Evaluating the Effectiveness of Deltamethrin and Permethrin in Insecticide Treated Nets

in the Northern Peruvian Amazon

by

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Thesis submitted in partial fulfillment of
the requirements for the degree of
Master of Science in the Duke Global Health Institute
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ABSTRACT

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Abstract

The Loreto Region of Peru has the highest malaria rates in the country. Insecticide treated nets are one of the best prevention malaria prevention methods. Deltamethrin and permethrin are two of the most commonly used insecticides for treating bed nets, however insects are known to develop resistance to these insecticides. This study aims to compare deltamethrin and permethrin in the Northern Peruvian Amazon. This study examines the effectiveness of deltamethrin and permethrin in decreases the number of the malaria vector, the *Anopheles* mosquito, all mosquitos, and all insects in homes that use insecticide treated nets that have been treated with either deltamethrin or permethrin. Insects were collected using CDC light traps that were hung next to bed nets. Regressions were run after collecting insects that examine the decrease in number of insects in the homes, and odds ratios were calculated looking at the odds of seeing a decrease in number of insects in homes that received either a deltamethrin or permethrin treated net. Deltamethrin resulted in a statistically significant greater decrease in overall number of insects than permethrin. The study also identified bed net washing practices and inadequate insecticide residual spraying of homes in the study as present behaviors that have been shown to contribute to insecticide resistance. This study provides insight as to whether deltamethrin or permethrin is a more effective insecticide in treated bed nets in the Northern Peruvian

Amazon as well as identifies behaviors that are present that can contribute to insecticide

resistance.

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1. Introduction

Peru is classified to have a moderate risk level for contracting malaria. About 85% of the malaria in Peru is caused by *Plasmodium vivax* and 15% is caused by *Plasmodium falciparum*[1]. *P. falciparum* is a more deadly form of malaria than *P. vivax* as *P. falciparum* is responsible for the majority of malaria related deaths world-wide *[2]*. The best malaria prevention methods include home insecticide residual spraying and insecticide treated bed nets. Sleeping under a bed net is one of the most effective ways of preventing malaria infection as it disrupts the process of mosquitos feeding on humans. While sleeping under an untreated bed net still helps reduce the risk of malaria, sleeping under an insecticide treated bed net is even more effective [3]. Insecticide treated bed nets are an important form of mosquito control and disease prevention. Insecticide treated nets have been credited with a roughly fifty percent reduction in malaria cases worldwide from 2000 to 2008 [4, 5]. Insecticide treated nets are considered one of the most effective forms of malaria prevention, and have been shown to reduce the frequency and severity of malaria worldwide. Insecticide treated nets can reduce the clinical episodes of malaria caused by both *P. falciparum* and *P. vivax* by about fifty percent in areas where these bed nets are highly employed [6]. Insecticide treated bed nets have been shown to have an effect similar to herd immunity in that if a few homes in a community have bed nets, there is an overall protective effect for the entire community as the overall number of insects in the area decreases, which results in

lower transmission of malaria or other insect transmitted diseases and fewer cases of

these disease [7].

Furthermore, insecticide treated nets are generally an effective malaria

prevention method due to the fact that they are cost effective, easy to distribute, and

easy to use. Many governments of countries with high malaria transmission subsidize,

give vouchers for, or completely pay for bed net distribution throughout the country [8].

Some of these nets are considered long lasting and are thought to last for potentially up

to a year or more. Finally, studies have been performed to figure out how to increase

the uptake of insecticide treated nets in communities. As a result, bed nets are sold in

many different colors so that they can be personalized and appear to be decorative. This

has helped increase the amount of insecticide treated bed nets being used and used

properly [9].

While insecticide nets have greatly contributed to the lower number of malaria

cases over the last decade, insecticide treated nets run the risk of contributing to insect

resistance to insecticides, and thus these nets may become less effective over time [10].

This resistance makes not only the insecticide treated nets less effective, but also lowers

the effectiveness of the indoor spraying of homes leaving people more vulnerable to

malaria. Resistance to the chemicals used to treat insecticide treated nets is becoming

more prevalent in areas where bed nets and intensive fumigation campaigns are utilized

to control insect prevalence and insect transmitted diseases.

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Fumigating homes with insecticides is also effective against malaria transmission. However, for this method to truly be effective, studies from East Africa have shown that roughly eighty percent of homes in an area need to be sprayed [11]. This requires either a strictly regimented governmental program or a high level of responsibility among local residents. Unfortunately, many governments do not spray the required eighty percent of homes and local resident are often unable to either do it themselves due to difficulty obtaining the necessary materials [11]. Combining the use of both insecticide treated nets and indoor residual spraying of homes is considered the most effective way of lowering malaria incidence rates.

Permethrin and deltamethrin are two of the most common insecticides that are used to treat bed nets, and they are also both commonly used in insecticide residual spray campaigns. They are both part of the pyrethroid class of chemicals. Insecticides that are a part of this chemic class are the only insecticides that are approved for human usage, and are two of the most commonly used insecticides for treating insecticide treated bed nets. Both permethrin and deltamethrin work by disrupting the normal function of the nervous system of insects [12, 13]. However, the human body breaks these insecticides down quickly, and as a result, there are no long- or short-term negative health effects from these insecticides for humans [12, 13].

