.7g/kg or 200mg/m^2) was fixed onto plywood panels (0.5m x 0.5m) and four of these were fitted on the inside at the top of the 4 interior walls of the experimental chicken shed. The total area covered was 1m^2. In addition 4 plywood panels (0.5m x 0.5m) were sprayed with microencapsulated l-cyhalothrin (Karate Zeon 50 CS, Syngenta, Huddersfield, UK; 20mg a.i.m^{-2}) which were then fitted inside

at the tops of the 4 walls of the experimental chicken shed. The total area treated was $1m^2$ and the insecticide was applied at the dosage required by the Brazilian MoH VL control handbook[5].

For experiments 3 and 4 a single layer of Olyset® Plus netting (Sumitomo Chemical Company UK PLC, London, UK) polyethylene netting impregnated with permethrin [2.0% w/w ($20g\ kg^{-1} \pm 5g\ kg^{-1}$) about 800mg of permethrin/m^2] combined with piperonyl butoxide (PBO) as a pyrethroid synergist [1.0% w/w ($10g\ kg^{-1} \pm 2.5g\ kg^{-1}$) about 400mg of PBO m^{-2}] was fixed onto a plywood panel (1m x 1m) and placed inside the real chicken shed. A plywood panel (1m x 1m) sprayed with a-cypermethrin (Alfatek 200 SC, Rogama) ($20mg\ m^{-2}$)[5] was placed inside the chicken shed.

Experimental design

Experiment 1

To compare the lethal effect of insecticide-treated netting with the lethal effect of insecticide spraying over a 24-hour period we conducted an experiment over 4 nights at the 2 houses A and B (Fig. 1). Two pairs of traps were used at each house and the position of the traps within a pair was swapped on the second night to control for any positional bias (1 replicate). The trapping cycle was repeated on subsequent nights. The experimental design gave a total of 32 possible trap catch data points from 8 replicates. Replicates were excluded from the analysis unless a complete set of data was obtained for the replicate (for example if one or more of the traps did not function correctly on any pair of nights) thus we obtained 28 trap-catch data points from 7 experimental replicates.

Experimental chicken sheds were placed in the residential gardens of each of the two houses (A and B) prior to sunset (approximately 18:00). The experimental chicken sheds in each pair were 3m apart and the 2 pairs in each household were 5m from each other. The two households were 25m apart. Each pair of experimental chicken sheds consisted of one fitted with sprayed insecticide treated wooden panels and the other one fitted with insecticide impregnated netting treated wooden panels.

The following morning (approximately 14h after the traps were placed) the HP traps and attached Barraud cages were removed and the chickens released. The number of live and dead female and male *Lu. longipalpis* sand flies in each Barraud cage were then counted.

The live sand flies were transferred to plastic holding pots (9.5cm diam, 8cm deep) with a nylon netting top. The base of the holding pot was filled with Plaster of Paris (0.5cm deep) dampened to maintain humidity. A piece of cotton wool, soaked in a solution of 20% sucrose and 50% honey syrup, was placed on the top of each holding pot as a sugar source for the sand flies. The pots were placed on a layer of moistened filter paper in the bottom of a Styrofoam box (28cm L x 24cm W x 35cm D) and covered with a dark cloth and kept for an additional 10h after which the number of live and dead, male and female sand flies in each pot were counted again.

Thus, we noted the total number of male and female sand flies collected as well as the total number of males and females that were dead 24h after the traps were placed.

Experiment 2

To compare the lethal effect of insecticide treated netting with residual spraying over an extended period of time (16-weeks) two pairs of traps were again placed at each of the 2 houses used in experiment 1 and trapping was performed over 4 nights at each of 3 timepoints; week 1 (in May), week 8 (in July) and week 16 (in September). In total 8 replicates for each time point, were attempted. However, as before, replicates were excluded from the analysis if they were partially completed. Thus, we had 6 replicates at T=0, 7 replicates at T=8 weeks and 8 replicates at T=16 weeks (total = 21 replicates = 84 data points).

When not in use the insecticide treated panels were removed from the experimental chicken sheds and kept uncovered and thus exposed to the prevailing weather conditions.

Experiments 3 and 4

To compare the effect of sprayed insecticide with netting insecticide treatments on *Lu. longipalpis* in real chicken sheds two longitudinal intervention studies[19] were carried out during an 2-week period from 06/05/2019–17/05/2019 (experiment 3) and a 5-week period from 27/08/2019–24/09/2019 (experiment 4). The distances between experimental sites varied from 82 metres (between house 1 and 2), to 3,110 metres (between house 1 and 6). In experiment 3 seven chicken sheds and 6 replicates were used for each insecticide treatment; spray or netting. In experiment 4 the number of chicken sheds was reduced to 4 and the number of replicates increased to 10 to reduce variability.

On the first night of the experiment an HP trap (without a light) and a pheromone lure (ca. 30cm from the trap) was placed inside each of the chicken sheds at 6pm. The trap and pheromone remained in position in the chicken shed overnight. The next morning, approximately 12 hours later, the cages containing the sand flies collected overnight were removed and the number of sand flies (male and female, dead and alive) was recorded. Live sand flies were removed from the collection cage and placed in a pot and held for a further 12 hours. On the evening after the first night of trapping a $1m^2$ (1 x 1m) insecticide treated wooden board (treated with either Olyset Plus netting or a-cypermethrin spray) was added to the chicken shed about 30 cm from the HP trap and pheromone. A fresh collection cage was attached to the HP trap. The next morning the overnight sand fly collection was removed, and sand flies processed as before. The insecticide treatment, pheromone lure and HP trap were also removed from the chicken shed.

Thus at the end of the period, we noted the total number of male and female sand flies collected as well as the total number of males and females that were dead after 24h both before and after the application of the intervention.

Chickens were present throughout each experiment (average±sem=14.6±3.3 per shed for experiment 3 and 20.0±3.7 per shed for experiment 4).

During the experiment the chicken sheds were visited twice with an interval of 7 days between the first visit and the second visit.

Control Data

To obtain control data on the lethal effect of capturing, transferring and holding *Lu. longipalpis* in a plastic pot, we placed an HP trap, with a tungsten light only, beside the real chicken shed of each of the 2 study houses. The sand flies that were collected were counted and placed in holding pots in the same way as the *Lu. longipalpis* exposed to the different insecticide treatments. Their mortality was recorded at the same time intervals as for the experiments described above. To avoid causing mortality through handling species identity was determined at the end of the experiment.

