

Impediments to Malaria Elimination

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Abstract

The following study was done on the impediments of malaria suppression that are hindering researchers from eliminating malaria. The main impediments being discussed in the study are population densities of areas as well as the number of anopheles that have the ability to transmit malaria in the area. The mosquitos being looked at in reference to malaria were the *Anopheles gambiae* and the *Anopheles funestus* due to their ability to transmit malaria causing countless deaths. The study was done both in a laboratory setting which took place in Lusaka Zambia, and in a research setting where information was gathered using databases. The laboratory environment consisted of the collection of wild mosquitos and the research involved acquiring census data on the population densities and malaria incidence of various wards in Zambia. The results of the study show that there is a correlation between the population Density of an area and the malaria incidence as well as the number of mosquitos that transmit malaria in the area and the malaria incidence. This allows researcher to have an area of focus in malaria elimination since it is evident that some areas pose a higher risk of malaria.

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Introduction

In many developing nations, high mosquito populations can be detrimental to societies, leading to an increase amount of disease. Mosquitoes are responsible for several vector borne diseases that can have a huge affect on people's health, and in many cases are fatal. According to the World Health Organization, over 1 million people die from mosquito-borne diseases each year and 20 million people are affected by them. Diseases such as Malaria, Dengue, Yellow fever and Zika have riddled populations around the world causing countless deaths. 90% of all mosquito related disease cases are in Africa due to its high mosquito populations. Therefore, there is an urgent need to better understand how to control mosquito populations in Africa to eradicate diseases such as malaria.

Researchers have developed several techniques to combat mosquitos in hopes of stopping Malaria. Malaria suppression techniques such as the use of indoor residual spraying (IRS) have been found to be effective in the short term when implemented into areas with high mosquito populations. The technique involves spraying insecticide from a large container inside buildings such as houses where there is a large mosquito human interaction (Hamainza, Digani, Chanda, 2/18/16). Other techniques include using insecticide treated nets (ITN) to solve the problem but these are only applicable inside areas where people sleep like houses so they only provide protection for a limited amount of time leaving (CDC division of parasitic diseases 8/15). Specifically a study was done by researchers in Uganda to evaluate the resurgence of malaria months after discontinued Indoor residual spraying. The study was conducted after repeated indoor residual spraying for a number of years, the rate of malaria incidence for the 18 months

after it was discontinued was being monitored, The study showed that each month the malaria incidence increased by about 4% until reaching its previous levels which shows it is only a short term and not fully effective solution for malaria elimination (Raof, Mpimbaza 8/1/17). Several studies like this one have proved techniques like IRS to be effective to some extent in lowering malaria but not fully suppressing it which allows it come back up in big numbers.

Studies on the effectiveness of several malaria elimination techniques often counter problems as there are many confounding factors that can be a hindrance to malaria suppression. Studies like *Resurgence of Malaria Following Discontinuation of Indoor Residual Spraying of Insecticide in an Area of Uganda With Previously High-Transmission Intensity* exemplify how there is frequently a reemergence of malaria months after treatments. This is due to the many impediments to malaria suppression which is something not explored in research but can hold valuable information regarding how to truly stop mosquitos related vector borne disease such as malaria. Due to the complexity of the vector cycle humans actually have the ability to be carriers of the malaria plasmodium when bitten by a mosquito and for a period they can be secondary malaria host allowing for mosquitoes to bite them and further spread malaria (CDC disease control and elimination, 11/18). Factors like these are often overlooked in studies but these impediments to malaria suppression are just as important.

The purpose of this study was to discuss and analyze the impediments of malaria elimination in Lusaka, Zambia. The mosquitos being studied are the *Anopheles gambiae* and the *Anopheles funestus* both being prominent vectors that transmit malaria. The Impediments being discussed are the relationship between the number to *Anopheles* in an area and the population density of that area in correlation to the malaria incidence. The hypothesis was areas with a high

prevalence of malaria transmitting strains of *Anopheles*-- *funestus* and *gambiae*-- and a high population density will have higher malaria incidence.

