THESIS

GEOSPATIAL ANALYSES OF CHILDHOOD MALARIA FOLLOWING REPEATED VILLAGE-WIDE IVERMECTIN ADMINISTRATIONS: SECONDARY ANALYSES FOR THE RIMDAMAL PILOT STUDY

Submitted by

Chelcie A. Barnett

Department of Environmental and Radiological Health Sciences

In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Summer 2017

Master's Committee:

Advisor: Sheryl Magzamen

Brian Foy
Micah Hahn

ABSTRACT

GEOSPATIAL ANALYSES OF CHILDHOOD MALARIA FOLLOWING REPEATED VILLAGE-WIDE IVERMECTIN ADMINISTRATIONS: SECONDARY ANALYSES FOR THE RIMDAMAL PILOT STUDY

Malaria has long been a major public health concern, with historic roots dating back thousands of years. This febrile disease is caused by a parasite that is transmitted among vertebrates by mosquitoes. Over the past century, global eradication programs have focused on minimizing populations of the insect vectors, and administering treatments to people infected, especially young children and pregnant women, as they are the most vulnerable to suffering severe complications. Overall, these programs have decreased the geographic distribution and global disease burden; however, malaria remains a major problem in regions where these efforts have been unsuccessful. In 2015, there were an estimated 214 million cases throughout the world, resulting in approximately 438,000 deaths; however, over 3 billion people are living at risk of becoming infected with malaria. Widespread use of the few available effective insecticides and anti-malarial drugs has conferred resistance in both parasitic and mosquito species, decreasing the effectiveness of current interventions. As anti-malarial resistance and insecticide resistance spread, the need for novel malaria interventions becomes more urgent.

One novel approach to combatting malaria was pilot-tested by researchers in the Department of Microbiology, Immunology and Pathology at Colorado State University. The Repeated Ivermectin Mass Drug Administration to control Malaria, or the RIMDAMAL study, evaluated the safety and effectiveness of repeated village-wide administrations of an anti-parasitic drug to prevent malaria in children $\leq$ 5 years old. The RIMDAMAL study was a

randomized trial carried out in Burkina Faso, a small tropical country in West Africa. Ivermectin (IVM) is a common anti-parasitic used around the world to prevent and treat parasitic diseases. Recent evidence has demonstrated that IVM is toxic to malaria-transmitting mosquitoes, and can inhibit the propagation of some life stages of malaria parasites. Initial analyses of the RIMDAMAL data found significantly fewer childhood malaria cases in intervention villages that received repeated IVM administrations, compared to control villages.

This study is a geospatial analysis of the RIMDAMAL data to provide further insight as to how this intervention could be implemented. There were two study aims for this research: 1) identify significant clustering of high and low childhood malaria incidence within each study village; and 2) identify significant clustering of high and low childhood malaria incidence throughout the entire study region. In total, eight villages were enrolled in the study, four of which served as controls, while the other four received the intervention. Residents of each village live in concessions, or compounds of extended family. Geospatial coordinates were collected for each concession within a study village, along with data on the participants within each concession. Using this data, incidence density of malaria among children 5 years old or younger was calculated at the concession level. Concessions were mapped, and spatial clustering of incidence density values was evaluated using the Getis-Ord Gi* (G-I-star) spatial autocorrelation statistic. To evaluate within village clustering, each of the eight study villages were analyzed individually, and between village clustering was evaluated by analyzing the entire study region.

Within each village, several “hot spots,” or statistically significant clusters of high malaria incidence density values were recognized during analyses with max clustering, at the 95% confidence level. Statistically significant clusters of low incidence density were identified in one study village during the analysis with max clustering. The proportion of concessions identified as significant clusters varied by village, ranging from 12% to 91.3%. There seems to be no trend in

clustering patterns seen within each village; some villages had randomly distributed hot or cold spots, while others appeared more clustered.

The spatial clustering patterns in the whole study region are more telling. Max clustering occurs in a bimodal pattern with two peaks; at 2,100 meters and 10,000 meters. The clustering patterns that occur indicate regions of similar malaria incidence. The proximity and locations of these villages may imply the RIMDAMAL protocol has regional impacts. Additional research is needed to evaluate how to most effectively implement this intervention to protect against malaria.

ACKNOWLEDGEMENTS

I am extremely grateful for the people in my life who have supported me over the last few years, and helped see me through the completion of this project. I would like to especially extend my thanks and appreciation to my committee, good friends, and family. I could not have gotten here without them.

TABLE OF CONTENTS

LIST OF ACRONYMS

ACD- Active case detection

ACT- Artemisinin-based combination therapy

BMGF- the Bill and Melinda Gates Foundation

CM- Centre MURAZ

CSR- Complete spatial randomness

CSU- Colorado State University

DALY(s)- Disability-Adjusted Life Year(s)

DDT- Dichloro-diphenyl-trichloroethane

ID- Incidence Density

IPTp- Intermittent Preventative Treatment during Pregnancy

IRB- Institutional Review Board

IRS- Indoor residual spraying

IRSS- Institute de Recherche en Sciences de la Santé

ITN(s)- Insecticide-treated bed nets

IVM- Ivermectin

LMIC- Low- and middle-income countries

MDA- Mass drug administration

MDG(s)- Millennium Development Goal(s)

NAI- Natural acquired immunity

NTDs- Neglected tropical diseases

RBM- Roll Back Malaria

RIMDAMAL- Repeat Ivermectin Mass Drug Administrations for Control of Malaria

SMC- Seasonal malaria chemoprevention

UNDP- United Nations' Development Programme

UNICEF- United Nations Childrens' Fund

WHO- World Health Organization

WGS- World Geodetic System

I. INTRODUCTION

"It is essentially a political disease- one which affects the welfare of whole countries; and the prevention of it should therefore be an important branch of public administration." –Sir Ronald Ross, The Prevention of Malaria, *1910* [1]

Overview

This chapter briefly introduces the motivations for and purpose of this research study. This includes a brief background on malaria, research questions addressed through the study, and outlines the research parameters.

Background

Evidence suggests malaria has been a major infectious disease for hundreds of thousands of years, and was first documented as a public health concern approximately 4,700 years ago. [2-4] It was once endemic on all continents except Antarctica, and has one of the highest estimated global burdens of disease throughout history. [4, 5] Although the geographic distribution has drastically decreased with intense intervention and control programs over the past century, malaria is still a major contributor to the burden of disease in countries where it is endemic, with an estimated 214 million cases and 438,000 deaths in 2015. [3] Highly endemic countries are mostly low and middle-income, and located in tropical regions of Africa and Southeast Asia. [2, 3] Poverty has been implicated as both a contributing factor to malaria burden, and a barrier to its control, as interventions and treatments can be expensive for countries with limited resources. [3, 4, 6-9] More than 70% of all deaths from malaria occur in children less than five years old, making infants and young children an especially vulnerable group. Globally, this tropical disease is ranked in the top five leading causes of death among

children under five, along with preterm birth complications, pneumonia, birth asphyxia, and diarrheal diseases. [3, 10, 11]

Currently, control programs include methods to limit vector-human interactions and medicinal treatments for those infected. Insecticide-treated bed nets (ITNs) and indoor residual spraying (IRS) of insecticidal chemicals within dwellings are the most common methods to mitigate the abundance of mosquito vectors. Some regions also practice larval control through modifying vector habitats, using larvicidal chemicals, or predatory fish to consume larvae. [3] Effective antimalarial treatments are currently limited to four main drug classes: the aminoquinolines, such as quinine, chloroquine, and primaquine; the antifolate compounds, such as pyrimethamine and sulfadoxine; artemisinin and its derivatives; and hydroxynaphthoquinones, such as atovaquone. [2, 3, 12] Recent campaigns and programs to control and decrease the global burden of malaria have involved the widespread distribution of these insecticidal and antimalarial chemicals. Though the estimated burden has decreased over the past several decades, prevalence of insecticide-resistance among mosquito vectors, and antimalarial-resistance among parasites is increasing. This development of resistance is decreasing the overall effectiveness of current treatments, and has the potential to reverse the impacts of recent mitigation efforts. [3, 9, 13, 14] Decreasing global trends of disease burden, along with increasing resistance to treatments makes now a pivotal time for exploring innovative and novel approaches to controlling malaria.

Purpose of the Study

Geospatial analyses allow researchers to visualize and identify spatial patterns that exist within a dataset, while providing a set level of confidence through statistical tests. Identification of spatial clusters of similar values can provide significant insight on factors that may be contributing to the formation of those clusters. One of the earliest examples of the successful use of this type of analysis dates back to 1854, when John Snow mapped cases of cholera

during an outbreak in London, and was able to identify a common water pump as the source. [15] Mapping and spatially analyzing disease outbreaks is becoming increasingly popular, especially as geographic information systems (GIS) software advances.

The purpose for this research was to explore how the use of a novel malaria intervention method might impact the spatial distribution of malaria incidence density among children aged 5 years and younger. More specifically, this study aimed at determining whether statistically significant clustering of high and low malaria incidence density values occurred, and how patterns of clusters formed within and between villages. Understanding how this control method might impact the spatial distribution of malaria density is an important step in understanding its overall effectiveness. This research can also generate hypotheses for future studies, and provide insight for efficient implementation of this intervention in the future.

Aims & Methods

Study Aim 1- To identify and evaluate patterns of spatial clustering of childhood malaria incidence within each study village.

The spatial autocorrelation statistic, Getis-Ord Gi* (G-I-star) was utilized to identify significant clusters of high and low malaria incidence among children $\leq$ 5 years old. For each village, the Gi* analysis was conducted at several distances, starting with a short distance, and incrementally increasing until a maximum distance was reached. The starting distances, incremental increases, and maximum distances varied by village based on their differing geographic extents.

Study Aim 2- To explore whether the Repeated Ivermectin Mass Drug Administrations for Control of Malaria (RIMDAMAL) protocol has an impact on the regional distribution of childhood malaria incidence by evaluating spatial clustering throughout the entire study region.

The Gi* statistic was also used to identify clusters of high and low malaria incidence density among children $\leq$ 5 years old throughout the entire study region. Much like in Study Aim

1, the Gi* analysis was conducted at several distances, beginning with a relatively short distance, increasing incrementally, and ending at a maximum distance. The primary difference between the methods for these aims is that Aim 1 evaluates clustering within each individual village, while Aim 2 evaluates clustering when considering incidence density values in neighboring villages.

Study Parameters

The data used to conduct this research was collected as part of a pilot, single blinded, parallel assignment, cluster randomized trial in the Sud-Ouest administrative region of Burkina Faso. The RIMDAMAL pilot study evaluated the effectiveness of a novel approach to minimize vector abundance. Ivermectin is an endectocide, or parasiticide, used globally to treat various parasitic infections. [16] Several observational and experimental studies have demonstrated that *Anopheles* mosquitoes experience adverse physiological effects, and sometimes death, after ingesting IVM in the blood of a treated person or animal. [15, 17-22] There is also evidence that the drug has an impact on certain life stages of malaria parasites, though the exact mechanisms and life stages that may be impacted are not well-characterized. [20] The goal of the RIMDAMAL study was to determine whether repeated village-wide administrations of this common anti-parasitic drug would significantly lower the cumulative incidence of malaria among children aged five years and younger. Most children five years old and younger were not tall enough to receive the intervention, but malaria incidence in these children was the outcome of interest. Most older children and adults received the intervention, and the protective effects were measured in younger children.

Initial analyses for the RIMDAMAL study revealed significant differences in cumulative incidence of malaria between children in control villages and children in intervention villages. Statistical methods for the RIMDAMAL study included a Poisson-distribution regression to compare malaria incidence between the study arms, and additional subgroup and adjusted

analyses based on significant findings. These analyses showed that the mean number of malaria episodes per child was 20% lower in the intervention arm of the study compared to the control arm. This effect was even stronger among the 4 and 5-year-old children who were also tall enough to receive IVM, with 44% reduction in incidence among the intervention arm compared to the control arm. [23]

To better understand potential spatial extent of the RIMDAMAL protocol, this study was conducted utilizing geospatial analysis methods. The Getid-Ord Gi* statistic was used to identify significant clusters of high and low childhood malaria incidence. Analyses were conducted within each village, and between villages, using incidence density of concessions, or households, as the attribute values. In total, there were 106 concessions with 263 children ≤ 5 years old in the four control villages, and 127 concessions with 327 children ≤ 5 years old in the four intervention villages.

II. LITERATURE REVIEW

Overview

This chapter provides a review of current literature pertaining to the various aspects of malaria that are important to understanding this project and analysis. This includes background on the significance of malaria in human populations, the biology and epidemiology of the disease, and the challenges encountered with current mitigation and treatment efforts.

Historical Perspectives

Malaria is one of the most archaic diseases that still plagues the modern world. Evidence of the pathogen can be traced back 15 to 20 million years ago, in the form of a fossilized mosquito preserved in amber, and heavily infected with a strain of malaria parasite closely related to those which infect humans today. [24] Though this suggests the parasites have been infecting vertebrates through mosquito vectors for millions of years, the earliest evidence of the disease in humans was discovered in tissue samples from 5,500-year-old Egyptian mummies. [25] Nearly 800 years after those malaria-infected remains were mummified, symptoms characteristic of the febrile illness were first referenced in the ancient Chinese medical documents, *Nei Ching* ("Canon of Medicine," 2700 BCE). [2, 4] Following this initial documentation, references to malaria were recorded by various ancient civilizations, including an implication that the disease played a substantial role in the collapse of the Roman Empire. [4, 26-29]

Until the mid-twentieth century, malaria was endemic on all continents, except Antarctica, with the greatest burden concentrated in tropical and subtropical climates. There are several historical examples of outbreaks that were so terrible in these regions, colonization and development projects were postponed or abandoned altogether. [1, 4, 30-32] Near the end of the

nineteenth century, two major discoveries regarding the transmission of malaria provided better understanding of how to combat it. In 1880, Dr. Alphonse Laveran, a French military doctor, identified a protozoal parasite in the blood of a febrile patient as the causative agent of malaria. Then, in 1897, Sir Ronald Ross, a British medical officer with the Indian Medical Service, demonstrated the transmission of malarial parasites by mosquitoes. Both men were later awarded the Nobel Prize in Medicine, Ross in 1902 and Laveran in 1907.[2] After Ross identified mosquitoes as the route of transmission, vector mitigation and sanitation programs were implemented and had a dramatic impact on development projects in tropical regions. One of the first major projects completed as a result of these mosquito control programs was the Panama Canal, constructed between 1905 and 1910. [2, 4, 27, 28, 31] Revered as a construction miracle in the early twentieth century, the successful completion of the canal demonstrated to the world that mosquito control programs could drastically minimize the burden of a disease that had previously been near impossible to control.

Regional campaigns for eradication first began in the 1940s, and by 1955, had developed into the Global Malaria Eradication Program. [4] Through these efforts, most European countries, North America, and regions of Central and South America experienced vastly reduced morbidity and mortality. However, eradication attempts were met with much less success in sub-Saharan Africa, the most heavily burdened region in the world. After encountering challenges implementing control programs in Africa, the global initiative to eradicate malaria slowly lost its ardor, and by 1969, efforts were halted. [2, 4]

Over the next two decades, extensive research provided additional knowledge on economic and cultural aspects of the disease, as well as better-developed tools to combat it; however, the decreased allotment of resources for intervention resulted in a steady increase of the global malaria burden. By the 1990s, the urgency of the problem brought mitigation efforts back into focus. The World Health Organization (WHO) convened for a global Ministerial Conference on Malaria in 1992, where ambassadors from around the world assessed the

worsening situation and strategized methods to once again work toward eradication. [33] These efforts further progressed in 1998 when WHO partnered with UNICEF, UNDP, and the World Bank, to form Roll Back Malaria (RBM), a platform through which actions against malaria could be efficiently coordinated globally.[6] The RBM partnership has since grown to include over 500 partners including malaria-endemic countries, development partners, and other organizations and foundations aimed at minimizing malaria. Collectively, these entities work together to upscale malaria-control programs to the country level, while ensuring the most efficient use of all resources. [6, 34]

In 2000, the global initiative to control malaria was further strengthened during the Millennium Assembly of the United Nations, where major world leaders gathered to discuss international values, principles and objectives for the twenty-first century. One of the major results of this general assembly was the establishment of the eight Millennium Development Goals (MDGs), which aimed at reducing barriers to development and addressing different facets of extreme poverty around the world. [8] World leaders in attendance at the Assembly recognized malaria as a barrier to development, and a disease that perpetuates poverty. It was therefore included as a component for MDG-6, with the specific goal of halting and reducing its incidence rate by 2015. [8] Though some malaria-endemic countries managed to make great strides toward achieving MDG-6 targets, the disease is still ranked among the top causes of death and disability in low and middle income countries (LMIC). [10]

Global Malaria Burden

According to the World Malaria Report released by WHO, there were an estimated 214 million cases and 438,000 deaths from malaria in 2015, with 3.2 billion, or 44% of the world's population, living in areas at-risk for infection. [3] These numbers are drastic improvements when compared to the 1900 estimates of over 3 million deaths and 77% of people living at-risk. Along with overall mortality, the geographic distribution of malaria has also decreased substantially

over the last century, from an estimated 140 countries at risk for epidemics in 1900, to 88 countries in 2010. [9] However, this has resulted in a disproportionately high concentration of malaria burden in LMIC, mostly in Africa, Southeast Asia, and Eastern Mediterranean regions. [3] The vast majority of the global burden is concentrated in sub-Saharan Africa, where an estimated 88% of all global cases and 90% of all global deaths from malaria occur each year. [3]

This overwhelming global burden of malaria is more accurately portrayed using disability-adjusted life years (DALYs), a metric expressed as the potential number of healthy years of life lost due to illness, disability, or premature death. In 2012, it was estimated that malaria was responsible for 26,359,000 DALYs in low income countries, or approximately 3,114 years of life lost or spent disabled per 100,000 people. People who live in extreme poverty and are constantly ill are unable to work to support themselves or their families. When vast regions are poverty-stricken and highly burdened by malaria, the economic development and welfare of entire countries suffer. Between 1965 and 1990, the average growth of income per capita in countries with severe malaria was an estimated 0.4%, less than one-fifth the average growth in other countries. [7] This difference is not entirely attributable to high malaria incidence, but evidence suggests malaria plays at least some role in the perpetuating cycle of poverty in highly endemic regions. The cost to control malaria is also extremely high, especially for LMIC, with an estimated $12 billion being spent annually by endemic countries in Africa alone. [7, 34]

Modern medicine and technology have dramatically decreased the case fatality rate of malaria, with less than a half million deaths last year, despite over 200 million estimated cases. When treated promptly and correctly, malaria is generally curable. Symptoms often present much like influenza, with the patient suffering from fever, chills, sweats, headaches, body aches, and nausea and vomiting. [2, 4] When a person is infected with a particularly virulent strain, when an infection is left untreated, or after repeated and chronic malarial infections over time, the disease may manifest in a more severe and complicated manner. Symptoms of a severe case may include neurologic abnormalities, anemia, acute kidney failure, or several other life-

threatening conditions that require immediate medical attention. [2, 4, 26, 30] Complicated cases can often be avoided through preventative measures and timely treatment. Despite increased efforts to control malaria, millions of people around the world still lack access to the services they need to prevent and properly treat infections. [3]

Although the overall number of deaths due to malaria have decreased dramatically, 306,000 of the 438,000 deaths (70%) in 2015 occurred in children 5 years old or younger. [3] As people living in high-risk regions are repeatedly infected, they begin to develop a strong immune system that minimizes the severity of disease over time and reduces their chance of dying. However, young children have not yet developed this strong immunity and are highly vulnerable to malaria morbidity and mortality. It is estimated that 5-10% of all children born in tropical regions of Africa will die of malaria before reaching their fifth birthday. [3, 4] Preventing these deaths could be achieved through better access to prevention and treatment methods, and innovative approaches that offer highly effective and cost-efficient protection to the most vulnerable at-risk populations.

Biology and Ecology

Malaria is a complicated disease, requiring complex interactions among multiple life forms for successful propagation. Evolutionary evidence suggests that over the course of millions of years, these interactions and life cycles have been perfected, making control of the disease difficult at best. [4, 26, 31, 32, 35-37] The pathogen responsible for causing malaria is a collection of parasitic protozoans, belonging to the *Plasmodium* genus. There are approximately 100 species that are responsible for causing this febrile disease in various animals, five of which are known to infect humans: *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi*. [2, 38] *P. vivax* and *P. falciparum* tend to be the most widespread, and are responsible for the vast majority of global malaria morbidity and mortality. *P. falciparum* is the most virulent, resulting in severe clinical manifestations and complications. Though it is present in tropical and subtropical

regions worldwide, this species is primarily found in Africa, where it is responsible for 80-90% of all malaria cases. [2, 4] In contrast, *P. vivax* is rarely seen in Africa, but is the predominant species found in Asia and the Americas. This species tends to cause milder illness and fewer fatalities than *P. falciparum*, but because it is the predominant species in densely populated regions, a greater proportion of people are at risk of being infected with *P. vivax*. [2] *P. malariae*, *P. ovale* and *P. knowlesi* are considerably less prevalent, causing fewer than 10% of all cases. *P. malariae* can be found in most tropical and subtropical regions, while *P. ovale* is generally only seen in Africa. [2, 4] *P. knowlesi* is found in Southeast Asia, but is unique compared to the other four human strains because it is naturally a simian pathogen, most commonly found in long-tailed and pig-tailed macaques. This zoonotic species tends to induce rapid disease onset, resulting in severe clinical manifestations, and can be fatal in humans. Recently, *P. knowlesi* was discovered to be the most prevalent form of malarial infection in Malaysia. [39]

Plasmodium parasites require both a vertebrate host and invertebrate vector to complete their complex life cycle due to temperature-dependent stages of development. When a mosquito takes a blood meal from a malaria-infected human, they also ingest the sexually immature *Plasmodium* gametocytes. [26, 40] This initiates sexual development and maturation stages of the life cycle that occur within the invertebrate host, as the parasite travels from the gut of the mosquito to the salivary glands. Though the length of this process varies depending on vector and parasitic species, and environmental conditions, it is generally estimated that this invertebrate life cycle takes 10-18 days. [2, 26]

When female mosquitoes take blood meals from humans or other vertebrates, they also inject saliva and anticoagulants to keep blood from clotting while feeding. Mature *Plasmodium* parasites within infected salivary glands are also injected into the vertebrate, thus beginning the other half of the parasite's complex life cycle. In human hosts, malarial parasites travel to the liver, where they will infect hepatocytes, and rapidly divide. The parasitic life stages produced by this rapid asexual division either infect new liver cells, or enter the bloodstream to infect

erythrocytes. During this process, *P. vivax* and *P. ovale* also produce dormant life forms that can persist in the liver and upon reactivation, cause relapses of malaria symptoms for up to several years after the primary infection has been cleared. [2] A parasite that infects an erythrocyte will undergo several developmental transformations, before it eventually lyses the cellular host, releasing toxins and sexually immature gametocytes into the bloodstream. It is the release of the toxins that causes the cyclical fevers and chills characteristic of malarial infection, with symptoms recurring every two to three days, depending on the parasitic species and how long it takes the host's immune system to clear the infection. The gametocytes released are then ingested by mosquitoes, reinitiating the *Plasmodium* life cycle in the vector. [2, 26, 40]

Malarial parasites are transmitted among humans by female mosquitoes belonging to the *Anopheles* genus. There are just over 400 different *Anopheles* species that can be found on all continents except Antarctica, though only about 40 are capable of transmitting *Plasmodium*. [3] The primary vector species seen throughout the world vary depending on geographic location and environmental preferences of the mosquitoes. In tropical regions of Africa, some of the most common species found include *An. gambiae*, *An. funestus*, and *An. arabiensis*; while *An. barbirostris* and *An. culicifacies* tend to be some of the dominant vector species in Southeast Asia. [41, 42]

Survival and successful propagation of these disease vectors are dependent on ideal environmental conditions, such temperature and rainfall. These preferred conditions vary for each species, but all anophelines have four life stages: egg, larvae, pupae, and adult. [2] On average, the time from anopheline eggs being laid in an aqueous environment until they reach adulthood ranges from 5 to 14 days. The adult stage of *Anopheles* mosquitoes is the longest, and is the only stage that is medically important for transmission of malaria. In fact, only female adult anophelines are medically important, since males do not take blood meals. Males only live for about one week, feeding on nectar and other sources of sugar, and aggregating in swarms near water for breeding. Females can live for one month or longer in captive laboratory settings,

but likely live for only 1-2 weeks in nature. They too feed on sugar sources for energy, but require a blood meal for proper egg development. [2]

Despite the vast differences between invertebrate and vertebrate biological processes, *Plasmodium* have evolved over millions of years to efficiently utilize both types of host to produce one of the most successful and persistent diseases known to man. Evidence suggests the parasites may have even evolved mechanisms that alter feeding behaviors of infected anophelines. [35, 43-46] The evolutionary history between *Anopheles* and *Plasmodium* has resulted in some unique symbiotic relationships, and researchers are attempting to understand how knowledge of these relationships could be used to better control global malaria epidemics.

Naturally Acquired Immunity & Disease Transmission

Despite the complex symbiotic relationships malaria vectors and parasites have evolved over millions of years, the efficacious transmission of disease still relies on a vertebrate host, such as a human. Fortunately, millions of years of selective pressure has also lead to the evolution of complex immune responses, such as the naturally acquired immunity (NAI) people develop over years of exposure to malaria parasites. The earliest scientific explanation of NAI dates back to 1900, when Robert Koch deduced that this type of protection against malaria is acquired after long, uninterrupted periods of heavy exposure to the parasite. [47] Despite having scientifically recognized its existence for over a century and understanding the primary features, the underlying mechanisms of NAI are still poorly understood. Age is one of the main factors believed to be highly correlated with immune responses to malaria parasites. Research has shown that children living in endemic regions are generally at high risk for infection by the time they reach three to four months old. They remain at high risk for severe morbidity and mortality until they reach about five years old, when risk of illness decreases, even with high parasitemia. [47] Though children adapt the ability to manage severe disease at a young age, the solid, protective effects of NAI are not usually in effect until the onset of puberty. From adolescence

onward, people living in malaria-endemic regions rarely experience severe disease as their immune responses are able to maintain low parasite loads; however, the cumulative incidence of blood parasitemia within these populations is often near 100%. [47, 48]

Although nearly all adolescents and adults living in malaria-endemic regions have detectable blood parasite levels, they are not usually responsible for transmitting gametocytes to *Anopheles* mosquitoes. The presence of gametocytes within an infected human's blood is necessary for the parasite to be transmitted to a mosquito, and most often, the effects of NAI keep parasite loads low enough that gametocytes are rarely produced. Children with high parasitemia and who have not developed the protective effects of NAI (usually between five and fifteen years old) are most often the culprits of transmitting *Plasmodium* gametocytes to disease vectors. [47] Gaining more knowledge about how parasitemia and gametocyte production are controlled through immune processes can provide potential for future intervention methods.

Prevention, Treatment, and Challenges

There are currently several prevention and treatment methods in use to minimize the global burden of malaria. Many of the preventative methods target the vector to minimize transmission rates. Two of the most widespread methods used in highly endemic regions are insecticide-treated bed nets (ITNs) and indoor residual spraying (IRS). Larval control is another vector mitigation strategy to minimize malaria, and is used by 48 countries around the world. Methods to control vector larvae include habitat modification, chemical larvicides, and biological techniques, such as using fish to prey on larvae.

The mass distribution of ITNs is considered one of the most important preventative measures due to their high success rates and overall cost-effectiveness. Currently, pyrethroids are the only approved insecticide for ITNs, because they have relatively low health impacts on mammals, but are highly toxic to insects. [2] Universal access to and use of ITNs in malaria-endemic countries has become a common goal for many malaria control programs. [3] As

reported in the 2015 World Malaria Report, the proportion of at-risk populations sleeping under ITNs has increased dramatically since 2000, from less than 2% to 55%, and 68% of all children aged 5-years or younger. Despite these improvements, there is a considerable amount of work that remains to reach the goal of 100% ITN coverage.

IRS involves spraying the insides of homes with insecticides, effectively coating all surfaces to kill any potential disease vectors that contact treated surfaces. The most common insecticidal agent used for IRS is dichloro-diphenyl-trichloroethane (DDT); however, other organophosphates are also used, as well as pyrethroids and carbamates. [2, 3] The use of DDT IRS was once a very popular method to reduce the burden of malaria, but has slowly been losing popularity due to the negative health and environmental impacts associated with DDT. Today, IRS is far less common than ITNs, with coverage peaking in 2010 at 5.7% of the at-risk population protected by this method. These rates have since been declining, with only 3.4% coverage in 2014. In countries where IRS is the primary control method, coverage is as high as 70%. [3]

Preventative therapies for malaria are currently only administered to the most vulnerable among those living at risk for malaria; specifically, pregnant women and young children. The WHO recommends intermittent preventative treatment during pregnancy (IPTp) for women in endemic regions. The primary therapy given to pregnant women is a combination of the sulfonamide antibiotic, sulfadoxine; and the antiprotozoal, pyrimethamine, collectively known as sulfadoxine-pyrimethamine. It is suggested that all women receive a dose during each antenatal care visit following the first trimester, and receive at least three doses during their entire pregnancy to greatly reduce their risk of experiencing adverse malarial episodes. As of 2014, 36 African countries had adopted this policy and an estimated 52% of eligible women received at least one dose. [3] Seasonal malaria chemoprevention (SMC) is the administration of full treatment courses of antimalarial medications in up to four monthly doses to children in regions

with high seasonal transmission. Currently the WHO recommends this policy for 15 countries, six of which have adopted it. [3]

In most cases, the recommended treatment of malaria is artemisinin-based combination therapies (ACT). Artemisinin is the active ingredient in the Qinghao plant, first documented for its anti-fever properties in 340 C.E., and isolated by Chinese scientists in 1971. [2] For nearly two centuries, quinine was the only known antimalarial treatment. The medicinal properties of quinine were first documented during the seventeenth century, when indigenous Peruvian tribes told Spanish missionaries of the bark from the cinchona tree, and its use to cure fevers. [2, 49] In 1934, chloroquine was discovered, and by 1946 it was recognized as a safe and effective treatment method for malaria. Extensive use of quinine and chloroquine has contributed to widespread parasitic resistance to these drugs, reducing their overall effectiveness. [49] Chloroquine is used primarily to treat *P. vivax* in regions where resistance has not been identified; quinine is rarely used, except in severe or critical cases.

Despite the global initiates to combat malaria, there are several barriers that prevent effective implementation of necessary intervention methods. Two of the most significant concerns are the expanding geographic distribution of vector resistance to insecticides, and parasitic strains resistant to antimalarial drugs. [3, 14, 26, 32, 35, 49, 50] Current intervention strategies rely heavily on a limited number of insecticides and antimalarial medications, making resistance to these defenses an immediate concern. There are also major gaps in intervention coverage, as millions of people living at risk of malaria do not have proper access to prevention and treatment services. [3] Countries with the greatest malaria burden tend to have low national income and poor medical infrastructure, making the dissemination of interventions in these regions difficult.

Malaria in Burkina Faso

With an estimated land area slightly larger than the state of Colorado, Burkina Faso is a relatively small, tropical country in Western Africa. It is home to approximately 19.5 million people of varying ethnic sub-groups; however, collectively citizens are referred to as Burkinabé. Once a French colony, Burkina Faso achieved independence in 1960, but has since experienced various military coups, political corruption, and has struggled to establish a stable government. This political instability, along with few national resources and a weak industrial base has hindered overall economic growth. [51] In 2015, Burkina Faso ranked 200 of 217 sovereign nations recognized by the World Bank for their gross national income (GNI), which was estimated at $660 (USD) per capita. [52] Within the country, the estimated unemployment rate is 77%, nearly 50% of the population lives below the poverty line, and the poorest 10% of households account for 2.9% of income, while the wealthiest 10% account for 32.2% of the country's income. [51]

Approximately 70% of the country's population is estimated to reside in rural villages [52], often in structures known as concessions. A concession is a compound that may include several small huts in which extended family members reside and share resources. Though the overall compositions of concessions may vary drastically within villages, a typical extended family unit includes a man, his wife or wives, their children, and sometimes the man's or his wife's older parents. Generally, there is a common central kitchen hut, surrounded by smaller sleeping huts: one for the man of the family; one for each wife, which she shares with her young children; and one for the animals. Older children often sleep on their own or with the animals. Rural villages are composed of clusters of these concessions, surrounded by the agricultural fields farmed by the villagers, and range drastically in size and population density.