Data analysis

Experiment 1

Prior to analysis data were checked for normality by Shapiro-Wilk and Kolmogorov-Smirnov tests. Comparisons between the numbers of sand flies (male and female) caught in experimental chicken sheds treated with insecticide impregnated netting and the numbers caught in the chicken sheds sprayed with residual insecticide was made by Wilcoxon signed-rank test. The numbers of males and females dead 24h after exposure to the 2 treatments was compared by Wilcoxon signed-rank test. Differences in numbers due to night of testing and location of testing were assessed using a Kruskal-Wallis test.

Experiment 2

The data were tested for normality using both Kolmogorov-Smirnov and Shapiro-Wilk tests. Comparisons of sprayed insecticide and netting insecticide treatments on the numbers of sand flies (total, male and female) caught in each treatment and their mortality after 24h were made. Numbers trapped and dead after 24h were compared using the Wilcoxon signed-rank test. The effect of time (month when the experiment was performed) on the effectiveness of either netting or spray insecticide treatment was investigated with a Kruskal-Wallis test (non-parametric equivalent of a 1-way ANOVA). The effect of any interaction between the treatment and month was also investigated using a Kruskal Wallis test.

Experiment 3

As the data set for experiment 3 was small, a non-normal distribution was assumed. Comparisons of sprayed insecticide and netting insecticide treatments on the numbers of sand flies (total, male and female) caught in each treatment and their mortality after 24h were made. Numbers trapped and dead after 24h were compared using the Wilcoxon signed-rank test. Comparison of the proportions of *Lu. longipalpis* collected and dead at 24h were made by Fishers exact test.

Experiment 4

The data obtained in experiment 4 was tested for normality and subsequently analysed by non-parametric Mann-Whitney test to compare the absolute numbers of male and female *Lu. longipalpis* trapped and dead after 24h in the sprayed and netting treated real chicken sheds. Comparison of the proportions of *Lu. longipalpis* collected and dead at 24h were made by Fishers exact test.

Control traps

The numbers of sand flies that were trapped in each of the 4 months (Jan, May, July and Sept) in the absence of insecticide treatment were tested for normality using Shapiro-Wilk and Kolmogorov-Smirnov tests. Sex differences in the numbers caught and dead at 24h were compared using Wilcoxon's signed-rank test. Differences in numbers between houses were also tested using Wilcoxon's signed-rank test. Differences in numbers between months were tested using a Kruskal-Wallis test.

Results

Experiment 1

In total 538 (mean±sem; 9.6±1.1) Lu. longipalpis [393 (14.0±1.6) males and 145 (5.2±1.0) females] were collected in 14 nights of trapping in both spray and netting treated experimental chicken sheds (Table 1). The overall ratio of males:females was 2.7:1.

Insert Table 1

Trapping data for night 1 (N1) house A1 and night 2 (N2) house A1 was not included because the CDC trap failed on N1. More male flies were caught than female flies, across all traps and locations (393 *vs* 145; Mann-Whitney U-test P=0.001).

More male flies were caught in netting treated sheds than in spray treated sheds (257 *vs* 136, Wilcoxon signed-rank test, P=0.018) however, the numbers of females caught in net treated sheds compared to spray treated sheds (87 *vs* 58) was not statistically significant (P=0.410).

All flies caught in the spray treated sheds were dead at 24 hours. However, in the netting treated sheds 97% of females (87 *vs* 84, ns) and 88% of males (257 *vs* 225, Wilcoxon Signed Ranks Test P=0.043) were dead at 24 hours. Overall the night of testing (Kruskal-Wallis test P=0.148) and the location of the traps (Kruskal-Wallis test P=0.251) had no significant effect on the outcomes.

The results of this experiment suggest that spraying and netting treatments were equally effective at killing females although spraying may be more efficient at killing males than netting. However, as a significantly greater number of males were caught in netting treated sheds (257 vs 136, Wilcoxon signed-rank test P<0.018) the netting treated sheds therefore produced the greatest male mortality (225 *vs* 136, Wilcoxon signed-rank test P<0.028).

Experiment 2

In total 2034 (mean±sem; 12.1±1.0) *Lu. longipalpis* [1441 (17.5±1.8) males and 593 (7.1±0.6) females] were collected during 42 nights in three 1-week trapping periods in May, July and Sept 2015 (summary data Table 2 and full data Additional file 2). The overall ratio of males:females was 2.4:1 and significantly more males than females were caught in both the net and spray treated experimental chicken sheds (Wilcoxon signed-rank test, $P<0.001$, for both).

Insert Table 2

Neither netting or spray treated experimental chicken sheds preferentially caught either male or female *Lu. longipalpis* (Wilcoxon signed rank test, $P=0.188$) and there was no indication that any month was more effective than the others for trapping males or females for either of the insecticide treated (netting or spray) traps (Kruskal-Wallis test, $P=0.174$).

The average mortality for the netting treated sheds was 85% and for spray treated sheds 96%. There was no significant difference in the numbers of *Lu. longipalpis* dead at 24h in the netting (1013) or spray (834) treated experimental sheds (Wilcoxon signed-rank test, $P=0.110$).

The age of the treatment (netting and spray) had a significant effect on the number of dead *Lu. longipalpis* at 24h (Kruskal-Wallis, $P=0.000$). This effect was notable in the netting treated traps for both males ($P=0.000$) and females ($P=0.002$). However, in spray treated sheds age of the treatment was only a significant factor for females ($P=0.014$) but not for males ($P=0.24$). The effect was most obvious in September when the proportion of males and females that were dead at 24h was approximately 60% in the net traps compared to compared to 90% in the spray traps. (Table 2, Fig 2).

Fig. 2 Mean number of *Lutzomyia longipalpis*, females and males, captured and dead after 24 hrs in May, July and August, after exposure to either a-cypermethrin impregnated netting or l-cyhalothrin residual spray.

Insert Figure 2

Although there was no significant difference in the numbers of *Lu. longipalpis* trapped throughout the experiment, the effectiveness of the netting in September appeared to be reduced compared to the spray. Thus, there was a significant reduction in the proportion of *Lu. longipalpis* females dead at 24h in the netting treatment compared to spray (i.e. 64% *vs* 96%; Kruskall Wallis $P=0.000$) and also for males (89% *vs* 100%; Kruskall Wallis $P=0.000$). (By comparison in both May and July almost 100% of the sand flies caught were dead at 24h).

