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Predictive Factors for Households Positive for Lymphatic Filariasis after TAS-2/3
in Burkina Faso, Haiti, and Nepal

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An abstract of

A thesis submitted to the Faculty of the

Rollins School of Public Health of Emory University

in partial fulfillment of the requirements for the degree of

Master of Public Health

in Executive Master of Public Health

2021

Abstract

Predictive Factors for Households Positive for Lymphatic Filariasis after TAS-2/3 in Burkina Faso, Haiti, and Nepal

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Lymphatic Filariasis (LF) is a vector-borne parasitic disease. In 2000, the World Health Organization (WHO) launched the Global Programme to Eliminate Lymphatic Filariasis (GPELF). Under WHO guidance, endemic countries implemented multiple rounds of mass drug administration (MDA) to interrupt LF transmission across their communities. The goal is to reduce transmission to a level where transmission is no longer sustainable, eventually resulting in eliminating LF as a public health problem. After several years of MDA, a surveillance program called Transmission Assessment Survey (TAS) is repeated 3 times, spanning 4-6 years. TAS-1 is used to determine when MDA can be stopped. Subsequently, TAS-2 and TAS-3 are used to monitor and evaluate that LF prevalence has not exceeded the threshold in the absence of chemotherapeutic intervention. Despite passing TAS benchmarks, many countries are finding LF-positive children who would have lived the majority of their lives during MDA. LF-positive children indicate that transmission is on-going, albeit at low levels. Some regions have failed TAS-2 or TAS-3, indicating that interpretation of previous TAS results did not consider the full meaning of persistent LF-positive children at levels below the threshold. There is concern that isolated TAS-cases represent transmission "hotspots" or micro-foci. It is possible that TAS methodology is not sensitive enough, nor has enough predictive value, to consistently spotlight areas of focal transmission. If focal transmission is not detected, recrudescence of LF could occur. TAS-2/3 follow-up studies investigate the epidemiology associated with TAS-cases in regions that have passed TAS. This thesis seeks to determine if LF-positive children identified during TAS are indicators of "hotspots" in their communities. Specifically, the hypothesis tested is that households of TAS-cases are located within micro-foci with other households that have LF-positive individuals. The question tested is whether distance to the TAS-case household affects the likelihood that another household has at least one LF-positive member. logistical regression analysis on a sample population from Burkina Faso, Haiti and Nepal indicated that distance did not affect the likelihood that a household is LF-positive. However, the analysis yielded other predictive factors that could be applied to better surveille LF during TAS-2/3 follow-up.

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CHAPTER 1: INTRODUCTION

I. Introduction and Rational

Lymphatic Filariasis (**LF**) is a vector-borne neglected tropical disease caused by three parasitic filarial worms: *Wuchereia bancrofti*, *Brugia malayi* and *Brugia timori*. Mature worms reside in the lymphatic system of humans and impede lymphatic vessel function. Acute and recurrent symptoms of LF include swelling proximal to the affected site, pain, and fever lasting up to 2 weeks (Lourens & Ferrell, 2019). Mating female worms shed larvae, termed microfilarea (**Mf**), into the blood stream. However, Mf is not observed in all infected individuals. When present, kidney damage is observed in up to 45% of cases with bancroftian LF and is also common in Brugia cases (InfoNTD, 2021; Dreyer, Dreyer, & Piessens, 1999). Adult worms can cause long-term damage of lymphatic vessels and surrounding tissue leading to extreme swelling of proximal limbs (lymphedema) or proximal genitalia, usually at the testicular sac (hydrocele). Lymphedema and hydrocele are chronic physical disabilities and lead to psychological and social problems. Between 2007-2019, 1.1 million lymphoedema cases and 0.55 million hydrocele cases were reported to the World Health Organization (**WHO**) (WHO, 2020b).

An estimated 51 million people (95%CI: 42.9-63.4) were infected with a lymphatic filarial worm in 2018 (NTD Collaborators, 2020). Approximately, 70% of cases were in south-east Asia, 20% in Africa and 0.7% in America (NTD Collaborators, 2020). Subsequent estimations for 2019 indicate that 56% of cases were men (IHME, 2020). Hydrocele caused the majority of years lived with disability (**YLD**) due to LF in 2019. Overall, the estimated burden on health was 1.63 million disability-adjusted life years (**DALY**) (IHME, 2020).