Burkina Faso is classified as very high risk for major infectious diseases, including HIV/AIDS, typhoid fever, and various tropical diseases. In terms of malaria, it is one of the most highly burdened countries. In 2013, there were nearly 5.5 million confirmed cases, and 5,600

confirmed deaths; however, due to the lack of surveillance programs, data on malaria in Burkina Faso are limited, and these figures are likely only a fraction of the true burden. [3] With 100% of the country's 19.5 million residents living at high risk for infection, the WHO recommends the most aggressive intervention methods, including ITNs, IRS, larval control, and preventative therapies for pregnant women and children. [3] Currently, all recommended intervention methods have been adopted in Burkina Faso, except the use of SMC for children. Though these control efforts seem to have decreased the estimated incidence of malaria from 600 cases per 1,000 people in 2000, to approximately 400 cases per 1,000 people by 2014, the burden of malaria remains high.

The biological profile of malaria in Burkina Faso is similar to that found throughout endemic regions in Africa, with *P. falciparum* being the only parasitic species, while *An. gambiae*, *An. funestus*, and *An. arabiensis* are the major vectors present. [3] Prior to the 1990's, malaria control programs were basically non-existent in Burkina Faso. However, in less than three decades of large, up-scaled approaches, high rates of insecticide-resistance and antimalarial-resistance have been observed, and the overall effectiveness of ITNs seems to be decreasing. [14] Loss of current intervention effectiveness, gaps in intervention coverage, poor medical infrastructure, and the lack of resources for mitigation put the Burkinabé at risk of experiencing increased incidence and mortality of malaria in the coming decades. These challenges with controlling this disease in Burkina Faso demonstrate the necessity for innovative and cost-effective interventions to reduce its overall burden.

Mass Drug Administrations & Ivermectin

A mass drug administration (MDA) is a large-scale public health initiative in which the entire population in a specified geographic region is treated for a certain disease. Most commonly, high-coverage MDAs are implemented to treat neglected tropical diseases (NTDs), a class of diseases that tend to be highly debilitating, despite low overall mortality rates. [16] They

are often highly prevalent in LMIC in tropical and subtropical regions, where control efforts are minimal due to the lack of adequate medical resources and infrastructure to combat them. These diseases can have devastating medical consequences, such as blindness due to onchocerciasis, and excessive swelling in lower limbs due to lymphatic filariasis. These diseases often result in billions of dollars lost in worker productivity, and negative impacts on child development and maternal health annually. [16] At present, apart from a few specific circumstances, the World Health Organization (WHO) recommends against using these public health initiatives to control malaria. Past efforts suggest MDA of antimalarials do not efficiently limit the disease in the long term, and mass distribution of these drugs may provide significant selective pressure for the development of resistance. [53]

Endectocides are antiparasitic drugs that can effectively treat both parasites that live within the host and on the outside of the host's body. Ivermectin (IVM) is a common endectocide used throughout the world to treat and prevent various parasitic infections, and is one of the primary drugs given during MDAs to control NTDs. [22, 54] Since the 1980's, IVM has been recognized as a toxic compound to *Anopheles* mosquitos taking blood meals from treated mammals. [17-19, 21, 54, 55] At lethal concentrations, IVM works by targeting and hyperpolarizing the membrane potential of postsynaptic neurons and muscle fibers in arthropods. [19] This causes a flaccid paralysis and weakness, that often leads to the insect's death. When blood concentrations are sub-lethal to feeding *Anopheles* mosquitoes, IVM has been reported to reduce egg hatch rate, length of progeny survival, and the overall reproductive rate. [18, 19] Along with its mosquitocidal effects, IVM has recently been shown to inhibit production of sporozoites within the guts of vectors. [20] Additionally, results of the RIMDAMAL study indicated children five years old and younger who received repeated IVM treatments experienced a stronger protective effect than other children in the treatment villages who did not receive the intervention. The exact mechanisms by which IVM disrupts the parasitic life cycle is not well understood and requires additional research. Ivermectin is an inexpensive, effective, and safe drug that protects

millions of humans and animals from parasitic diseases, and recent significant findings demonstrate its potential to minimize the spread of malaria.

Conclusion

Few diseases have been as devastating to human populations throughout history as malaria. In the last century, efforts to eradicate the disease have drastically reduced its global incidence and distribution. However, with billions still living at risk of infection, malaria remains a major global health concern. Preventative interventions are primarily focused on minimizing human interactions with mosquito vectors using insecticides, and only a few effective treatments are available to treat those infected. These limited intervention strategies are slowly losing their effectiveness as malaria parasites and vectors are developing resistance around the world. Recently, IVM was implicated as a potential method to control malaria, as it has lethal effects on the vector and can inhibit parasite development. If malaria is ever to be eradicated, novel interventions, such as the use of IVM, must be explored, especially as current methods are becoming less potent.

III. METHODS

Overview

This chapter describes methods used to geospatially analyze the childhood malaria incidence for the RIMDAMAL study. The chapter begins with an explanation of the methodology used in the original study, and then describes how the data were analyzed to achieve the aims of the geospatial analyses.

RIMDAMAL: A Brief Synopsis of the Original Study

The Repeat Ivermectin Mass Drug Administrations for Control of Malaria (RIMDAMAL) pilot study was conducted in the Sud-Ouest administrative region (Figure 12, Appendix A) of Burkina Faso in 2015. This was a single-blinded, parallel assignment, cluster randomized trial designed to test the safety and efficacy of repeated doses of IVM to minimize the burden of malaria among children aged five years and younger. The RIMDAMAL protocol was designed to test the assumption that individuals repeatedly treated with IVM would maintain blood concentrations levels that are lethal to the feeding *Anopheles* mosquitoes, which would therefore lower the overall abundance of malaria vectors, and decrease the number of new infections acquired, especially among children. To best evaluate the effectiveness of this intervention, all eligible Bukinabé residents in the eight study villages (Map of study villages: Figure 13, Appendix B) were asked to participate in this study.

Participants were deemed ineligible to receive IVM if they a) had a height of 90 centimeters (approximately 3 feet) or shorter; b) were currently pregnant; c) were currently breastfeeding an infant within one week of birth; or d) known to have travelled to regions endemic for *Loa loa.* In other words, mostly very young children and pregnant or postpartum women were not eligible. Within the four control villages, there were 1,265 residents, of which 1,055 were deemed eligible to receive the intervention. The four intervention villages consisted

of 1,442 residents total, of which 1,180 were deemed eligible. All eligible participants were given IVM at the beginning of the 18-week rainy season. For participants in the control arm, this was the only administration of IVM they received; whereas, participants in the intervention arm received an additional 5 IVM administrations at 3 week intervals. The estimated participation rate for the first IVM administration was 79%, but participation rates steadily decreased over the five successive administrations (75%, 73%, 74%, 72%, and 70% respectively).

The outcome of interest was the cumulative incidence of malaria in children ≤ 5 years old within the study villages, which was evaluated through active case detection (ACD). Although most children ≤ 5 years old were not tall enough to receive the intervention, there was a small group (121 children total; 22%) who were treated at least once with IVM. There were four nurses working with the clinical data collection team, each assigned to one control village and one intervention village. Every two weeks, nurses visited their assigned villages at least three times to collect incidence data. Nurses would ask questions regarding the general health of all children since their previous visit to the villages. If a child was suspected of being infected or had a recent history of febrile illness, a small blood sample was collected from the child and used for a rapid diagnostic test (RDT) and a slide smear. Diagnostically confirmed, uncomplicated cases were treated as per the national malaria treatment guidelines, and any complicated cases or other adverse health outcomes were reported to study physicians for treatment or referral to the community health center. In total, study participants were followed for approximately 18 weeks. Additionally, geospatial data were collected for each concession within all study villages, using the WGS 1984 coordinate system. In total, there were 149 concessions within the four control villages, and 156 within the four intervention villages.

The RIMDAMAL study was made possible through the collaboration of researchers at Colorado State University (CSU) and three entities in Burkina Faso, including the Institution de Recherche en Sciences de la Santé (IRSS), Centre MURAZ (CM), and the Ministry of Health (MoH). Funding for this original study was provided by the Bill and Melinda Gates Foundation

(BMGF) Grand Challenges Exploration grant. Methods for RIMDAMAL were approved by the CSU Institutional Review Board (IRB) and the Comite d'Ethique d'IRSS in Burkina Faso, and this trial is registered at ClinicalTrials.gov (NCT02509481).

Geospatial Analyses

Evaluating the geospatial clustering of infectious disease occurrences is not a new epidemiologic method; however, it has been gaining popularity as spatial analytical methods and technology improve. There are several spatial autocorrelation statistics and techniques available to identify significant clusters, including Geary's C, Moran's I, the Getis-Ord general Gi statistic, and the Getis-Ord Gi*. [15] Both Geary's C and Moran's I statistics determine whether spatial data are clustered or dispersed. These global autocorrelation statistics can be useful for evaluating trends and where clustering occurs; however, they provide no indication as to whether clusters are similarly high values or similarly low values. The general G statistic, also known as the high/low clustering tool, can be useful in identifying whether high or low values cluster; however, the presence of clustering of both high and low values within a dataset tend to have a cancelling-out effect. The Gi* statistic, also referred to as hotspot analysis, can identify clustering of both high and low values within one dataset. Although both Gi and Gi* compare local attribute values to global attribute values, calculation of the Gi statistic excludes the attribute value at the feature being analyzed, while the calculation of Gi* includes the value of the feature being analyzed. Since RIMDAMAL was a pilot study, no prior knowledge existed on the potential impact such an intervention could have on the regional distribution of malaria among children. Gi* allows more flexibility in analysis by differentiating between clusters of low and high values, and thus was chosen to explore these spatial patterns. All geospatial analyses for this research were completed using ArcGIS 10.3.[56]

Currently, there are only a handful of studies that have utilized the Gi* statistic to evaluate clustering of infectious diseases. These studies have evaluated the geospatial

distribution of human cases of malaria [57, 58], cutaneous leishmaniasis [59], scarlet fever [60], and *Salmonella* [61]; as well as cases of Hendra virus in horses [62], and co-infection of Flanders and West Nile viruses in mosquitoes. [63] In each of these studies, the Gi* analyses were used to identify and evaluate significant spatial clusters throughout varying geographic regions, including large urban centers, counties, provinces, and an entire country; all of which had a study population of 900,000 people or more. These studies were conducted primarily for the purposes of disease surveillance and risk estimation. In contrast, the RIMDAMAL study includes eight small villages with only 2,707 participants in total, and provides a unique opportunity to spatially evaluate the impacts of a novel intervention for malaria control.

Gi* is calculated by dividing the sum of the attribute values for neighboring attributes, by the sum of all attribute values in the study area. The equation to calculate Gi* for a given concession (*i*) with a specified distance (*d*) is:

$$G_i*(d) = \frac{\Sigma\, w_{ij}(d)\, x_j}{\Sigma\, x_j}$$

Where x_j is the incidence density value for a neighboring concession (*j*), and $w_{ij}(d)$ is the weight for the target-neighbor pair at the specified distance. The weight is a binary variable in that 1 indicates a feature within distance *d* of the target feature *i*, and 0 indicates a feature outside of that distance. Statistical significance for this method is evaluated using a Z-score test. The equation to calculate the Z score for each concession is given as:

$$Z(G_i*) = \frac{G_i^* - E(G_i^*)}{\sqrt{Var(G_i^*)}}$$

Where $E(G_i^*)$ is the expected value for Gi*, and $Var(G_i^*)$ is variance of Gi*. The expected Gi* values are calculated as follows:

$$E(G_i^*) = \frac{\Sigma\, w_{ij}(d)}{n-1}$$

Where *n* is equal to the total number of concessions. The null hypothesis for Gi* analysis is that no spatial clustering of attribute values exists, or complete spatial randomness (CSR).

There are two inherent statistical problems when performing spatial analyses: spatial dependency and multiple hypothesis testing. When evaluating the relationships of objects in space, it is important to understand that objects nearer to each other tend to be more similar than objects that are farther away. Spatial dependence is the concept that some statistically significant clustering can be explained due to the non-random spatial distribution of some feature or process. Pattern analysis statistics, like Gi*, are calculated at each feature using attribute values of neighboring features. Near features tend to share neighbors, creating an overlap that exacerbates spatial dependency, and can increase the rate of type I errors, or falsely rejecting the null hypothesis.

Multiple testing is also known as multiple comparisons, and is a common problem in statistical analysis methods that simultaneously test several hypotheses. When using spatial autocorrelation statistics such as Gi*, simultaneous hypothesis tests are performed for each feature within the dataset during one analysis. Much like spatial dependence, multiple testing increases the chance of type I errors, resulting in the artificial inflation of statistical significance.

ArcGIS provides the option of correcting for spatial dependence and multiple testing by applying the False Discovery Rate (FDR) correction, which reduces the critical p-value threshold. The FDR correction is a Boolean parameter that tends to be less conservative than the classical Bonferroni and Sidak corrections, and works by ignoring features with the largest (weakest) p-values based on the false positive estimate for the dataset.

Aim 1- Within Village Analyses

Within village analyses evaluated spatial clustering of high and low childhood malaria incidence density values for each study village. Incidence density was calculated at each concession by dividing the total number of positively diagnosed malarial episodes in children ≤ 5 years old, by the sum of days each child was followed, and multiplied by 100 days (# malarial episodes per 100 person-days). For each village, several Gi* analyses were conducted at varying distances, starting with a short specified distance, incrementally increasing, and

finishing when the maximum distance for each village was reached. These distances varied by village based on its geographic extent and distances between concessions. The starting distance was determined based on the minimum distance that would include at least two neighboring concessions for each concession being analyzed. This distance ranged from 25 meters for control village B (1B[1]), to 250 meters for intervention village A (2A). Distances for analyses were increased by the same amount in each village, and ranged from 25 meters in village 1B to 150 meters in village 2A. Analyses were run at increased distances until the maximum threshold distance was reached for each study village, as indicated by an error when running an analysis at a distance greater than the geographic extent of the study region.

For each specified distance within the study villages, an analysis was first performed without the FDR correction applied. If any statistically significant clusters of high incidence density (hot spots) or low incidence density (cold spots) occurred, an analysis was conducted at the same distance, but with the FDR correction applied to determine statistical significance corrected for spatial dependence and multiple testing.

Aim 2- Between Village Analyses

For the between village analyses, clustering of high and low incidence density values (# malarial episodes/ 100 person-days) was evaluated at the concession level, much like the analytical methods utilized for Aim 1. Several Gi* analyses were performed at varying distances throughout the entire study region to compare these incidence density values among all study villages. The first analysis was performed at 100 meters, and distances were incrementally increased by 500 meters to 4,100 meters. After 4,100 meters an analysis was performed at 6,000 meters, and incrementally increased by 2,000 meters until the maximum distance was reached. The maximum distance error occurred during analyses attempts at 14,000 meters and 13,500 meters, with the final analysis successfully occurring at 13,000 meters. These

[1] Abbreviated village codes indicate study arm (1=control; 2=intervention) and village label (A-D). For example, control village C=1C, and intervention B=2B

incremental distances were chosen based on the geographic extent and distances between villages. An analysis was first performed at each distance without the FDR correction applied, and if statistically significant hot or cold spots were identified, the analysis was performed with the FDR correction applied.

Geospatial Hypotheses

Lack of existing literature on the spatial clustering of childhood malaria incidence made hypothesizing geospatial results in terms of this intervention difficult; however, some spatial scenarios were postulated prior to analyses. Though results within individual villages would not be comparable, clustering trends were suspected to occur among control villages and intervention villages. For example, it was hypothesized that hot spots in intervention villages might be more likely to occur around the village edges, while cold spots might occur near the center. Since this intervention worked by decreasing the abundance of infected vectors, this clustering trend was postulated on the assumption vectors within the village mostly experienced the lethal effects of IVM, while vectors on the outskirts of the village experienced lesser lethal effects, and continued to transmit the parasites. Furthermore, significant clusters of high and low malaria incidence in control villages would appear more randomly distributed.

The major hypothesis postulated for the between village analyses was that intervention villages would be more likely to have concessions identified as significant cold spots, while control villages would be more likely to have concessions identified as hot spots. It was also believed that if this intervention has the potential to provide regional protection, some control village concessions within closer proximity to intervention villages would potentially be identified as significant cold spots.

Additional Statistical Methods

T -tests were also performed to compare mean incidence density values of concessions identified as significant clusters, to those not identified as significant clusters. These analyses

were performed within each village and between villages for the Gi* results with maximum clustering. The mean incidence density of the concessions identified as hot spots was compared to the mean incidence density of the concessions identified as having no statistical difference from their expected value. For analyses resulting in cold spots, t-tests were also performed comparing the mean of cold spot concessions to the mean of the concessions identified as not statistically different than expected. The primary goal of these tests was to determine if concessions were identified as significant clusters because of the spatial distribution of malaria incidence, or because their incidence densities were significantly higher or lower, ignoring the spatial distribution. Failure to reject the null hypothesis implied no significant difference between the incidence densities of concessions identified as significant clusters and other concessions. Rejecting the null hypothesis indicated a significant difference existed between incidence densities of significant clusters and other concessions, which could have biased the geospatial results. These additional statistical methods were completed using *R*. [64]

IV. RESULTS

Overview

This chapter presents the results derived through analytical methods described in the previous chapter. Descriptive data for the study villages are presented here, along with maps constructed through the geospatial analyses. All tables and figures are presented at the end of the chapter.

Descriptive Results

In total, there were eight villages evaluated for this research; four control villages, and four intervention villages. There were 1,265 total participants enrolled in the control arm, of which 263 (20.8%) were children aged 5 years or younger. Within the four intervention villages, 327 (22.7%) of the 1,442 total participants were children $\leq$ 5 years old. Since analyses were conducted on malaria incidence density among children $\leq$ 5 years old, concessions with zero children five-years-old or younger were excluded. In the control arm, there were 106 of 149 (71.1%) concessions analyzed, and in the intervention arm, 127 of 156 (81.4%) concessions were analyzed. Basic descriptive statistics for the study villages and the entire study region are provided in Table 1, along with mean incidence density, estimated village area, and estimated population density. Mean incidence density (ID) among control villages was 2.09 cases per 100 person-days (range= 1.73- 2.34), while the mean ID among intervention villages was 1.65 cases per 100 person-days (range= 1.5- 2.29). Study villages ranged drastically in size (from just under 5 acres in village 1B, to over 650 acres in village 2A) and population density (from 0.45 persons per acre, to 41.08 persons per acre).

Study Aim 1- Within Village Analyses

Results for each village are described within the text, and then presented with a color-coded table displaying the analysis results, the statistical details of the analysis with maximum clustering, and a map displaying the spatial distribution of the clusters. Within all study villages, multiple significant clusters of high childhood malaria incidence density (hot spots) were identified. Significant clusters of low childhood malaria incidence density (cold spots) were identified in only one village at maximum clustering, village 1A.

Control Village A (1A)- There were 321 participants in village 1A, 58 (18.1%) of which were children[2], residing in 17 of 20 concessions (85%). Mean ID was 2.33 cases per 100 person-days, the third largest of all study villages. Village 1A was the fourth largest (111 acres), with the sixth largest population density (2.89 persons/acre) (Table 1). The initial Gi* analysis was performed at 200 meters, and the specified distance was increased by 100-meter increments until the maximum of 700 meters was reached (Table 2). Maximum clustering occurred at 400 meters with the FDR correction applied, and identified a total of sixteen (94.1%) significant clusters; five (29.4%) cold spots and eleven (64.7%) hot spots (Table 3; significant cold spots highlighted in blue; significant hot spots highlighted in orange; significant p values are bolded and italicized). In total 57 (98.3%) children were residing in concessions identified as significant clusters; 15 (25.9%) in cold spots, and 42 (72.4%) in hot spots. All hot spots appeared clustered near the village center, while all cold spots appeared clustered on the north-eastern edge (Figure 1).

Control Village B (1B)- In village 1B, 48 of the 205 participants were children (23.4%), residing in 24 of the 48 concessions (50%). The mean ID was 1.73 cases per 100 person-days, the lowest of all control villages, fifth lowest of all study villages. At 4.99 acres, this was the smallest village, but had the largest population density (41.08 persons/acre) (Table 1). The initial analysis

[2] Throughout the results, "children ≤ 5 years old" will be referenced as "children."

was conducted with a set distance of 25 meters, increasing by 25-meter increments, and reaching a maximum distance of 125 meters (Table 4). Maximum clustering occurred during the final analysis at 125 meters and with the FDR correction applied, and nine (37.5%) concessions were identified as significant hot spots (Table 5; significant hot spots highlighted in orange; significant p values are bolded and italicized). There were 17 (35.2%) children residing in hot spot concessions. All hot spots appeared clustered near the center of the village (Figure 2).

Control Village C (1C)- There were 216 participants residing in village 1C, 43 (19.9%) of which were children. Of the 32 concessions, 23 (71.9%) housed children. Mean ID was 1.93 cases per 100 person-days, the fourth highest of all study villages. Village 1C was the second largest with approximately 480 acres, but had the lowest population density (0.45 persons/acre) (Table 1). The initial geospatial analysis in Village 1C was conducted at 200 meters, with incremental increases of 100 meters, until reaching the maximum distance of 1,700 meters (Table 6). Maximum clustering occurred during the final analysis at 1,700 meters and with the FDR correction applied. A total of 13 concessions (56.5%) were identified as significant hot spots (Table 7; significant hot spots highlighted in orange; significant p values are bolded and italicized), where 24 children (55.8%) resided. These hot spots appeared generally clustered near the center of the village (Figure 3).

Control Village D (1D)- With 523 residents, village 1D was the second most populous of the study villages. There were 114 children (21.8%) total, and 42 (85.7%) of the 49 concessions housed these children and were included in analyses. Village 1D had the greatest mean ID at 2.34 cases per 100 person-days. It was the third largest village (176 acres), with the fifth largest population density (2.97 persons/acre) (Table 1). Analyses began at 100 meters, increased by 100-meter increments, and reached a maximum distance at 1,300 meters (Table 8). Maximum clustering occurred during the analysis at 1,000 meters with the FDR correction applied, with a total of 31 (73.8%) concessions identified as significant hot spots (Table 9; significant hot spots highlighted in orange; significant p values are bolded and italicized). There were 89 (78.1%)

children residing in hot spot concessions, and the spatial distribution of the hot spots appeared random (Figure 4).

Intervention Village A (2A)- There were 533 participants in village 2A, making it the most populous of the study villages. Of the participants, 128 (24%) were children living in 46 (90.2%) of the 51 concessions. The mean ID was 1.64 cases per 100 person-days, which ranked as the seventh highest, or second to the lowest. With 652.8 acres, village 2A was also the largest of all the study villages, and it had the second lowest population density (0.82 persons/acre) (Table 1). The initial geospatial analysis in Village 2A was conducted at 250 meters, with incremental increases of 150 meters, until reaching the maximum distance of 1,900 meters (Table 10). The final analysis at 1,900 meters, with the FDR correction applied, resulted in maximum clustering, identifying a total of 42 (91.3%) concessions as significant hot spots, the highest number of hot spots observed within one village (Table 11; significant hot spots highlighted in orange; significant p values are bolded and italicized). In total, 113 (88.3%) of the children resided in the concessions identified as significant clusters. The four concessions that were statistically no different than their expected Gi* values appeared on the western edge of the village (Figure 5).

Intervention Village B (2B)- In village 2B, there were 390 participants, 98 (25.1%) of which were children. Of the 58 total concessions, 45 (77.6%) housed children. Village 2B had the lowest mean ID at 1.5 cases per 100 person-days. It was the third smallest village (44.7 acres), but had the second highest population density (8.72 persons/acre) (Table 1). Analyses in village 2B began at 100 meters, and incrementally increased by 50 meters until the maximum distance of 350 meters was reached (Table 12). The final analysis at 350 meters with the FDR correction applied identified 22 (48.9%) significant hot spots, the maximum number of significant clusters identified in this village (Table 13; significant hot spots highlighted in orange; significant p values are bolded and italicized). There were 46.9% (46) of the children living in hot spot concessions. The general spatial distribution of these hot spots appeared grouped near the center of the village (Figure 6).

Intervention Village C (2C)- Village 2C had 398 participating residents, and 66 (16.6%) were children, the lowest proportion of children of all study villages. Children lived in 25 (69.4%) of the 36 concessions, and the mean ID of 1.68 cases per 100 person-days was the third lowest of all study villages. Village 2C was the fourth smallest (76.6 acres) with the third highest population density (5.2 persons/acre) (Table 1). The initial Gi* analysis was conducted at 100 meters, incrementally increasing by 100 meters, until the maximum distance of 700 meters was reached (Table 14). Maximum clustering occurred during the final analysis at 700 meters, with the FDR correction applied. In total, three (12%) concessions were identified as significant hot spots, and housed four (6.1%) of the children (Table 15; significant hot spots highlighted in orange; significant p values are bolded and italicized). The significant hot spots appeared grouped in the northern half of the village (Figure 7).

Intervention Village D (2D)- In village 2D, there were 121 total participants residing in eleven concessions. Children made up 28.9% (35) of the population, and they resided in all eleven concessions (100%). The mean ID was 2.29 cases per 100 person-days, the highest mean ID of all intervention villages, and second highest overall. With 28.6 acres, it was the second smallest village, with the fourth highest population density (4.23 persons/acre) (Table 1). Analyses in village 2D began with a set distance of 75 meters, which incrementally increased by 75 meters, until the maximum distance of 300 meters was reached (Table 16). Maximum clustering occurred during the final analysis at 300 meters with the FDR correction applied, and identified two concessions as significant hot spots (Table 17; significant hot spots highlighted in orange; significant p values are bolded and italicized). The two hot spots appeared centrally located within the village (Figure 8).

Study Aim 2- Between Village Analyses

The whole study population was 2,707 participants, 590 (21.8%) of which were children. Participants resided in 305 concessions, 233 (76.4%) of which housed children and were

included in analyses. The study region spanned an area of approximately 66,500 acres, with a population density of 0.27 persons per acre (based on the average population density of Burkina Faso[10]). The specified distance for the first Gi* analysis was set at 100 meters, and then incrementally increased by 500 meters to 4,100 meters. The analysis distance was then increased to 6,000 meters, and then incrementally increased by 2,000 meters until reaching 12,000 meters, and the final analysis in the whole study region was conducted at 13,000 meters (Table 18).

Statistically significant hot and cold spots appeared throughout most of the analyses, and clustering first appeared to peak during analyses at 1,600/ 2,100 meters (same results) with 122 (52.4%) concessions identified as significant clusters, 69 (29.6%) of which were cold spots, and 53 (22.7%) of which were hot spots. There were 146 (24.8%) children living in concessions identified as significant cold spots, and 149 (25.3%) children living in hot spots. Clustering peaked for a second time and reached its max during analysis at 10,000 meters, with 163 (70%) concessions identified as significant clusters, 62 (26.6%) were cold spots, and 101 (43.4%) were hot spots. There were 184 (31.2%) children living in the concessions identified as cold spots, and 219 (37.1%) children living in hot spot concessions. Table 18 lists the numbers of significant clusters identified during each analysis, and Figure 9 represents this data graphically. The detailed results of the analyses during which clustering peaked are displayed in Table 19 (significant cold spots are highlighted with blue; significant hot spots are highlighted with orange; significant p values are bolded and italicized).

The analysis results at 2,100 meters show that all concessions in villages 1B and 2B were identified as significant cold spots, while all concessions in villages 1D and 2D were identified as significant hot spots (Figure 10). Villages 1B and 2B are neighboring villages, as are villages 1D and 2D. During the analysis at 10,000 meters, all concessions in neighboring villages 1A and 2A (except one concession in 2A) were identified as significant cold spots. All concessions in villages 1B, 1C, 2B, and 2D (except one concession in 1C and one concession

in 2D) were identified as significant hot spots (Figure 11). The cold spot villages are neighbors in the north-eastern region of the study area, somewhat geographically isolated from many the other study villages. The hot spot villages seem to be grouped near each other, while the statistically insignificant villages appear near edges of the study region and seem to be farthest from most the other villages (Figure 9). As an additional reference for visualizing the spatial distribution of the study region, Appendix B contains a map of the general location of each study village (Figure 13).

T-test Results

Results from the t-tests within each individual village are displayed in Table 20. Village 1A was unique in that it was the only study village where max clustering results included the identification of both hot and cold spots. Since there was only one value for statistically insignificant concessions in village 1A, one-sample t-tests were conducted to evaluate whether the mean ID values of significant concessions were equal to the ID value of the insignificant concession. In other words, one t-test evaluated whether the mean ID of cold spot concessions (1.01 cases per 100 person-days) was equal to 1.65, while another t-test evaluated whether the mean ID of hot spot concessions (2.83 cases per 100 person-days) was equal to 1.65, the ID value of the statistically insignificant concession. For all other villages, only hot spot concessions were identified, and more than one insignificant cluster existed, so two-sample t-tests were conducted. One t-test yielded a significant p value, indicating the mean ID of hot spot concessions in village 1A is statistically different than 1.65 cases per 100 person-day (Table 20).