The results suggest that initially (during May and July) the a-cypermethrin impregnated netting and l-cyhalothrin residual spray were equally good at killing both male and female sand flies. However, the effectiveness of the netting treatment deteriorated over time so that in September significantly fewer male and female sand flies were killed by the compared to the spray treatment (Kruskal Wallis Test $P=0.004$).

Experiment 3

In total 948 (mean±sem; 19.8±5.8) *Lu. longipalpis* [795 (33.1±11.0) males and 153 (6.4±1.5) females] were collected before and after intervention in real chicken sheds during 12 nights in May 2019 (Table 3).

Insert Table 3

The overall ratio of males:females was 5.2:1. In the a-cypermethrin impregnated netting treated chicken sheds 418 (mean±sem; 17.4±5.6) *Lu. longipalpis* in total [344 (28.7±10.3) males and 74 (6.2±1.7) females] were collected before and after the intervention. In the l-cyhalothrin spray treated chicken sheds 530 (mean±sem; 22.1±10.4) *Lu. longipalpis* in total [451 (37.6±19.9) males and 79 (6.6±2.6) females] were collected before and after the intervention.

Before the netting intervention 235 males and 37 females were caught and afterwards 109 (proportion pre-intervention catch: post-intervention catch = 0.46) males and 37 (1.0) females. Before the spray intervention 285 males and 51 females were caught and afterwards 166 (0.58) males and 28 (0.55) females.

Significantly more males than females were collected prior to the interventions (Wilcoxon signed-rank test; netting P=0.005, spray P=0.005) and similarly, after the interventions, significantly more males than females were collected (Wilcoxon-signed-rank test; netting P=0.044, spray P=0.029).

In this experiment only the netting intervention increased the proportion of females killed. The spray intervention did not increase the proportion of either males or females killed.

Netting treatment, male Lu. longipalpis

Before the netting intervention was applied the proportion of male flies dead after 24h compared to the number collected was 70% (164/235). After the intervention the proportion of males flies dead at 24h was not significantly different 79% (85/109) (Fisher's exact test P=0.122).

Netting treatment, female Lu. longipalpis

Before the netting intervention was applied the proportion of female flies dead after 24h compared to the number collected was 60% (22/37) and after the intervention the proportion of females flies dead was marginally significantly greater 81% (30/37) (Fisher's exact test P=0.042).

Spray treatment, male Lu. longipalpis

Before the spray intervention the proportion of male flies dead after 24h compared to the number collected was 66% (187/285) and afterwards the proportion of males flies dead was significantly lower, 55% (92/166) (Fisher's exact test P=0.032).

Spray treatment, female *Lu. longipalpis*

Before the spray intervention was applied the proportion of female flies dead after 24h compared to the number collected was 73% (37/51) and after the intervention the proportion of females flies dead was not significantly different 54% (15/28) (Fisher's exact test *P*=0.089).

Significantly less males were killed in chicken sheds treated with netting or spray than were caught (Wilcoxon Signed Ranks Test; mean caught = 17.6±6.2 *vs* mean dead = 13.0±5.4 *P*=0.026). (Wilcoxon Signed Ranks Test; mean caught = 31.8±24.6 *vs* mean dead = 17.2±12.2 *P*=0.042). There was no significant difference in the numbers of females killed and numbers caught in either intervention.

This result suggests that the insecticide treatments were having a greater effect on the females than the males.

The total number of males was significantly greater than the number of females for both netting and spray treated sheds (Wilcoxon Signed Ranks Test: *P*<0.05). Although the data set was small there was no indication that the house location contributed to the experimental outcome (Kruskal Wallis Test; *P*=ns).

Experiment 4

In total 1073 (mean±sem; 13.4±1.7) *Lu. longipalpis* [788 (19.7±2.9) males and 285(7.1±0.9) females] were collected before and after intervention during a 20-night period from August to September 2019 (Table 4). The overall ratio of males:females was 2.8:1.

Insert Table 4

In the boxes assigned to the a-cypermethrin impregnated netting treatment, 519 (mean±sem; 13.0±2.7) *Lu. longipalpis* in total [395 (19.8±4.9) males and 124 (6.2±1.1) females] were collected before and after the intervention. In the boxes assigned to the l-cyhalothrin spray treatment, 554 (mean±sem; 13.9±2.0) *Lu. longipalpis* in total [393 (19.7±3.3) males and 161 (8.1±1.4) females] were collected before and after the intervention. Thus, the numbers of *Lu. longipalpis* (males and females and proportions) were similar for both the netting and spray treated elements of the experiment.

Application of the netting intervention significantly increased the proportions of both males and females dead after 24 h compared to before the intervention. The spray intervention also significantly increased the proportion of females dead after 24h but did not affect the proportion of males dead after 24h.

Netting treatment, male Lu. longipalpis

Before the netting intervention the proportion of male flies dead after 24h compared to the number collected was 56% (154/277). After the intervention the proportion of males flies dead was significantly greater, 86% (102/118) (Fisher's exact test P<0.0001).

Netting treatment, female Lu. longipalpis

Before the netting intervention the proportion of female flies dead after 24h compared to the number collected was 60% (44/73) and after the intervention the proportion of female flies dead was marginally significantly greater 80% (41/51) (Fisher's exact test *P*=0.0194).

Spray treatment, male Lu. longipalpis

Before the spray intervention the proportion of male flies dead after 24h compared to the number collected was 62% [97/156]) and after the intervention the proportion of male flies dead after 24h compared to the number collected (70% [167/237]) (Fisher's exact test; *P*=0.0997).

Spray treatment, female Lu. longipalpis

The proportion of female flies dead after 24h compared to the number collected before the spray intervention (43% [33/76]) and was not significantly different to the proportion of females flies dead after the intervention (72% [61/85]) (Fisher's exact test *P*=0.0004).

Overall these results indicate that the netting and spray insecticide interventions have similar impacts on the population of *Lu. longipalpis* that are caught in the traps.

Control traps

Both *Lu. longipalpis* (98.4%) and *Evandromyia cortelezzii* (1.6%) were trapped in the control traps placed in houses A and B, however, only the numbers of *Lu. longipalpis* are reported in Table 5.