Permethrin and deltamethrin were two of the first pyrethroid insecticides used in areas with high levels of malaria and were originally very effective in lowering infections of malaria by repelling and killing the Anopheles mosquito [14]. In recent decades, insect resistance to the pyrethroid class of chemicals has been noted across many countries [14, 15]. This resistance has been found in many countries with similar disease profiles, climate conditions, and malaria prevention and control measures to Peru. Furthermore, there is some recent evidence for resistance to permethrin in some areas of the Peruvian Amazon [16]. However, no study has compared the efficacy of deltamethrin and permethrin in the Peruvian Amazon.

The main aim of this study was to determine the effectiveness of deltamethrin and permethrin, the two most commonly used insecticides to treated insecticide treated bed nets, in repelling the malaria vector, *Anopheles* mosquitos, in the Northern Peruvian Amazon. Permethrin and deltamethrin are both part of the pyrethroid class of chemicals. Insecticides that are a part of this chemic class are the only insecticides that are approved for human usage, and are two of the most commonly used insecticides for treating insecticide treated bed nets. Both permethrin and deltamethrin work by disrupting the normal function of the nervous system of insects, but neither insecticide has any major health effects for humans [12, 13]. In recent decades, insect resistance to the pyrethroid class of chemicals has been noted across many countries [14]. This resistance has been found in many countries with similar disease profiles, climate

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conditions, and malaria prevention and control measures to Peru. Permethrin resistance has been found in Africa, and Asia; furthermore, there is some recent evidence for resistance to permethrin in some areas of the Peruvian Amazon [15, 16]. However, no study has compared the efficacy of deltamethrin and permethrin in the Peruvian Amazon.

A secondary aim of this study was to gain deeper insight into the insect repelling behaviors used by people living in the Loreto Region of Peru and to see if any of these practices are potentially detrimental to the use of insecticide effectiveness in the area.

2. Methods

2.1 Study Setting

Alto Amazonas is one of the provinces in Loreto, the northern most region of Peru (Figure 1). Located in the Amazon jungle, the climate is both hot and humid throughout the year. Average temperatures throughout the year are steady around 90F[17]. During June and July, the months during which this study occurred, the average rainfall is about 100mm per month[17]. Roughly 120,000 people live in this region, with 64,000 living in Yurimaguas, the largest city in the region where no *Anopheles* exist so the malaria risk is minimal [18]. This study took place in two communities in this region, Pampa Hermosa and Santa Lucia (Figure 1).

This area of Peru is considered to have a high level of transmission of malaria, and roughly 95% of reported cases come from the Loreto Region[19, 20]. In 2015, there were roughly 850,000 suspected cases of malaria in Peru; however only 65,000 of these cases were confirmed [21]. This is thought to be due to the major form of malaria in Peru being *P. vivax*, which is less deadly and most likely leads to underreporting of cases as people are less likely to go to the health clinic since few people in Peru die from malaria [1]. Since 2012, the number of reported cases of malaria has doubled in Peru, and quintupled in the Loreto region [20].

Pampa Hermosa is a periurban community, with a main highway running through the middle of the community. The community is 40.1 kilometers southwest of

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Yurimaguas, the main city in the Alto Amazonas province. As of 2010, the population was 8.700 [22]. Agricultural work and palm oil harvesting are the major economic activities in this community. Of the households in Pampa Hermosa that participated in this study, the average household income was reported to be between 300 and 500 soles per month, which is roughly 92 to 154 USD per month.

Santa Lucia is an urban community that is 20.6 kilometers west of Yurimaguas. There are no population statistics from the Peruvian census for Santa Lucia, but Santa Lucia is a smaller community than Pampa Hermosa [22]. There is one road leading into Santa Lucia, and there is a river to the North of the community. Both the road and the river are used to bring resources into the community, and both are used for human movement as well. Agricultural work is the main economic activity in this community. Of the twenty households in Santa Lucia that participated in this study, the average income was reported to be between 100 and 300 soles per month, which is roughly 31 to 92 USD per month.

A list of mosquitos and other insects that were caught in these two communities throughout the study is attached in Appendix B.

A. Pampa Hermosa

B. Santa Lucia

Figure 1: Locations of Participating Households in the Two Communities.

2.2 Sampling

Two communities, Pampa Hermosa and Santa Lucia, within the Alto Amazonas were used in this study. Twenty households in each community participated in the study for a total of forty households. Households were eligible to participate in the study if at least one family member in the household had contracted malaria within the

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last six months. This selection criteria was chosen based on the fact that *Anopheles*

mosquitos are difficult to trap, and if somebody in the family had contracted malaria in

the past six months, we knew that the family had some kind of contact with *Anopheles*

mosquitos which could lead to a better ability to trap *Anopheles* mosquitos.

The sample size of forty homes was chosen so as to see a decrease of twenty

percent of insects. This effect size was chosen based on effect sizes seen in other studies

[23, 24]. No similar studies have been performed in South America so this effect size

was chosen based off of the results of similar studies in other regions.

2.3 Procedures

CDC light traps were set up in each of the forty homes for four nights each. A

bottle containing a mixture of water, sugar, and yeast was hung alongside the CDC light

traps [25, 26]. This mixture produces CO_2, which attracts *Anopheles* mosquitos to the

area of the CDC light trap [26-28]. A packet of the chemical octenol was also attached to

the trap as this chemical produces a smell that acts as an attractant for the *Anopheles*

mosquito [26, 29-32]. Anopheles mosquitos are a difficult species to catch and the

combination of the CDC light trap, CO_2 producing mixture, and the octenol are

considered the best way to trap the *Anopheles* [29, 33-35]. The CDC light traps were hung

directly next to a bed of one of the household family members. The bed used was

always up against an outside wall of the home as these areas have a higher

concentration of insects due to gaps in the walls.