T-tests were also conducted based on geospatial results for the between village analyses. Two-sample t-tests were conducted comparing both cold spots to statistically insignificant concessions, and hot spots to statistically insignificant concessions. Both t-tests were performed for the two distances at which clustering peaked during between village

analyses. The t-test results for cold spots at both 2,100 meters and 10,000 meters indicate there is no evidence of a statistical difference between mean ID of cold spots and mean ID of insignificant concessions. However, t-test results for hot spots at both 2,100 meters and 10,000 meters indicate a statistical difference does exist between mean ID of hot spots and mean ID of insignificant spots. (Table 21)

Figures & Tables

Table 1- Descriptive Results for All Study Villages and Entire Study Region

Village	Total # Participants	# Children ≤ 5 (% total)	Total # Concessions	# Concessions w/ children ≤ 5 (% total)	Mean Incidence Density (cases/ 100 person-days)	Area (acres)*	Population Density (persons/ acre)
Control 1A	321	58 (18.1)	20	17 (85)	2.23	111	2.89
Control 1B	205	48 (23.4)	48	24 (50)	1.73	4.99	41.08
Control 1C	216	43 (19.9)	32	23 (71.9)	1.93	480.6	0.45
Control 1D	523	114 (21.8)	49	42 (85.7)	2.34	176	2.97
All Controls	1265	263 (20.8)	149	106 (71.1)	2.09	N/A	N/A
Intervention 2A	533	128 (24)	51	46 (90.2)	1.64	652.8	0.82
Intervention 2B	390	98 (25.1)	58	45 (77.6)	1.50	44.7	8.72
Intervention 2C	398	66 (16.6)	36	25 (69.4)	1.68	76.6	5.20
Intervention 2D	121	35 (28.9)	11	11 (100)	2.29	28.6	4.23
All Interventions	1442	327 (22.7)	156	127 (81.4)	1.65	N/A	N/A
Entire Study Region	2707	590 (21.8)	305	233 (76.4)	1.85	66,570**	0.27***

Note- *Approximate area of study region based on area of polygon (Google Maps) encapsulating all concessions within individual villages. **Approximate area of the map of the study region. ***Based on the average population density of Burkina Faso[10]

Table 2- Analysis Outcomes for Control Village A

			Concession ID*																	Number of significant clusters
Attempt	Specified Distance (meters)	FDR Correction Applied (Y/N)	01	02	03	04	05	06	07	08	09	10	12	13	14	15	16	18	20	
1	200	N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	C	C	C	NS	NS	NS	NS	3
2	200	Y	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0
3	300	N	H	H	H	H	H	NS	H	H	H	H	C	C	C	C	H	NS	C	15
4	300	Y	H	H	H	H	H	NS	NS	NS	H	H	C	C	C	C	H	NS	C	13
5	400	N	H	H	H	H	H	H	H	NS	H	H	C	C	C	C	H	H	C	16
6	400	Y	H	H	H	H	H	H	H	NS	H	H	C	C	C	C	H	H	C	16
7	500	N	H	H	NS	NS	H	H	NS	NS	H	H	NS	C	NS	C	H	H	C	11
8	500	Y	H	H	NS	NS	H	NS	NS	NS	H	H	NS	C	NS	C	H	H	C	10
9	600	N	H	H	NS	NS	NS	NS	NS	H	H	H	NS	NS	NS	NS	H	H	H	8
10	600	Y	H	H	NS	NS	NS	NS	NS	H	H	H	NS	NS	NS	NS	H	H	NS	7
11	700	N	NS	H	NS	NS	NS	H	NS	H	H	NS	NS	NS	NS	NS	NS	H	NS	5
12	700	Y	NS	H	NS	NS	NS	H	NS	H	H	NS	NS	NS	NS	NS	NS	H	NS	5

* Includes only concessions with children ≤ 5

C= Cold Spot (95% Confidence) | NS= Not Statistically Significant | H= Hot Spot (95% Confidence)

Table 3- Analysis Results with Max Clustering in Control Village A; 400 meters with FDR correction applied

Concession	# Participants	# Children ≤ 5	Incidence Density	Gi* Z Score	Gi* P Value
01	44	8	1.80	3.26	***0.001***
02	43	10	2.64	3.26	***0.001***
03	26	5	3.14	3.26	***0.001***
04	12	2	2.89	3.26	***0.001***
05	34	8	2.89	3.26	***0.001***
06	5	1	3.31	2.15	***0.032***
07	13	2	2.89	2.30	***0.022***
08	11	1	1.65	1.19	0.236
09	5	1	2.48	3.26	***0.001***
10	7	2	2.89	3.26	***0.001***
11	12	0	Not included in analysis		
12	31	5	1.51	-2.13	***0.033***
13	20	4	1.47	-3.26	***0.001***
14	5	2	0	-3.40	***<0.001***
15	16	2	1.65	-3.26	***0.001***
16	13	2	2.89	2.55	***0.011***
17	4	0	Not included in analysis		
18	8	1	3.31	2.40	***0.016***
19	5	0	Not included in analysis		
20	7	2	0.41	-3.09	***0.002***

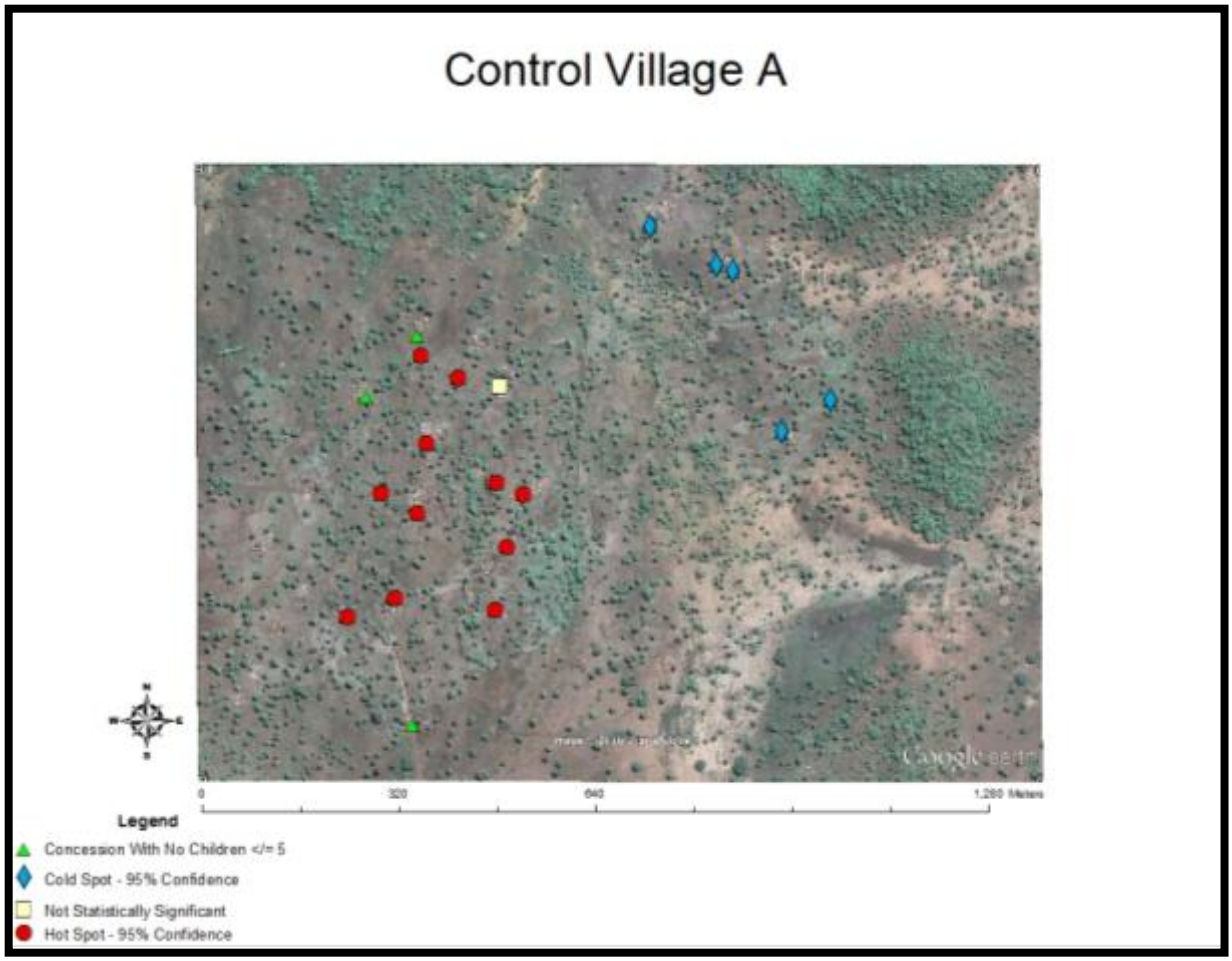

Figure 1- Map of Analysis Results with Max Clustering for Control Village A: Total of 16 concessions (94.1%) were identified as significant clusters during analysis at 400 m and with FDR correction applied. There were 11 hot spots identified, and 5 cold spots identified. All hot spots appeared in the main region of the village, while all cold spots appeared in a clump of concessions on the north-eastern border of the village.

Table 4- Analysis Outcomes for Control Village B

			Concession ID*																									Number of signficant clusters
Attempt	Specified Distance (meters)	FDR Correction Applied (Y/N)	01	02	03	04	06	08	10	11	15	16	22	23	24	26	27	30	32	34	38	39	41	42	47	49		
1	25	N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS		0
2	50	N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS		0
3	75	N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS		0
4	100	N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	H	H	NS	NS	NS	NS	NS	NS	NS	H	NS		3
5	100	Y	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	H	H	NS	NS	NS	NS	NS	NS	NS	H	NS		3
6	125	N	H	H	NS	H	NS	NS	NS	NS	NS	NS	NS	H	H	H	H	NS	NS	NS	NS	NS	NS	NS	H	H		9
7	125	Y	H	H	NS	H	NS	NS	NS	NS	NS	NS	NS	H	H	H	H	NS	NS	NS	NS	NS	NS	NS	H	H		9

* Includes only concessions with children ≤ 5

C= Cold Spot (95% Confidence) | **NS= Not Statistically Significant** | **H= Hot Spot (95% Confidence)**

Table 5- Analysis Results with Max Clustering in Control Village B; 125 meters with FDR correction applied

Concession	# Participants	# Children ≤ 5	Incidence Density	Gi* Z Score	Gi* P Value
01	5	1	0.83	>1.96	***<0.001***
02	3	1	0.83	>1.96	***<0.001***
03	3	2	2.48	NULL	NULL
04	5	2	3.72	>1.96	***<0.001***
05	1	0	Not included in analysis		
06	7	1	0.83	NULL	NULL
07	6	0	Not included in analysis		
08	9	4	2.08	NULL	NULL
09	3	0	Not included in analysis		
10	6	3	2.48	NULL	NULL
11	4	2	3.31	NULL	NULL
12	2	0	Not included in analysis		
13	5	0	Not included in analysis		
14	5	0	Not included in analysis		
15	5	1	0.83	0.53	0.60
16	7	2	0.41	0.99	0.32
17	2	0	Not included in analysis		
18	2	0	Not included in analysis		
19	3	0	Not included in analysis		
20	4	0	Not included in analysis		
21	1	0	Not included in analysis		
22	6	2	2.07	NULL	NULL
23	13	4	0.98	>1.96	***<0.001***
24	6	1	3.31	>1.96	***<0.001***
26	10	4	1.03	>1.96	***<0.001***
27	3	1	3.31	>1.96	***<0.001***
28	4	0	Not included in analysis		
29	2	0	Not included in analysis		
30	4	1	1.09	1.47	0.14
31	3	0	Not included in analysis		
32	8	2	1.24	1.47	0.14
33	2	0	Not included in analysis		
34	6	3	2.20	1.47	0.14
35	3	0	Not included in analysis		
36	1	0	Not included in analysis		

37	1	0	Not included in analysis		
38	4	2	0	1.47	0.14
39	4	2	1.24	1.47	0.14
40	1	0	Not included in analysis		
41	5	1	3.33	1.47	0.14
42	6	3	0.52	1.21	0.23
43	1	0	Not included in analysis		
44	5	0	Not included in analysis		
45	2	0	Not included in analysis		
46	2	0	Not included in analysis		
47	3	1	2.48	>1.96	***<0.001***
48	5	0	Not included in analysis		
49	7	2	0.83	>1.96	***<0.001***

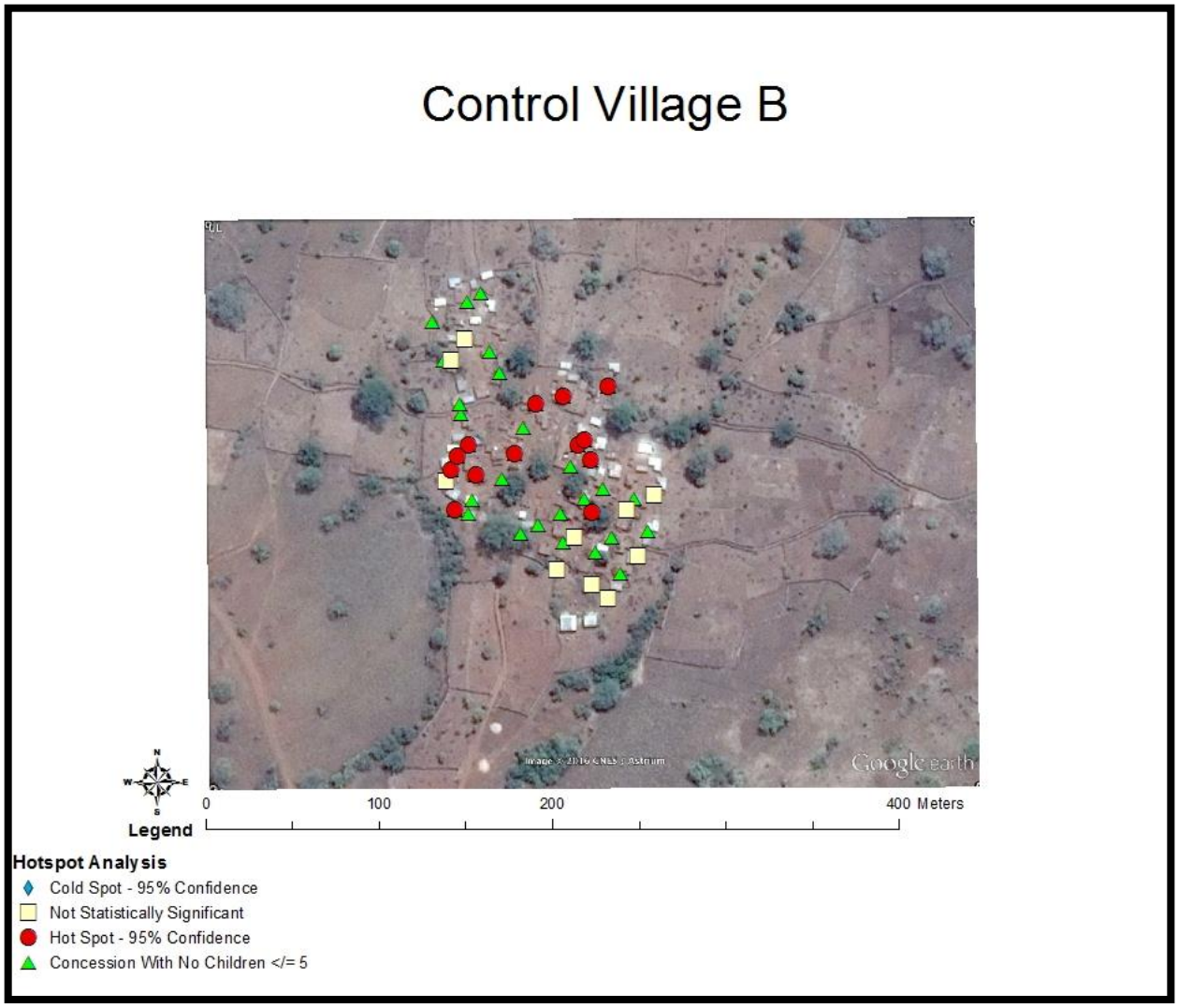

Figure 2- Map of Analysis Results with Max Clustering for Control Village B: Total of 9 concessions (37.5%) were identified as significant hot spots at distance of 125 m, with FDR correction applied. In general, the hot spots seem grouped near the center of the village.

Table 6- Analysis Outcomes for Control Village C

			Concession ID*																								Number of significant clusters
Attempt	Specified Distance (meters)	FDR Correction Applied (Y/N)	01	03	05	06	07	08	09	10	12	13	14	16	17	19	21	23	24	26	28	29	30	31	32	
1	200	N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0
2	300	N	NS	NS	NS	NS	NS	NS	NS	C	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	1
3	300	Y	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0
4	400	N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0
5	500	N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0
6	600	N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0
7	700	N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0
8	800	N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0
9	900	N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0
10	1000	N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	H	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	1
11	1000	Y	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	H	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	1
12	1100	N	NS	NS	NS	NS	NS	NS	NS	NS	NS	H	H	NS	NS	H	NS	NS	NS	NS	H	NS	NS	NS	H	5
13	1100	Y	NS	NS	NS	NS	NS	NS	NS	NS	NS	H	H	NS	NS	H	NS	NS	NS	NS	H	NS	NS	NS	H	5
14	1200	N	NS	NS	NS	NS	NS	NS	NS	NS	NS	H	H	NS	NS	H	NS	NS	NS	NS	H	NS	H	NS	H	6
15	1200	Y	NS	NS	NS	NS	NS	NS	NS	NS	NS	H	H	NS	NS	H	NS	NS	NS	NS	H	NS	H	NS	H	6
16	1300	N	NS	NS	NS	NS	NS	NS	NS	NS	H	H	H	NS	NS	H	NS	NS	H	NS	H	NS	H	NS	H	8
17	1300	Y	NS	NS	NS	NS	NS	NS	NS	NS	H	H	H	NS	NS	H	NS	NS	H	NS	H	NS	H	NS	H	8
18	1400	N	NS	NS	NS	NS	NS	NS	NS	H	H	H	H	NS	NS	H	NS	NS	H	NS	H	NS	H	NS	H	9
19	1400	Y	NS	NS	NS	NS	NS	NS	NS	H	H	H	H	NS	NS	H	NS	NS	H	NS	H	NS	H	NS	H	9
20	1500	N	H	NS	H	NS	H	H	NS	H	H	H	H	NS	NS	H	NS	NS	H	NS	H	NS	H	NS	H	13
21	1500	Y	H	NS	H	NS	H	H	NS	H	H	H	H	NS	NS	H	NS	NS	H	NS	H	NS	H	NS	H	13
22	1600	N	H	NS	H	NS	H	H	NS	H	H	H	H	NS	NS	H	NS	NS	H	NS	H	NS	H	NS	H	13
23	1600	Y	H	NS	H	NS	H	H	NS	H	H	H	H	NS	NS	H	NS	NS	H	NS	H	NS	H	NS	H	13
24	1700	N	H	NS	H	NS	H	H	NS	H	H	H	H	NS	NS	H	NS	NS	H	NS	H	NS	H	NS	H	13
25	1700	Y	H	NS	H	NS	H	H	NS	H	H	H	H	NS	NS	H	NS	NS	H	NS	H	NS	H	NS	H	13

* Includes only concessions with children ≤ 5

C= Cold Spot (95% Confidence) | NS= Not Statistically Significant | H= Hot Spot (95% Confidence)

Table 7- Analysis Results with Max Clustering in Control Village C; 1,700 meters with FDR correction applied

Concession	# Participants	# Children ≤ 5	Incidence Density	Gi* Z Score	Gi* P Value
01	6	1	1.63	>1.96	***<0.001***
02	4	0	Not included in analysis		
03	8	2	2.03	NULL	NULL
04	5	0	Not included in analysis		
05	4	2	1.63	>1.96	***<0.001***
06	9	2	2.03	NULL	NULL
07	7	1	3.33	>1.96	***<0.001***
08	5	1	1.63	>1.96	***<0.001***
09	7	1	1.63	NULL	NULL
10	6	1	0.81	>1.96	***<0.001***
11	4	0	Not included in analysis		
12	3	1	1.67	>1.96	***<0.001***
13	5	1	0.81	>1.96	***<0.001***
14	11	4	1.42	>1.96	***<0.001***
15	7	0	Not included in analysis		
16	8	2	1.22	-1.02	0.31
17	4	2	2.06	-1.02	0.31
18	4	0	Not included in analysis		
19	7	1	1.63	>1.96	***<0.001***
20	5	0	Not included in analysis		
21	5	2	1.65	NULL	NULL
22	4	0	Not included in analysis		
23	11	2	2.03	NULL	NULL
24	4	1	2.30	>1.96	***<0.001***
25	6	0	Not included in analysis		
26	7	3	2.71	0.56	0.58
27	3	0	Not included in analysis		
28	6	1	4.07	>1.96	***<0.001***
29	9	2	3.25	NULL	NULL
30	12	3	1.93	>1.96	***<0.001***
31	6	1	1.63	NULL	NULL
32	24	6	1.37	>1.96	***<0.001***

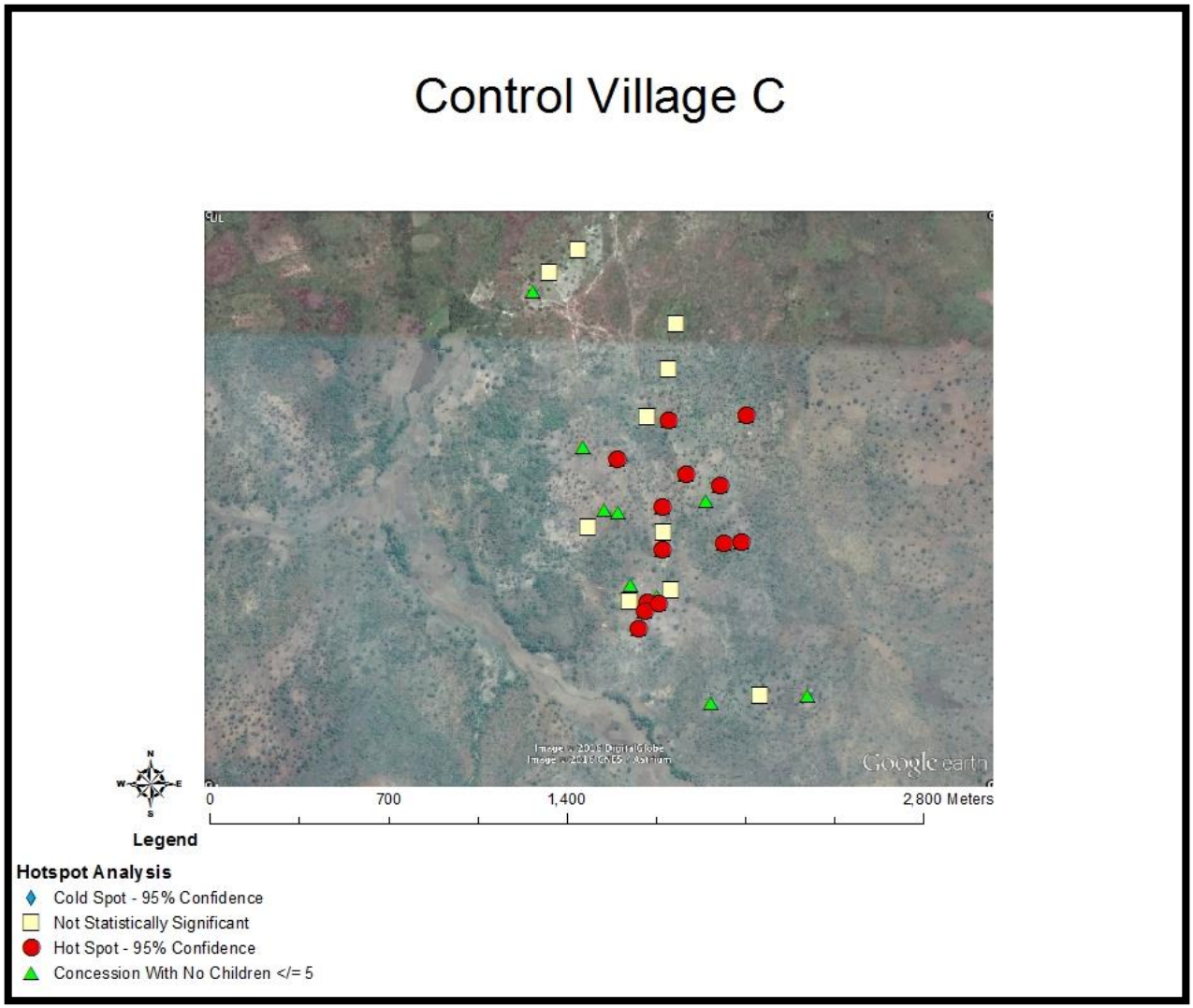

Figure 3- Map of Analysis Results with Max Clustering for Control Village C: During analysis at 1,700 m and with FDR correction applied, a total of 13 concessions (56.5%) were identified as significant hot spots. These hot spots appear to generally cluster centrally in the village.

Table 8- Analysis Outcomes for Control Village D

			Concession ID*																																										Number of significant clusters
Attempt	Specified Distance (meters)	FDR Correction Applied (Y/N)	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	33	34	35	36	38	39	40	41	42	43	45	47	
1	100	N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	C	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	C	NS	NS	NS	NS	NS	2
2	100	Y	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0
3	200	N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	C	NS	NS	NS	NS	NS	1
4	200	Y	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0
5	300	N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	C	NS	NS	NS	NS	NS	1
6	300	Y	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0
7	400	N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	C	NS	NS	NS	NS	NS	1
8	400	Y	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0
9	500	N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	H	H	NS	H	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	3
10	500	Y	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0
11	600	N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	H	NS	NS	NS	H	NS	H	H	NS	H	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	H	NS	NS	NS	NS	NS	NS	NS	H	7
12	600	Y	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0
13	700	N	NS	H	H	NS	H	H	NS	H	NS	H	NS	NS	NS	NS	H	NS	H	H	H	NS	NS	H	H	NS	NS	NS	NS	NS	NS	H	NS	H	NS	NS	NS	NS	NS	NS	NS	NS	NS	H	15
14	700	Y	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0
15	800	N	H	H	H	H	H	H	H	H	H	H	NS	H	NS	H	H	H	H	H	H	NS	H	H	H	H	H	H	H	NS	NS	H	H	H	H	NS	NS	NS	NS	NS	NS	NS	NS	H	29
16	800	Y	H	H	H	H	H	H	H	H	H	H	NS	H	NS	H	H	H	H	H	H	NS	H	H	H	H	H	H	H	NS	NS	H	H	H	H	NS	NS	NS	NS	NS	NS	NS	NS	H	29
17	900	N	H	H	H	H	H	H	H	H	H	H	NS	H	NS	NS	H	H	H	H	H	NS	H	H	H	H	H	H	H	H	H	H	H	H	NS	NS	NS	NS	NS	NS	NS	NS	NS	H	29
18	900	Y	H	H	H	H	H	H	H	H	H	H	NS	H	NS	NS	H	H	H	H	H	NS	H	H	H	H	H	H	H	H	H	H	H	H	NS	NS	NS	NS	NS	NS	NS	NS	NS	H	29
19	1000	N	H	H	H	H	H	H	H	H	H	H	H	H	NS	NS	H	H	H	H	H	NS	H	NS	H	H	H	H	H	H	H	H	H	H	NS	NS	NS	NS	NS	NS	NS	H	H	H	31
20	1000	Y	H	H	H	H	H	H	H	H	H	H	H	H	NS	NS	H	H	H	H	H	NS	H	NS	H	H	H	H	H	H	H	H	H	H	NS	NS	NS	NS	NS	NS	NS	H	H	H	31
21	1100	N	NS	H	H	NS	H	H	NS	H	H	NS	H	H	H	NS	H	H	H	H	H	NS	H	NS	H	H	H	NS	H	H	H	H	H	H	NS	NS	NS	H	NS	NS	H	H	H	H	29
22	1100	Y	NS	H	H	NS	H	H	NS	H	H	NS	H	H	H	NS	H	H	H	H	H	NS	H	NS	H	H	H	NS	H	H	H	H	H	H	NS	NS	NS	H	NS	NS	H	H	H	H	29
23	1200	N	NS	H	H	NS	H	NS	NS	H	NS	NS	H	H	H	NS	H	H	H	H	H	NS	H	NS	H	NS	H	NS	H	H	H	H	H	H	NS	NS	NS	H	NS	NS	H	H	H	H	26
24	1200	Y	NS	H	H	NS	H	NS	NS	H	NS	NS	NS	H	NS	NS	H	H	H	H	H	NS	H	NS	H	NS	H	NS	H	H	H	H	NS	H	NS	NS	NS	H	NS	NS	H	H	H	H	23
25	1300	N	NS	H	H	NS	H	NS	NS	H	NS	NS	H	H	H	NS	H	H	H	H	H	NS	H	NS	H	NS	H	NS	H	H	H	H	H	H	NS	NS	NS	H	NS	NS	H	H	H	H	26
26	1300	Y	NS	H	H	NS	H	NS	NS	H	NS	NS	NS	H	NS	NS	H	H	H	H	H	NS	H	NS	H	NS	H	NS	H	H	H	H	H	H	NS	NS	NS	H	NS	NS	H	H	H	H	24

* Includes only concessions with children ≤ 5

C= Cold Spot (95% Confidence) NS= Not Statistically Significant H= Hot Spot (95% Confidence)

Table 9- Analysis Results with Max Clustering in Control Village D; 1,000 meters with FDR correction applied

Concession	# Participants	# Children ≤ 5	Incidence Density	Gi* Z Score	Gi* P Value
01	9	0	Not included in analysis		
02	12	3	2.48	2.12	***0.034***
03	12	3	2.82	>1.96	***<0.001***
04	9	2	2.60	2.12	***0.034***
05	13	1	1.65	2.12	***0.034***
06	14	3	2.83	>1.96	***<0.001***
07	25	5	1.85	2.12	***0.034***
08	6	2	4.13	2.12	***0.034***
09	8	2	1.24	2.12	***0.034***
10	6	2	2.48	2.12	***0.034***
11	15	3	3.60	2.12	***0.034***
12	6	3	0.56	2.12	***0.034***
13	19	6	3.19	2.12	***0.034***
14	18	6	2.88	-0.06	0.950
15	9	1	0.83	NULL	NULL
16	11	4	2.08	>1.96	***<0.001***
17	26	6	1.96	>1.96	***<0.001***
18	12	5	2.36	>1.96	***<0.001***
19	30	3	2.91	>1.96	***<0.001***
20	35	9	2.50	>1.96	***<0.001***
21	6	2	2.02	NULL	NULL
22	15	3	1.51	>1.96	***<0.001***
23	13	3	0.32	NULL	NULL
24	12	2	2.10	2.12	***0.034***
25	15	3	3.07	2.12	***0.034***
26	8	2	2.92	2.12	***0.034***
27	7	1	1.67	2.12	***0.034***
28	7	2	2.08	>1.96	***<0.001***
29	4	1	2.50	>1.96	***<0.001***
30	15	5	1.48	>1.96	***<0.001***
31	8	2	2.92	>1.96	***<0.001***
32	3	0	Not included in analysis		
33	8	2	2.14	2.12	***0.034***
34	3	1	1.67	>1.96	***<0.001***
35	7	2	2.50	NULL	NULL

36	6	1	2.50	NULL	NULL
37	4	0	Not included in analysis		
38	6	1	2.50	-0.60	0.549
39	11	2	2.92	-0.60	0.549
40	12	2	0.42	-0.41	0.685
41	12	3	3.06	-0.60	0.549
42	15	2	2.92	-0.60	0.549
43	4	1	1.67	>1.96	***<0.001***
44	5	0	Not included in analysis		
45	4	1	4.17	>1.96	***<0.001***
46	2	0	Not included in analysis		
47	7	1	4.17	>1.96	***<0.001***
48	5	0	Not included in analysis		
49	4	0	Not included in analysis		

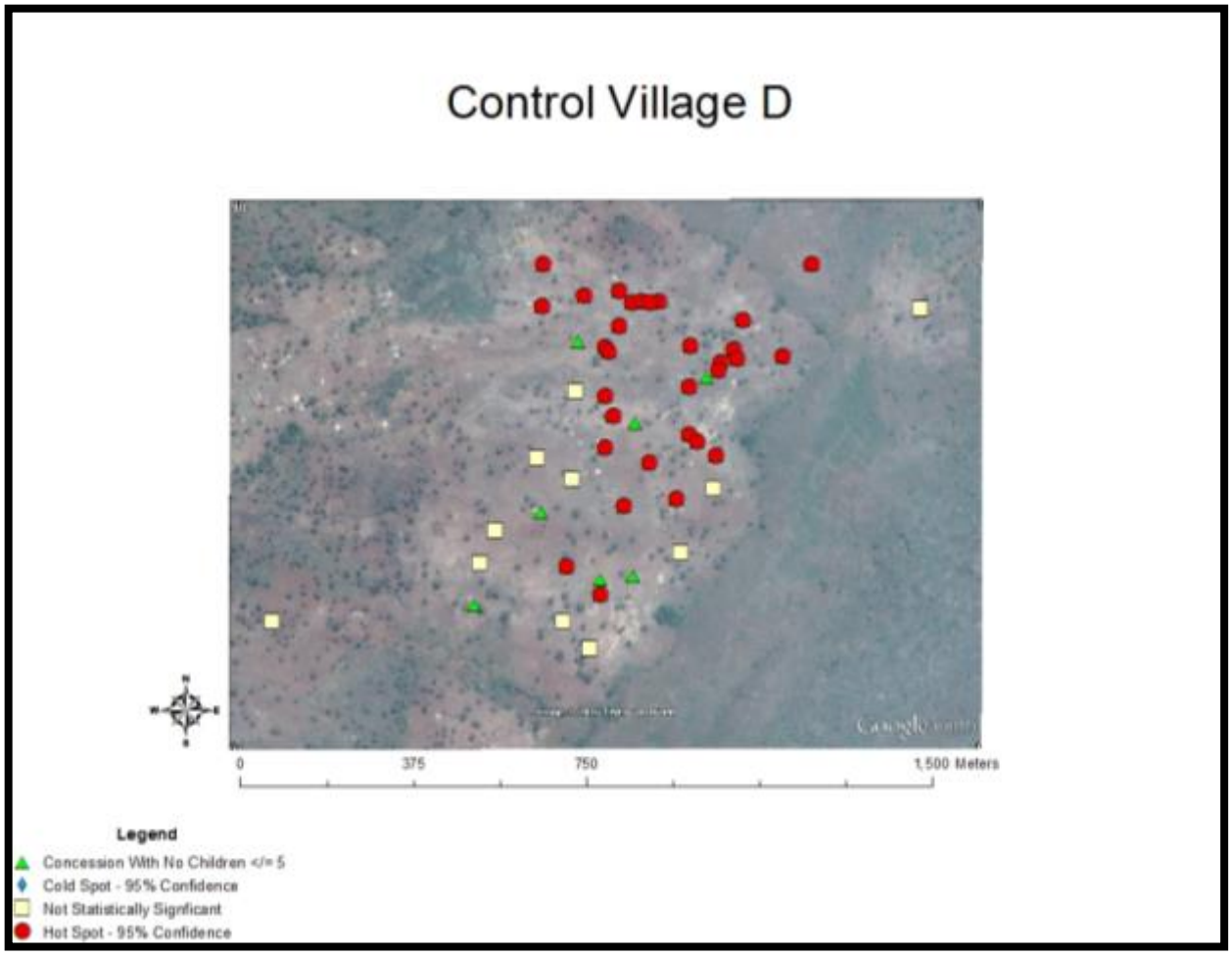

Figure 4- Map of Analysis Results with Max Clustering for Control Village D: At a distance of 1,000 m and with the FDR correction applied, 31 concessions (73.8%) were identified as significant hot spots. These hot spots appear clustered in northern and central regions of the village.