Insert Table 5

In total 414 (mean±sem; 6.5±0.9) *Lu. longipalpis* [266 (8.3±1.5) males and 148 (4.6±0.8) females)] were collected in a total of 16 nights of trapping at both sites. Neither the male nor female trap counts were normally distributed (Shapiro-Wilk P=0.001; Kolmogorov-Smirnov P=0.003). Although fewer male flies were trapped in September than in the other months (Kruskal-Wallis *P*=0.033), more males than females were trapped in both houses (Wilcoxon signed-rank test: house A, *P*=0.012, house B, *P*=0.037). Also, there was no significant difference (Wilcoxon signed-rank test) in numbers of flies caught in house A compared to house B. The overall ratio of males:females caught was 1.8:1.

The control experiment showed that trapping was not a significant cause of mortality to either male or female *Lu. longipalpis*; we found no significant difference in the number of males (72%) or females (68%) alive after 24h compared to the numbers initially caught (Fisher's exact test on a 2x2 sex-mortality table *P*=0.43, and chi-square test *P*=0.40).

Discussion

In these experiments we showed that insecticide treated netting was as effective as sprayed insecticide overall in killing female and male *Lutzomyia longipalpis* sand flies in both experimental and real chicken sheds when co-located with synthetic sex aggregation pheromone (*S*)-9-methylgermacrene-B. Insecticide

treated netting is widely available as it is used in bed-nets for malaria control and although there are concerns over its misuse[36] the potential of insecticide impregnated materials including netting has also been recognised and evaluated for use in crop protection[37, 38]. The experiments did not differentiate between the effectiveness of the different insecticides we investigated only the mode of their delivery; either in a spray or in a netting formulation.

The insecticides that we used are widely used for vector control in Brazil and Africa countries. The l-cyhalothrin and a-cypermethrin are recommended by the Brazilian MoH for *Lu. longipalpis* control[5]. The Interceptor® netting used in experiments 1 and 2 is a surface treated, controlled release formulation of a-cypermethrin used in Brazil for malaria vector control[39] and Tanzania against *Anopheles gambiae*[40]. Olyset Plus netting is a LLIN in which the active ingredients permethrin and piperonyl butoxide are impregnated into the polyethylene fibres of the netting during manufacture and is currently being evaluated for use against malaria vectors in Africa where insecticide resistance is a problem[41].

In experiment 1 with experimental chicken sheds we found that the numbers of females collected and dead at 24h was not statistically different in insecticide sprayed sheds compared to the netting treated sheds. By contrast significantly more males were caught (and dead at 24h) in the netting treated sheds compared to the spray treated sheds. The differential effect of insecticide on males compared to females has been previously observed in other studies[18, 42]. The difference has been ascribed to the relatively smaller size of the males making them more susceptible to the insecticides than females[42]. However, it may also be that in our experiments these differences are related to the different behaviours of the males and females in the proximity of hosts as well differences in the “knock-down” effect of the insecticide treatments. Males rest on the surfaces near the blood meal source where they defend territories and when females enter these territories they mate[15, 43]. Thus, the males are likely to be in contact with the insecticide treated surfaces for longer than the females and thus may be disproportionately affected by the insecticide. The spray treatment has a greater knockdown effect on both males and females but because the females are not in contact with the insecticide treated surface for as long as males, they may pick up a lower dose of insecticide which makes them more likely to be caught in the trap. In our study, we only accounted for those *Lu. longipalpis* that entered the suction traps, we did not count those sand flies that might have been affected by insecticide and knocked down before entering the trap. Given the irregular construction of the real chicken sheds and the multiple opportunities for evasion of capture after exposure to insecticide the methodology utilised in other studies[44] would seem impractical although it could potentially provide very useful information in the experimental chicken shed experiments if the collecting sheet was separated from the chicken.

Another possible explanation for the reduced number of *Lu. longipalpis* in the spray (compared to netting) treated chicken sheds (both experimental and real) may be related to the relative repellent effect of the insecticides i.e. the spray treatment moved sand flies away from the HP traps. Repellence can be an advantage of pyrethroids, which is useful in reducing insect contact with the individual and thus offering personal protection against bites[45]. However, in a community vector control program the repellent effect of spraying may be to divert the sand flies to untreated places.

In experiment 2 initially the same pattern of catches and lethality was observed as in experiment 1, i.e. there was no significant difference between the netting and spray treatments the difference in the effect of the treatments on both females and males became more pronounced over time when the a-cypermethrin netting became significantly less effective at killing both males and females compared to the l-cyhalothrin spray treatment. As initially the netting was as effective as spray for the first 2 months the deterioration was likely related to the effectiveness of the netting rather than a change in the response of the sand flies to the traps. However, it is unclear why the a-cypermethrin netting became less effective over time in our experiments. In field-scale evaluations this netting when used indoors has been found to be durable and effective against the malaria vectors, *Anopheles culicifacies* in India *[46]* and *A. gambiae* in Tanzania where 80% of the nets met WHOPES Phase III activity criteria at 36 months[40]. In our experiments the netting and l-cyhalothrin spray treatments remained in the experimental chicken sheds throughout the day and between experimental replicates were stored outside where they were exposed to UV light, fluctuating heat, humidity and rainwater. Although there is some evidence to suggest that a-cypermethrin degrades in UV[47] other studies have shown that repeated exposure to UV did not reduce the efficacy of the Interceptor netting[48]. In addition, the surface on which the insecticide is applied plays a significant role in determining the effectiveness of the insecticide treatment[42, 49, 50]. In addition, although the present study did not evaluate this aspect, as the netting was placed on a plywood substrate, its efficacy could have been affected by either the possible exposure to fungal growth encouraged by the proximity to damp wood or the interaction between the l-cypermethrin and the constituents of the plywood.

Experiments 3 and 4 demonstrated that when carried out in real chicken sheds the effect of the netting insecticide was similar to that of the sprayed insecticide. This suggests that the approach of treating real chicken sheds with insecticide treated netting to reduce sand flies is potentially valuable.