During the first two nights of insect trapping, an untreated bed net was hung over the bed next to the CDC light trap. Insects were trapped from 6 pm until 6 am the next morning. During the second two nights of insect trapping, a bed net treated with either permethrin or deltamethrin was hung over the bed next to the CDC light trap, and the insects were trapped from 6 pm until 6 am the next morning as this is the active feeding time of the *Anopheles* mosquito. Ten homes in each community received a bed net treated with permethrin, and ten homes in each community received a bed net treated with deltamethrin. At the end of the four nights, study participants kept the treated net for future use.

Every morning, the insects were collected, killed, and counted. The insects were sorted into three categories: number of *Anopheles*, total number of mosquitos, and total number of insects.

Furthermore, one member from each household completed a survey. This survey collected data such as demographic data, GPS location, and insect repelling behaviors. This survey was used to identify practices that could potentially help lead to insecticide resistance in the area. The survey was administered in Spanish by one of the study staff members, and took approximately twenty to thirty minutes to complete. The survey is included in Appendix A.

Figure 2: Study Design Diagram

2.4 Measures

2.4.1 Insect Abundance

After the insects were trapped and killed, they were sorted into groups and counted. The groups consisted of *Anopheles,* other mosquitos, and other insects. *Anopheles* mosquitos are easily identifiable due to the white stripes on their legs. Insect counts were spot checked by an entomologist.

2.4.2 Meteorological Conditions

Temperature and humidity were taken three times throughout the collection period of 6 pm until 6 am every night. These data points were recorded every day at 6 pm when the light traps were set up, at midnight which was the midway point of the collection, and at 6 am when the light traps were turned off. Rainfall was also measured, however there was never any rainfall during the collection periods.

2.4.3 Household Mosquito Control Practices

One member of each household was asked to participate in a survey to collect socio-demographic information from the two communities where this study took place. Questions regarding the household, income, bed net usage, and other insect control and prevention behaviors were asked. Study personnel who were fluent in Spanish administered the survey. The questions used in the survey had not been previously validated in the area. The survey is listed in Appendix A.

2.5 Analysis

A linear regression model was used to compare the change in number of insects collected after implementation of a bed net treated with either permethrin or deltamethrin. The model used also contained data for the GPS location of the household, temperature, and humidity. This model was used with the change in number of *Anopheles* mosquitos collected, total number of mosquitos collected, and total number of insects collected as the dependent variables. Odds ratios, from a logistic

regression, for the odds of seeing a decrease in *Anopheles,* total number of mosquitos, and total number of insects based on the insecticide used to treat the bed nets.

Finally, data from the demographic survey was analyzed to evaluate areas for potential future focus regarding mosquito and malaria control efforts. Survey participants were asked questions regarding their personal and household ITN usage patterns and how often and with which insecticide their homes had been sprayed. Participants were also asked to elaborate on any other mosquito control measures they employed.

2.6 IRB Approval

This study had IRB approval from Cayetano Heredia University, and the IRB protocol number is SIDISI 66647.

3. Results

The insects were categorized into three categories: *Anopheles,* total mosquitos, and total insects. The counts for each of the three categories found in each community can be seen in Table 1.

Table 1: Insect Counts by Community

	Total Anopheles	Total Mosquitos	Total Insects
Pampa Hermosa			
Control Period	8	654	2682
With Deltamethrin	7	346	1143
With Permethrin	0	161	1222
Santa Lucia			
Control Period	10	878	6245
With Deltamethrin	8	319	1814
With Permethrin	13	474	2674
Total	46	2832	15780

The insects were spot checked by an entomologist, and identified by species. The breakdown for the species collected in Pampa Hermosa can been see in Table 4. The insects collected in Santa Lucia were too degraded due to heat exposure for identification.

3.1 Change in Anopheles

After switching from an untreated bed net to a bed net treated with deltamethrin, there was a mean increase of 0.2 *Anopheles* over a two-night collection period, as indicated in Figure 2 which shows the distribution of change in Anopheles per household by the insecticide used. After switching from an untreated bed net to a bed net treated with permethrin, there was a mean increase in 0.3 *Anopheles* over the two-night collection period, as indicated in Figure 2. This change in number of *Anopheles* is not statistically significant (p=.788) as indicated in Table 1.

Figure 3: Change in Anopheles Mosquitos by Treatment.

Table 1 lists the variables considered in the model used to predict the change in number of *Anopheles* mosquitos due to the use bed nets treated with either deltamethrin or permethrin. Only variables with a p-value less than 0.05 had a significant effect on the change in number of *Anopheles* mosquitos. The p-values for all the variables considered in the model are shown in Table 1.

Table 2: Beta Coefficients and p-values for Model Variables for Change in Anopheles

	β	p-value	95% CI
Permethrin vs. Deltamethrin	.128	0.788	-.831, 1.086
Temperature at 6pm	-.0379	0.494	-.149, .0733
Temperature at 12 am	--	--	--
Temperature at 6 am	--	--	--
Humidity at 6 pm	3.653-8	0.229	-2.40e-8, 9.69e-8
Humidity at 12 am	--	--	--
Humidity at 6 am	--	--	--
Deltamethrin (intercept)	3.082	0.647	-10.466, 16.631

-- indicates variables that were omitted due to collinearity

A logistic regression was performed to find the odds ratios for seeing a decrease in *Anopheles,* and the resulting odds ratios are shown in Table 2. The treatment of the bed net did not have a significant effect (p=.867) on the odds ratio for seeing a decrease. A decrease was defined as a negative change in number of *Anopheles*, while an increase in *Anopheles* or no change in number of *Anopheles* were both classified as a non-decrease.