Question: what are the impediments to malaria elimination?

Methods/ Materials

Organism Studied

The *Anopheles gambiae* and *Anopheles funestus*, were the 2 breeds of mosquito being studied due to their ability to transmit malaria causing thousands of deaths in Zambia each year. Both the *Anopheles Gambiae* and *funestus* are native to Zambia and thrive off the blood of mammals which can be detrimental to the human populations of Zambia. The vector cycle for the *Gambiae* and *funestus* operates much differently from other types of mosquitoes found across Africa which makes them exponentially more dangerous. In the vector cycle of these mosquitos a person who is carrying malaria can be bitten by the *Gambiae* or *funestus* who does not carry malaria; following this the mosquito that was bitten also becomes a carrier that can spread malaria quickly. The *gambiae* and *funestus* effectively turn anyone they infect into a contagious body since that person can be bitten by many other *gambiae* or *funestus* without malaria and can cause them to carry the disease and further spread it. Due to the nature of the vector cycle the *gambiae* and *funestus* have the ability to disrupt and infect huge populations of humans who don't carry malaria. Other breeds of mosquito also exist in Zambia such as the *Culex pipiens*, *Anopheles rufipes*, *Anopheles coustani*, *Anopheles squamosus* and *Anopheles implexus* but they do not transmit malaria.

Study Site

Since mosquitoes are such a complex organism to study, the research took place at multiple sites. The entire field study took place in Zambia, Africa, since it has an abundance of mosquitoes and diseases like malaria are of large concern. The research was done both in the field at various mosquito collection sites within the different provinces of Zambia, and in the lab in Lusaka, Zambia. The field research involved collecting wild mosquitoes, some of which carried malaria. The collection of the wild mosquitoes was done by my mentor while I worked in the lab since the process of collection involves actually camping in the bush where there was both a threat of animals and a huge risk of actually getting malaria. Although I wasn't with my mentor during the collection of the mosquitoes I was in frequent communication with him. I was debrief on how the mosquitoes were collected in its entirety; After the collection, a portion of the live mosquitoes were transferred back to the lab for identification and rearing. During collection mosquitoes are collected using 3 techniques: CDC light traps, Human Landing Catches and pyrethrum spray. CDC light traps can be hung in trees near bodies of water to lure the mosquitoes in with the light before trapping them in its capsule, Human Landing Catches, which I also used in the lab, allow live mosquitoes to be sucked into a tube and transferred into a holding container before being transferred to the lab. The final method, Pyrethrum spray, was an insecticide used to "knock down" mosquitoes so that they were able to be collected. All three methods that are used are done mainly near mosquito birthing sites which are located in areas of still water accompanied by the appropriate climate being between 27-33 degrees Celsius with

humidity. During this particular collection CDC light traps were used, After the collection, a portion of the live mosquitoes were transferred back to the lab for identification and rearing.

A large portion of the research occurred in the insectary at the Zambia National Malaria Elimination Center. The lab is where rearing and identifying occurred of mosquitoes (both wild, and captivity born). The environment in the insectary was controlled to mimic the temperature and humidity mosquitoes would be subject to in the wild. This was accomplished using a powerful electric heater which kept the rooms between 27C- 33C (about 80F-91F) at all times. The reason for the large range is the larvae and adult mosquitoes require different temperatures. In addition, to create an environment within the lab that was similar to the hot humid climate of Zambia Damp towels were hung over the tops of the cages to keep the room humid. The mosquitoes were kept in various areas around the lab depending on different factors such as levels of maturation. The larvae were kept in Petri dishes which were filled with distilled water, each dish had a handwritten tag on it which showed the day the larvae was hatched to give an indication of the stage of development of that particular batch. Every morning, the pupae were transferred into different dishes based on their development and maturity, this was done so that there wasn't pupae in different stages of development living amongst one another. The pupae were fed fish food into the dishes, they were fed this was until they matured and turned into adults which took a few days. Once they were adults they were moved to 1x1 ft enclosures with nets at the end for easy access. Each day, the adults were fed a sugar water solution of 90% water and 10% sugar. To do this, cotton balls were dipped into the solution and distributed to the 20+ cages of adult mosquitoes of different species.