In both sets of experiments, in the experimental and real chicken sheds, the netting performed as well as the spray treatments. The concentration of insecticide that the sand flies were exposed to in the different treatments was variable and does not appear to have affected this outcome. Generally, the amount of insecticide that the sand flies were exposed to in the spray treatments was greater than that in the impregnated nets. Although 1 m^2 of Olyset Plus®, netting impregnated with permethrin (20g/kg) has 800 mg of ai it is released over the lifetime of the net which can be up to 3 years or longer. Therefore, the amount of insecticide present on the surface at any given time is very small. By comparison $1m^2$ of a-cypermethrin spray treated surface had 40mg of a.i. Similarly, $1m^2$ of Interceptor® netting treated with a-cypermethrin has 200mg of a.i. bound to the surface of the netting fibres and this is used over 3 years or longer, it is not replenished from a central reservoir during the lifetime of the netting as with impregnated netting. The microencapsulated l-cyhalothrin treatment had 20mg of a.i. In addition, there are differences in the effectiveness of the different insecticides for example permethrin is 10x more potent than l-cyhalothrin.

According to the World Health Organisation LLINs are cost-effective for reducing the transmission and burden of malaria[51]. However, evidence for the conventional use of insecticide impregnated bed nets to

reduce VL transmission is contradictory. A small scale trial in Bangladesh showed a 66.5% reduction of VL cases[52], in Sudan the use of insecticide treated bed nets reduced VL by 59%[53] and the use of Olyset Plus impregnated netting showed a significant reduction in incidence rate (4.78% to 0.37%) of cutaneous leishmaniasis in a hyperendemic area in Turkey[54]. However a large scale community trial carried out in India and Nepal contradict these findings and suggest that large scale usage of long-lasting insecticidal nets provided no additional protection against visceral leishmaniasis compared with existing control practice[55]. A significant problem with the conventional use for personal use of insecticide impregnated netting in VL control is that most female sand flies (>50%) bite in the early evening (18:00-21:00) meaning that the majority of the human population is not sleeping under, and thus is not protected by the nets [54-56].

The possibility of using permethrin-impregnated netting as an alternative to a micro-encapsulated l-cyhalothrin surface treatment against female *Lu. longipalpis* was first tested in a laboratory study[27]. The study showed that Olyset netting was initially as effective as -cyhalothrin spray treatment. However, whereas the netting remained nearly 100% lethal 24 h post exposure for 12 months the effectiveness of the residual insecticide declined to approximately 74% over 6 – 12 months. The reduction in the effectiveness of the residual spray treatment was similar to that observed against the cutaneous leishmaniasis vector *Lu. verrucarum* when sprayed on outside walls in Peru[57] and Olyset Plus netting has been shown to remain fully active for at least 1 year in field conditions[54]. It was also noted that initially the immediate mortality of the netting was significantly lower than that of the l-cyhalothrin spray. The netting killed on average 13.5%±1.3 (mean ± SEM) of exposed sand flies immediately following 1 h of exposure whereas the spray killed 56%±3.9. However, the immediate lethality of the netting remained relatively constant over the 12 months of the study whereas the immediate lethality of the spray treatment decreased to around 10–12% over 12 months. In practice the Brazilian MoH recommend that insecticide spray treatment is reapplied after 3 to 4 months[5].

This study showed for the first time in field experiments that long-lasting insecticide impregnated netting could be used against *Lu. longipalpis* sand flies as an alternative to residual insecticide spraying. The approach is potentially cost-effective, simple to apply and can be combined with synthetic sex/aggregation pheromone to provide a readily accessible intervention measure for leishmaniasis control[58].

Other studies have demonstrated the efficacy of impregnated netting and other materials against endophilic sand flies[59, 60]. However, this strategy, directed against exophilic *Lu. longipalpis* abundant in peridomestic environments is a new approach. The possible population effects of the use of netting against sand flies are still unknown and more detailed studies involving bioassays and susceptibility tests comparing the same insecticides and concentrations in residual spray and impregnated nets, as well as longer lasting field experiments in sand flies resting sites (e.g. chicken sheds) are essential to understand the effectiveness of these strategies in controlling leishmaniasis. In any case, for leishmaniasis control, the regular spraying of all potential aggregation sites in Brazil, particularly in rural communities is impractical[61].

More extensive trials that co-locate insecticide impregnated net and synthetic sex/aggregation pheromone are required to determine the effectiveness of this approach in an “attract-and-kill” strategy to improve sand fly control. A trial of synthetic (±)-9-methylgermacrene-B[25] formulated in a long-lasting controlled release device[26,27] co-located with microencapsulated l-cyhalothrin in chicken roosting sites significantly reduced *Lu. longipalpis* densities, canine *Leishmania* parasite infection incidence, tissue loads and canine seroconversion incidence [23] indicating the potential of this strategy for reducing disease incidence however replacing the sprayed insecticide application with a netting application could improve the cost of the application and therefore coverage.

Conclusions

The main objective of these experiments was to investigate the feasibility of using netting rather than spraying as an insecticide treatment in chicken sheds alongside *Lu. longipalpis* synthetic sex aggregation pheromone. The application of insecticide treated netting for *Lu. longipalpis* control has several potential important advantages over residual insecticide spraying, these include; accurate dose control, ease of application (reduced training with no requirement for specialist spraying equipment), personnel and environmental safety, reduced costs and longevity of treatment. The application of a single piece of insecticide impregnated netting (or other insecticide pre-treated surface) along with synthetic sex pheromone could provide a cost-effective means of *Lu. longipalpis* control. Our results indicate that netting has the potential to replace spraying as a means of delivering insecticide for vector control and consequent reduction of disease transmission. However, more work is needed to investigate the long-term effectiveness of this strategy as part of a control program applied in animal sheds and other *Lu. longipalpis* aggregation sites, in combination with synthetic sex aggregation pheromone.

Abbreviations

ANA: Agência Nacional de Águas; APA: Área de Preservação Ambiental; GC/MS: gas chromatography-mass spectrometry; GV: Governador Valadares; HP: Hoover Pugedo; ITN: Insecticide-impregnated/treated netting; IBGE: Instituto Brasileiro de Geografia e Estatistica; MoH: Ministry of Health; PBO: piperonyl butoxide; SIRGAS: Sistema de Referência Geocêntrico; UTM: Universal Transverse Mercator; VL: visceral leishmaniasis.

Declarations

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Ethics approval and consent to participate

The project, including the involvement of householders, was reviewed and approved by the Faculty of Health and Medicine Ethical Review Committee (FHMREC15125) at Lancaster University. This study was carried out in accordance with the guidelines of the Animals in Science Regulation Unit (ASRU) and in compliance with the Animals (Scientific Procedures) Act (ASPA) 1986 (amended 2012) regulations and was consistent with UK Animal Welfare Act 2006 and The Welfare of Farmed Animals (England) Regulations 2007 and 2010. Oral consent was obtained from the Governador Valadares health authority (CCZ) to conduct the study within their administrative jurisdiction and from the householders for use of their animals and property.