Table 3: Odds Ratios for A Decrease in *Anopheles*

	OR	p-value	95% CI
Permethrin vs. Deltamethrin	.9862	0.867	.151, 4.913
Temperature at 6pm	.845	0.282	.622, 1.149
Temperature at 12 am	--	--	--
Temperature at 6 am	--	--	--
Humidity at 6 pm	1	0.016*	.999, 1
Humidity at 12 am	--	--	--
Humidity at 6 am	--	--	--
Deltamethrin (intercept)	1.23e10	0.222	7.69e-7, 1.95e26

-- indicates variables that were omitted due to collinearity
* indicates statistical significances

3.2 Change in Total Mosquitos

Households given a bed net treated with deltamethrin showed a mean decrease

of 8.8 total mosquitos over the two-night collection period after switching to the

deltamethrin treated net from an untreated to a treated bed net as shown in Figure 3.

Households given a bed net treated with permethrin shows a mean decrease of 10.5 total

mosquitos over a two-night collection period after switching from an untreated to a

treated bed night as shown in Figure 3. This difference in change in number of

mosquitos due to the treatment is not statistically significant (p=.906).
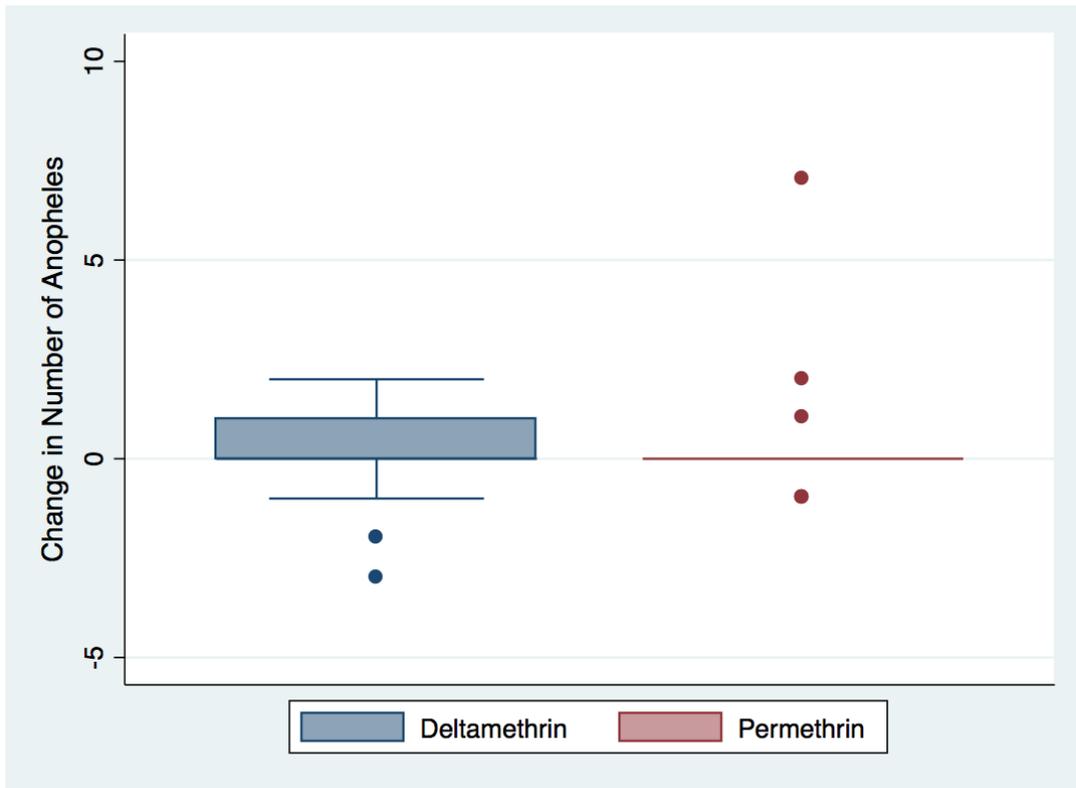
Figure 4: Change in Total Number of Mosquitos by Treatment

Table 2 lists the variables considered in the model to predict the change in number of total mosquitos due to bed nets treated with either deltamethrin or permethrin. Only variables with a p-value of less than 0.05 had an effect on the change in number of total mosquitos. Table 2 shows the p-values for all the variables in the model

Table 4: Beta Coefficients and p-values for Model Variables for Change in Mosquitos

	β	p-value	95% CI
Permethrin vs. Deltamethrin	-1.607	0.906	-29.023, 25.812
Temperature at 6pm	.137	0.931	-3.043, 3.318
Temperature at 12 am	--	--	--
Temperature at 6 am	--	--	--
Humidity at 6 pm	7.45e-8	0.931	-1.65e-6, 1.80e-6
Humidity at 12 am	--	--	--
Humidity at 6 am	--	--	--
Deltamethrin (intercept)	-25.819	0.893	-413.369, 361.731

-- indicates variables that were omitted due to collinearity

A logistic regression was performed to find the odds ratios for seeing a decrease in total number of mosquitos, and the resulting odds ratios are shown in Table 4. The treatment of the bed net did not have a significant effect (p=.162) on the odds ratio for seeing a decrease. A decrease was defined as a negative change in number of mosquitos, while both an increase in mosquitos or no change in number of mosquitos were both classified as a non-decrease.