Figure 1, female pure strain anopheles gambiae laying eggs in water



Figure 1 Female pure strain anopheles laying eggs in water Figure 2, newly hatched larvae in pupae stage



Figure 3, adult mosquitoes in cages



Figure 4, identified wild mosquitoes in capsules

In addition to the field, data was collected within the lab through mosquito identification. In this process a high powered light microscope was used to distinguish between various breeds

of mosquitoes based on physical characteristics. *Anopheles* can be distinguished between other types of mosquitos based on the way their larvae move in water and the way adults sit on a wall, Adult *Anopheles* have long slender legs which causes them to appear slanted when sitting on a wall. In addition, when in water Anopheles pupae tend to lie vertically while other breeds lie on a slant or even horizontally. Distinguishing between the many different types of Anopheles requires the help of a light microscope which portraits the mosquitoes in minute details. In the Identification stage distinctions were made based on qualitative observations such as color patterns, shape of legs, proboscis appearance, the amount of hair on the body and tuffs, and quantitative observations such as the size of the proboscis, wings and legs.

The population density and malaria incidence statistics for the various districts were determined based on the 2010 census that was taken in Zambia and recorded by the ministry of health. The data regarding the populations of the districts list first collected for 2010 using Zambian databases and then converted two probable populations for 2018 based on projected population growth for each district(Zambia portal 6/11).

Data analysis

For the data analysis, Zambian census data was used for population and malaria incidence so it needed to be converted into present projected numbers to increase the validity of the findings. The last census for Zambian population and malaria incidence was in 2010 and the data was extremely limited due to some populations being in rural poor income areas where many people were living in close quarters. The populations being used were 6 districts in the

various provinces of Zambia being that they needed to match up with the collecting areas where the various mosquitoes were caught but they were not limited to the collecting areas.

District	Province
serenje	central
kalushi	copperbelt
chadiza	eastern
kawamba	luapula
isoka	muchinga
Mpika	muchinga

From here data was collected for the populations of 6 districts across Zambia some of which were the districts used from mosquito collection, to establish a population density for the areas. Once again since the census was in 2010 we used a projected population growth chart from the official Zambia data portal for each of the specific districts in order to approximate the current populations. For example for the serenje district the growth rate was 2.2 so the projection showed exponential growth for 8 years for the 2018 population. For the districts the area sizes were determined based on the same census although no new growth rates needed to be established since the sizes of the areas stayed the same. After this we took each population in each district and divided it by the size of the area to establish the number of people per kilometer (Population density) this was later used in the full analysis.

The malaria incidence for each district was determined using the census for each district which provided information on the number of people with malaria per thousand.

For the analysis both scatter plots and bar graphs were used to show correlation between population density and the malaria incidence.

Results

		Mosquito collection data				
Catchment area	An. Gambiae	An. Funestus	Culex	An Squamosus	An Ruffies	Total
Chibale	0	0	6	0	0	6
Kabamba	0	0	0	0	0	0
Kaseba	0	8	0	0	0	8
Lumpampa	0	24	10	0	0	34
Mulembo	0	0	0	0	0	0
Nchimishi	0	13	1	12	1	27

The above chart shows the 6 collection sites across Zambia followed by the different types of mosquito collected from each site. The model organisms being studied are the *Anopheles Gambiae* and *Funestus* because they are the ones that transmit malaria, the other breeds of mosquito in the chart pose no malaria risk to humans.