Consent for publication

Not applicable

Availability of data and materials

All data generated or analysed during this study are included in this published article and its additional information files.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

VAB designed experiments 1 and 2, acquired and interpreted the data, drafted and substantially revised the manuscript. CFS designed all experiments and acquired the data. DG analysed and interpreted the data. RPB conceived the study and designed the experiments. JGCH conceived the study, designed the experiments, interpreted the data, drafted and substantially revised the manuscript. All authors read, contributed to and approved the final manuscript.

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Tables

Table 1 Number of male and female *Lu. longipalpis* collected in experimental chicken sheds treated with either a-cypermethrin impregnated netting or l-cyhalothrin residual spray and their mortality after 24 hours.

		a-cypermethrin netting				l-cyhalothrin spray			
N	H	collected		dead at 24h		collected		dead at 24h	
		♀	♂	♀	♂	♀	♂	♀	♂
N1	A2	31	11	21	11	8	1	8	1
N1	B1	17	8	16	8	16	3	16	3
N1	B2	30	0	17	0	12	2	12	2
N2	A2	16	1	13	1	11	1	11	1
N2	B1	19	7	19	7	9	8	9	8
N2	B2	25	4	20	3	10	1	10	1
N3	A1	5	1	5	1	12	11	12	11
N3	A2	28	25	28	25	3	1	3	1
N3	B1	21	6	21	6	14	6	14	6
N3	B2	28	8	28	6	15	11	15	11
N4	A1	19	6	19	6	1	0	1	0
N4	A2	0	0	0	0	9	9	9	9
N4	B1	10	6	10	6	12	1	12	1
N4	B2	8	4	8	4	4	3	4	3
total		257	87	225	84	136	58	136	58
		18.4	6.2	16.1	6.0	9.7	4.1	9.7	4.1
±sem		2.6	1.7	2.2	1.7	1.2	1.1	1.2	1.1

N = nights (1, 2, 3 or 4) = on which data were collected; H = house (A or B) and pair (1 or 2) in which the collection was made; collected ♀ and ♂ = the number of *Lu. longipalpis*, male and female collected by the HP trap (fitted with a pheromone lure and without a light) during each night in each trap; dead at 24h ♀ and ♂ = the number of males and females that were dead after 24 hrs; total = total number of *Lu. longipalpis* ♀ and ♂ collected during the trapping period; = mean number collected on each night; ±sem = ± standard error of the mean.

Table 2. Summary of number of male and female *Lu. longipalpis* caught in the experimental chicken sheds treated with either a-cypermethrin impregnated netting or l-cyhalothrin residual spray over a 5-

month period in experiment 2.

		a-cypermethrin impregnated netting				l-cyhalothrin residual spray			
		collected		dead at 24h		collected		dead at 24h	
		□	□	□	□	□	□	□	□
May	total	280	107	276	100	151	67	151	67
		23.3	8.9	23.0	8.3	12.6	5.6	12.6	5.6
	±sem	8.8	2.2	8.8	2.0	1.3	1.2	1.3	1.2
July	total	339	108	323	101	256	107	255	106
		24.2	7.7	23.1	7.2	18.3	7.6	18.2	7.6
	±sem	4.6	1.6	4.5	1.5	3.2	1.3	3.2	1.3
Sept	total	231	103	162	51	184	101	163	92
		14.4	6.4	10.1	3.2	11.5	6.3	10.2	5.6
	±sem	2.6	1.6	1.6	1.1	3.0	1.4	2.2	1.3

total = the total number of *Lu. longipalpis*, male and (female) collected by the HP trap during each month; total dead 24 hr = the total number dead after 24 hrs; = daily mean catch; ±sem = ± standard error of the mean.

Table 3. Number of male and female *Lu. longipalpis* trapped in a longitudinal intervention trial (experiment 3) in an HP suction trap baited with pheromone (no light) in real chicken sheds treated with either a-cypermethrin impregnated netting or l-cyhalothrin residual spray.

		before intervention				after intervention							
		no insecticide				a-cypermethrin netting				l-cyhalothrin spray			
	H	collected		dead at 24h		collected		dead at 24h		collected		dead at 24h	
		□	□	□	□	□	□	□	□	□	□	□	□
R1	1	6	0	4	0	2	2	1	1	-	-	-	-
	4	24	7	12	4	20	7	17	7	-	-	-	-
	6	24	5	19	3	14	5	6	2	-	-	-	-
R2	2	44	7	31	4	21	6	20	5	-	-	-	-
	3	3	1	2	0	12	0	9	0	-	-	-	-
	5	134	17	96	11	40	17	32	15	-	-	-	-
total*		235	37	164	22	109	37	85	30	-	-	-	-
		39.2	6.2	27.3	3.7	18.2	6.2	14.2	5.0	-	-	-	-
±sem		19.9	2.5	14.4	1.7	5.2	2.4	4.6	2.3	-	-	-	-
R1	3	8	8	5	3	-	-	-	-	5	2	5	1
	5	226	32	144	27	-	-	-	-	130	16	66	7
R2	1	8	1	6	1	-	-	-	-	7	1	5	0
	4	30	5	19	1	-	-	-	-	12	3	6	3
	6	1	2	1	2	-	-	-	-	5	0	4	0
	7	12	3	12	3	-	-	-	-	7	6	6	4
total#		285	51	187	37	-	-	-	-	166	28	92	15
		47.5	8.5	31.2	6.2	-	-	-	-	27.7	4.7	15.3	2.5
±sem		35.9	4.8	22.7	4.2	-	-	-	-	20.5	2.4	10.1	1.1

R=experimental replicate; H=household in which the chicken shed was located; before intervention=number of male and female *Lu. longipalpis* trapped when no insecticide treatment was present (1st night); after intervention=number of male and female *Lu. longipalpis* trapped when either a-cypermethrin netting or l-cyhalothrin spray insecticide treatment was present in the chicken shed (2nd night). Total* is the total number of sand flies caught and then dead after 24h in all chicken sheds and on all nights after the a-cypermethrin netting intervention and total# is the total number of sand flies caught and dead after 24h in all chicken sheds and on all nights after the l-cyhalothrin spray intervention. = mean number of *Lu. longipalpis* collected on each night; ±sem = ± standard error of the mean.