Table 10- Analysis Outcomes for Intervention Village A

Attempt	Specified Distance (meters)	FDR Correction Applied (Y/N)	Concession ID*																																														Number of significant clusters
			02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	42	43	44	45	47	48	49	50	51	
1	250	N	H	NS	H	H	H	NS	H	H	H	NS	NS	C	NS	NS	NS	C	C	NS	NS	NS	C	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	11
2	250	Y	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0
3	400	N	H	NS	H	H	NS	NS	NS	NS	H	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	C	C	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	H	NS	NS	NS	7
4	400	Y	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0
5	550	N	NS	NS	NS	H	NS	NS	NS	NS	H	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	C	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	C	NS	NS	H	NS	NS	NS	NS	5
6	550	Y	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0
7	700	N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	C	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	H	NS	NS	NS	NS	2
8	700	Y	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0
9	850	N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0
10	1000	N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	C	NS	1
11	1000	Y	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0
12	1150	N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	H	NS	NS	NS	NS	NS	NS	NS	NS	H	H	H	H	H	NS	NS	NS	H	H	H	NS	NS	NS	H	NS	10
13	1150	Y	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	H	NS	NS	NS	NS	NS	NS	NS	NS	H	H	H	H	H	NS	NS	NS	H	H	H	NS	NS	NS	H	NS	10
14	1300	N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	H	H	NS	H	H	NS	NS	H	NS	H	NS	NS	H	H	H	H	H	H	H	H	H	H	H	NS	NS	NS	NS	NS	17
15	1300	Y	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	H	H	NS	H	H	NS	NS	H	NS	H	NS	NS	H	H	H	H	H	H	H	H	H	H	H	NS	NS	NS	NS	NS	17
16	1450	N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	H	H	H	NS	H	H	H	H	H	H	H	H	H	H	NS	H	H	H	H	H	H	H	H	H	H	H	H	NS	NS	H	NS	NS	26
17	1450	Y	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	H	H	H	NS	H	H	H	H	H	H	H	H	H	H	NS	H	H	H	H	H	H	H	H	H	H	H	H	NS	NS	H	NS	NS	26
18	1600	N	NS	NS	NS	NS	NS	H	NS	NS	NS	NS	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	NS	H	H	H	H	H	H	H	H	H	H	H	H	NS	NS	NS	H	H	33
19	1600	Y	NS	NS	NS	NS	NS	H	NS	NS	NS	NS	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	NS	H	H	H	H	H	H	H	H	H	H	H	H	NS	NS	NS	H	H	33
20	1750	N	NS	NS	NS	NS	H	H	H	H	NS	H	H	H	H	H	H	H	H	NS	H	H	H	H	H	H	H	H	H	H	NS	H	H	H	H	H	H	H	H	H	H	H	H	NS	H	NS	H	H	37
21	1750	Y	NS	NS	NS	NS	H	H	H	H	NS	H	H	H	H	H	H	H	H	NS	H	H	H	H	H	H	H	H	H	H	NS	H	H	H	H	H	H	H	H	H	H	H	H	NS	H	NS	H	H	37
22	1900	N	H	NS	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	NS	H	H	H	H	H	H	H	H	H	H	NS	H	H	H	H	H	H	H	H	H	H	H	H	NS	H	H	H	H	42
23	1900	Y	H	NS	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	NS	H	H	H	H	H	H	H	H	H	H	NS	H	H	H	H	H	H	H	H	H	H	H	H	NS	H	H	H	H	42

* Includes only concessions with children ≤ 5

C= Cold Spot (95% Confidence) | NS= Not Statistically Significant | H= Hot Spot (95% Confidence)

Table 11- Analysis Results with Max Clustering in Intervention Village A; 1,900 meters with FDR correction applied

Concession	# Participants	# Children ≤ 5	Incidence Density	Gi* Z Score	Gi* P Value
01	5	0	Not included in analysis		
02	7	2	3.25	>1.96	***<0.001***
03	26	6	1.63	NULL	NULL
04	8	1	2.44	>1.96	***<0.001***
05	16	4	1.64	>1.96	***<0.001***
06	15	4	2.64	>1.96	***<0.001***
07	7	1	0	>1.96	***<0.001***
08	10	2	2.85	>1.96	***<0.001***
09	18	3	3.42	>1.96	***<0.001***
10	19	3	2.71	>1.96	***<0.001***
11	3	1	3.25	>1.96	***<0.001***
12	13	4	0.81	>1.96	***<0.001***
13	17	3	1.90	>1.96	***<0.001***
14	10	2	0.41	>1.96	***<0.001***
15	6	1	0.81	>1.96	***<0.001***
16	5	2	0	>1.96	***<0.001***
17	5	1	1.63	>1.96	***<0.001***
18	5	2	0.81	>1.96	***<0.001***
19	7	1	0	0.25	0.80
20	10	0	Not included in analysis		
21	4	0	Not included in analysis		
22	7	2	1.80	>1.96	***<0.001***
23	5	1	3.25	>1.96	***<0.001***
24	5	1	0	>1.96	***<0.001***
25	10	2	0.81	>1.96	***<0.001***
26	4	1	0	>1.96	***<0.001***
27	6	1	3.25	>1.96	***<0.001***
28	13	3	0.70	>1.96	***<0.001***
29	5	2	0.84	>1.96	***<0.001***
30	11	4	1.92	>1.96	***<0.001***
31	39	7	1.24	>1.96	***<0.001***
32	10	5	1.14	NULL	NULL
33	31	8	1.96	>1.96	***<0.001***
34	6	2	0.82	>1.96	***<0.001***
35	6	1	1.67	>1.96	***<0.001***

36	11	4	2.29	>1.96	***<0.001***
37	6	2	1.67	>1.96	***<0.001***
38	23	6	3.65	>1.96	***<0.001***
39	15	3	1.67	>1.96	***<0.001***
40	6	3	0.83	>1.96	***<0.001***
41	4	0	Not included in analysis		
42	14	4	2.21	>1.96	***<0.001***
43	8	3	1.68	>1.96	***<0.001***
44	9	2	0.84	>1.96	***<0.001***
45	5	2	1.67	>1.96	***<0.001***
46	1	0	Not included in analysis		
47	14	3	1.39	1.64	0.10
48	12	4	2.71	>1.96	***<0.001***
49	20	5	1.67	>1.96	***<0.001***
50	7	2	1.48	>1.96	***<0.001***
51	4	2	2.08	>1.96	***<0.001***

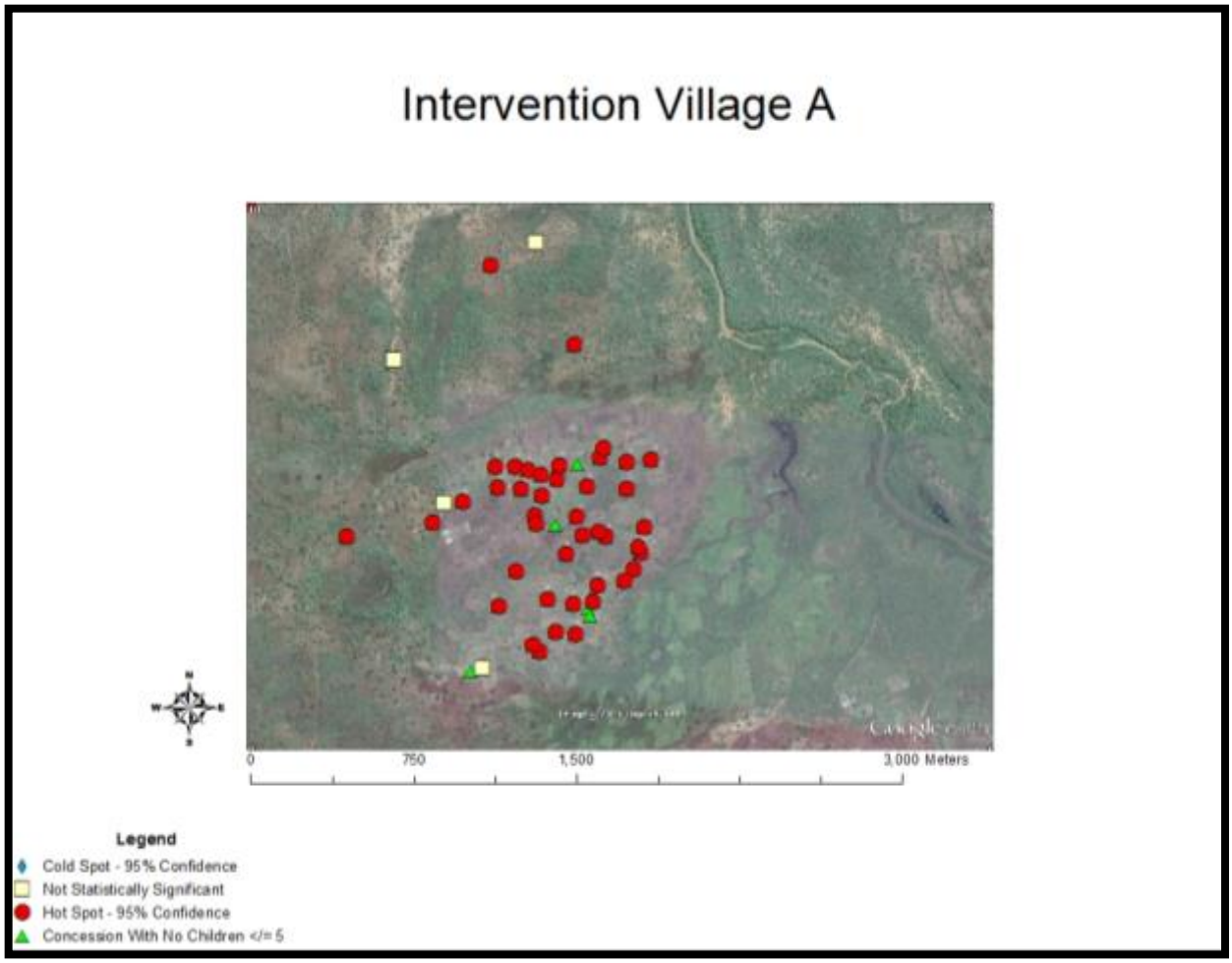

Figure 5- Map of Analysis Results with Max Clustering for Intervention Village A: The analysis at 1,900 m and with the FDR correction applied identified 42 concessions (91.3%) as significant hot spots. The four concessions identified as statistically insignificant appear on the northern and western edges of the village.

Table 12- Analysis Outcomes for Intervention Village B

			Concession ID*																																													Number of significant clusters
Attempt	Specified Distance (meters)	FDR Correction Applied (Y/N)	01	02	03	05	06	08	09	10	11	12	13	14	15	16	17	18	19	21	22	23	26	27	28	29	30	31	32	34	35	36	38	39	40	41	42	44	45	46	47	48	49	53	54	55	57	
1	100	N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	H	NS	NS	NS	NS	NS	H	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	2
2	100	Y	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0
3	150	N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	H	H	H	NS	NS	NS	NS	NS	NS	NS	3
4	150	Y	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0
5	200	N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0
6	250	N	NS	NS	NS	NS	NS	NS	NS	NS	H	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	H	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	2
7	250	Y	NS	NS	NS	NS	NS	NS	NS	NS	H	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	H	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	2
8	300	N	H	NS	NS	NS	NS	NS	NS	NS	H	H	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	H	NS	NS	NS	NS	H	NS	NS	NS	H	H	H	NS	NS	NS	H	NS	H	H	H	H	NS	NS	NS	13
9	300	Y	H	NS	NS	NS	NS	NS	NS	NS	H	H	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	H	NS	NS	NS	NS	H	NS	NS	NS	H	H	H	NS	NS	NS	H	NS	H	H	H	H	NS	NS	NS	13
10	350	N	H	NS	NS	NS	H	NS	H	H	H	H	NS	NS	NS	NS	H	NS	NS	NS	H	H	NS	H	NS	NS	NS	NS	H	NS	NS	NS	H	H	H	H	NS	H	H	H	H	H	H	H	NS	NS	NS	22
11	350	Y	H	NS	NS	NS	H	NS	H	H	H	H	NS	NS	NS	NS	H	NS	NS	NS	H	H	NS	H	NS	NS	NS	NS	H	NS	NS	NS	H	H	H	H	NS	H	H	H	H	H	H	H	NS	NS	NS	22

* Includes only concessions with children ≤ 5

C= Cold Spot (95% Confidence) | **NS= Not Statistically Significant** | **H= Hot Spot (95% Confidence)**

Table 13- Analysis Results with Max Clustering in Intervention Village B; 350 meters with FDR correction applied

Concession	# Participants	# Children ≤ 5	Incidence Density	Gi* Z Score	Gi* P Value
01	6	1	3.39	>1.96	***<0.001***
02	8	2	0.82	NULL	NULL
03	13	3	1.37	NULL	NULL
04	5	0	Not included in analysis		
05	11	3	3.55	NULL	NULL
06	6	2	1.64	>1.96	***<0.001***
07	5	0	Not included in analysis		
08	5	2	0.41	NULL	NULL
09	8	2	1.64	>1.96	***<0.001***
10	9	1	0	>1.96	***<0.001***
11	7	3	2.73	>1.96	***<0.001***
12	5	2	0.41	>1.96	***<0.001***
13	9	1	1.64	NULL	NULL
14	11	4	1.23	-0.14	0.89
15	8	3	1.10	1.54	0.12
16	9	4	1.43	1.54	0.12
17	8	1	0.82	>1.96	***<0.001***
18	11	3	1.92	1.54	0.12
19	5	2	1.23	1.54	0.12
20	7	0	Not included in analysis		
21	6	3	2.04	1.54	0.12
22	4	1	0.82	>1.96	***<0.001***
23	9	3	2.46	>1.96	***<0.001***
24	2	0	Not included in analysis		
25	5	0	Not included in analysis		
26	10	2	0.82	NULL	NULL
27	7	3	1.91	>1.96	***<0.001***
28	5	1	0	NULL	NULL
29	4	1	2.46	NULL	NULL
30	8	3	0.82	NULL	NULL
31	9	5	0.89	NULL	NULL
32	7	2	0.82	>1.96	***<0.001***
33	4	0	Not included in analysis		
34	10	2	1.64	0.28	0.78
35	6	2	0.41	NULL	NULL

36	5	2	2.49	NULL	NULL
37	2	0	Not included in analysis		
38	7	2	1.23	>1.96	***<0.001***
39	6	2	2.87	>1.96	***<0.001***
40	7	4	1.23	>1.96	***<0.001***
41	3	1	3.28	>1.96	***<0.001***
42	7	1	0	-0.89	0.38
43	11	0	Not included in analysis		
44	8	2	1.64	>1.96	***<0.001***
45	9	4	1.43	>1.96	***<0.001***
46	5	2	2.05	>1.96	***<0.001***
47	7	2	1.05	>1.96	***<0.001***
48	6	2	2.46	>1.96	***<0.001***
49	7	2	2.87	>1.96	***<0.001***
50	3	0	Not included in analysis		
51	1	0	Not included in analysis		
52	2	0	Not included in analysis		
53	6	2	1.23	>1.96	***<0.001***
54	9	1	0	NULL	NULL
55	10	1	0	NULL	NULL
56	8	0	Not included in analysis		
57	4	1	3.28	1.54	0.12
58	5	0	Not included in analysis		

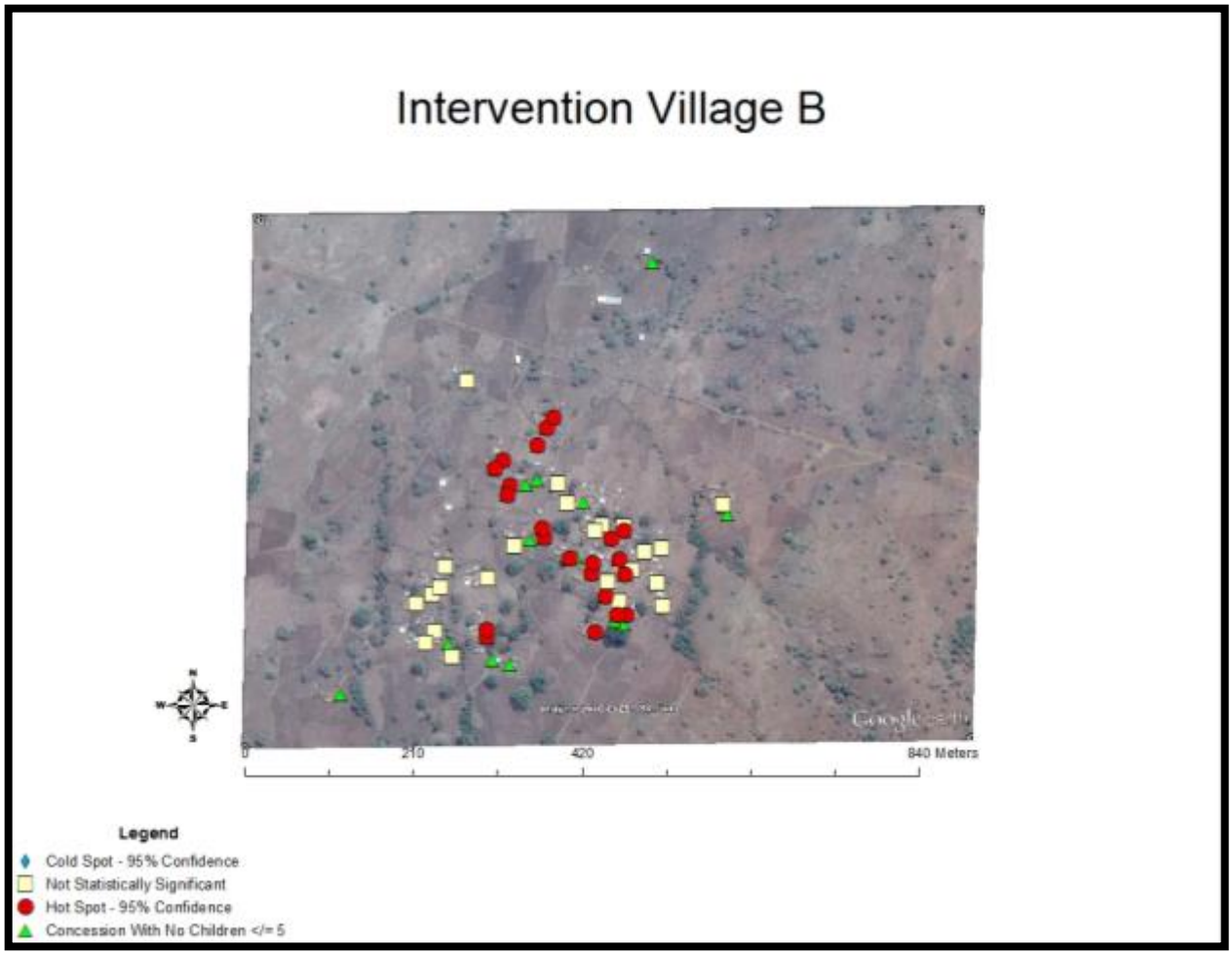

Figure 6- Map of Analysis Results with Max Clustering for Intervention Village B: This analysis, conducted at 350 m with the FDR correction applied, identified 22 concessions (48.9%) as significant hot spots. These hot spots appear grouped near the center of the village.

Table 14- Analysis Outcomes for Intervention Village C

			Concession ID*																												Number of significant clusters
Attempt	Specified Distance (meters)	FDR Correction Applied (Y/N)	01	02	06	07	09	11	12	14	15	19	20	21	22	23	25	26	27	28	29	30	31	32	33	34	36				
1	100	N	C	C	NS	C	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	H	NS	NS	NS	NS	NS	NS	NS	NS	NS				4
2	100	Y	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS				0
3	200	N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS				0
4	300	N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS				0
5	400	N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS				0
6	500	N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS				0
7	600	N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	H	NS	NS	NS	NS	NS	NS	NS	NS	NS				1
8	600	Y	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	H	NS	NS	NS	NS	NS	NS	NS	NS	NS				1
9	700	N	NS	NS	NS	NS	H	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	H	NS	NS	NS	NS	H	NS	NS	NS	NS				3
10	700	Y	NS	NS	NS	NS	H	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	H	NS	NS	NS	NS	H	NS	NS	NS	NS				3

* Includes only concessions with children ≤ 5

C= Cold Spot (95% Confidence) | NS= Not Statistically Significant | H= Hot Spot (95% Confidence)

Table 15- Analysis Results with Max Clustering for Intervention Village C; 700 meters with FDR correction applied

Concession	# Participants	# Children ≤ 5	Incidence Density	Gi* Z Score	Gi* P Value
01	10	2	1.02	0.66	0.51
02	8	1	1.61	0.66	0.51
03	3	0	Not included in analysis		
04	3	0	Not included in analysis		
05	2	0	Not included in analysis		
06	10	3	0	0.66	0.51
07	29	3	1.61	0.66	0.51
08	10	0	Not included in analysis		
09	6	1	0	>1.96	***<0.001***
10	3	0	Not included in analysis		
11	6	1	0.81	0.66	0.51
12	41	5	1.46	0.66	0.51
13	7	0	Not included in analysis		
14	22	7	1.62	0.66	0.51
15	15	1	3.23	0.66	0.51
16	7	0	Not included in analysis		
17	5	0	Not included in analysis		
18	2	0	Not included in analysis		
19	29	8	1.81	0.66	0.51
20	9	1	2.42	0.66	0.51
21	13	5	1.76	0.66	0.51
22	12	1	2.42	0.66	0.51
23	14	3	2.18	0.66	0.51
24	2	0	Not included in analysis		
25	17	4	2.02	NULL	NULL
26	4	2	2.54	>1.96	***<0.001***
27	5	1	3.23	NULL	NULL
28	15	2	1.21	NULL	NULL
29	8	1	2.42	NULL	NULL
30	6	1	1.61	NULL	NULL
31	3	1	2.42	>1.96	***<0.001***
32	8	2	1.21	NULL	NULL
33	13	3	1.61	NULL	NULL
34	33	5	0.61	NULL	NULL
35	1	0	Not included in analysis		

36	17	2	1.14	-0.06	0.95

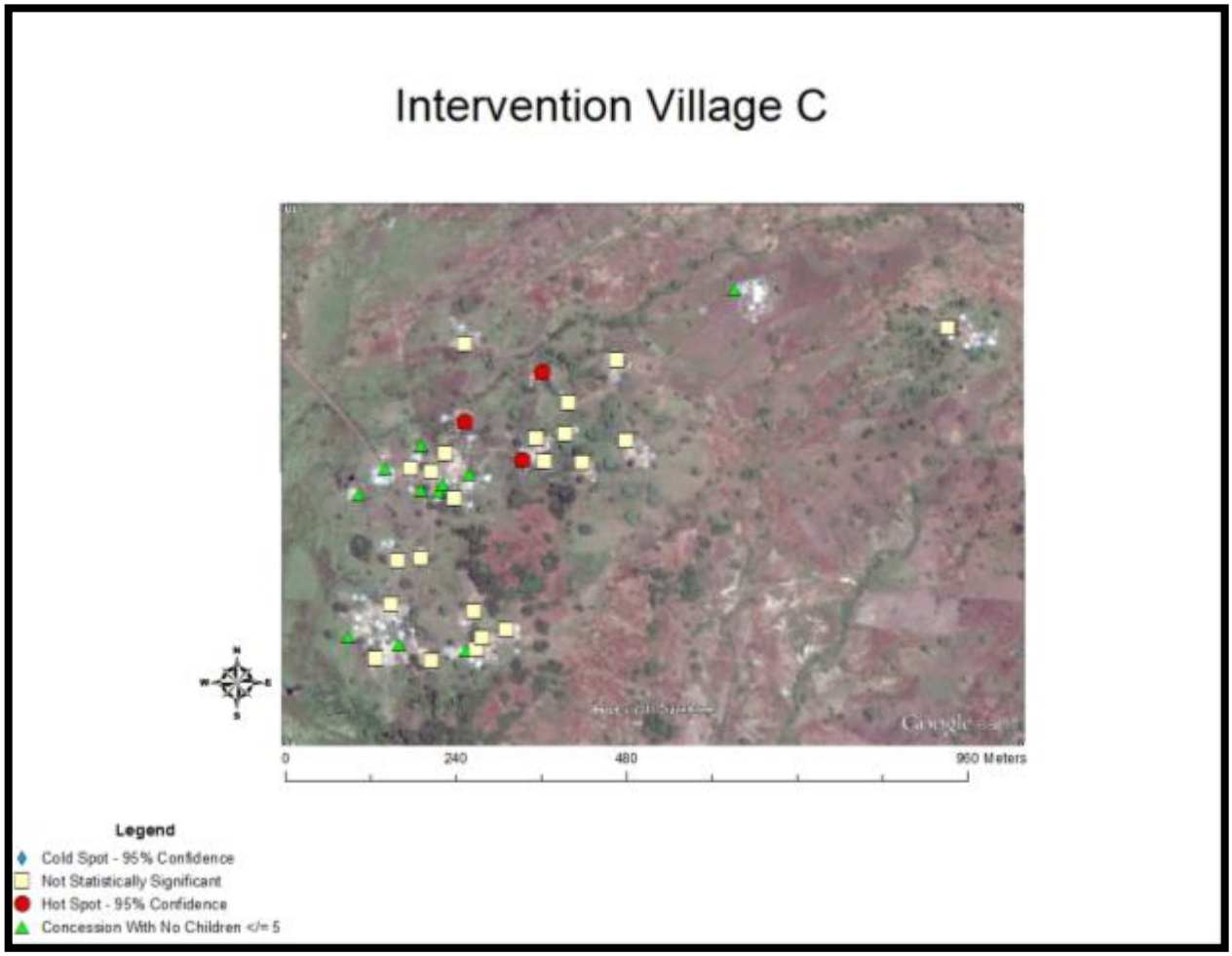

Figure 7- Map of Analysis Results with Max Clustering for Intervention Village C: This analysis was set at a distance of 700 m with the FDR correction applied, and identified 3 concessions as significant hot spots (12%). These hot spots appear grouped in the northern half of the village.

Table 16- Analysis Outcomes for Intervention Village D

			Concession ID*											Number of significant clusters
Attempt	Specified Distance (meters)	FDR Correction Applied (Y/N)	01	02	03	04	05	06	07	08	09	10	11	
1	75	N	NS	NS	NS	NS	NS	NS	NS	NS	H	H	NS	2
2	75	Y	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0
3	150	N	NS	NS	NS	NS	NS	NS	NS	NS	NS	H	H	2
4	150	Y	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0
5	225	N	NS	NS	NS	C	NS	NS	NS	NS	NS	NS	H	2
6	225	Y	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0
7	300	N	NS	NS	NS	NS	NS	H	H	NS	NS	NS	NS	2
8	300	Y	NS	NS	NS	NS	NS	H	H	NS	NS	NS	NS	2

* Includes only concessions with children ≤ 5

C= Cold Spot (95% Confidence) | NS= Not Statistically Significant | H= Hot Spot (95% Confidence)

Table 17- Analysis Results with Max Clustering for Intervention Village D; 300 meters with FDR correction applied

Concession	# Participants	# Children ≤ 5	Incidence Density	Gi* Z Score	Gi* P Value
01	17	3	1.49	-1.60	0.11
02	15	7	2.46	-1.60	0.11
03	16	5	2.60	-0.22	0.82
04	14	5	2.14	-0.22	0.82
05	10	2	1.63	-0.22	0.82
06	6	2	2.03	>1.96	***<0.001***
07	8	2	1.63	>1.96	***<0.001***
08	6	2	1.63	0.61	0.54
09	12	2	2.85	0.61	0.54
10	9	3	4.32	0.61	0.54
11	8	2	2.47	0.86	0.39

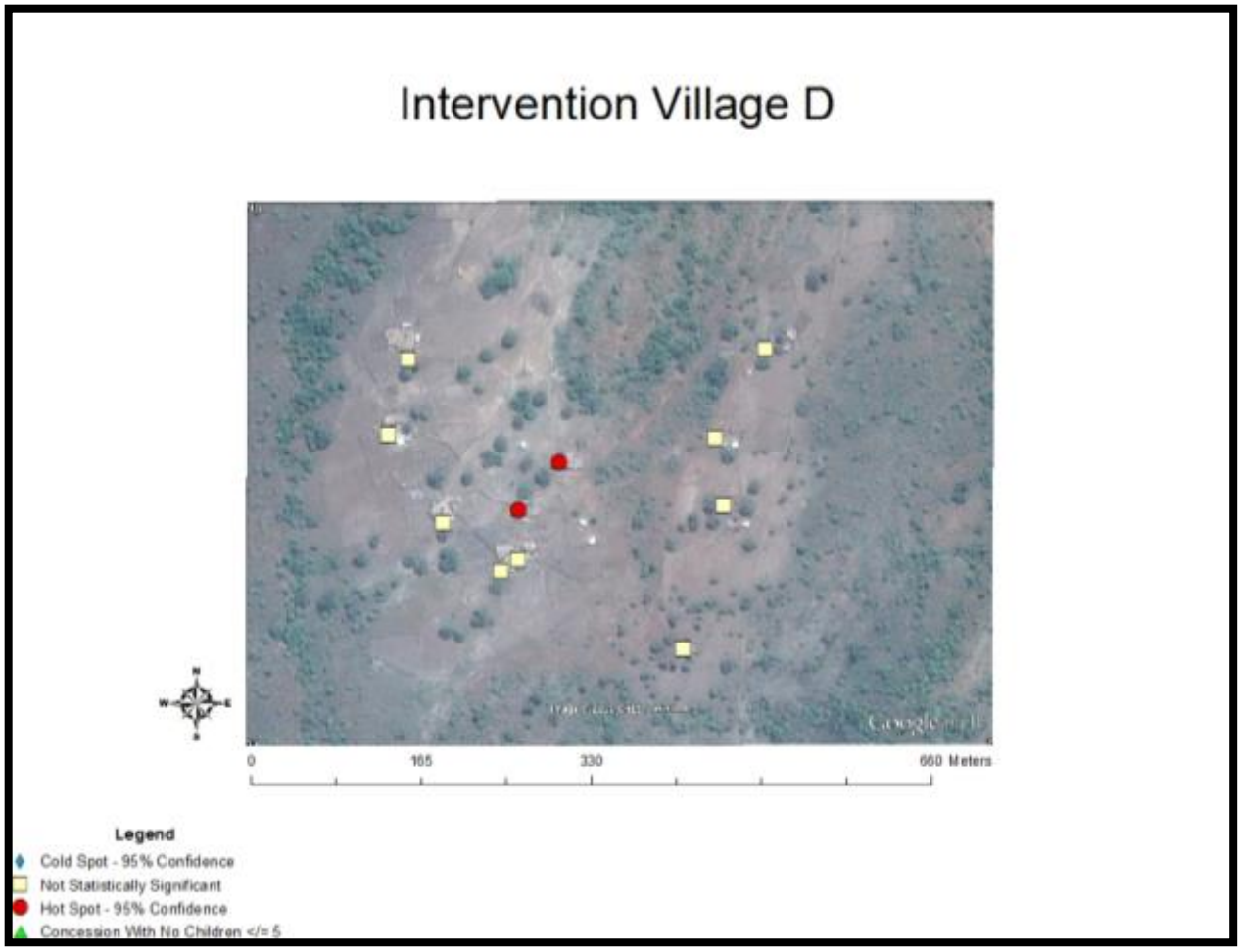

Figure 8- Map of Analysis Results with Max Clustering for Intervention Village D: This analysis was conducted with a set distance of 300 m and the FDR correction applied. A total of 2 concessions were identified as significant hot spots (18.2%). These hot spots seem centrally located within the village.