In 1997, the World Health Organization (**WHO**) resolved to eliminate LF as a public health problem by the year 2020 under resolution WHA50.29. Therefore, in 2000, the Global Programme to Eliminate Lymphatic Filariasis (**GPELF**) was established to 1) interrupt LF transmission through mass drug administration (**MDA**) in endemic countries and 2) manage and prevent lymphoedema and hydrocele through the morbidity management and disability prevention (**MMDP**) program. The GPELF designated 72 endemic countries in need of MDA, amounting to 1.4 billion people at risk (Mathew et al., 2020). With guidance from the WHO, endemic countries began yearly MDA campaigns and surveillance for LF. By 2019, 17 of the GPELF countries obtained validation from the WHO as being free of LF as a public health problem, 50 were still undergoing MDA and 3 hadn't yet started MDA (WHO, 2020b). Significant progress was made in the Americas and Southeast Asia where 80% and 70% of targeted regions, respectively, no longer require MDA. However, 70% of targeted regions in Africa are still in need of MDA.

Recent epidemiological studies predicted that many countries would not reach the 2020 goal, mostly due to inaccurate estimates of baseline prevalence, programmatic complications due to co-endemicity with other parasitic worms and sub-optimal diagnostics (NTD Collaborators, 2020). Unfortunately, MMDP progress also lagged behind. Therefore, the timeline to achieve GPELF goals for 82% of endemic countries was extended to 2030 (WHO, 2020a).

Extension of GPELF allows for review and optimization of WHO guidelines, specifically, for monitoring and evaluation (**M&E**). Early on, M&E methods were complex, involving combinations of diagnostic tools and different age groups (Lammie et al., 2020). In 2011, the WHO introduced the Transmission Assessment Survey (**TAS**) as a simpler methodology for uniform M&E (WHO, 2011). TAS is primarily a decision-making tool to determine if MDA is no longer required to interrupt LF transmission. TAS measures LF prevalence of 6–7-year-olds. These children would have lived most of their lives under MDA with minimal chance of acquiring LF. If prevalence is at or below the target threshold, then the first TAS, "TAS-1", is passed and MDA can be stopped for that region. Subsequently, TAS is used two more times to confirm that prevalence is sustained at or below the threshold, 2-3 years apart. When all targeted regions in the country passed TAS-3 then the country can apply for validation from the WHO.

II. Problem Statement

Endemic countries have conducted 4,203 TAS in geographically defined evaluations units (**EUs**) between 2011-2019 (WHO, 2020b). In 2019 alone, 305 TAS were conducted but 10 failed at TAS1, 1 failed at TAS-2, and 3 failed at TAS-3. There has been numerous documented failed TAS prior to 2019 (Goldberg et al., 2019; Lau et al., 2020). Failed TAS-2/3 indicate that transmission is on-going at a level that allows LF prevalence in children to increase since the previous TAS. Furthermore, failed TAS-2/3 are the result of an opportunity lost in predicting recrudescence of LF through the current M&E methodology, applied in the previous TAS. One reason for failed TAS-2/3 could be that the methodology is not sufficiently sensitive to detect small pockets of significant on-going transmission, "micro-foci", in a region with low prevalence. TAS measures prevalence in an EU through cluster-sampling using schools or communities as the primary sampling unit. When most clusters have zero LF-positive children, the implications for the few clusters that have positives is dampened in the favorable TAS result. The community represented by a TAS-case in a cluster may have significant on-going transmission. Without follow-up on TAS-cases, there is no information to support or reject than an LF micro-foci is located in a community.

Many EUs pass TAS despite identifying positive cases. WHO recommends that TAS-cases receive treatment. WHO also suggests follow-up investigation of TAS-cases by confirming LF positivity via a more definitive diagnostic test and performing a follow-up survey in the community represented by TAS-cases. However, there isn't yet evidence-based guidance on how to conduct follow-up surveillance of TAS cases from a *passed* TAS. This thesis seeks to provide information on how to increase the positive predictive value of TAS follow-up surveys by assuming that TAS-cases represent micro-foci in their communities.