Table 4. Longitudinal trapping experiment: numbers of male and female *Lu. longipalpis* trapped in a pheromone baited modified HP trap in real chicken sheds treated with either a-cypermethrin impregnated netting or l-cyhalothrin residual spray.

		before intervention				after intervention							
		no insecticide				a-cypermethrin netting				l-cyhalothrin spray			
	H	collected		dead at 24h		collected		dead at 24h		collected		dead at 24h	
		□	□	□	□	□	□	□	□	□	□	□	□
	1	35	13	22	5	-	-	-	-	35	13	18	8
R1	2	3	0	2	0	-	-	-	-	6	3	4	1
	3	10	2	2	2	10	3	8	3	-	-	-	-
	4	32	8	14	4	8	6	5	4	-	-	-	-
	1	86	9	39	3	28	7	27	3	-	-	-	-
R2	2	20	7	19	7	1	1	0	1	-	-	-	-
	3	13	5	8	1	-	-	-	-	17	7	10	5
	4	15	17	3	0	-	-	-	-	29	11	17	4
	1	67	15	40	9	18	5	18	5	-	-	-	-
R3	2	8	0	2	0	-	-	-	-	1	1	0	1
	3	19	10	13	6	10	3	8	3	-	-	-	-
	4	23	19	17	10	-	-	-	-	62	18	40	11
	1	26	11	20	8	-	-	-	-	39	12	38	12
R4	2	6	0	5	0	3	1	1	1	-	-	-	-
	3	7	1	4	1	-	-	-	-	22	5	19	5
	4	26	17	18	(0	23	12	23	12	-	-	-	-
	1	19	6	17	5	-	-	-	-	14	13	14	13
R5	2	2	2	2	2	1	2	0	1	-	-	-	-
	3	7	4	2	3	-	-	-	-	12	2	7	1
	4	9	3	2	1	16	11	12	8	-	-	-	-
total[+]		433	149	251	77								
mean		21.7	7.5	12.6	3.9								
±sem		21.2	6.2	11.7	3.5								
total*		277	73	154	44	118	51	102	41				
mean		27.7	7.3	15.4	4.4	11.8	5.1	10.2	4.1				

±sem	8.8	1.8	4.5	1.1	2.9	1.2	3.1	1.1				
total[#]	156	76	97	33					237	85	167	61
mean	15.6	7.6	9.7	3.3					23.7	8.5	16.7	6.1
±sem	3.2	2.2	2.6	1.1					5.7	1.8	4.2	1.5

R=experimental replicate; H=house in which the chicken shed was located; before intervention=number of male and female *Lu. longipalpis* collected when no insecticide treatment was present (1st night); after intervention=number of male and female *Lu. longipalpis* collected when either a-cypermethrin netting or l-cyhalothrin spray insecticide treatment was present in the chicken shed (2nd night). Total^{+} is the total number of sand flies collected and dead after 24h in all chicken sheds and on all nights prior to intervention. Total* is the total number of sand flies collected and dead after 24h in all chicken sheds and on all nights after the a-cypermethrin netting intervention and total$^{\#}$ is the total number of sand flies collected and dead after 24h in all chicken sheds and on all nights after the l-cyhalothrin spray intervention.

Table 5. Control trap catches. Numbers of male and female *Lu. longipalpis* collected in HP suction traps (with light bulb) at 2 houses without insecticide treatment.

	House A				House B			
collection	collected		dead at 24h		collected		dead at 24h	
date	□	□	□	□	□	□	□	□
26/01	13	9	0	0	12	4	2	0
27/01	8	6	3	2	6	7	2	2
28/01	4	1	0	0	4	7	3	7
29/01	4	1	3	0	4	0	0	0
Total	29	17	6	2	26	18	7	9
	7.3	4.3	1.5	0.5	6.5	4.5	1.8	2.3
±sem	2.1	2.0	0.9	0.5	1.9	1.7	0.6	1.7
12/05	33	16	13	9	6	0	0	0
13/05	26	14	6	11	2	1	1	1
14/05	14	14	4	0	15	4	8	2
15/05	17	4	4	0	2	4	0	0
Total	90	48	27	20	25	9	9	3
	22.5	12	6.8	5	6.3	2.3	2.3	0.8
±sem	4.3	2.7	2.1	2.9	3.1	1.0	1.9	0.5
07/07	34	12	8	3	5	0	1	0
08/07	1	12	1	3	2	2	0	0
09/07	13	6	3	2	2	4	0	1
10/07	5	1	4	1	1	1	1	1
Total	53	31	16	9	10	7	2	2
	13	7.8	4.0	2.3	2.5	1.8	0.5	0.5
±sem	7.3	2.7	1.5	0.5	0.9	0.9	0.3	0.3
13/09	4	2	2	1	3	1	1	0
14/09	2	2	0	0	8	3	0	0
15/09	2	0	1	0	4	4	1	1
16/09	1	1	0	0	9	5	2	0
Total	9	5	3	1	24	13	4	1

	2.3	1.3	0.8	0.3	6.0	3.3	1.0	0.3
±sem	0.6	0.5	0.5	0.3	1.5	0.9	0.4	0.3

Collection date = the date on which the sand flies were collected; House (A or B) = the house in which the collection was made; and collected = the number of *L. longipalpis*, male and female collected by the HP trap each night in each trap; and dead at 24h = the number of males and females that were dead after 24 hrs; total= total number of *Lu. longipalpis* and collected during the trapping period;
 = mean number of *Lu. longipalpis* collected on each night; ±sem = ± standard error of the mean.