Table 18- Analysis Attempts for Whole Study Region

Attempt	Specified Distance (meters)	FDR Correction Applied (Y/N)	# Significant Cold Spots	# Significant Hot Spots	Total # Significant Clusters
1	100	N	17	6	23
2	100	Y	0	0	0
3	600	N	51	48	99
4	600	Y	50	45	95
5	1,100	N	70	42	112
6	1,100	Y	70	42	112
7	1,600	N	69	53	122
8	1,600	Y	69	53	122
9	2,100	N	69	53	122
10	2,100	Y	69	53	122
11	2,600	N	69	58	127
12	2,600	Y	51	58	109
13	3,100	N	26	44	70
14	3,100	Y	24	43	57
15	3,600	N	24	41	65
16	3,600	Y	0	41	41
17	4,100	N	0	33	33
18	4,100	Y	0	25	25
19	6,000	N	0	0	0
20	8,000	N	63	10	73
21	8,000	Y	63	9	72
22	10,000	N	63	101	164
23	10,000	Y	62	101	163
24	12,000	N	0	143	143
25	12,000	Y	0	142	142
26	13,000	N	0	145	145
27	13,000	Y	0	145	145
All analyses included 233 concessions with children ≤ 5 years old					

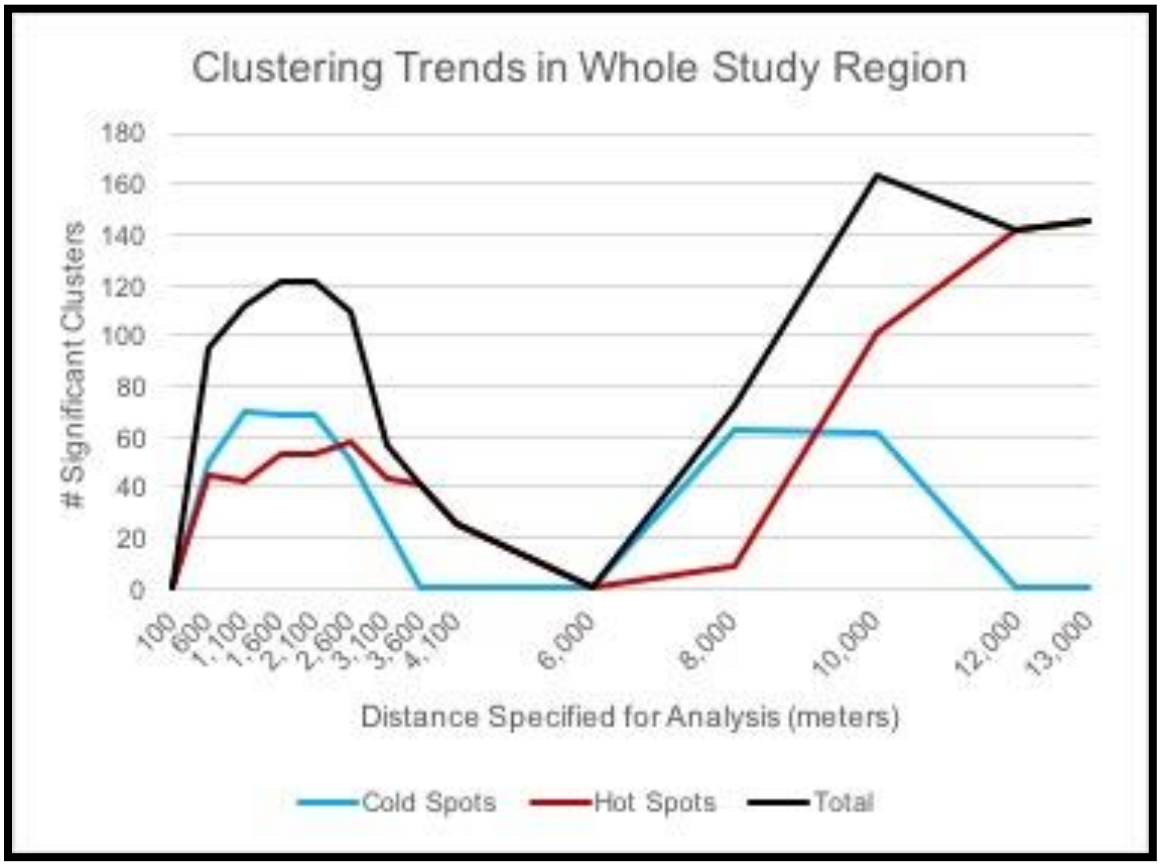

Figure 9- The graph displays the number of clusters that appeared in each analysis conducted for the whole study region. There appears to be two primary peaks, or a bimodal distribution of clustering, which occurred during analyses at 2,100 meters and 10,000 meters.

Table 19- Side-by-side Analyses Results for Max Clustering Peaks in Whole Study Region; 2,100 meters and 10,000 meters, both with FDR correction applied

Concession	Incidence Density (cases/ 100 person-days)	Analysis at 2,100 meters		Analysis at 10,000 meters	
		Gi* Z Score	Gi* P Value	Gi* Z Score	Gi* P Value
1A 01	1.80	-0.35	0.72	-2.32	***0.02***
1A 02	2.64	-0.70	0.49	-2.32	***0.02***
1A 03	3.14	-0.84	0.40	-2.32	***0.02***
1A 04	2.89	-0.54	0.59	-2.32	***0.02***
1A 05	2.89	0.35	0.72	-2.32	***0.02***
1A 06	3.31	0.53	0.60	-2.32	***0.02***
1A 07	2.89	0.11	0.91	-2.32	***0.02***
1A 08	1.65	0.35	0.72	-2.32	***0.02***
1A 09	2.48	-0.40	0.69	-2.32	***0.02***
1A 10	2.89	0.34	0.73	-2.32	***0.02***
1A 12	1.51	0.53	0.60	-2.44	***0.01***
1A 13	1.47	-0.18	0.86	-2.36	***0.02***
1A 14	0	-0.17	0.86	-2.36	***0.02***
1A 15	1.65	-1.34	0.18	-2.49	***0.01***
1A 16	2.89	-0.73	0.46	-2.32	***0.02***
1A 18	3.31	-0.36	0.72	-2.32	***0.02***
1A 20	0.41	-1.36	0.17	-2.36	***0.02***
1B 01	0.83	-2.75	***0.01***	>1.96	***<0.001***
1B 02	0.83	-2.75	***0.01***	>1.96	***<0.001***
1B 03	2.48	-2.75	***0.01***	>1.96	***<0.001***
1B 04	3.72	-2.75	***0.01***	>1.96	***<0.001***
1B 06	0.83	-2.75	***0.01***	>1.96	***<0.001***
1B 08	2.08	-2.75	***0.01***	>1.96	***<0.001***
1B 10	2.48	-2.75	***0.01***	>1.96	***<0.001***
1B 11	3.31	-2.75	***0.01***	>1.96	***<0.001***
1B 15	0.83	-2.75	***0.01***	>1.96	***<0.001***
1B 16	0.41	-2.75	***0.01***	>1.96	***<0.001***
1B 22	2.07	-2.75	***0.01***	>1.96	***<0.001***
1B 23	0.98	-2.75	***0.01***	>1.96	***<0.001***
1B 24	3.31	-2.75	***0.01***	>1.96	***<0.001***
1B 26	1.03	-2.75	***0.01***	>1.96	***<0.001***
1B 27	3.31	-2.75	***0.01***	>1.96	***<0.001***
1B 30	1.09	-2.75	***0.01***	>1.96	***<0.001***

1B 32	1.24	-2.75	***0.01***	>1.96	***<0.001***
1B 34	2.20	-2.75	***0.01***	>1.96	***<0.001***
1B 38	0	-2.75	***0.01***	>1.96	***<0.001***
1B 39	1.24	-2.75	***0.01***	>1.96	***<0.001***
1B 41	3.33	-2.75	***0.01***	>1.96	***<0.001***
1B 42	0.52	-2.75	***0.01***	>1.96	***<0.001***
1B 47	2.48	-2.75	***0.01***	>1.96	***<0.001***
1B 49	0.83	-2.75	***0.01***	>1.96	***<0.001***
1C 01	1.63	1.25	0.21	>1.96	***<0.001***
1C 03	2.03	1.25	0.21	>1.96	***<0.001***
1C 05	1.63	1.25	0.21	>1.96	***<0.001***
1C 06	2.03	1.25	0.21	>1.96	***<0.001***
1C 07	3.33	1.25	0.21	>1.96	***<0.001***
1C 08	1.63	1.25	0.21	>1.96	***<0.001***
1C 09	1.63	0.40	0.69	>1.96	***<0.001***
1C 10	0.81	1.25	0.21	>1.96	***<0.001***
1C 12	1.67	1.25	0.21	>1.96	***<0.001***
1C 13	0.81	1.06	0.29	>1.96	***<0.001***
1C 14	1.42	0.44	0.66	>1.96	***<0.001***
1C 16	1.22	0.40	0.69	>1.96	***<0.001***
1C 17	2.06	0.40	0.69	-0.85	0.40
1C 19	1.63	0.31	0.75	>1.96	***<0.001***
1C 21	1.65	1.25	0.21	>1.96	***<0.001***
1C 23	2.03	1.31	0.19	>1.96	***<0.001***
1C 24	2.30	1.25	0.21	>1.96	***<0.001***
1C 26	2.71	1.25	0.21	>1.96	***<0.001***
1C 28	4.07	1.33	0.18	>1.96	***<0.001***
1C 29	3.25	0.40	0.69	>1.96	***<0.001***
1C 30	1.93	0.40	0.69	>1.96	***<0.001***
1C 31	1.63	0.40	0.69	>1.96	***<0.001***
1C 32	1.37	0.40	0.69	>1.96	***<0.001***
1D 02	2.48	3.97	***<0.001***	0.53	0.60
1D 03	2.82	3.97	***<0.001***	0.53	0.60
1D 04	2.60	3.97	***<0.001***	0.53	0.60
1D 05	1.65	3.97	***<0.001***	0.53	0.60
1D 06	2.83	3.97	***<0.001***	0.53	0.60
1D 07	1.85	3.97	***<0.001***	0.56	0.57
1D 08	4.13	3.97	***<0.001***	0.53	0.60
1D 09	1.24	3.97	***<0.001***	0.56	0.57

1D 10	2.48	3.97	***<0.001***	0.56	0.57
1D 11	3.60	3.97	***<0.001***	0.53	0.60
1D 12	0.56	3.97	***<0.001***	0.50	0.62
1D 13	3.19	3.97	***<0.001***	0.56	0.57
1D 14	2.88	3.97	***<0.001***	0.52	0.61
1D 15	0.83	3.97	***<0.001***	0.53	0.60
1D 16	2.08	3.97	***<0.001***	0.53	0.60
1D 17	1.96	3.97	***<0.001***	0.53	0.60
1D 18	2.36	3.97	***<0.001***	0.53	0.60
1D 19	2.91	3.97	***<0.001***	0.53	0.60
1D 20	2.50	3.97	***<0.001***	0.53	0.60
1D 21	2.02	3.97	***<0.001***	0.53	0.60
1D 22	1.51	3.97	***<0.001***	0.53	0.60
1D 23	0.32	3.97	***<0.001***	0.53	0.60
1D 24	2.10	3.97	***<0.001***	0.53	0.60
1D 25	3.07	3.97	***<0.001***	0.56	0.57
1D 26	2.92	3.97	***<0.001***	0.53	0.60
1D 27	1.67	3.97	***<0.001***	0.53	0.60
1D 28	2.08	3.97	***<0.001***	0.53	0.60
1D 29	2.50	3.97	***<0.001***	0.53	0.60
1D 30	1.48	3.97	***<0.001***	0.53	0.60
1D 31	2.92	3.97	***<0.001***	0.53	0.60
1D 33	2.14	3.97	***<0.001***	0.56	0.57
1D 34	1.67	3.97	***<0.001***	0.53	0.60
1D 35	2.50	3.97	***<0.001***	0.53	0.60
1D 36	2.50	3.97	***<0.001***	0.53	0.60
1D 38	2.50	3.97	***<0.001***	0.53	0.60
1D 39	2.92	3.90	***<0.001***	0.53	0.60
1D 40	0.42	3.49	***<0.001***	0.53	0.60
1D 41	3.06	3.90	***<0.001***	0.53	0.60
1D 42	2.92	3.90	***<0.001***	0.53	0.60
1D 43	1.67	3.97	***<0.001***	0.53	0.60
1D 45	4.17	3.97	***<0.001***	0.53	0.60
1D 47	4.17	3.97	***<0.001***	0.53	0.60
2A 02	3.25	-1.64	0.10	-2.32	***0.02***
2A 03	1.63	-0.53	0.60	-2.32	***0.02***
2A 04	2.44	-1.64	0.10	-2.32	***0.02***
2A 05	1.64	-1.64	0.10	-2.32	***0.02***
2A 06	2.64	-1.64	0.10	-2.32	***0.02***

2A 07	0	-1.64	0.10	-2.32	***0.02***
2A 08	2.85	-1.64	0.10	-2.32	***0.02***
2A 09	3.42	-1.45	0.15	-2.32	***0.02***
2A 10	2.71	-1.64	0.10	-2.32	***0.02***
2A 11	3.25	-1.64	0.10	-2.32	***0.02***
2A 12	0.81	-1.64	0.10	-2.32	***0.02***
2A 13	1.90	-1.66	0.10	-2.47	***0.01***
2A 14	0.41	-1.88	0.06	-2.47	***0.01***
2A 15	0.81	-0.83	0.41	-2.32	***0.02***
2A 16	0	-1.34	0.18	-2.32	***0.02***
2A 17	1.63	-1.06	0.29	-2.32	***0.02***
2A 18	0.81	-0.83	0.41	-2.32	***0.02***
2A 19	0	-0.53	0.60	-2.83	***0.00***
2A 22	1.80	-0.72	0.47	-2.32	***0.02***
2A 23	3.25	-0.53	0.60	-2.43	***0.02***
2A 24	0	-1.70	0.09	-2.44	***0.01***
2A 25	0.81	-0.70	0.48	-2.32	***0.02***
2A 26	0	-1.16	0.24	-2.44	***0.01***
2A 27	3.25	-0.86	0.39	-2.36	***0.02***
2A 28	0.70	-1.16	0.24	-2.49	***0.01***
2A 29	0.84	-0.53	0.60	-2.44	***0.01***
2A 30	1.92	-0.83	0.41	-2.32	***0.02***
2A 31	1.24	-0.53	0.60	-2.32	***0.02***
2A 32	1.14	-0.53	0.60	-2.32	***0.02***
2A 33	1.96	-0.53	0.60	-2.18	***0.03***
2A 34	0.82	-0.53	0.60	-2.32	***0.02***
2A 35	1.67	-0.53	0.60	-2.32	***0.02***
2A 36	2.29	-0.53	0.60	-2.32	***0.02***
2A 37	1.67	-0.53	0.60	-2.32	***0.02***
2A 38	3.65	-0.53	0.60	-2.32	***0.02***
2A 39	1.67	-0.70	0.48	-2.32	***0.02***
2A 40	0.83	-0.70	0.48	-2.32	***0.02***
2A 42	2.21	-0.53	0.60	-2.32	***0.02***
2A 43	1.68	-0.53	0.60	-2.32	***0.02***
2A 44	0.84	-0.53	0.60	-2.32	***0.02***
2A 45	1.67	-0.53	0.60	-2.32	***0.02***
2A 47	1.39	-1.64	0.10	-2.39	***0.02***
2A 48	2.71	-0.96	0.34	-2.32	***0.02***
2A 49	1.67	-0.53	0.60	-2.43	***0.02***

2A 50	1.48	-0.53	0.60	-2.36	***0.02***
2A 51	2.08	-0.53	0.60	-2.03	0.04
2B 01	3.39	-2.75	***0.01***	>1.96	***<0.001***
2B 02	0.82	-2.75	***0.01***	>1.96	***<0.001***
2B 03	1.37	-2.75	***0.01***	>1.96	***<0.001***
2B 05	3.55	-2.75	***0.01***	>1.96	***<0.001***
2B 06	1.64	-2.75	***0.01***	>1.96	***<0.001***
2B 08	0.41	-2.75	***0.01***	>1.96	***<0.001***
2B 09	1.64	-2.75	***0.01***	>1.96	***<0.001***
2B 10	0	-2.75	***0.01***	>1.96	***<0.001***
2B 11	2.73	-2.75	***0.01***	>1.96	***<0.001***
2B 12	0.41	-2.75	***0.01***	>1.96	***<0.001***
2B 13	1.64	-2.75	***0.01***	>1.96	***<0.001***
2B 14	1.23	-2.75	***0.01***	>1.96	***<0.001***
2B 15	1.10	-2.75	***0.01***	>1.96	***<0.001***
2B 16	1.43	-2.75	***0.01***	>1.96	***<0.001***
2B 17	0.82	-2.75	***0.01***	>1.96	***<0.001***
2B 18	1.92	-2.75	***0.01***	>1.96	***<0.001***
2B 19	1.23	-2.75	***0.01***	>1.96	***<0.001***
2B 21	2.04	-2.75	***0.01***	>1.96	***<0.001***
2B 22	0.82	-2.75	***0.01***	>1.96	***<0.001***
2B 23	2.46	-2.75	***0.01***	>1.96	***<0.001***
2B 26	0.82	-2.75	***0.01***	>1.96	***<0.001***
2B 27	1.91	-2.75	***0.01***	>1.96	***<0.001***
2B 28	0	-2.75	***0.01***	>1.96	***<0.001***
2B 29	2.46	-2.75	***0.01***	>1.96	***<0.001***
2B 30	0.82	-2.75	***0.01***	>1.96	***<0.001***
2B 31	0.89	-2.75	***0.01***	>1.96	***<0.001***
2B 32	0.82	-2.75	***0.01***	>1.96	***<0.001***
2B 34	1.64	-2.75	***0.01***	>1.96	***<0.001***
2B 35	0.41	-2.75	***0.01***	>1.96	***<0.001***
2B 36	2.49	-2.75	***0.01***	>1.96	***<0.001***
2B 38	1.23	-2.75	***0.01***	>1.96	***<0.001***
2B 39	2.87	-2.75	***0.01***	>1.96	***<0.001***
2B 40	1.23	-2.75	***0.01***	>1.96	***<0.001***
2B 41	3.28	-2.75	***0.01***	>1.96	***<0.001***
2B 42	0	-2.75	***0.01***	>1.96	***<0.001***
2B 44	1.64	-2.75	***0.01***	>1.96	***<0.001***
2B 45	1.43	-2.75	***0.01***	>1.96	***<0.001***

2B 46	2.05	-2.75	***0.01***	>1.96	***<0.001***
2B 47	1.05	-2.75	***0.01***	>1.96	***<0.001***
2B 48	2.46	-2.75	***0.01***	>1.96	***<0.001***
2B 49	2.87	-2.75	***0.01***	>1.96	***<0.001***
2B 53	1.23	-2.75	***0.01***	>1.96	***<0.001***
2B 54	0	-2.75	***0.01***	>1.96	***<0.001***
2B 55	0	-2.75	***0.01***	>1.96	***<0.001***
2B 57	3.28	-2.75	***0.01***	>1.96	***<0.001***
2C 01	1.02	-0.94	0.35	0.53	0.60
2C 02	1.61	-0.94	0.35	0.53	0.60
2C 06	0	-0.94	0.35	0.53	0.60
2C 07	1.61	-0.94	0.35	0.53	0.60
2C 09	0	-0.94	0.35	0.53	0.60
2C 11	0.81	-0.94	0.35	0.53	0.60
2C 12	1.46	-0.94	0.35	0.53	0.60
2C 14	1.62	-0.94	0.35	0.49	0.62
2C 15	3.23	-0.94	0.35	0.49	0.62
2C 19	1.81	-0.94	0.35	0.49	0.62
2C 20	2.42	-0.94	0.35	0.49	0.62
2C 21	1.76	-0.94	0.35	0.53	0.60
2C 22	2.42	-0.94	0.35	0.53	0.60
2C 23	2.18	-0.94	0.35	0.53	0.60
2C 25	2.02	-0.94	0.35	0.53	0.60
2C 26	2.54	-0.94	0.35	0.53	0.60
2C 27	3.23	-0.94	0.35	0.53	0.60
2C 28	1.21	-0.94	0.35	0.53	0.60
2C 29	2.42	-0.94	0.35	0.53	0.60
2C 30	1.61	-0.94	0.35	0.53	0.60
2C 31	2.42	-0.94	0.35	0.53	0.60
2C 32	1.21	-0.94	0.35	0.53	0.60
2C 33	1.61	-0.94	0.35	0.53	0.60
2C 34	0.61	-0.94	0.35	0.53	0.60
2C 36	1.14	-0.94	0.35	0.53	0.60
2D 01	1.49	4.12	***<0.001***	2.94	***<0.001***
2D 02	2.46	4.25	***<0.001***	2.91	***<0.001***
2D 03	2.60	4.42	***<0.001***	2.91	***<0.001***
2D 04	2.14	4.12	***<0.001***	2.91	***<0.001***
2D 05	1.63	4.12	***<0.001***	2.70	***0.01***
2D 06	2.03	4.42	***<0.001***	3.35	***<0.001***

2D 07	1.63	4.42	***<0.001***	2.19	***0.03***
2D 08	1.63	4.11	***<0.001***	2.19	***0.03***
2D 09	2.85	4.42	***<0.001***	2.16	***0.03***
2D 10	4.32	4.25	***<0.001***	1.88	0.06
2D 11	2.47	3.73	***<0.001***	>1.96	***<0.001***

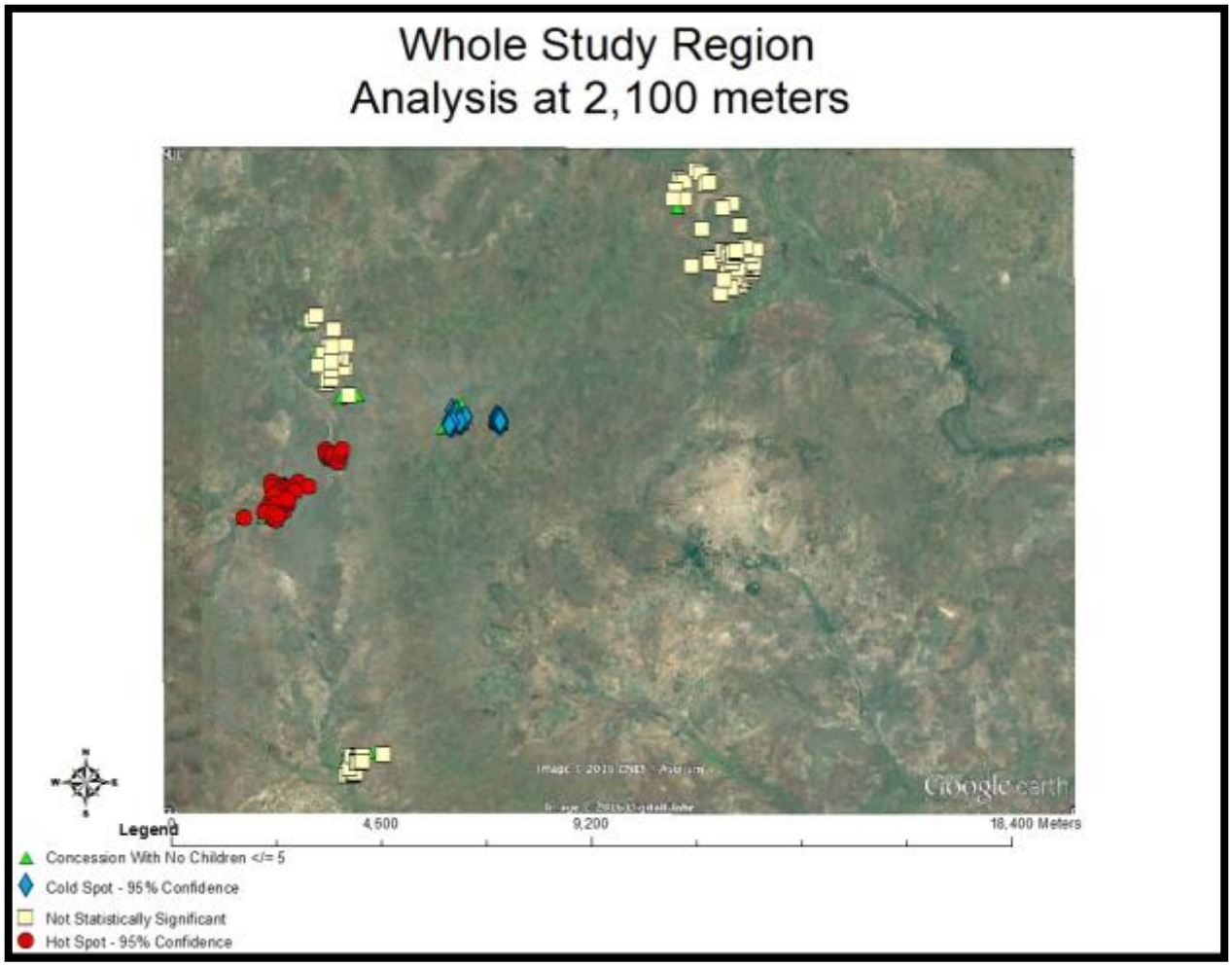

Figure 10- Map of Analysis Results at 2,100 meters for Whole Study Region: Analysis conducted with the FDR correction applied. All concessions in Control Village B and Intervention Village B were identified as significant cold spots, while all concessions in Control Village D and Intervention Village D were identified as significant hot spots.

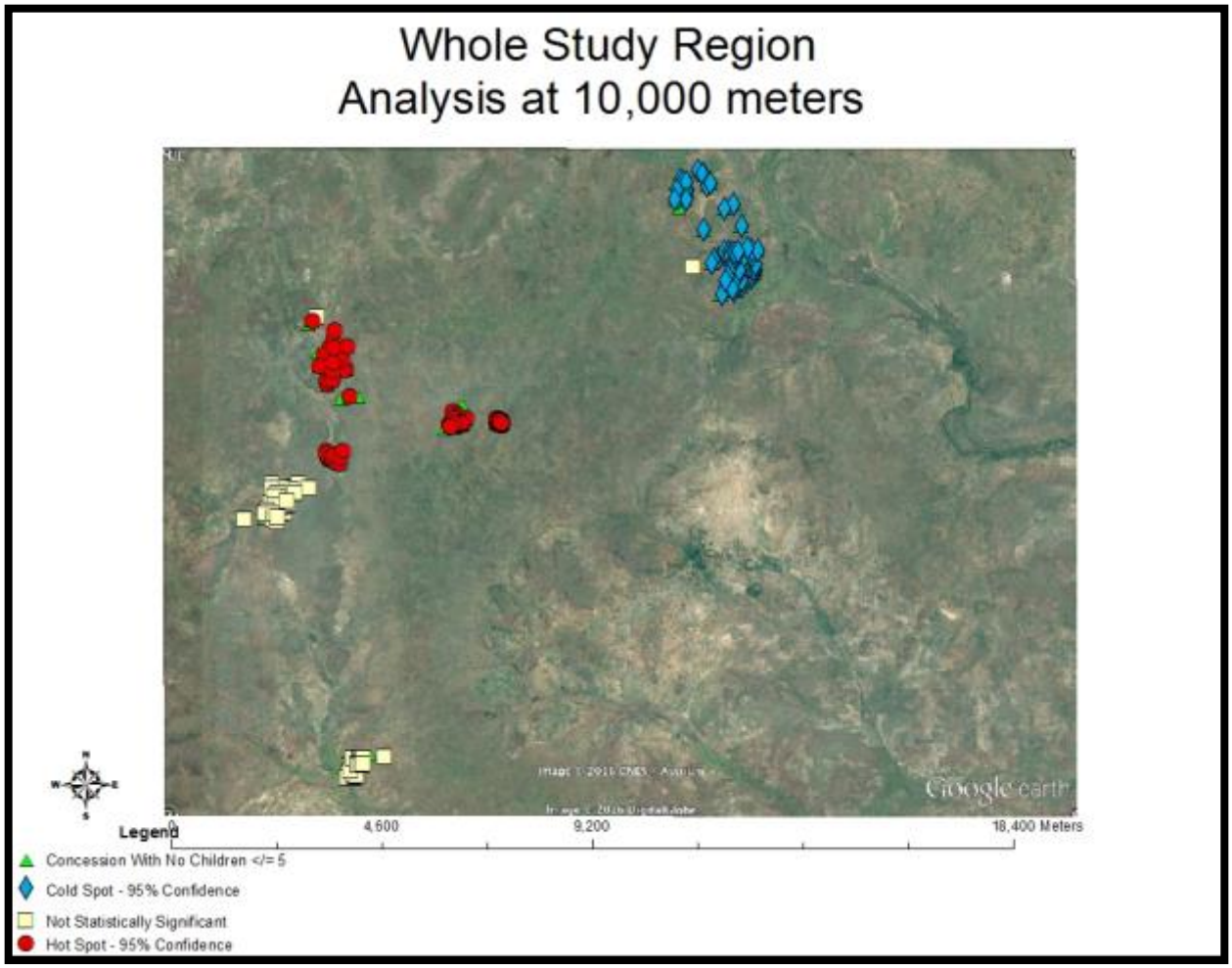

Figure 11- Map of Analysis Results at 10,000 meters for Whole Study Region: Analysis conducted with the FDR correction applied. All concession in Control Village A and all concessions except one in Intervention Village A were identified as significant cold spots. All concessions in Control Villages B and C (except one in C) and all concessions in Intervention Villages B and D (except one in D) were identified as significant hot spots.

Table 20- T-test Results for Individual Study Villages

Village	Concessions included in analyses	# Concessions Identified as Significant Clusters (%)		# Children ≤ 5 living in Significant Concessions (%)		Mean ID: Significant Concessions (cases/ 100 person-days)		Mean ID: Statistically Insignificant Concessions (cases/ 100 person-days)	T-test P Value***	
Control 1A*	17	16 (94.1)		57 (98.3)		2.23				
		COLD	HOT	COLD	HOT	COLD	HOT	1.65**	COLD	HOT
		5 (29.4)	11 (64.7)	15 (25.9)	42 (72.4)	1.01	2.83		0.13	***<0.001***
Control 1B	24	9 (37.5)		17 (35.2)		1.92		1.61	0.54	
Control 1C	23	13 (56.5)		24 (55.8)		1.86		2.02	0.61	
Control 1D	42	31 (73.8)		89 (78.1)		2.43		2.08	0.33	
Intervention 2A	46	42 (91.3)		113 (88.3)		1.7		1.04	0.16	
Intervention 2B	45	22 (48.9)		46 (46.9)		1.73		1.28	0.13	
Intervention 2C	25	3 (12)		4 (6.1)		1.65		1.68	0.98	
Intervention 2D	11	2 (18.2)		4 (11.4)		1.83		2.4	0.16	

Note- *Control Village 1A max clustering included identification of both hot and cold spots, unlike all other villages where only hot spots were identified. Results for village 1A are displayed in a manner to differentiate between hot and cold spots. **This ID value is not a mean as there was only one concession not identified as significant in village 1A. This value is the ID calculated for that one concession. ***The t tests performed for village 1A evaluated whether mean ID of cold spots and mean ID of hot spots were statistically different than the ID value of the one statistically insignificant concession (1.65); whereas, t tests for other villages compared significant hot spots ID means to means of insignificant concessions.

Table 21- T-test Results for Whole Study Region

Specified Distance of Analysis	Concessions included in analyses	# Concessions Identified as Significant Clusters (%)		# Children ≤ 5 living in Significant Concessions (%)		Mean ID: Significant Concessions (cases/ 100 person-days)		Mean ID: Statistically Insignificant Concessions (cases/ 100 person-days)	T-test P Value	
		COLD	HOT	COLD	HOT	COLD	HOT		COLD	HOT
2,100 meters	233	69 (29.6)	53 (22.7)	146 (24.8)	149 (25.3)	1.58	2.33	1.80	0.15	***<0.001***
10,000 meters	233	62 (26.6)	101 (43.4)	184 (31.2)	219 (37.1)	1.79	1.71	2.12	0.06	***0.01***

V. DISCUSSION

Overview

This chapter highlights and describes the significant results presented in the previous chapter. Overall conclusions, limitations and strengths of this study, and implications for future research are provided here.