Table 5: Odds Ratios for Decrease in Total Number of Mosquitos

	OR	p-value	95% CI
Permethrin vs. Deltamethrin	2.813	0.162	.660, 11.996
Temperature at 6pm	.924	0.362	.778, 1.096
Temperature at 12 am	--	--	--
Temperature at 6 am	--	--	--
Humidity at 6 pm	1	0.434	.999, 1
Humidity at 12 am	--	--	--
Humidity at 6 am	--	--	--
Deltamethrin (intercept)	1418.622	0.484	2.12e-6, 9.49e11

-- indicates variables that were omitted due to collinearity

3.3 Change in Total Insects

Figure 4 shows the change in total number of insects after switching from the untreated bed net to a bed net treated with either deltamethrin or permethrin. In terms of overall number of insects, households given a bed net treated with deltamethrin had an average decrease of 87.15 insects over a two night collection period, whereas households given a bed net treated with permethrin had an average decrease of 6.4 insects over the two night collection period (p=.020).
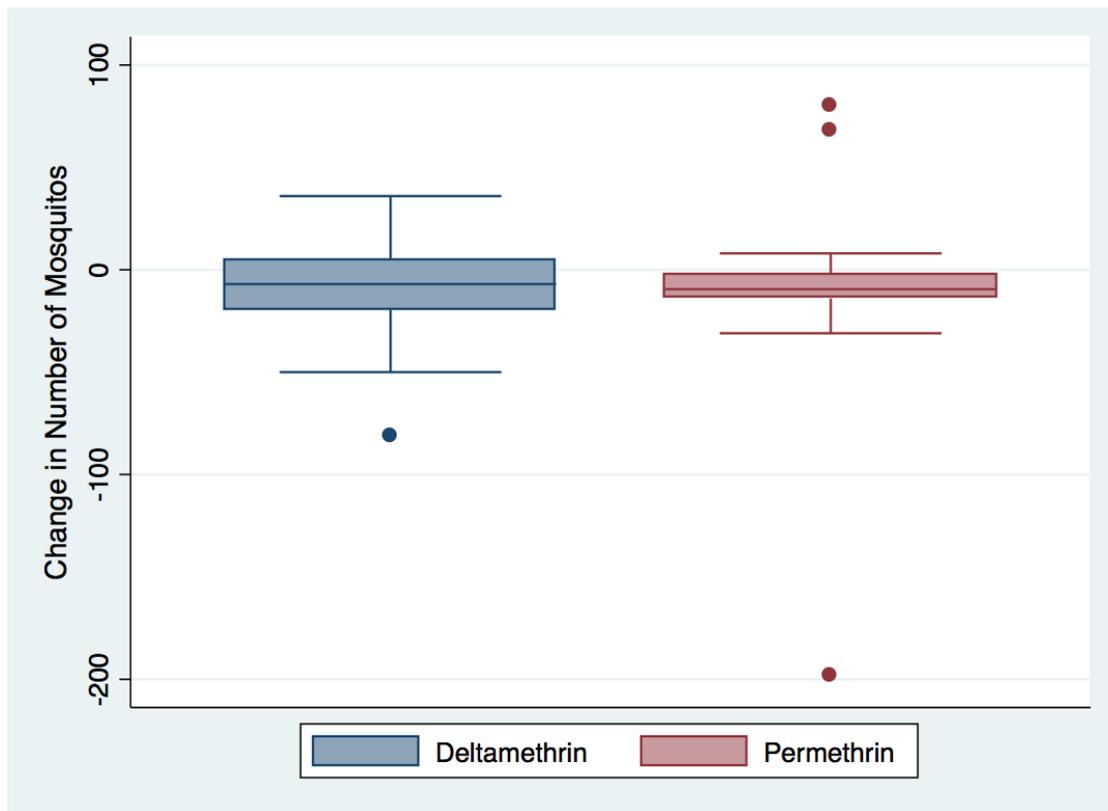
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Figure 5: Change in Number of Insects by Treatment

Table 3 lists the variables that were considered in the model to predict change in

number of total insects due to bed nets treated with either deltamethrin or permethrin.

Only variables with p-values less that 0.05 had a significant effect on the change in

number of overall insects. Table 3 shows the p-values for the variables considered in the

model.

Table 6: Beta Coefficients and p-values for Model Variables for Change in Insects

	β	p-value	95% CI
Permethrin vs. Deltamethrin	84.482	0.020*	14.392, 154.572
Temperature at 6pm	10.900	0.010*	2.769, 19.031
Temperature at 12 am	--	--	--
Temperature at 6 am	--	--	--
Humidity at 6 pm	2.29e-7	0.917	-4.14e-6, 4.73e-6
Humidity at 12 am	--	--	--
Humidity at 6 am	--	--	--
Deltamethrin (intercept)	-1435.153	0.006*	-2425.82, -444.465

-- indicates variables that were omitted due to collinearity
* indicates statistical significances

A logistic regression was performed to find the odds ratios for seeing a decrease in total number of insects, and the resulting odds ratios are shown in Table 6. The treatment of the bed net did not have a significant effect (p=.271) on the odds ratio for seeing a decrease. A decrease was defined as a negative change in number of insects, while both an increase in *Anopheles* or no change in number of insects were both classified as a non-decrease.