Figures

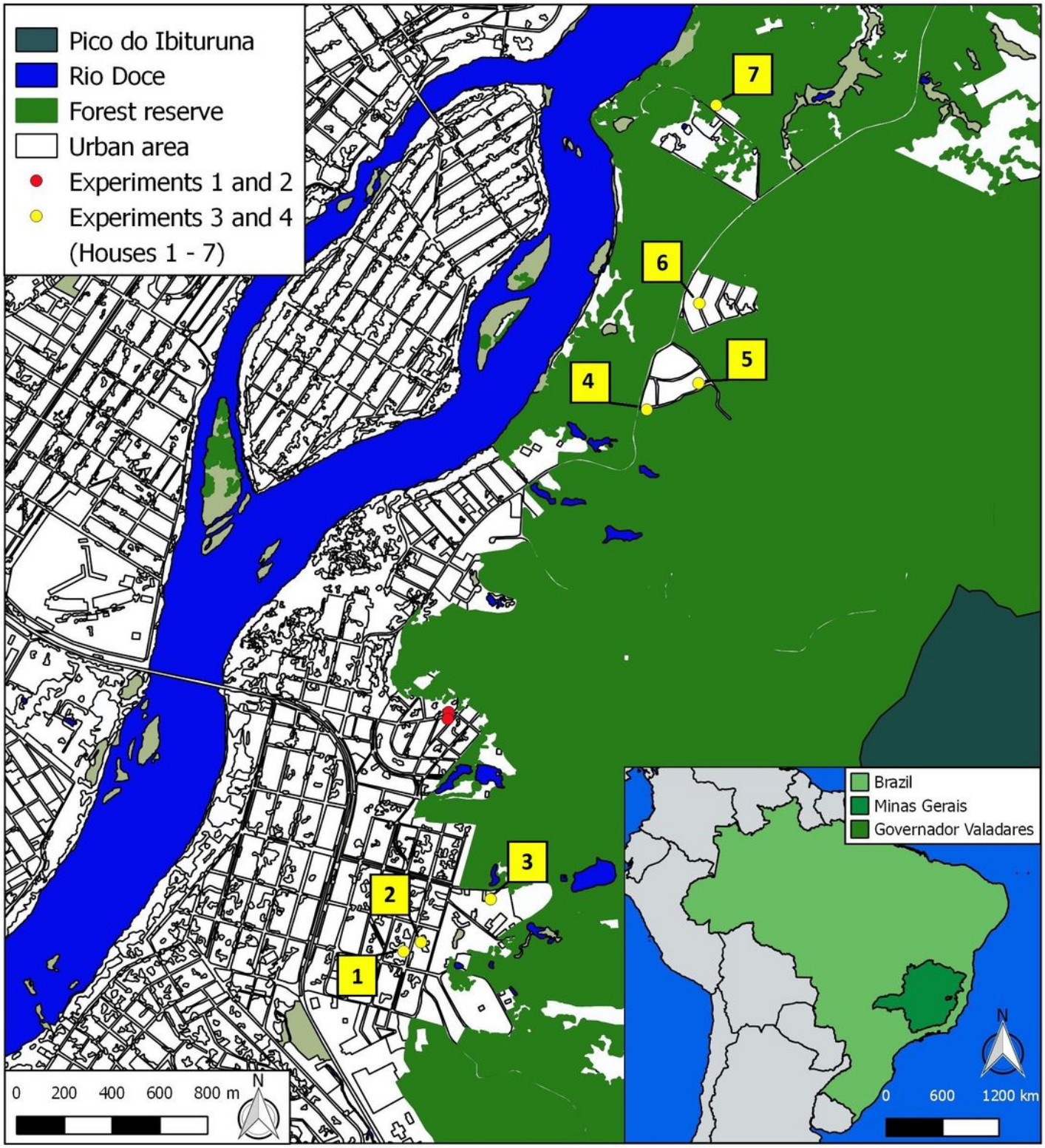

Figure 1

The locations for experiments 1 and 2 (red circles) and experiments 3 and 4 (yellow circles) are shown. The map was built using the Geographic information system QGIS 2.16. Cartographic bases were obtained from the Instituto Brasileiro de Geografia e Estatistica (IBGE) and Agência Nacional de Águas (ANA). Coordinates were assigned using The Universal Transverse Mercator (UTM) and the reference system SIRGAS 2000, zona 24S.

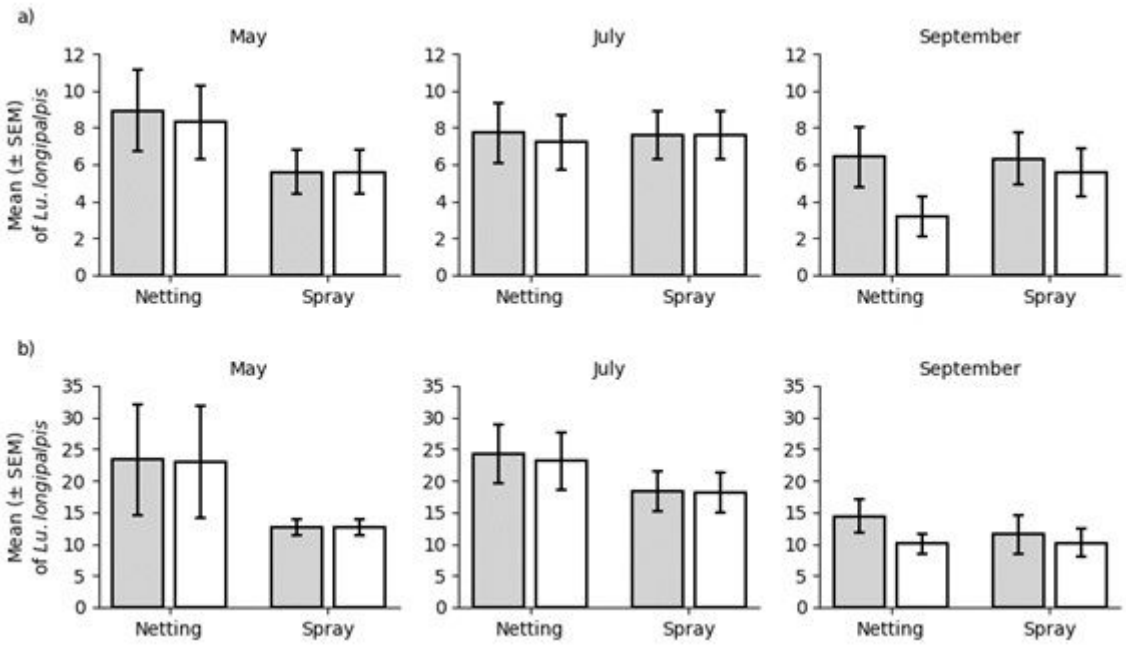

Figure 2

a) females and b) males, captured (grey bars) and dead after 24 hrs (white bars) during the trapping period. Netting = -cypermethrin impregnated netting treated experimental chicken sheds. Spray = -cyhalothrin residual spray treated experimental chicken sheds. Values shown are the mean number of females or males caught per day during the experiment ± standard error of the mean.

Supplementary Files

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