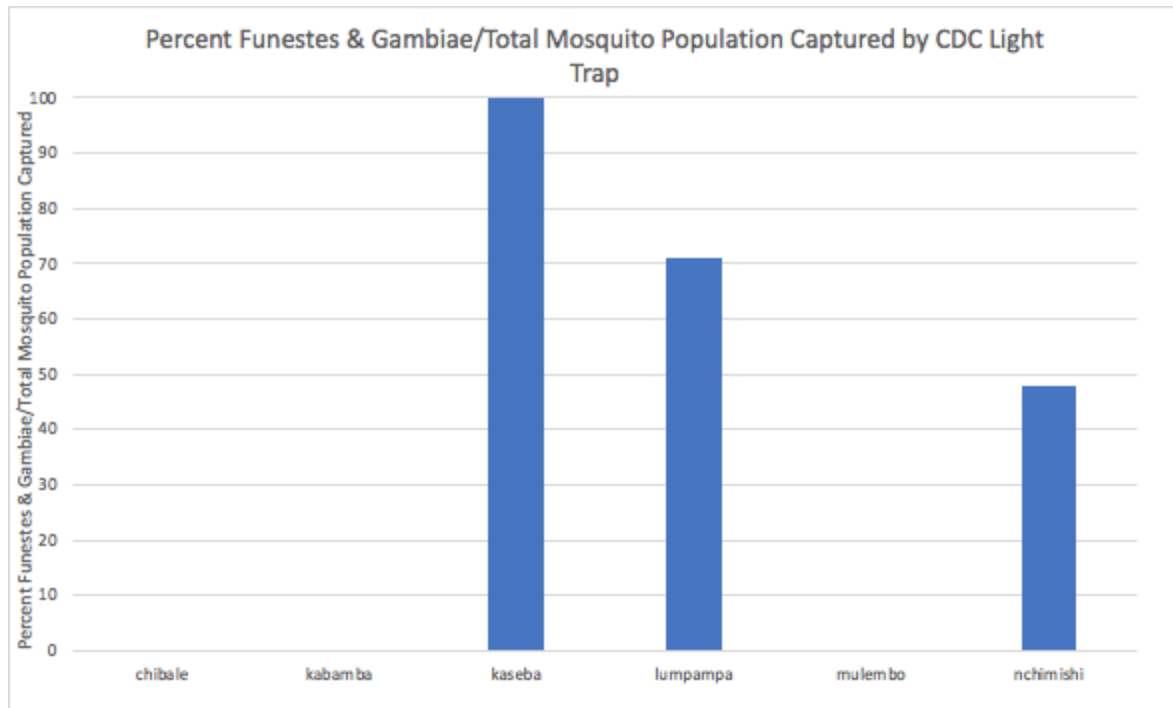
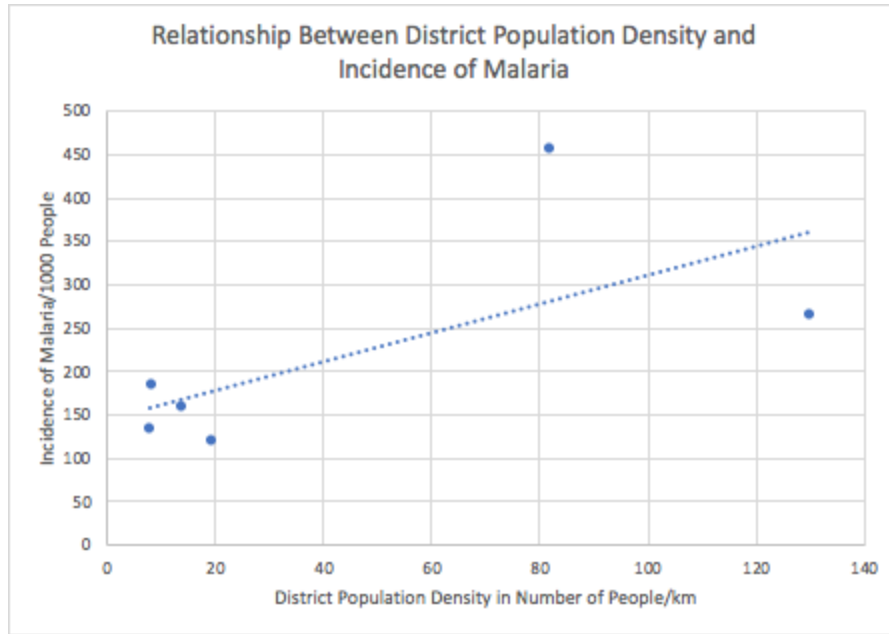