Table 7: Odds Ratios for A Decrease in Total Insects

	OR	p-value	95% CI
Permethrin vs. Deltamethrin	.447	0.217	.107, 1.873
Temperature at 6pm	.796	0.013*	.665, .954
Temperature at 12 am	--	--	--
Temperature at 6 am	--	--	--
Humidity at 6 pm	1	0.719	.999, 1
Humidity at 12 am	--	--	--
Humidity at 6 am	--	--	--
Deltamethrin (intercept)	6.61e11	0.014*	270.431, 1.62e21

--indicates variables that were omitted due to collinearity
* indicates statistical significance

3.4 Household Mosquito Control Practices Results

Answers from the survey were examined to find areas of insect repelling behaviors or patterns that coincide with known risk factors for the development of insecticide resistance. Three main areas of concern were identified: bed nets retreatment, bed net washing patterns and insecticide residual spraying patterns.

For the first area of concern, bed net retreatment, participants were asked if their bed net had ever been treated with an insecticide, which insecticide had been used to treat the bed net, if they retreated their bed nets, and how often they retreated the bed net. All forty homes reported that they did not know if their bed nets had ever been treated, and all forty homes reported that they had never treated their bed nets themselves.

For the first area of concern, bed net washing, study participants were asked if they washed their bed nets and how often they washed their bed nets. All forty

households that participated in the study reported washing their bed nets on a regular

basis, and the intervals of the washing of the bed nets are show in Figure 6.

Figure 6: Reported Frequency of Bed net Washing

For the second area of concern, insecticide residual spraying of homes, study

participants were asked if their homes had ever been sprayed, when the last spray

occurred, and if they knew which insecticide was used to spray their home. Of the forty

households in the study, only twenty-five of the homes reported that their homes had

been sprayed. The other fifteen homes reported that they believed that their homes had

been sprayed but either could not remember or say with certainty that their homes had

indeed been sprayed. Only nine homes reported that their homes had been sprayed in

the past six months; however only four knew the actual date that their home had been

sprayed. One of these homes had been sprayed in the last six months because family members had sprayed the home themselves. The homes that did not know when their home had last been sprayed reported that while they did not know the date of the last spray, their homes had not been sprayed in the last six months. All twenty-five households that had certainly been sprayed reported that they did not know which insecticide had been used to spray their homes; even the household that sprayed their own home did not know which insecticide had been used as the treatment.

4. Discussion

4.1 Deltamethrin vs. Permethrin

4.1.1 Change in Number of *Anopheles*

There was no significant change in number of *Anopheles* mosquitos by type of insecticide used on the bed net. The odds of seeing a decrease when comparing permethrin to deltamethrin was also insignificant (p=.867). There was a very small number of *Anopheles* caught over the duration of this study, and that contributes to the fact that there is no statistically significant data concerning the change in number of *Anopheles* mosquitos between households using a net treated with deltamethrin or permethrin after switching from an untreated net to a net treated with one of the insecticides. The number of households also contributes to this. The sample size of forty households was calculated as having enough power to detect at 20% decrease in the number of *Anopheles,* total number of mosquitos, and total number of insects.

However, a 20% decrease in *Anopheles* was not seen. This effect size was calculated based off effect sizes reported from other regions. It's possible that either the sample size was too small to see this effect size, or due to differing locations, this effect size will not be seen, and a sample size based off of a smaller effect size should have been calculated.

This model originally included the following variables: treatment of the bed net, latitude and longitude of the participating households, temperature at 6 pm, temperature at 12 am, temperature and 6 am, humidity at 6 pm, humidity at 12 am, and humidity at 6 am. However, due to multi-collinearity, the final model only included treatment of the bed net, latitude and longitude of the participating households, temperature at 6 pm, and humidity at 6 pm. This is also true for the models for the change in total number of mosquitos and total number of insects.

4.1.2 Change in Number of Total Mosquitos

There was no significant change in number of Anopheles mosquitos due to the treatment used on the bed net. In fact, none of the variables considered in the model had any statistically significant effect on the number of mosquitos caught during the collection periods. Just as with the change in number of *Anopheles*, there was a small number of total mosquitos trapped throughout the duration of the study. Again, the study was calculated to have enough power to detect a 20% decrease in total number of mosquitos. There was a decrease in number of total mosquitos, but it was smaller than

20%. This contributed to the fact that there is no statistically significant difference

between the change in number of mosquitos due to deltamethrin and permethrin. The

odds of seeing a decrease in total number of mosquitos when comparing permethrin to

deltamethrin was also insignificant (p=.162).