Conclusions

Study Aim 1- All study villages exhibited significant clustering of childhood malaria incidence density. In all villages except 1A and 1D, max clustering occurred during the analysis at the greatest distance tested. Village 1A was the only village in which statistically significant cold spots were identified at maximum clustering. The clustering patterns seen in this village could have been due to the spatial distribution of village concessions, as there seemed to be two separate groups of households. Most of the concessions were in a larger, centralized grouping of concessions, while a few were grouped to the northeast of the main cluster. All the concessions identified as significant hot spots were in the central group, and all the concessions in the small group were identified as significant cold spots. Max clustering occurred at 400 meters, but the two concessions that were farthest apart were approximately 1,000 meters apart. This means when Gi* statistics were calculated, only some neighboring concessions were included in the numerator. Village 1D also exhibited max clustering at a distance shorter than the max distance. It is difficult to interpret why these distances varied and why some villages experienced maximum clustering at shorter distances, and others at the greatest distance. The distances at which max clustering occurs is likely based on various village factors, such as geographic extents, population density, and other geographic characteristics.

There seems to be no overall trend of clustering patterns in the control or intervention villages. In most of the villages (1A, 1B, 1C, 2B and 2D) the spatial distributions of hot spots appear clustered near the center, while in village 2C, the hot spots appear clustered in the northern half, and the hot spots in 1D appear randomly distributed. Nearly all the concessions in 2A were identified as statistically significant hot spots. Although this seems unexpected, the results of this analysis were based on the incidence density values of only concessions within this village. The high number of hot spots in village 2A is likely due to some village characteristics that require further investigation. There were significantly more children in intervention villages who had zero reported malarial episodes, than in control villages. Figure 26 in Appendix D shows a map of the locations where children with zero cases resided. It appears village 2A had many children with zero cases distributed throughout the region. This distribution could have driven the high number of hot spots identified, or it could have been due to the population density, or geographic characteristics not evaluated in this study.

Results of geospatial analyses performed within one village cannot be compared to spatial patterns in other villages because analyses performed within each village were based solely on malaria incidence density values within the village being analyzed. Since the Gi* statistic utilizes attribute values at neighboring features to calculate the expected Gi* value, results from a study village can only be used to make statistical inferences about that same village. Despite the lack of comparability between study villages, within village analyses demonstrate significant spatial clustering of childhood malaria incidence. Researching and evaluating the factors that may be influencing formation of these clusters is an important next step in understanding how to most efficiently implement malaria control strategies.

Study Aim 2- The results of the between village analyses are more applicable to how the RIMDAMAL protocol may be utilized in the future. The purpose of this study aim was to evaluate whether this intervention appeared to have an impact on the spatial distribution of childhood malaria in the whole study region. Like the methods utilized for the within village analyses, these

analyses were conducted at varying distances. Figure 9 displays a graphical representation of the number of clusters that appeared at each analysis distance. The clustering trends appeared to form a bimodal distribution; the first peak occurred at 1,600/ 2,100 meters (results for these analyses were identical), and the second peak occurred at 10,000 meters. Much like a microbiologist adjusts the focus of a microscope to better visualize microorganisms, performing these spatial analyses at varying distances is like adjusting the focus to better uncover the clustering trends. The bimodal trend of this dataset is intriguing, and could be related to the intervention.

The analysis at 2,100 meters identified a total of 122 concessions (52.4%) as statistically significant clusters; 69 (29.6%) significant cold spots and 53 (22.8%) significant hot spots. All concessions in villages 1B and 2B were identified as significant cold spots, and all concessions in villages 1D and 2D were identified as significant hot spots. Villages 1B and 2B are close neighbors within 2,100 meters of each other, as are villages 1D and 2D. These results could imply characteristics of villages may influence results of their neighbors.

The analysis at 10,000 meters resulted in maximum clustering, with 163 (70%) total concessions identified as significant clusters, 62 (26.7%) significant cold spots, and 101 (43.4%) significant hot spots. Apart from one concession in 2A, all concessions in villages 1A and 2A were identified as significant cold spots. These villages are close neighbors and were geographically isolated from the primary cluster of villages. Apart from one concession in both 1C and 2D, all concessions in villages 1B, 1C, 2B, and 2D were identified as significant hot spots. These four villages were within 10,000 meters of each other. Although the results from this analysis identified more significant clusters than the analysis at 2,100 meters, the similar results among neighboring villages imply malaria incidence is similar in neighboring villages.

Evaluating the geospatial clustering patterns of the study region provides some insight as to how the RIMDAMAL protocol may regionally impact malaria in children $\leq$ 5 years old. Statistical analyses originally conducted for the RIMDAMAL study revealed significantly lower

incidences of childhood malaria in villages that received the intervention compared to control villages; however, spatial analyses did not entirely reflect these results. Two of the intervention villages were identified as statistically significant hot spots, indicating incidence density values within these villages were higher than expected, and the villages are surrounded by other villages with similarly high ID values. There was also one control village in which all concessions were identified as cold spots, indicating incidence density values that were significantly lower than expected. It is possible the RIMDAMAL intervention played a part in these spatial results; however, other factors may have also played a significant role. It is important to identify factors that may be driving the clustering pattern identified, and how those factors influence risk of malaria for children $\leq$ 5 years old.

T-test Results- In this study, t-tests were used to evaluate whether a statistically significant difference existed between mean incidence densities of significant clusters and concessions identified as statistically insignificant through spatial analyses. In other words, the t-tests were utilized to evaluate whether the statistically significant findings from spatial analyses were still significant when the spatial component was removed. In villages 1B, 1C, 1D, 2A, 2B, 2C, and 2D, t-tests compared the mean ID of hot spot concessions, to mean ID of statistically insignificant concessions. The results of all these analyses indicated no statistical difference in the means. This demonstrates that significant clustering of high childhood malaria incidence density was in fact due to the spatial distribution of the participant households.

The t-tests for village 1A were different than tests for the other villages because village 1A only had one concession identified as not statistically significant, and not a small subsample of concessions. The result of the t-test comparing mean ID of cold spots to the insignificant concession ID value indicated no statistical difference; however, the t-test comparing mean ID of hot spots to the insignificant concession ID value indicated a significant difference, with a p value of <0.001. The comparison of one data point to sample data is not an ideal statistical analysis, and these results may not accurately represent the comparison within this village.

For the whole study region, t-tests were performed for analyses at 2,100 meters and 10,000 meters; the distances at which clustering peaked. At both distances, the comparison of mean ID of cold spots to mean ID of insignificant concessions resulted in no statistical difference; however, the comparisons of mean ID of hot spots to the mean ID of insignificant clusters resulted in statistically significant differences. This could imply some of the concessions identified as significant hot spots during these analyses were identified because their ID values were truly higher than ID values throughout the rest of the study region. In other words, there could be some concessions within the study region that have statistically higher ID values, resulting in the identification of hot spot clusters. Some of the clusters may have been identified as hot spots because their ID values were truly higher than other concessions, and not because of their spatial distribution.

Limitations

There were various limitations in this study that could have had an impact on the results, but one of greatest limitations was the use of incidence density (ID) as the attribute value for evaluation. For the purposes of this study, ID was calculated in terms of malarial episodes per 100 person-days. Although this is a great method to evaluate the rate of disease transmission and standardize the unit of analysis, it does not accurately depict the complex transmission dynamics of malaria. As previously stated, young children typically five years and younger are the most vulnerable population at risk of severe malaria morbidity and mortality. As children age, their immune systems become stronger and are better able to fight malaria parasites, minimizing severity of the illness. Results from the original RIMDAMAL analyses supported this fact, and showed decreasing risk ratios with increasing age among children 2 to 5 years old. Typically, older children, between five-years-old and puberty, produce high enough parasite loads to transmit gametocytes to the vectors, which then go on to infect other persons. Evaluating ID among children 5-years-old and younger did not take into consideration the

variation of risk based on age and the immunity children were gaining over time due to repeated exposure.

Ultimately, evaluating spatial distribution of childhood malaria using ID could have introduced confounding bias in the results because the distribution of age throughout individual villages and the study region could have had a greater impact on the spatial clustering patterns identified than the intervention. For example, if a concession consisted of several very young children at higher risk of experiencing illness, there would likely be a high ID value, simply because the immune systems of the younger children would not have been well-developed enough to effectively combat infection. On the other hand, if a concession consisted of several four- or five-year-old children, there would likely be a lower ID value because the children's immune systems would be better equipped to fight infection. The distribution of these older children with lower risk of disease could have impacted the geospatial results of this study. One method that could potentially adjust for this bias would be to evaluate malaria incidence in each individual child, rather than at the concession level. Points representing individual children could be weighted by age, gender, or other factors that could impact malaria risk. This was not originally conducted for this study because GIS coordinates were recorded for each concession, and as such, the data were aggregated at these geographic points. Dispersing the markers or jiggering the coordinates slightly would allow for visualizing and evaluating the data at the individual child level.

Original analyses for the RIMDAMAL study found two significant variables that could also be confounding the spatial results: 1) a subgroup of children $\leq$ 5 years old in the intervention arm who were tall enough (>90 centimeters in height) to receive repeated IVM administrations experienced greater reduction in malaria incidence than other children in the intervention arm; and 2) there were significantly more children in the intervention arm that experienced zero malarial episodes throughout the study period than in the control arm. The spatial distribution of the children who received IVM administrations and the spatial distribution

of the children who experienced zero malarial episodes could be major driving factors in the overall distribution of childhood malaria incidences. To better visualize where these children resided within each village, maps were created denoting concessions where children received IVM (Appendix B) and concessions where children had zero malarial episodes (Appendix C). In some villages, concessions where these children lived were still identified as hot spots, while others were not. Although no analyses were performed on these variables, there seems to be no clear-cut geographic correlation between these variables and clustering of malaria incidence. It will be important for future analyses to evaluate how these and other household variables may impact the geographic distribution of childhood malaria. As previously mentioned, one method to adjust for these potential confounders could be to evaluate malaria incidence in individual children rather than aggregated concession data, utilizing weights based on how these factors could impact risk.

Another limitation was the use of only eight villages, despite the presence of dozens of villages scattered throughout the study region. Since malaria is transmitted by mosquitoes, it is unreasonable to rule out the possibility that these free-flying insects travelled from neighboring, untreated and unmeasured villages to infect children within study villages. This could have introduced bias in the results. Currently, there are no efficient methods to geographically track the feeding patterns of one mosquito, let alone millions, making this a difficult challenge to overcome. One option for minimizing these effects in future studies would be to include all villages within a set region. This would also provide a more accurate outlook on regional spatial clustering of childhood malaria following repeated mass administrations of IVM.

Another significant limitation is the lack of data regarding specific underlying environmental factors that could impact mosquito distribution and introduce confounding. For example, *Anopheles* species are dependent on standing water sources in which to lay their eggs. Although this could be a large water source, such as a pond or lake, many species have developed the ability to utilize even the smallest water sources, such as rainwater puddles, tire

tracks, and borrow pits. It is relatively simple to map profound water sources; however, these more minute sources are more difficult to include in spatial analyses. No environmental conditions were directly used in these analyses, but there could be unmeasured factors that are driving some of the spatial patterns identified.

Strengths

The unit of analysis in this study was individual concessions within each study village, of which there was a relatively large sample size. In total, there were 590 concessions with at least one child ≤ 5 years old, between all study villages. Of course, a larger sample size would more adequately demonstrate the farther-reaching spatial impacts of the RIMDAMAL protocol; however, as this was a pilot study, 590 concessions were more than adequate.

The utilization of the Gi* statistic to evaluate geospatial clustering of an infectious disease following the administration of an intervention has not yet been recorded in current published literature. Also, the existing publications have evaluated spatial clustering of diseases in study populations much larger than the entire population of the RIMDAMAL study. The successful use of Gi* to evaluate the geospatial impacts of an infectious disease intervention protocol, and its use within the small geographic regions evaluated in this study exhibit novel applications for its use, and further validate the role of spatial autocorrelation statistics in public health research.

Implications for Future Research

The RIMDAMAL study protocol evaluated a novel approach to minimize malaria incidence in children ≤ 5 years old, an age group that is highly vulnerable to severe complications of this ancient disease. As this was a pilot study, all results provide new information on the topic and generate hypotheses for future research. The most profound results of these spatial analyses provide some evidence that repeated mass drug administrations of IVM may alter the regional distribution of childhood malaria. If this is the case,

there is potential for this intervention to be given to a proportion of the population to provide regional protection. Further research is necessary to fully understand these impacts and how to most effectively distribute IVM to best combat childhood malaria.

There are various biological, ecological, environmental and behavioral factors that could have driven the geospatial clustering of childhood malaria in these study villages. For example, *Anopheles* mosquitoes tend to reside within human dwellings, bite at night, and tend to be more attracted to larger hosts. The fact that older children are often responsible for producing malarial gametocytes that propagate disease transmission is also a major factor that impacts risk for younger children. Future studies could better evaluate the magnitude of this factor by collecting blood samples from these older children to quantify gametocyte production. Evidence also suggests IVM inhibits significant parasitic life stages, so collecting gametocyte data could also provide insight as to how the RIMDAMAL protocol impacts parasitemia in older children less likely to exhibit disease.

Household variables such as bed net use and ratios of adults and older children to children $\leq$ 5 years old could drastically alter risk of contracting malaria within individual villages. Although this data was collected during the pilot study, these geospatial methods utilized a simple ID value for analyses, which did not take into consideration these variables. Using a more advanced statistical tool, such as geographically weighted regression, would allow for the evaluation of these variables and others, in addition to how they impact the spatial distribution of childhood malaria incidence.

Another unique finding from these geospatial analyses was the variation in distance at which maximum clustering occurred. The underlying factors that contributed to these distances were likely related to geographic characteristics that varied by village; however, they could have also been impacted by the intervention. Future RIMDAMAL studies can better evaluate the contributing factors by collecting qualitative data, such as recording vegetation, denoting major

hills or valleys that impact distribution of habitable structures, and average distance between households.

The primary hypothesis for the RIMDAMAL study was that repeated mass administrations of IVM would decrease vector abundance, and thus decrease transmission of malaria to young children. Although initial statistical analyses of the clinical data resulted in decreased disease incidence, there is no way to be sure this occurred by way of decreased vector abundance, as that data has not been analyzed. Since IVM has inhibitory effects on parasitic life stages important for propagation, it is possible this intervention worked by interrupting the parasitic life cycle, rather than the vector life cycle. These mechanisms could be evaluated in future studies by first collecting baseline vector and parasite data, including their distributions throughout the study region. Baseline data could then be used to understand how IVM impacts disease transmission in populations.

The results of initial statistical and geospatial analyses of the RIMDAMAL protocol are encouraging for the future of controlling malaria in highly endemic regions. This novel intervention is still new and requires additional research to fully understand its implications. Since initial geospatial results indicated significant clustering of childhood malaria occurred both within and between study villages, the next logical step is to evaluate the underlying factors that could be influencing these spatial patterns.

REFERENCES

1. Ross R. The Prevention of Malaria. New York: E.P. Dutton & Company; 1910.

2. Centers for Disease Control and Prevention (CDC). Malaria 2016 [updated March 24, 2016. Available from: http://www.cdc.gov/malaria/.

3. World Health Organization (WHO). World malaria report 2015. Geneva, Switzerland: World Health Organization; 2015. xxxv, 246 pages : p.

4. Carter R, Mendis KN. Evolutionary and historical aspects of the burden of malaria. Clin Microbiol Rev. 2002;15(4):564-94.

5. Wolfe ND, Dunavan CP, Diamond J. Origins of major human infectious diseases. Nature. 2007;447(7142):279-83.

6. Roll Back Malaria Parnership 2016 [updated 201609/02/2016]. Available from: http://www.rollbackmalaria.org/about/about-rbm/rbm-mandate.

7. Gallup JL, Sachs JD. The economic burden of malaria. American Journal of Tropical Medicine and Hygiene. 2001;64(1-2):85-96.

8. United Nations Development Group. United Nations Millennium Project: UN Secretary General; 2006 [updated December 31, 2006. Available from: http://www.unmillenniumproject.org/index.htm.

9. Hay SI, Guerra CA, Tatem AJ, Noor AM, Snow RW. The global distribution and population at risk of malaria: past, present, and future. Lancet Infect Dis. 2004;4(6):327-36.

10. Institute for Health Metrics and Evaluation (IHME). Global Burden of Diseases, Injuries, and Risk Factors Study. 2013.

11. Wang H, Naghavi M, Allen C, Barber RM, Bhutta ZA, Carter A, et al. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of

death, 1980–2015: a systematic analysis for the Global Burden of Disease Study 2015. The Lancet.388(10053):1459-544.

12. Na-Bangchang K, Karbwang J. Current status of malaria chemotherapy and the role of pharmacology in antimalarial drug research and development. Fundam Clin Pharmacol. 2009;23(4):387-409.

13. Adaptation to malaria the interaction of biology and culture. Greene LS, Danubio ME, editors. Amsterdam: Gordon and Breach Publishers; 1997. 409 p.

14. Toe KH, Jones CM, N'Fale S, Ismail HM, Dabire RK, Ranson H. Increased Pyrethroid Resistance in Malaria Vectors and Decreased Bed Net Effectiveness, Burkina Faso. Emerging Infectious Diseases. 2014;20(10):1691-6.

15. Mitchell A. The ESRI Guide to GIS Analysis; Volume 2: Spatial Measurements & Statistics. Redlands, CA: ESRI Press; 2005.

16. Webster JP, Molyneux DH, Hotez PJ, Fenwick A. The contribution of mass drug administration to global health: past, present and future. Philosophical Transactions of the Royal Society B: Biological Sciences. 2014;369(1645).

17. Alout H, Krajacich BJ, Meyers JI, Grubaugh ND, Brackney DE, Kobylinski KC, et al. Evaluation of ivermectin mass drug administration for malaria transmission control across different West African environments. Malar J. 2014;13:10.

18. Foley DH, Bryan JH, Lawrence GW. The potential of ivermectin to control the malaria vector Anopheles farauti. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2000;94(6):625-8.

19. Foy BD, Kobylinski KC, da Silva IM, Rasgon JL, Sylla M. Endectocides for malaria control. Trends Parasitol. 2011;27(10):423-8.

20. Kobylinski KC, Foy BD, Richardson JH. Ivermectin inhibits the sporogony of Plasmodium falciparum in Anopheles gambiae. Malar J. 2012;11:9.

21. Naz S, Maqbool A, Ahmad MUD, Anjum AA, Zaman S. Efficacy of Ivermectin for Control of Zoophilic Malaria Vectors in Pakistan. Pak J Zool. 2013;45(6):1585-91.

22. Sylla M, Kobylinski KC, Gray M, Chapman PL, Sarr MD, Rasgon JL, et al. Mass drug administration of ivermectin in south-eastern Senegal reduces the survivorship of wild-caught, blood fed malaria vectors. Malar J. 2010;9:10.

23. Foy BD, Haoues Alout, Jonathan A. Seaman, Sangeeta Rao, Hannah C. Slater, Roland Bougma, Abdoulaye Diabaté, A. Gafar V. Coulidiaty, Nöel Rouamba, Roch K. Dabiré. Repeat ivermectin mass drug administrations for control of malaria (RIMDAMAL): a pilot cluster-randomized trial to evaluate efficacy and risk of harms. 2016.

24. Poinar G. Culex malariager, N. sp (Diptera : Culicidae) from Dominican amber: The first fossil mosquito vector of plasmodium. Proc Entomol Soc Wash. 2005;107(3):548-53.

25. Nerlich AG, Schraut B, Dittrich S, Jelinek T, Zink AR. Plasmodium falciparum in ancient Egypt. Emerging Infectious Diseases. 2008;14(8):1317-9.

26. Malaria parasite biology, pathogenesis, and protection. Sherman IW, editor. Washington, DC: ASM Press; 1998. 575 p.

27. Cox FE. History of the discovery of the malaria parasites and their vectors. Parasites & Vectors. 2010;3(1):1-9.

28. Neghina R, Neghina AM, Marincu I, Iacobiciu I. Malaria, a Journey in Time: In Search of the Lost Myths and Forgotten Stories. Am J Med Sci. 2010;340(6):492-8.

29. Pappas G, Kiriaze IJ, Falagas ME. Insights into infectious disease in the era of Hippocrates. International Journal of Infectious Diseases. 2008;12(4):347-50.

30. Anderson W. Malaria Psychoses and Neuroses: Oxford, United Kingdom: Oxford University Press; 1927.

31. Macdonald G-. The epidemiology and control of malaria. London ;: Oxford University Press; 1957. 201 p.

32. Packard RM. The making of a tropical disease a short history of malaria. Baltimore, Md.: Johns Hopkins University Press; 2007. 296 p.

33. Kidson C. Global malaria challenge: the Amsterdam summit. The Southeast Asian journal of tropical medicine and public health. 1992;23(4):635-40.

34. Roll Back Malaria, World Health Organization. The Abuja Declaration and the Plan of Action. An Extract from the African Summit on Roll Back Malaria, Abuja. 2000;25.

35. Malaria genetic and evolutionary aspects. Dronamraju KR, Arese P, editors. New York, NY: Springer; 2006. 190 p.

36. Beet EA. Sickle cell disease in the Balovale District of Northern Rhodesia. East Afr Med J. 1946;23:75-86.

37. Flint J, Harding RM, Boyce AJ, Clegg JB. The population genetics of the haemoglobinopathies. Baillieres Clin Haematol. 1993;6(1):215-62.

38. Centers for Disease Control and Prevention (CDC). Parasites 2014 [updated March 5, 2014. Available from: http://www.cdc.gov/parasites/about.html.

39. Yusof R, Lau YL, Mahmud R, Fong MY, Jelip J, Ngian HU, et al. High proportion of knowlesi malaria in recent malaria cases in Malaysia. Malar J. 2014;13:9.

40. Malaria: Life Cycle of Plasmodium. New York: The McGraw Hill Companies, Inc.; 2006. p. 2:20.

41. Sinka ME, Bangs MJ, Manguin S, Chareonviriyaphap T, Patil AP, Temperley WH, et al. The dominant Anopheles vectors of human malaria in the Asia-Pacific region: occurrence data, distribution maps and bionomic précis. Parasites & Vectors. 2011;4(1):1-46.

42. Sinka ME, Bangs MJ, Manguin S, Coetzee M, Mbogo CM, Hemingway J, et al. The dominant Anopheles vectors of human malaria in Africa, Europe and the Middle East: occurrence data, distribution maps and bionomic precis. Parasites & Vectors. 2010;3:34.

43. Cohuet A, Harris C, Robert V, Fontenille D. Evolutionary forces on Anopheles: what makes a malaria vector? Trends Parasitol. 2010;26(3):130-6.

44. Githeko AK, Adungo NI, Karanja DM, Hawley WA, Vulule JM, Seroney IK, et al. Some observations on the biting behavior of Anopheles gambiae ss, Anopheles arabiensis, and Anopheles funestus and their implications for malaria control. Exp Parasitol. 1996;82(3):306-15.

45. Hurd H. Manipulation of medically important insect vectors by their parasites. Annu Rev Entomol. 2003;48:141-61.

46. Koella JC, Sorensen FL, Anderson RA. The malaria parasite, Plasmodium falciparum, increases the frequency of multiple feeding of its mosquito vector, Anopheles gambiae. Proc R Soc B-Biol Sci. 1998;265(1398):763-8.

47. Doolan DL, Dobaño C, Baird JK. Acquired Immunity to Malaria. Clin Microbiol Rev. 2009;22(1):13-36.

48. Kurtis JD, Mtalib R, Onyango FK, Duffy PE. Human resistance to Plasmodium falciparum increases during puberty and is predicted by dehydroepiandrosterone sulfate levels. Infection and Immunity. 2001;69(1):123-8.

49. Talisuna AO, Bloland P, D'Alessandro U. History, dynamics, and public health importance of malaria parasite resistance. Clin Microbiol Rev. 2004;17(1):235-+.

50. Killeen GF, Smith TA. Exploring the contributions of bed nets, cattle, insecticides, and excitorepellency to malaria control: a deterministic model of mosquito host-seeking behaviour and mortality. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2007;101(9):867-80.

51. Central Intelligence Agency. The World Factbook: Burkina Faso 2016 [updated October 7, 2016. Available from: https://www.cia.gov/library/publications/the-world-factbook/geos/uv.html.

52. The World Bank Group. DataBank: Burkina Faso. World Bank2016.

53. Global Malaria Programme. Recommendations- The role of mass drug administration, mass screening and treatment, and focal screening and treatment for malaria. World Health Organization website: World Health Organization; 2015 November 2015.

54. Pooda HS, Rayaisse JB, Hien DFD, Lefevre T, Yerbanga SR, Bengaly Z, et al. Administration of ivermectin to peridomestic cattle: a promising approach to target the residual transmission of human malaria. Malar J. 2015;14:12.

55. Chaccour C, Lines J, Whitty CJM. Effect of Ivermectin on Anopheles gambiae Mosquitoes Fed on Humans: The Potential of Oral Insecticides in Malaria Control. J Infect Dis. 2010;202(1):113-6.

56. Esri. ArcGIS. 10.3 ed. Redlands, CA: Esri; 2014.

57. Ahmad SS, Aziz N, Butt A, Shabbir R, Erum S. Spatio-temporal surveillance of water based infectious disease (malaria) in Rawalpindi, Pakistan using geostatistical modeling techniques. Environ Monit Assess. 2015;187(9):15.

58. Haque U, Overgaard HJ, Clements AC, Norris DE, Islam N, Karim J, et al. Malaria burden and control in Bangladesh and prospects for elimination: an epidemiological and economic assessment. The Lancet Global health. 2014;2(2):e98-105.

59. Abedi-Astaneh F, Hajjaran H, Yaghoobi-Ershadi MR, Hanafi-Bojd AA, Mohebali M, Shirzadi MR, et al. Risk Mapping and Situational Analysis of Cutaneous Leishmaniasis in an Endemic Area of Central Iran: A GIS-Based Survey. Plos One. 2016;11(8):16.

60. Mahara G, Wang C, Huo D, Xu Q, Huang F, Tao L, et al. Spatiotemporal Pattern Analysis of Scarlet Fever Incidence in Beijing, China, 2005-2014. International journal of environmental research and public health. 2016;13(1).

61. Varga C, Pearl DL, McEwen SA, Sargeant JM, Pollari F, Guerin MT. Area-level global and local clustering of human Salmonella Enteritidis infection rates in the city of Toronto, Canada, 2007-2009. BMC Infect Dis. 2015;15:359.

62. Smith C, Skelly C, Kung N, Roberts B, Field H. Flying-fox species density--a spatial risk factor for Hendra virus infection in horses in eastern Australia. PLoS One. 2014;9(6):e99965.

63. Lucero DE, Carlson TC, Delisle J, Poindexter S, Jones TF, Moncayo AC. Spatiotemporal Co-occurrence of Flanders and West Nile Viruses Within Culex Populations in Shelby County, Tennessee. J Med Entomol. 2016;53(3):526-32.

64. R Development Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2010.

65. File: Burkina Faso, administrative divisions: Wikimedia Commons; 2012 [Available from: https://commons.wikimedia.org/wiki/File:Burkina_Faso,_administrative_divisions_-_de_-_colored.svg.

APPENDICES

Appendix A- RIMDAMAL Study Region

Figure 12- This map of Burkina Faso displays the 13 administrative regions within the country.[65] The RIMDAMAL pilot study was conducted in the small area indicated by the red square in the Sud-Ouest region.

Appendix B- Approximate Locations of Study Villages

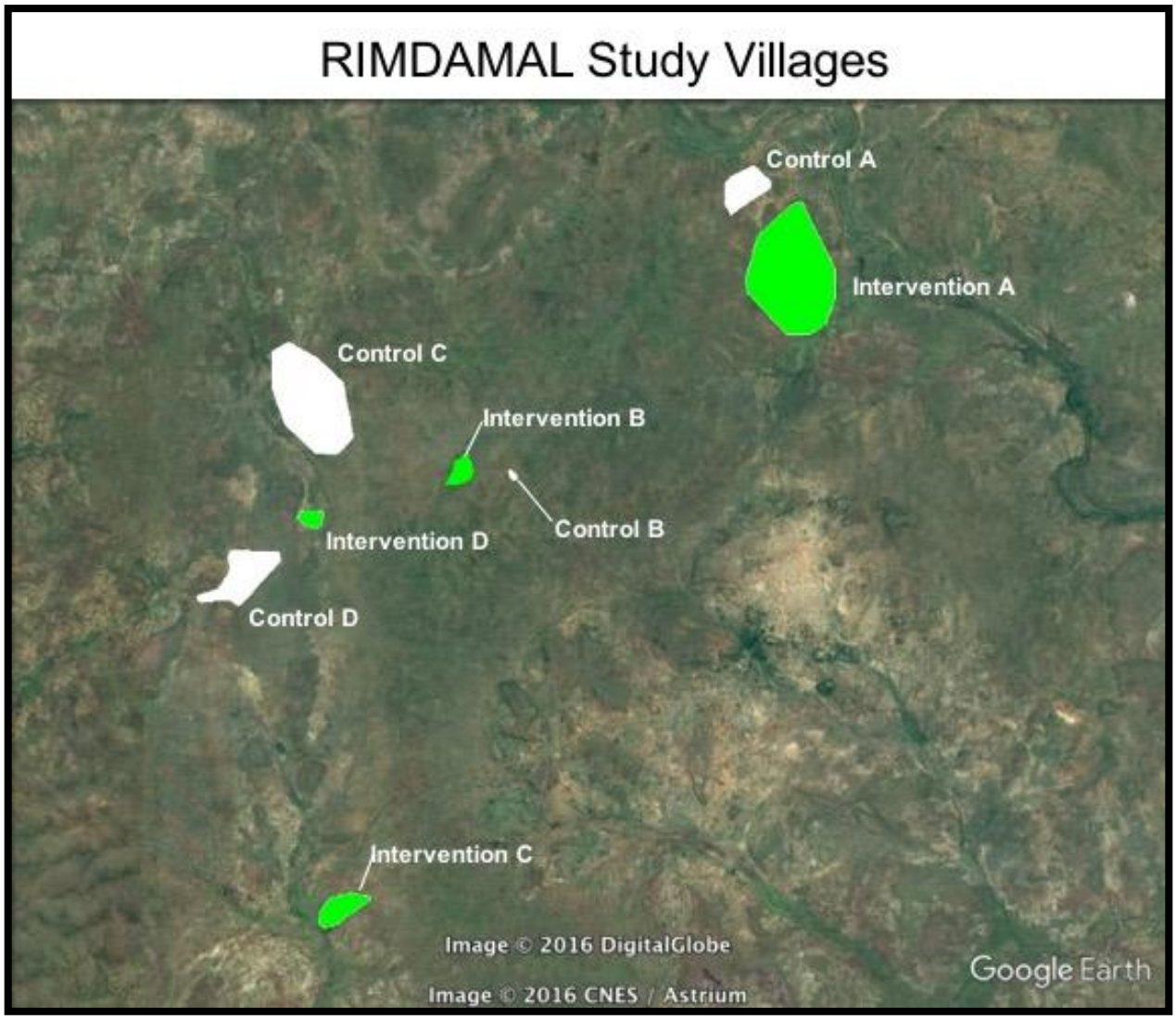

Figure 13- Map of the general locations of study villages with polygons representing the approximate geographic extents. White polygons represent control villages, and green polygons represent intervention villages.