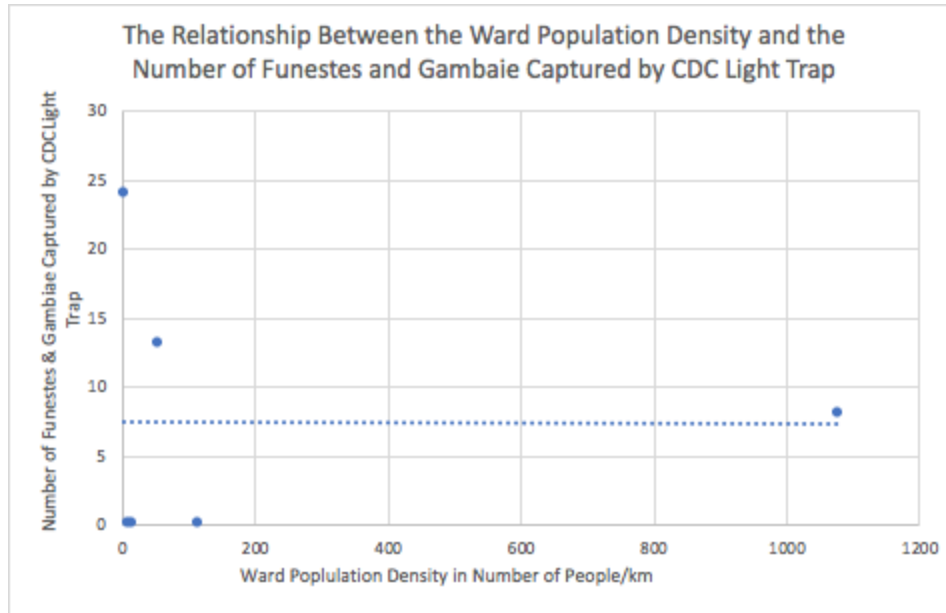
Figure # 5, percent *Funestes* and *Gambie* /total mosquito population captured by CDC light trap

The following Graph shows The *Anopheles Funestes* and *Gambiae* mosquitoes as a percentage of all the mosquitoes collected in the data collecting areas. It was intended to see if the presence of more mosquitos that have the ability to transfer malaria would have an impact on malaria incidence.