4.1.3 Change in Number of Total Insects

There was a statistically significant effect on the change in number of total insects

caught due to the treatment used to treat the bed net. The treatment used was the only

variable in the model that had a statistically significant effect on the change in number of

insects when switching from an untreated net to a treated net. Deltamethrin had a

statistically significant greater decrease in insects caught after switching from an

untreated net to a deltamethrin treated net than switching to a permethrin treated bed

net. Overall, deltamethrin had an average decrease of 87.15 insects over a two-night

collection period, whereas permethrin had an average decrease of 6.4 insects over the

two-night collection period. The odds of seeing a decrease in total number of insects

when comparing permethrin to deltamethrin was insignificant (p=.217)

4.2 Household Mosquito Control Practices

The demographic survey showed a few concerning behavioral patterns that

could lead to ineffective interventions involving ITNs. First, all of the households

reported regular washing of their bed nets. This is concerning because while washing

bed nets maintains good hygiene within the household, washing a bed net washes more

and more of the insecticide out with each successive wash. As the level of insecticide exposure constantly decreases without retreatment, insects can develop resistance to these insecticides [36]. Weekly washing for two and a half months has been shown to lead to a 50% reduction in the concentration insecticide left on the bed net [37]. In order to ensure long-term effectiveness of insecticide treated nets, the bed nets should not be washed or washed infrequently, or the bed nets should be regularly treated if they are washed often. Furthermore, insecticide treated nets should be retreated every six months at minimum. None of the forty households involved in the study knew if their bed net had ever been treated, and none of the homes had ever personally treated their bed nets. Retreating nets on a regular basis can help mitigate the effects of washing a bed net.

Second, only twenty-five of the forty households reported having their homes sprayed for insects. Of those twenty-five households, only nine had been treated in the past six months. Homes need to be sprayed roughly every six months for spray campaigns to be effective. If this praying schedule is not possible, spray campaigns should be done before peak rainy seasons as rainy season coincide with peak malaria incidence rates. Furthermore, none of the homes knew which insecticide had been used to spray their homes. The health posts in the areas also did not know which insecticides had been used in which areas. Officials at the DISA for the region reported that they had recently switched from using deltamethrin or permethrin to using malathion to

spray homes. However, they did not have exact dates as to when this change took place.

They reported that some areas were probably still being sprayed with deltamethrin or

permethrin, but they were unsure as to which areas. Spray campaigns, such as this, that

are irregular and inconsistent with the insecticide being used and the schedule of

spraying can lead to insect resistance. This is a situation that needs more attention and

should be tracked moving forward.

Insecticide treated nets are one of the best ways to prevent malaria as they are

cheap, easy to distribute, and easy to use; insecticide treated nets used in combination

with insecticide residual spraying is thought to be the most effective preventative

measure for malaria. These prevention methods need to be used correctly to both

effectively prevent malaria cases and help reduce the risk of insects developing

resistance to the insecticides being used.

4.3 Implications for Further Research

While there was no statistically significant difference between the effectiveness of

permethrin and deltamethrin when examining the change in number of *Anopheles*

mosquitos or change in number of all mosquitos, there was a statistically significant

difference between deltamethrin and permethrin. Research with a larger sample would

help bolster this finding, and may also find significant results for the change in *Anopheles*

mosquitos and overall number of mosquitos. Based on the effect size of the decrease in

number of mosquitos after switching form an untreated net to a treated net, performing

30

the study again using a sample size of 62 households could provide significant results. Genetic testing on insects would also need to be done to test confirm whether or not there is any genetic resistance to these two insecticides in the area.

5. Conclusion

This study is important because insecticide resistance is becoming more and more of a problem around the world, and this is one of the first studies to examine potential insecticide resistance in the Peruvian Amazon. This study shows that while there was statistically significant difference in the effectiveness of deltamethrin and permethrin when looking at the *Anopheles* mosquitos or total number of mosquitos, deltamethrin was statistically significantly more effective when looking at the total number of insects. This could indicate that there may be some resistance in the area. Further research with added sample size is necessary to confirm this finding.

Appendix A: Survey

Evaluación de Eficacia de Mosquiteros Impregnados para Prevenir Malaria y Leishmaniosis Cutánea en San Martin y Loreto, Perú

A. Información de fondo:

Nombre de la comunidad		Código de hogar		Fecha de visita	

PS	Lat:	_____._____ °	S	Long:	W_____.___ _____ °	Alt:	— _____ metros

Información de la Entrevista			
	Iti als	Vi sita 2	Vi sita 3
Fecha de visita:			
Resultados (códigos):			
Entrevistador:			
Encuestado (nombre):			
Hora de comienza:			
Hora de termino:			

Nombre de la cabeza económica	

Nombre de la pareja (de la cabeza)	

B. Información del Hogar:

1. Año la casa fue construido:	
2. Tipo de suelo (cemento, madera, tierra, otro:_____):	
3. Tipo de techo (palma, hierro corrugado, madera, otro:_____):	
4. Electricidad (sí o no):	Si / No
5. Si hay electricidad, por cuantas horas cada día?	
6. Piso elevado o en la tierra:	
7. Método de cocinar (fuego, cocina, otro):	
8. Área de cocinar está adentro o afuera:	
9. Numero de cuartos:	
10. Fuente de agua:	
11. Distancia a esta fuente (minutos):	minutos
12. Donde desecha la basura de alimento?	
13. Otra basura?	
14. Tiene esta casa una letrina?	Si / No
15. Si no, donde van al baño (letrina de una familia, bosque, cerca del rio)	

*Después de la entrevista, camine alrededor de la casa y buscar para envases de agua

B. Información del Hogar (Continua):

20. Numero de personas viviendo en esta casa:	
21. Numero de niños viven en esta casa:	
22. Numero de mosquiteros en la casa:	
23. Ha sido impregnado con un insecticida?	
24. En caso afirmativo, cuando fue el tratamiento mas reciénte?	
25. Lava su mosquitero?	
26. En caso afirmativo, que tan frecuentemente?	
27. Fecha del ultimo lavado?	
28. Numero de personas que durmieron en la casa anoche:	
29. Numero de personas que durmieron debajo de un mosquitero anoche:	
30. Numero de personas que no durmieron debajo de un mosquitero anoche:	
31. Ha fumigado su casa para insectos?	Si / No