Appendix C- Mapped Locations of Children Who Received IVM Within Each Village

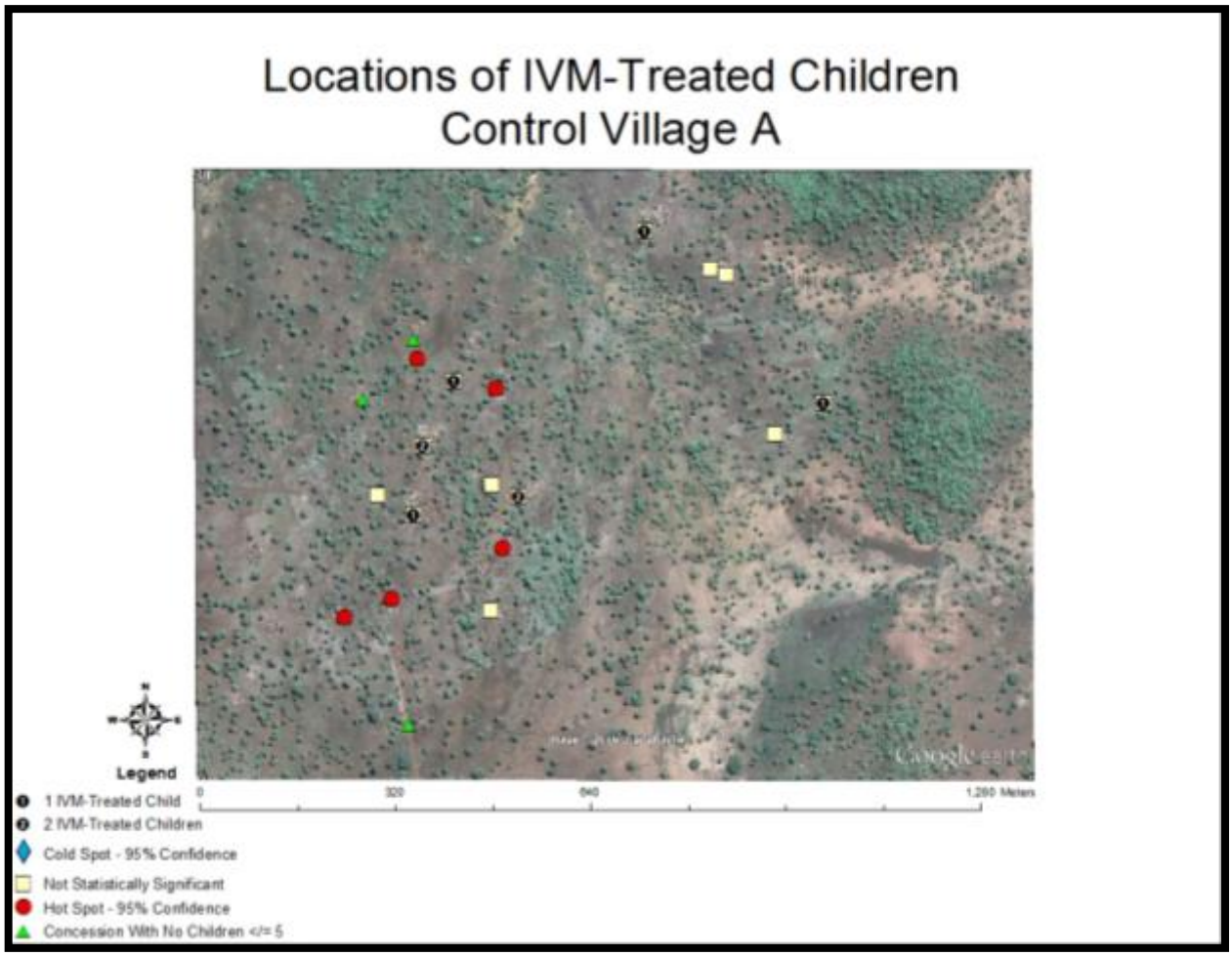

Figure 14- Locations of children ≤ 5 years old in Control Village A (1A) who received IVM at the beginning of the study period: There were four concessions where one child received dose(s) of IVM at the beginning of the study period, and two concessions where two children received IVM. None of the concessions where children ≤ 5 years old received IVM were found to be statistically significant hot spots during geospatial analyses.

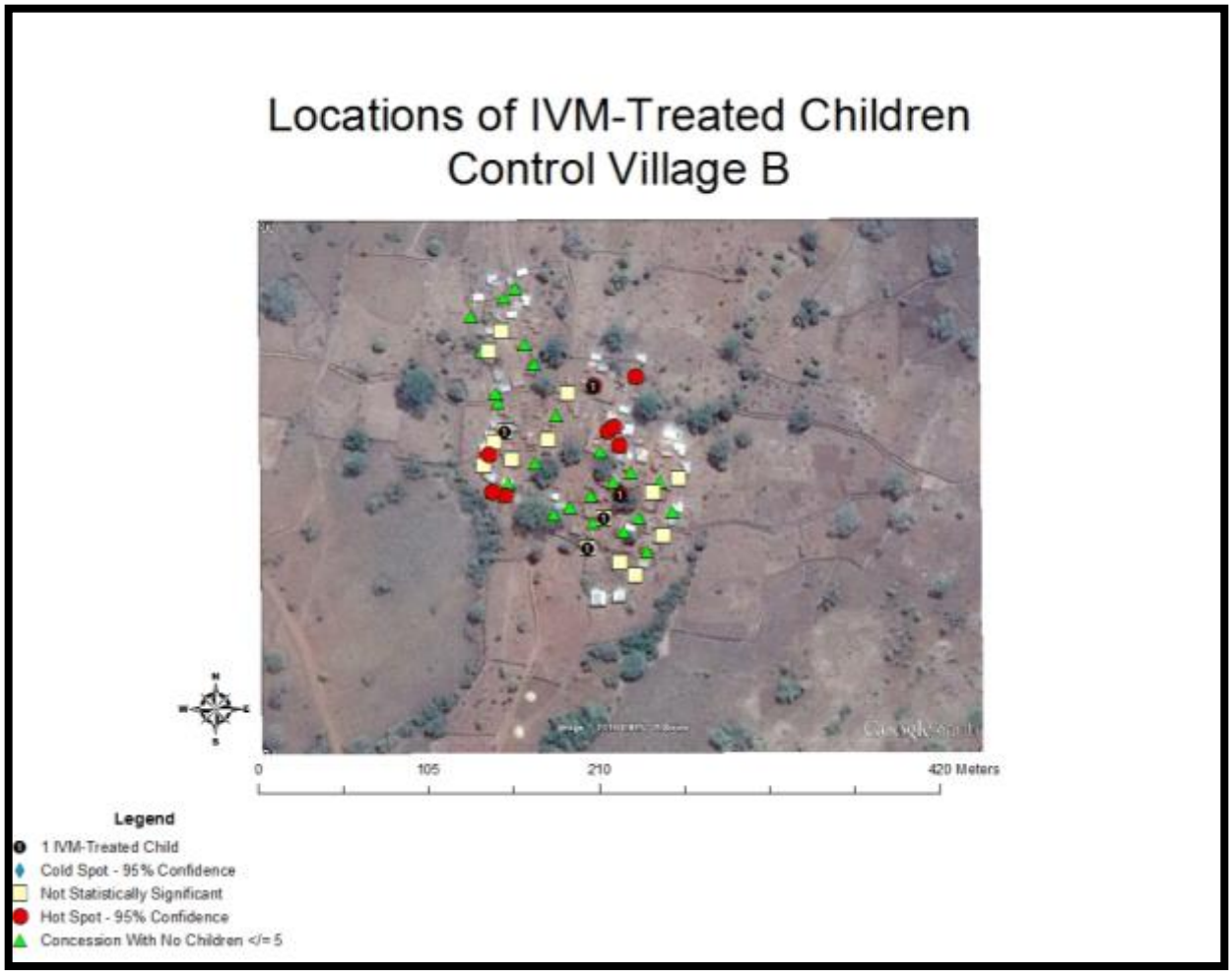

Figure 15- Locations of children ≤ 5 years old in Control Village B (1B) who received IVM at the beginning of the study period: In total, there were five concessions where one child received IVM dose(s) at the beginning of the study period. Of these five concessions, two were found to be significant hot spots during geospatial analyses.

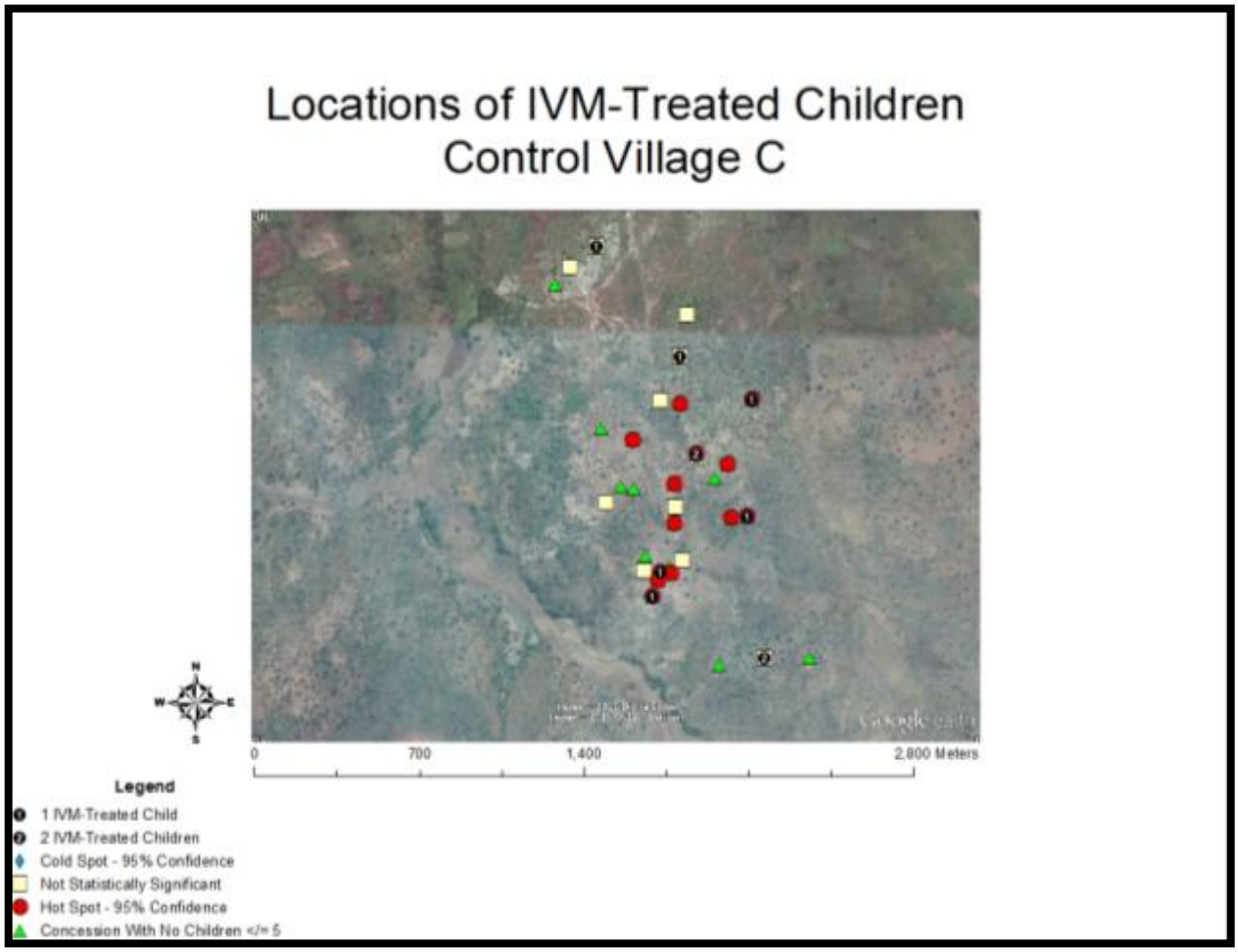

Figure 16- Locations of children ≤ 5 years old in Control Village C (1C) who received IVM at the beginning of the study period: There were six concessions where one child received IVM dose(s) at the beginning of the study period, four of which were found to be significant hot spots during geospatial analyses. There were also two concessions where two children were given IVM dose(s) at the beginning of the study, and one was found to be a significant hot spot.

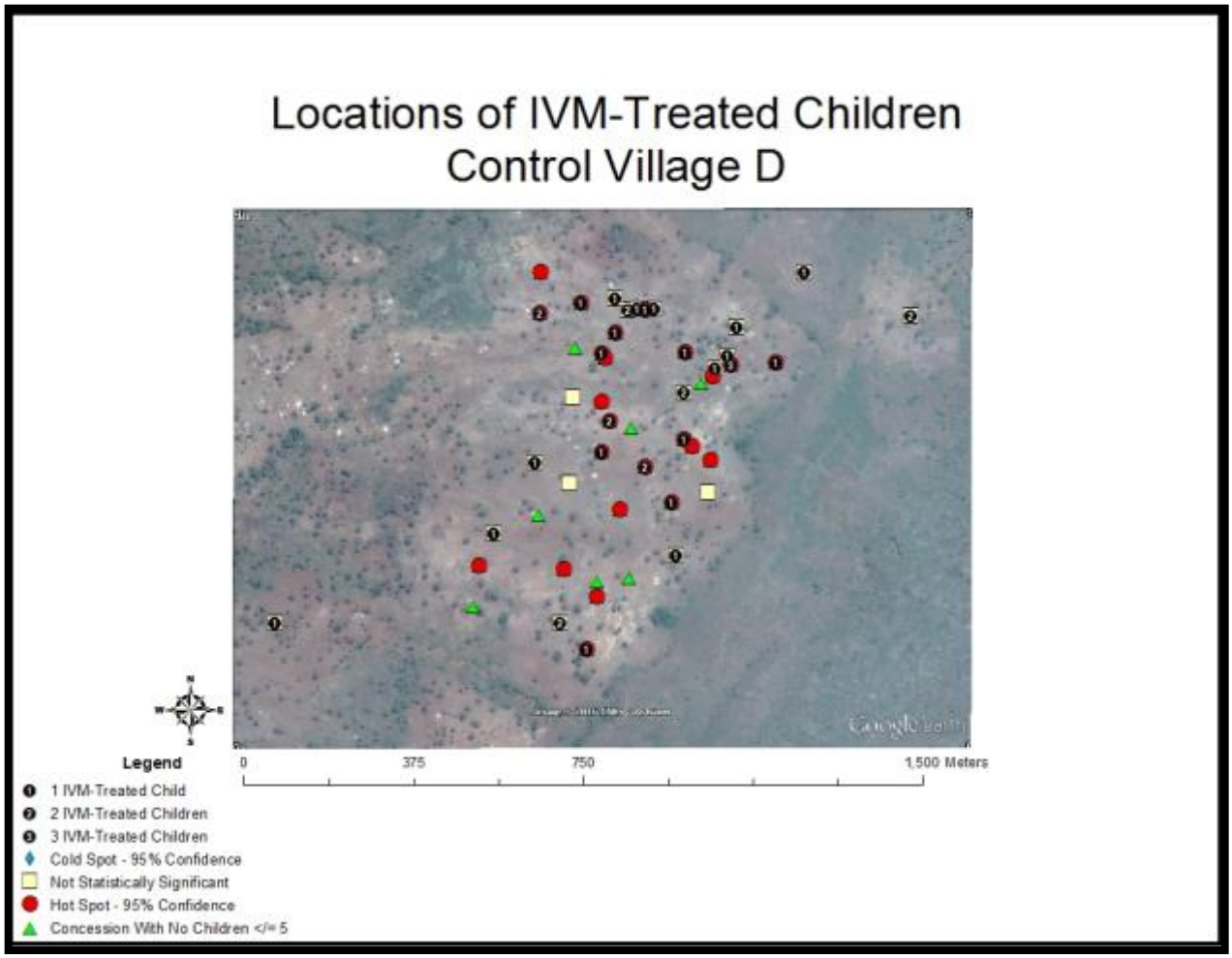

Figure 17- Locations of children ≤ 5 years old in Control Village D (1D) who received IVM at the beginning of the study period: There were twenty-one concessions where one child received IVM, seven concessions where two children received IVM, and one concession where three children received IVM. During geospatial analyses, ten of the 21 concessions where one child received IVM, three concessions of the seven where two children received IVM, and the concession where three children received IVM at the beginning of the study, were identified as significant hot spots.

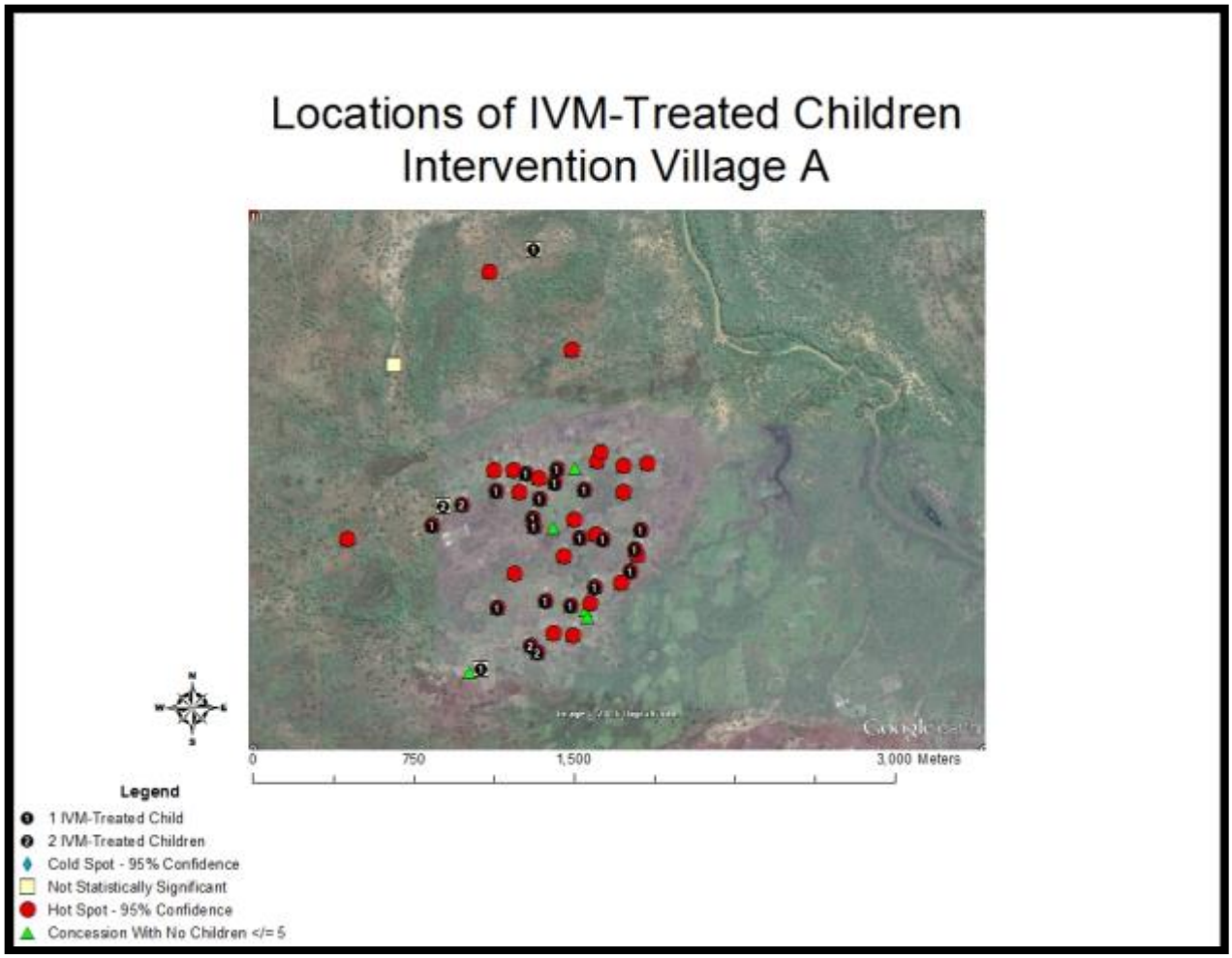

Figure 18- Locations of children ≤ 5 years old who received IVM in Intervention Village A (2A): There were twenty concessions where one child received the IVM intervention throughout the study period, and four concessions where two children received IVM. During geospatial analyses, eighteen of the twenty concessions where one child received IVM, three of the four concessions where two children received IVM were identified as significant hot spots.

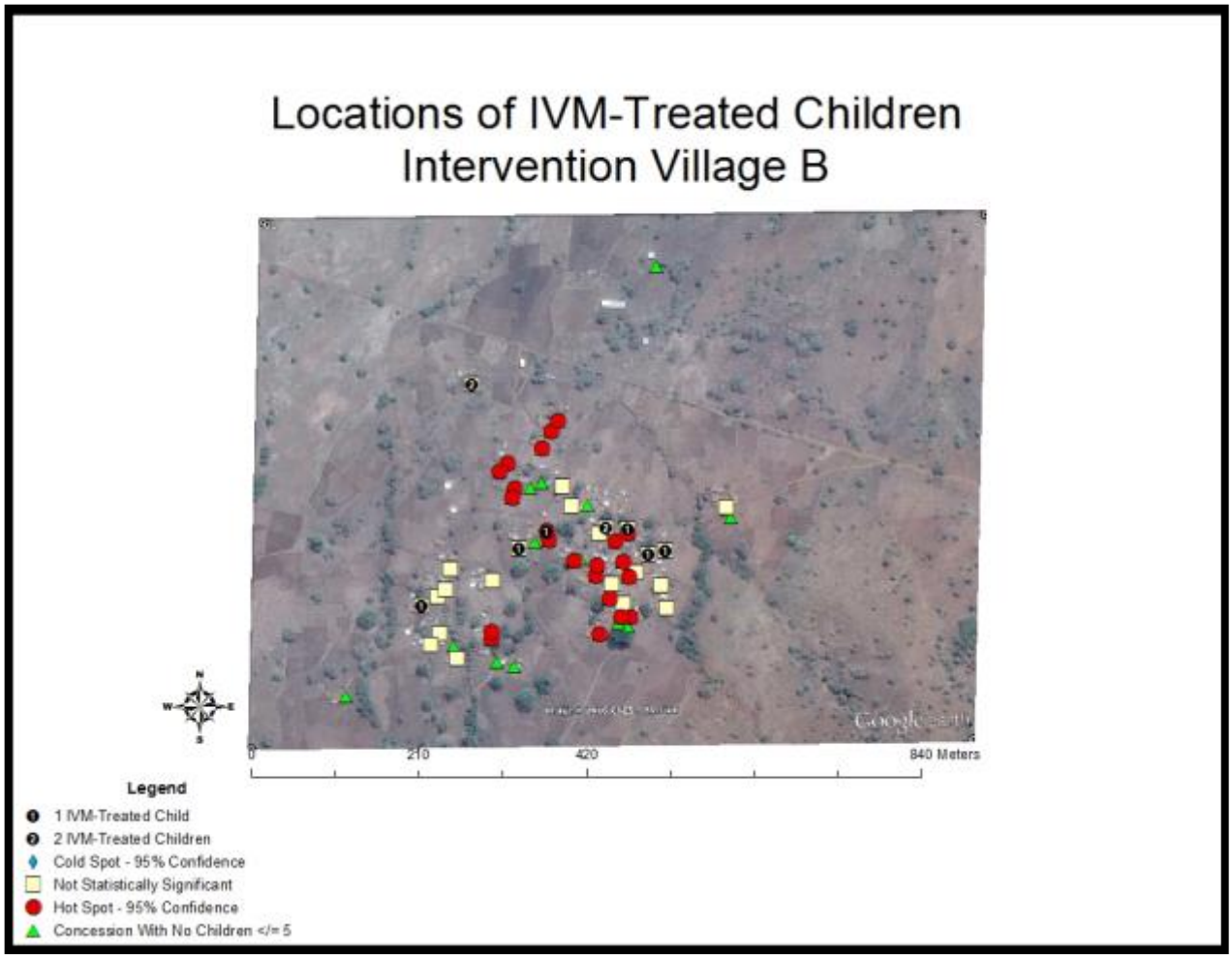

Figure 19- Locations of children ≤ 5 years old who received IVM in Intervention Village B (2B): There were a total of six concessions with one child who received the intervention, and two concessions with two children who received IVM. During geospatial analyses, only one concession with one child who received IVM was identified as a statistically significant hot spot.

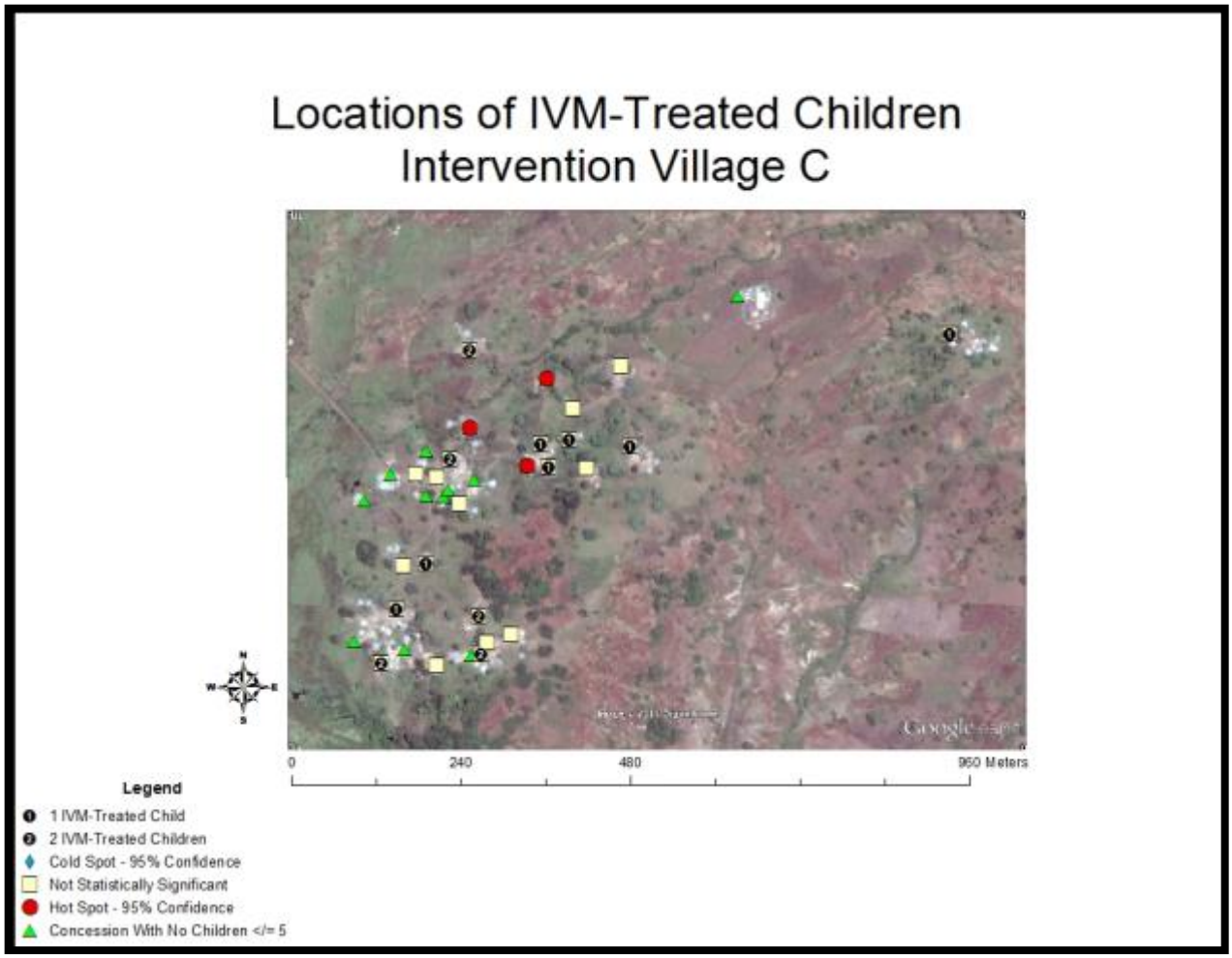

Figure 20- Locations of children ≤ 5 years old who received IVM in Intervention Village C (2C): There were seven concessions where one child received repeated IVM administrations throughout the study period, and five concessions where two children received IVM. During geospatial analyses, none of these concessions were identified as statistically significant hot spots.

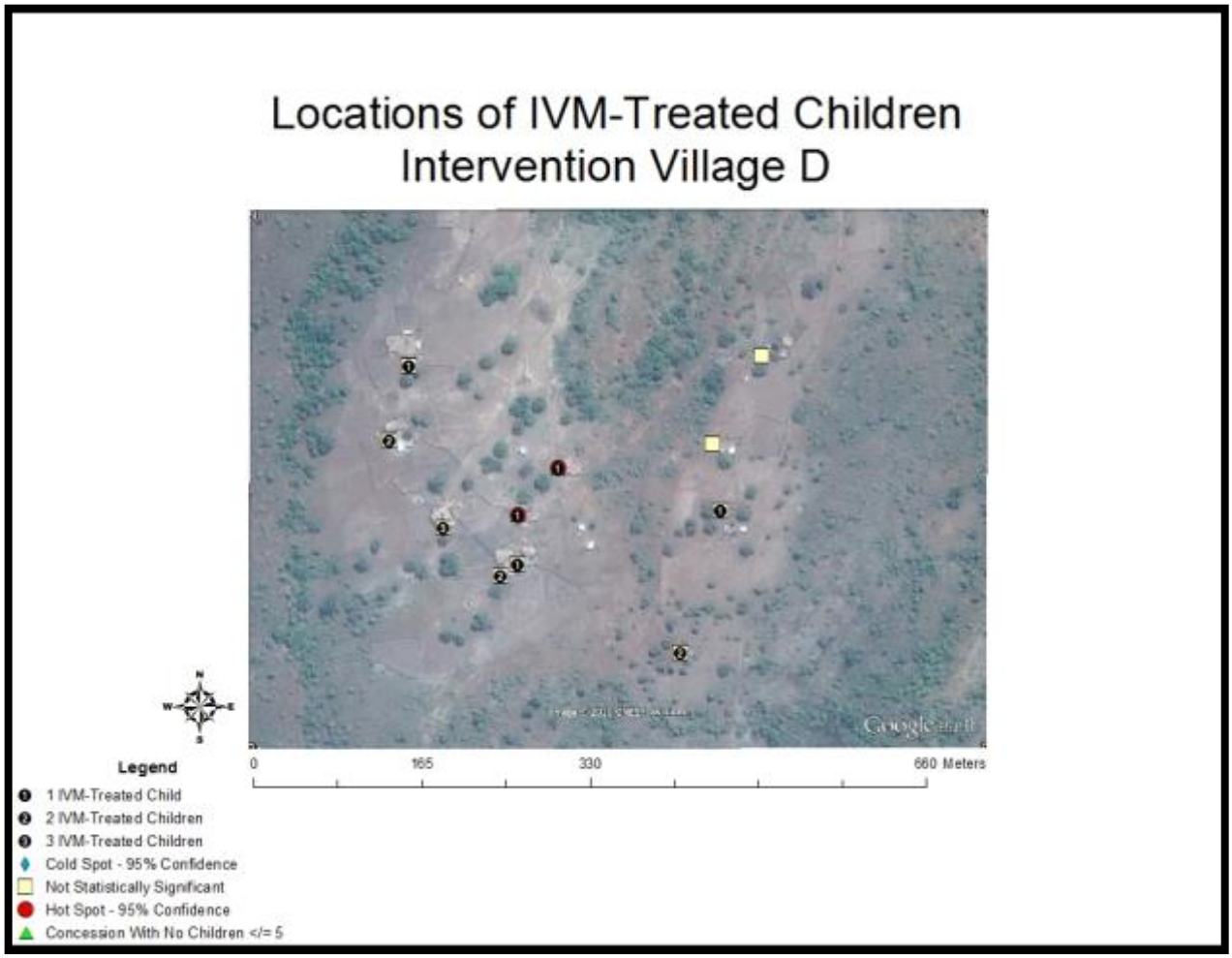

Figure 21- Locations of children ≤ 5 years old who received IVM in Intervention Village D (2D): There were five concessions where one child received repeated IVM administrations throughout the study period, three concessions where two children repeatedly received IVM, and one concession where three children repeatedly received IVM. During geospatial analyses, two of the concessions where one child received IVM were identified as the only two significant hot spots.