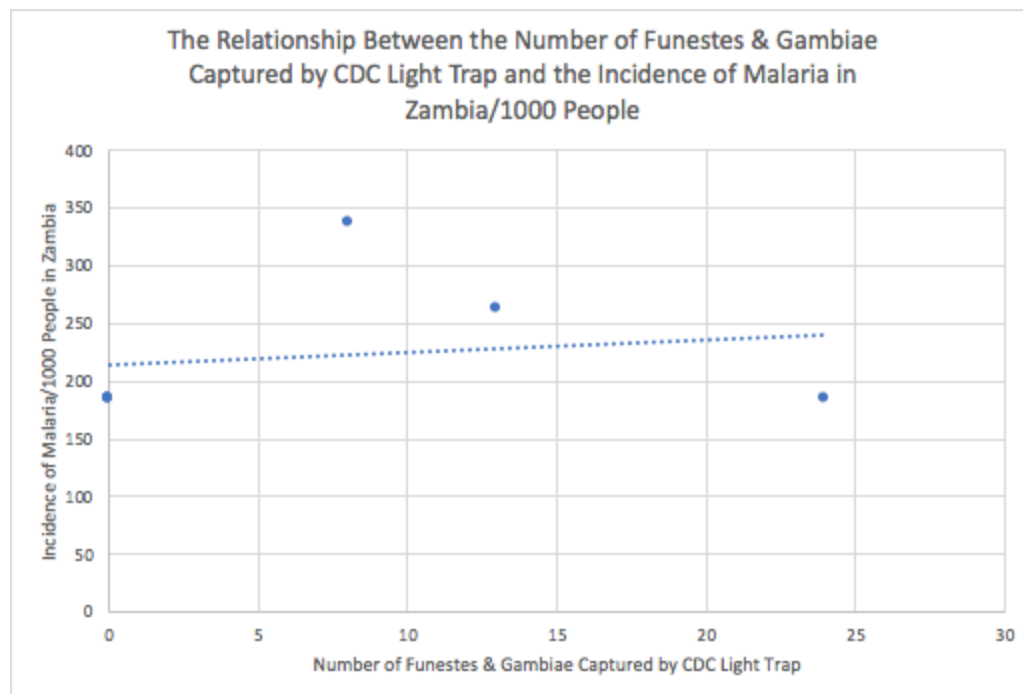
$$r=0.666$$

Figure 6, Relationship between district population density and incidence of malaria. The graph shows a strong correlation between the population density and the malaria incidence. In the Graph as the population density increases the malaria incidence also increases. The correlation coefficient for the graph is .666 which means there is a strong positive correlation.



$r = -0.007912$

Figure 7, The relationship between the ward population density and the number of Funestus and Gambiae captured by the CDC light trap. Take graph shows no correlation between the population density of wards and the number of malaria transmitting mosquitoes caught using CDC light traps. The correlation coefficient is $r = -0.007912$ which means no correlation.



$$r=0.16290705$$

Figure 8, The relationship between then number of Funestus and Gambiae captured by CDC like trap and the incidence of malaria in Zambia/1000 people. The graph shows a weak positive correlation between the number of malaria transmitting mosquitoes in that area and the malaria incidents of the areas. The correlation coefficient is .16290705 which indicates a weak positive correlation.

Discussion

The trends in the research is that as that as population density of the district increases the malaria incidence increases. This shows a direct relationship between the population density and the number of people getting malaria. The correlation between the two is due to the vector cycle allowing humans to become secondary malaria hosts which allows them to be bitten by another

mosquito which does not have malaria and cause it to become infected and further spread the malaria. If there are a lot of people in close quarters the chances of this occurring greatly increases. Another trend seen in the research is no correlation between the population densities of the wards and the number of *Funestus* and *Gambiae* in the areas. This shows that researchers have not yet identified this as a major issue and have not focused their efforts to malaria eliminations in these areas specifically. These areas should be considered high risk and focussed on rather than treated the same. The last trend is a weak positive correlation between the number of malaria transmitting mosquitoes caught in the area and the malaria incidence. This may be due to limitations in the collection techniques since a limited number of mosquitoes could be collected.

Some limitations Of the study including how limited the pool of data was four the various locations since some of them were small rural areas, this caused much of the data to be taken from the 2010 census been converted to projected amount is for 2018. Had there been exact census data from 2018 it would have greatly increase the validity of the research. In addition the collection technique was a limitation to the research. The mosquitoes were collected by CDC light traps which collects mosquitoes by causing them to fall into the trap. So there was likely many mosquitoes that were not accounted for since they did not fall into the trap, using better mosquito collection techniques would have also improved the research.

Based on my research a new question that arises is: Should new malaria elimination techniques be developed and implicated in areas with high population densities specifically? The reason for this question is the data established that areas with higher population densities are at

greater risk of malaria so should those places be treated differently in order to eliminate malaria completely stopping it from recurring.

Conclusion/Implications

The study was important because it outlined the impediments of malaria elimination and showed a correlation between these impediments and malaria incidence. The main impediments being discussed throughout the study were population density and the number of malaria transmitting vectors in the areas. For Both of these impediments a correlation was established which allows scientists to now focus efforts on ways to bypass these impediments now that they have been identified. The research is a step forward in the quest to start malaria which kills millions of people each year.

References

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