16. Cuales métodos usa para reducir el numero de zancudos en su casa? Una lista. *Para el entrevistador: espera y pregunta si algo mas?*	
17. Si usa insecticidas/repelentes, cuales? Una lista. *Para el entrevistador: espera y pregunta si algo mas?*	
18. *Numero de contenedores abiertos de agua adentro de la casa:	
19. *Numero de contenedores abiertos de agua alrededor de la casa (afuera):	

32. En su caso, cuando se fumigó? (las ultimas tres veces)	mm/yy	Fecha 1	Fecha 2	echa 3
		_ _/__	_ _/__	_ _/_ _
33. Han fumigado sus vecinos sus casa para insectos?				
34. Si no, porque piensa que no?				
35. Quien fue responsable por de fumigar su casa?		a. A su área	. A su casa	

36. Numero de mascotas o animales adentro su casa:				37. Numero animales en el área peridomestico:			
Animal	#	*Animal*		*Animal*		*Animal*	
a. Aves		f. Perros		a. Aves		f. Patos	
b. Cerdos		g. Pollos		b. Caballos		g. Perros	
c. Cuyes		h. Otro:____		c. Cerdos		h. Vacas	
d. Gatos		i. Otro: ____		d. Cuyes		i. Otro: _____	
e. Patos		j. Otro: ___		e. Gatos		j. Otro: _____	

B. *Información del Hogar (Continua):*

38. Nivel de educación mas alto alcanzado:	**a. Hombre**	**b. Mujer**

39. Edad:		
40. Por cuanto tiempo ha vivido en esta comunidad?		
41. En general, cual es el ingreso mensual total de este hogar?	< 100 soles 100-300 soles 300-500 soles 500-750 soles 750-1000 soles > 1000 soles	

Diagrama

Incluido la cama / área alrededor de la casa / donde esta la trama / distancia y altura de la trampa la distancia a la pared / ventana mas cerca

42. El mosquitero reemplaza una de la familia:	Si	/	No	
43. En case afirmativo, fue impregnado:	Si	/	No	
44. En caso afirmativo, cuando empezar a usarlo:	_____ MM / _____ YYYY			

	1	2	3	4
45. Hora de encender la trampa (PM):				
46. Hora de apagar la trampa (AM – mañana):				
47. Cuantas personas durmieron debajo del mosquitero anoche?				
48. Cuantas personas durmieron en la casa anoche?				
49. Hora de acostarse (debajo del mosquitero):				
50. Hora de despertarse (personas debajo del mosquitero):				

Appendix B: Further Statistics for Model Variables

Table 8: Counts of Spot Checked Mosquitos in Pampa Hermosa

	Count	Proportion of Insects Spot Checked
Anopheles benarrachi	14	.0156
Anopheles rangeli	1	.0011
Anopheles species unknown	5	.0056
Culex declarator	11	.0123
Culex theobaldi	37	.0413
Culex corniger	5	.0056
Culex vomerifer	1	.0011
Culex adamesi	1	.0011
Culex species unknown	379	.4230
Psorophora albigenus	1	.0011
Mansonia amazonensis	1	.0011
Mansonia species unknown	3	.0033
Uranotaenia species unknown	15	.0167
Ochlerotatus scapularis	2	.0022
Ochlerotatus species unknown	1	.0011
Coquillettidia juxtamansonia	10	.0112
Coquillettidia venezuelensis	315	.3516
Coquillettidia hermanoi	14	.0156
Coquillettida species unknown	80	.0893
Total	896	1.000

The insects from Santa Lucia were too heat damaged for similar identification.

Appendix C: Further Statistics for Model Variables

Table 9: Insect Counts by Community - Full Statistics

	Average	Standard Error	95% CI
Change in *Anopheles* in deltamethrin homes in Santa Lucia	.1	.433	-.881, 1.080
Change in *Anopheles* in permethrin homes in Santa Lucia	.3	.367	-.529, 1.129
Change in *Anopheles* in deltamethrin homes in Pampa Hermosa	-.2	.133	-.502, .101
Change in *Anopheles* in permethrin homes in Pampa Hermosa	.9	.737	-.767, 2.567
Change in Total Mosquitos in deltamethrin homes in Santa Lucia	-8.1	9.686	-30,010, 13.810
Change in Total Mosquitos in permethrin homes in Santa Lucia	-9.5	7.957	-27.500, 8.500
Change in Total Mosquitos in deltamethrin homes in Pampa Hermosa	-6.6	3.038	-13.471, .271
Change in Total Mosquitos in permethrin homes in Pampa Hermosa	-14.4	23.629	-67.854, 39.054
Change in Total Insects in deltamethrin homes in Santa Lucia	-17.6	21.878	-67.090, 31.890
Change in Total Insects in permethrin homes in Santa Lucia	6.2	11.255	-19.261, 31.662
Change in Total Insects in deltamethrin homes in Pampa Hermosa	-135.2	44.622	-236.142, 34.258
Change in Total insects in permethrin homes in Pampa Hermosa	-15.6	46.202	-102.115, 88.915

Table 10: Temperature and Humidity Statistics

	Average	Standard Error	95% CI	Min	Max
Temperature (Celsius) at 6 pm	28.796	.542	27.675, 29.917	26.111	33.333
Humidity (%) at 6 pm	78.292	2.650	72.812, 83.773	55	94

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