Appendix D- Mapped Locations of Children with Zero Malarial Infections Within Each Village

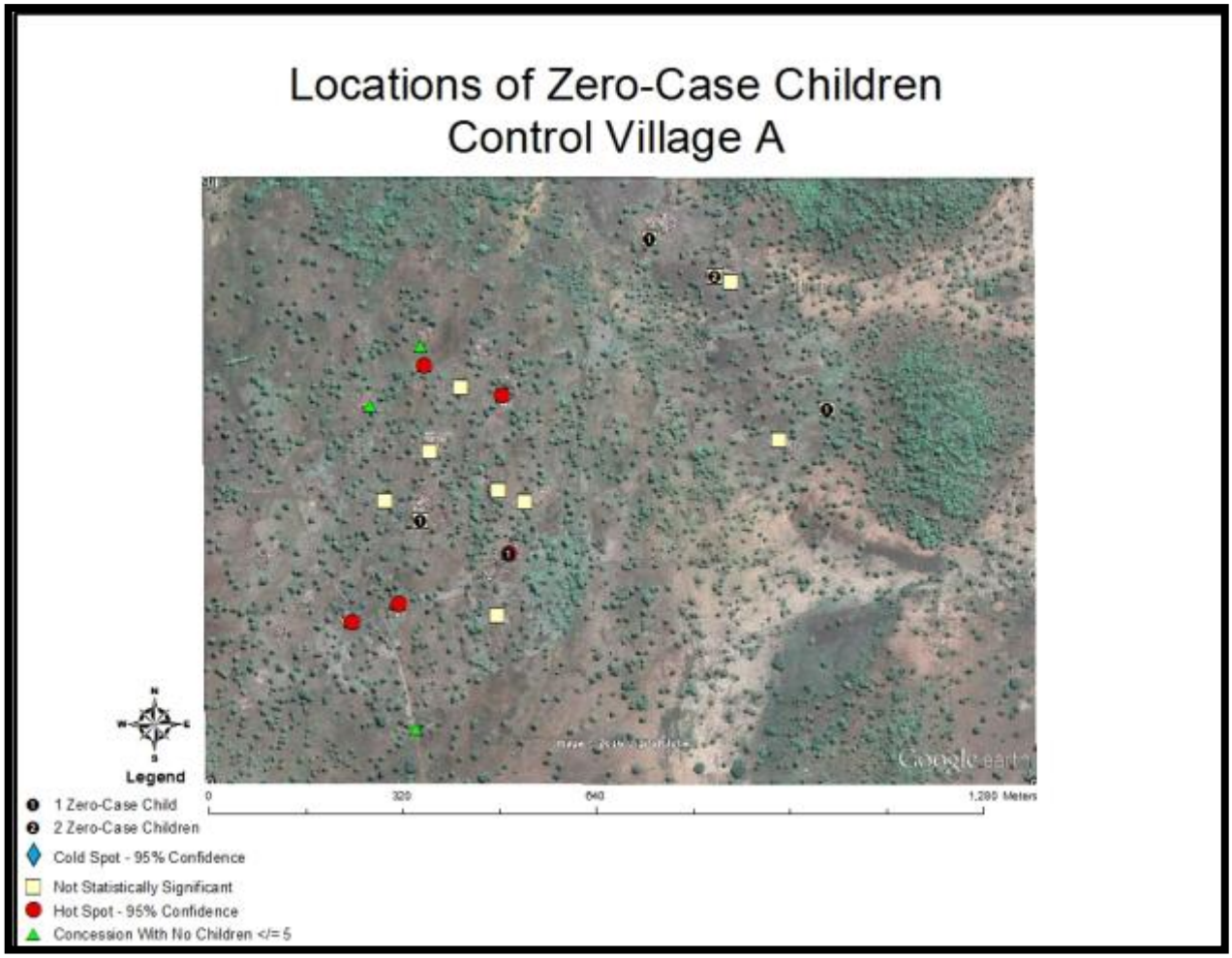

Figure 22- Locations of children ≤ 5 years old with zero reported malarial diagnoses throughout the study period in Control Village A (1A): In total, four concessions housed one child with zero reported diagnoses, and one concession had two children with zero diagnoses. During geospatial analyses, one of the concessions with one zero-case child was identified as a statistically significant hot spot.

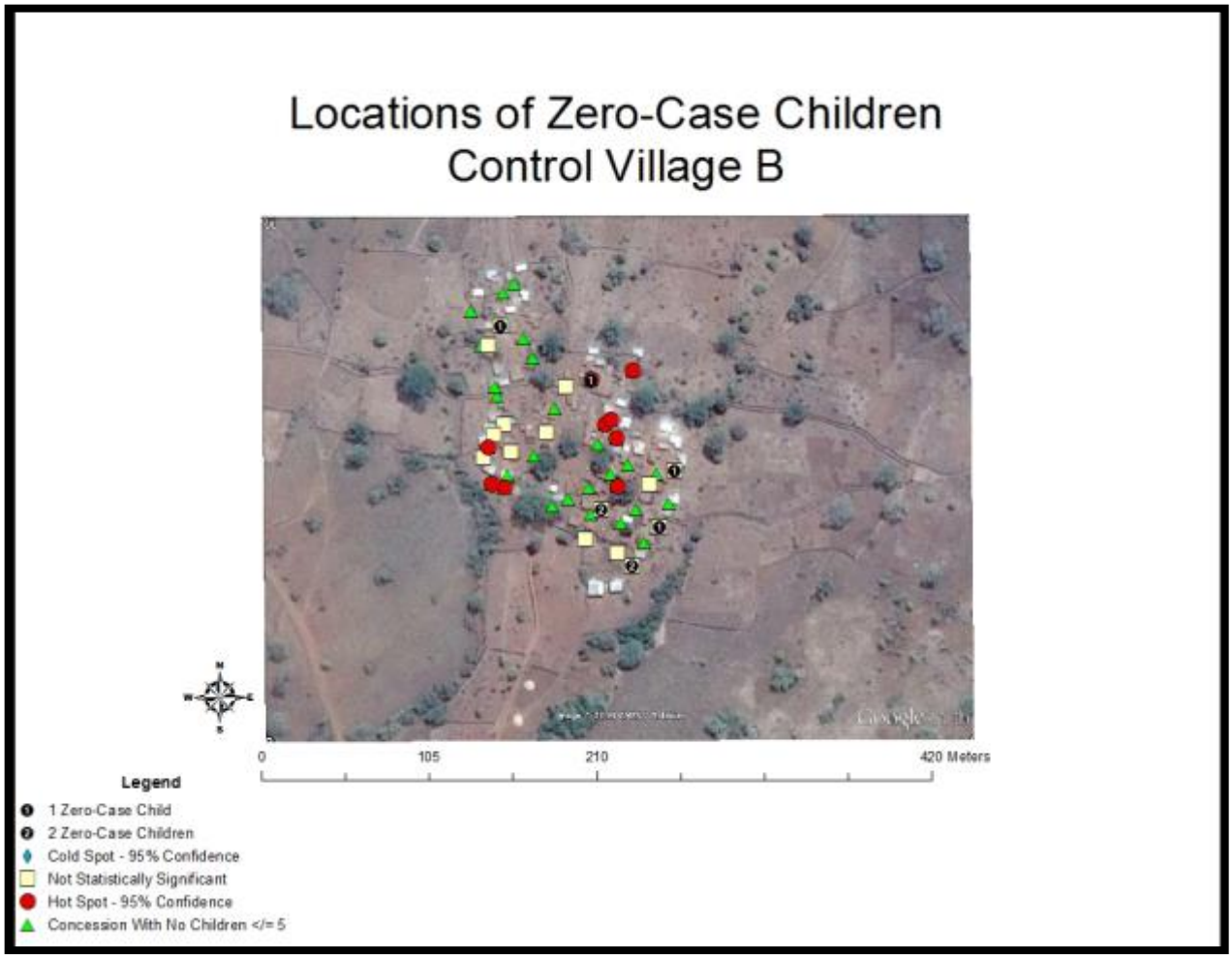

Figure 23- Locations of children ≤ 5 years old with zero reported malarial diagnoses throughout the study period in Control Village B (1B): In total, there were six concessions where children had zero reported diagnoses of malaria; four concessions with one child each, and two concessions with two children each. One of the concessions with one zero-case child was identified as a significant hot spot during geospatial analyses.

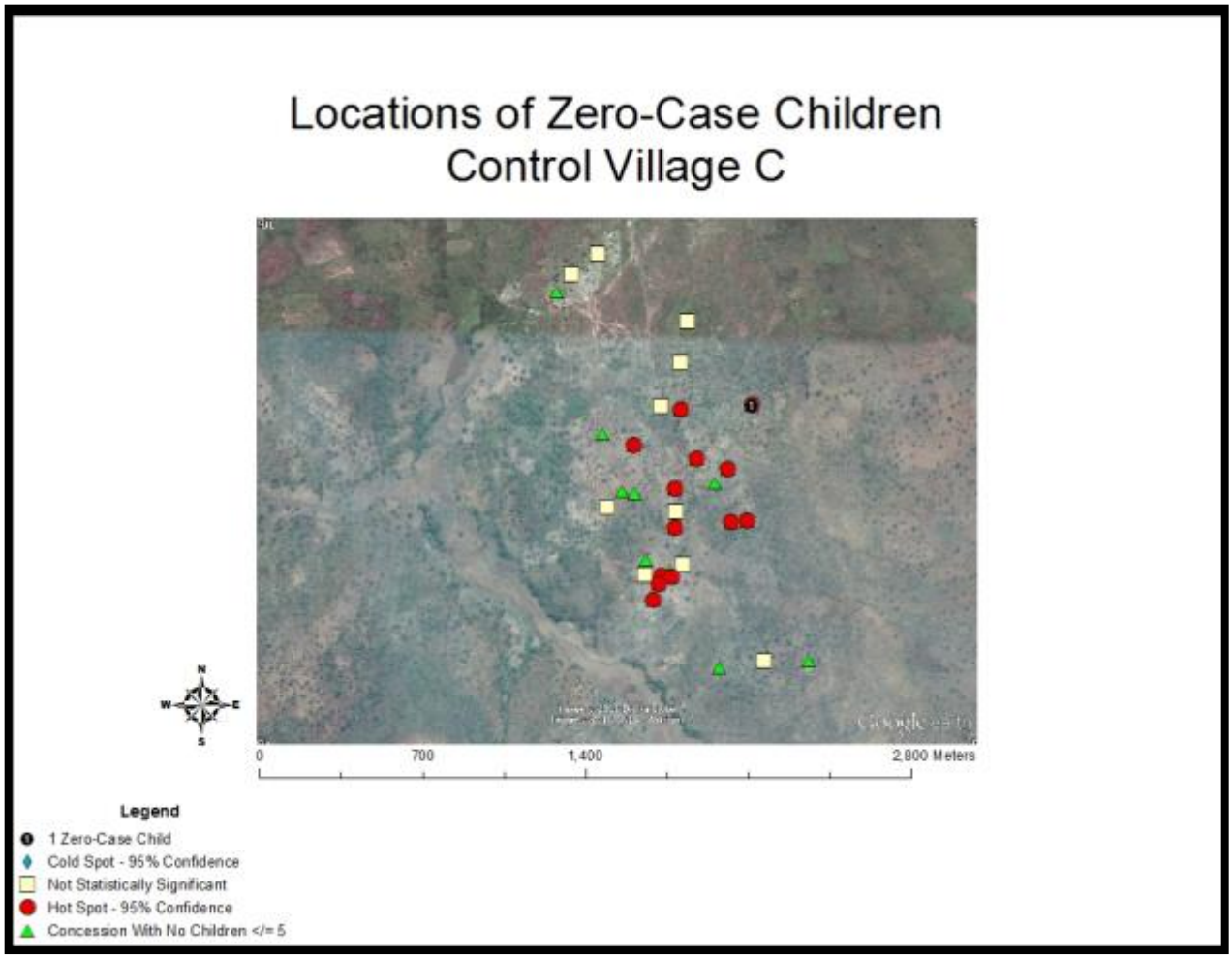

Figure 24- Locations of children ≤ 5 years old with zero reported malarial diagnoses throughout the study period in Control Village C (1C): One concession in village 1C had one child with zero reported diagnoses of malaria, and it was identified as a significant hot spot during geospatial analyses.

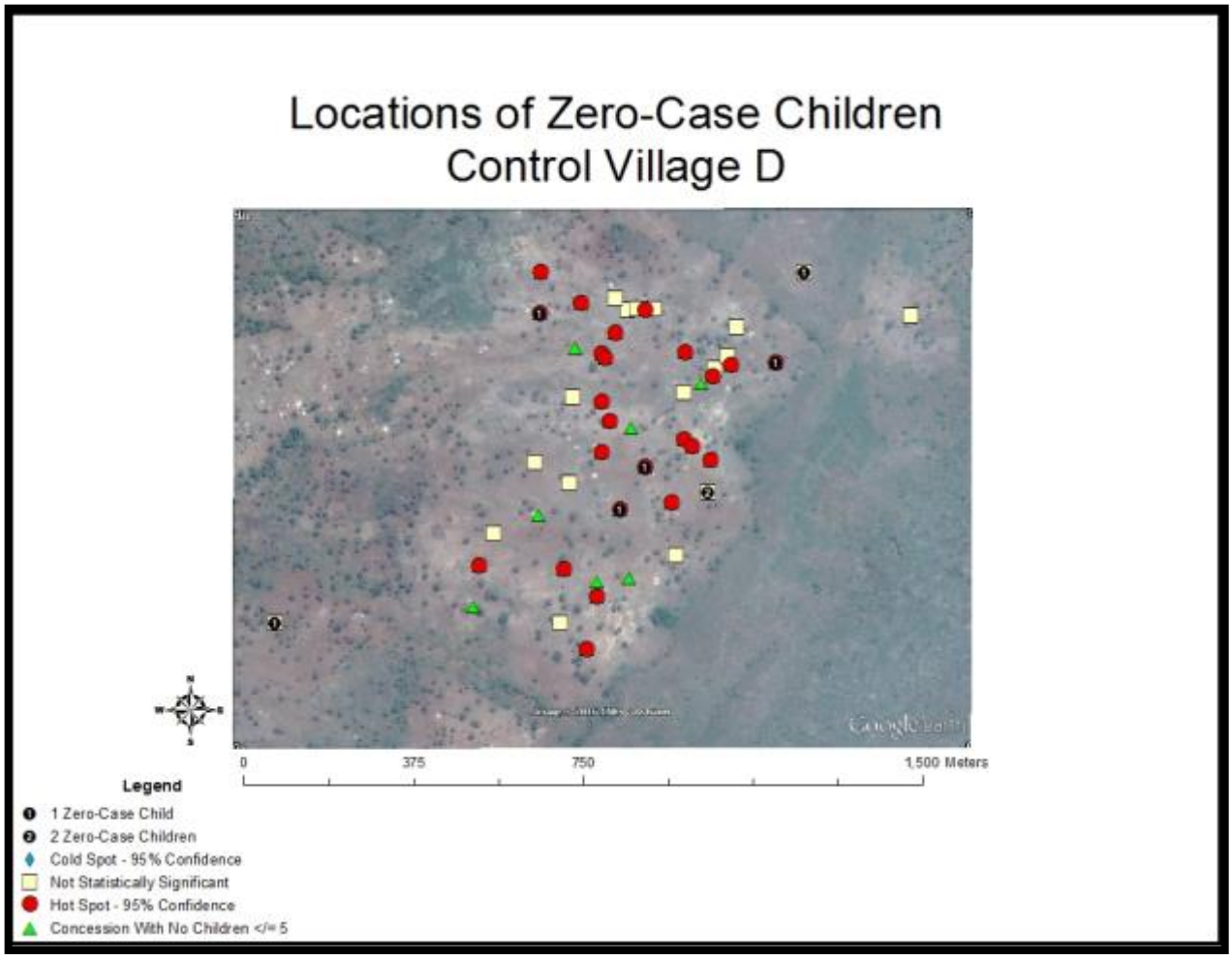

Figure 25- Locations of children ≤ 5 years old with zero reported malarial diagnoses throughout the study period in Control Village D (1D): There were seven total concessions with children who had zero reported diagnoses; six concessions with one child each, and one concession with two children. Four of the concessions with one zero-case child were identified as significant hot spots during geospatial analyses.

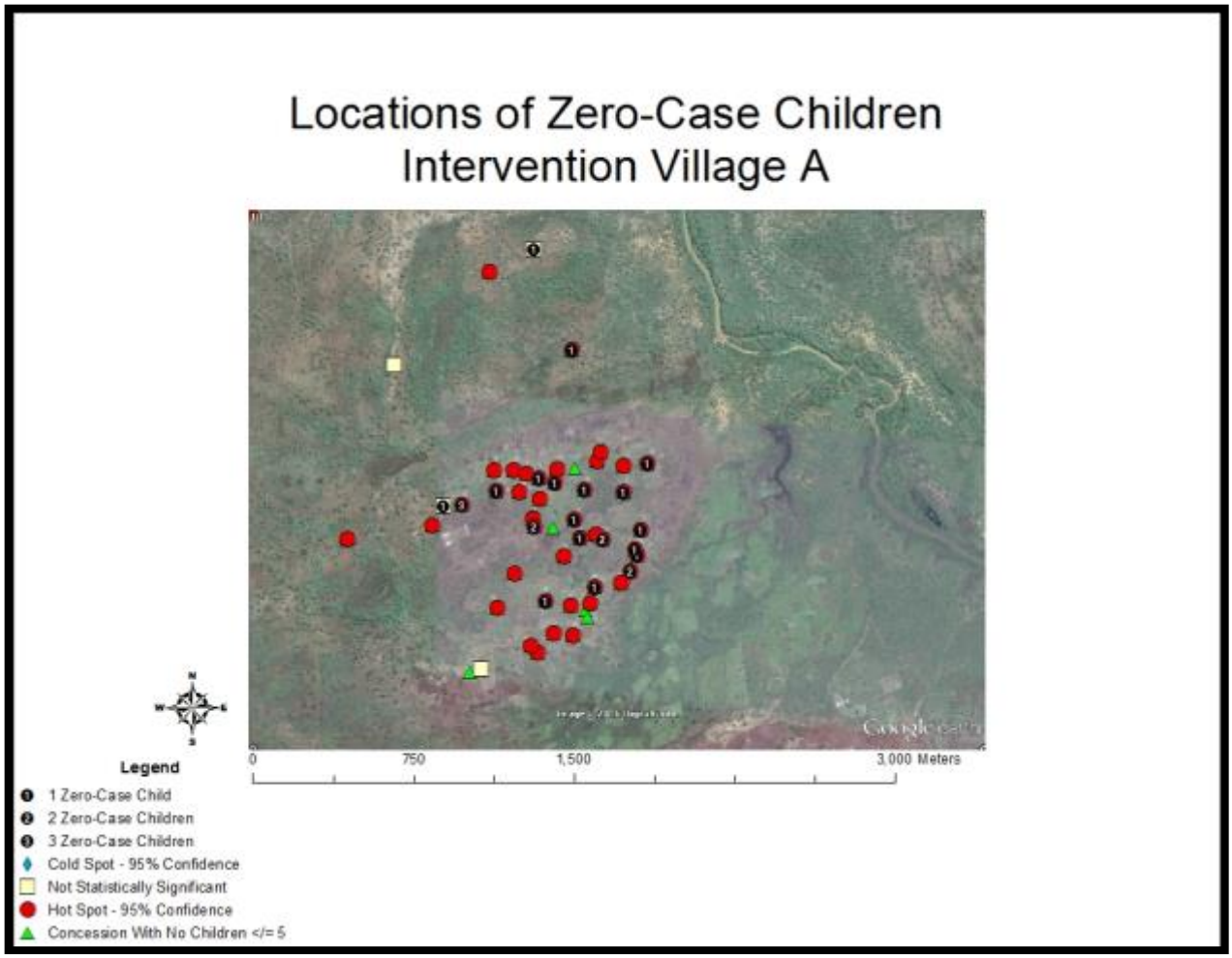

Figure 26- Locations of children ≤ 5 years old with zero reported malarial diagnoses throughout the study period in Intervention Village A (2A): There were a total of twenty concessions where children had zero reported diagnoses of malaria; sixteen of which had one child each, three of which had two children each, and one had three children with zero reported diagnoses. With the exception of two concessions with one zero-case child each, all of these concessions were identified as significant hot spots during geospatial analyses.

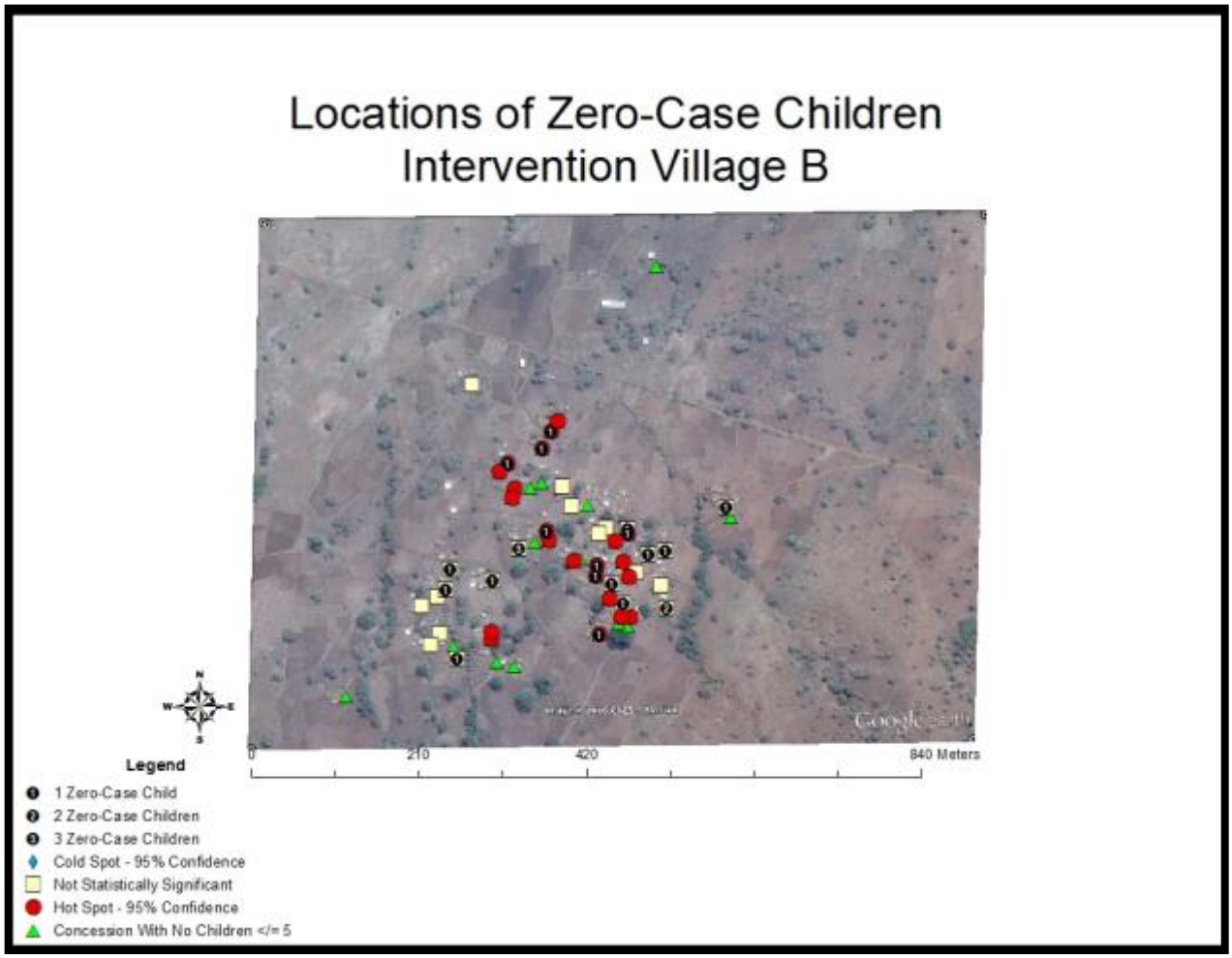

Figure 27- Locations of children ≤ 5 years old with zero reported malarial diagnoses throughout the study period in Intervention Village B (2B): There were twenty concessions with children who had zero malarial diagnoses in village 2B; eighteen concessions had one zero-case child each, one concession had two zero-case children, and one concession had three zero-case children. During geospatial analyses, eight of the concessions with one zero-case child were identified as statistically significant hot spots.

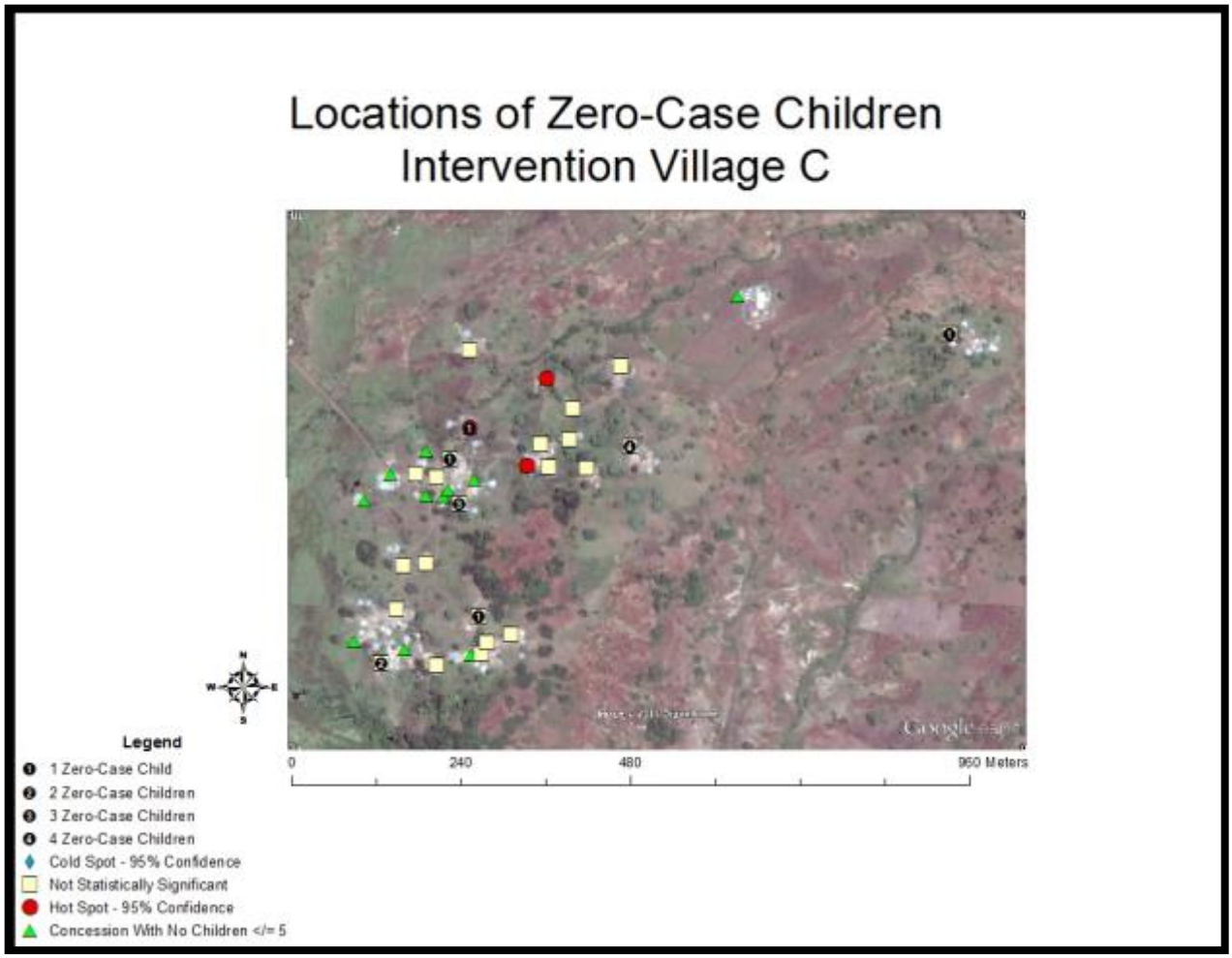

Figure 28- Locations of children ≤ 5 years old with zero reported malarial diagnoses throughout the study period in Intervention Village C (2C): There were seven concessions with children who had zero reported malarial diagnoses; four concessions had one zero-case child, one had two zero-case children, one had three zero-case children, and the final concession had four zero-case children. During geospatial analyses, one concession with one zero-case child was identified as a significant hot spot.

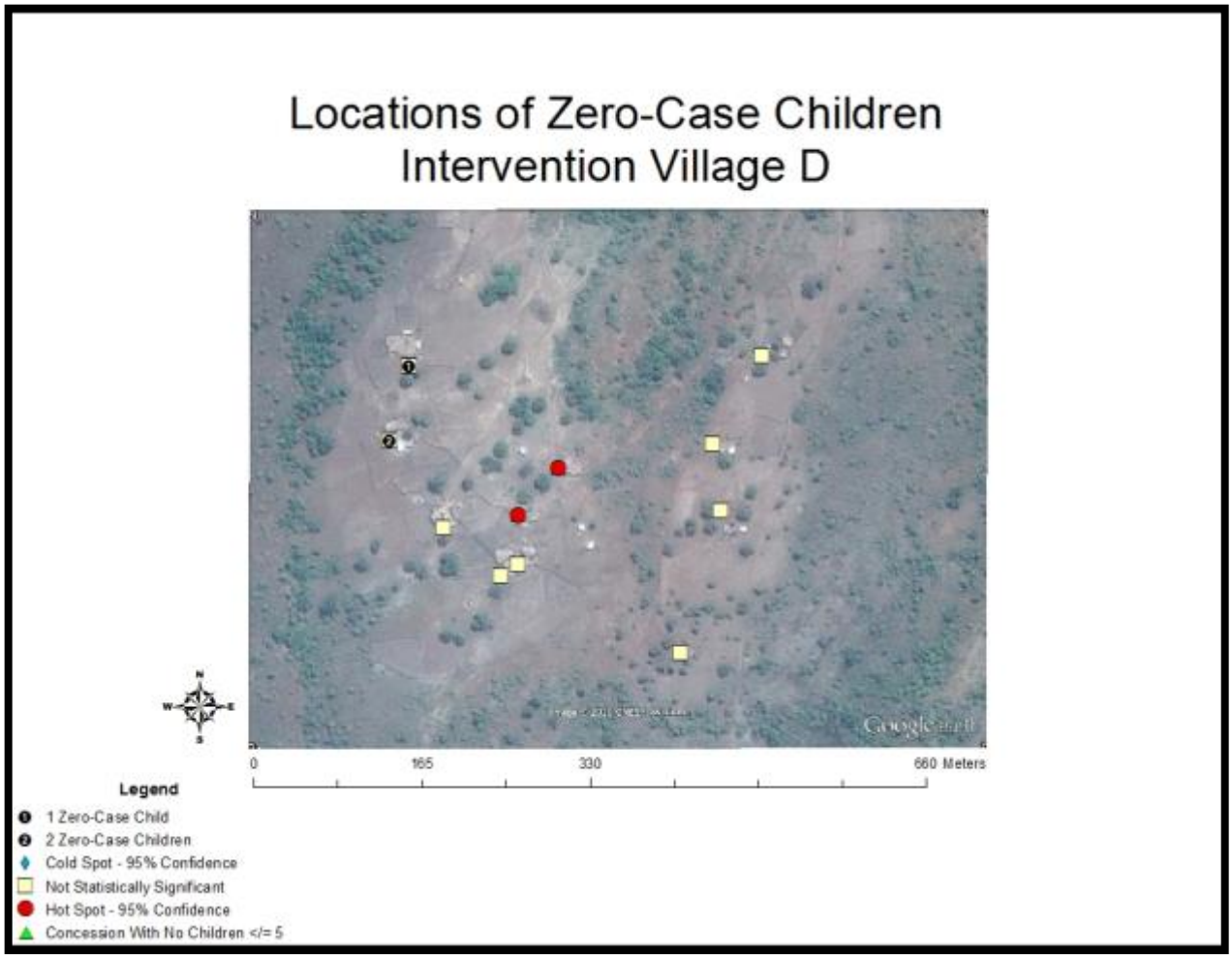

Figure 29- Locations of children ≤ 5 years old with zero reported malarial diagnoses throughout the study period in Intervention Village D (2D): There were two concessions with children who had zero malarial diagnoses; one with one child, and one with two children. Neither of these concessions were identified as a statistically significant hot spot during geospatial analyses.