III. Theoretical Framework

LF is a vector-borne parasitic disease. As a parasite, lymphatic worms require a definitive host for sexual reproduction (humans) and a vector host for asexual reproduction and transmission (mosquitoes). The parasite goes through various morphological stages through its life cycle (**Figure 1**). Mature worms mate in the lymphatic system and shed millions of larvae. Mf cross into blood vessels, exhibiting periodic circulation, either nocturnally or diurnally. Microfilariae are ingested by mosquitoes during their blood-feed on infected humans. In the vector, the parasite goes through several developmental stages while migrating from the gut to the proboscis (Gary & Lance, 2019). The human -infective stage of the parasite, L3 larvae, enters the skin when the mosquito takes its next blood meal on another mammal. It can take up to 2 years for microfilaremia to be apparent and for the transmission cycle to continue.

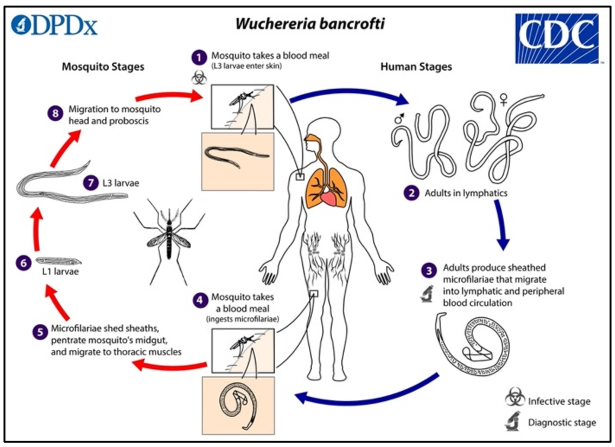

Figure 1: *W. bancrofti* life cycle. The life cycle of *Brugia spp.* is similar. (CDC, 2019).

The mosquito species of relevance depends on the region. In general, LF is transmitted by mosquitoes from 4 genera: *Aedes*, *Anopheles*, *Culex* and *Mansonia*. Anopheles, Culex, and Mansonia mosquitoes are night-biting insects, while some Aedes mosquitoes are active during the day and night (Gary & Lance, 2019). Most transmission occurs through the acquisition of nocturnally periodic Mf by mosquitoes, with the exception of *W. bancrofti* transmission by *Aedes* species in the South Pacific. Transmission therefore depends on the feeding habits, ecological setting, and flight range of infected vectors. For example, maximum flight of *Anopheles gambiae* is 10 km after a blood meal, but only 3 km when starved (Kaufmann & Briegel, 2004). It is therefore probable that significant transmission occurs at night and therefore in or around the household of infected individuals.

Previous work observed that the prevalence of LF-infected people is highest amongst members of TAS-case households compared to the surrounding community (Lau et al., 2020). Other work showed that households with LF-positive individuals tend to be within defined geospatial boundaries (Stanton et al., 2013; Lau et al., 2014) and are within small distances from each other (Drexler et al., 2012). Additionally, previous work showed that households with elevated LF-infected vectors inside the home, commonality in structural material, and elevated breeding site in the home were associated with "hotspot" communities. (Subramanian et al., 2017; Srividya, Subramanian, Sadanandane, Vasuki, & Jambulingam, 2018). Overall, these findings suggest that LF-positive individuals and vectors are concentrated in a geospatial pattern at the household-level and within communities.

IV. Purpose Statement

Based on the aforementioned arguments, this thesis assumes that households of TAS-cases are representative of a micro-focus in their community. The hypothesis of this thesis is that households with close proximity to a TAS-case are more likely to harbor an LF-positive individual than households further away in the same community during follow-up of TAS-2/3. The null hypothesis is that distance to TAS-index homes does not influence the probability of harboring an LF-positive individual during follow-up. The thesis will analyze data from a multi-country operational research project on follow-up to TAS-cases identified during *passed* TAS-2 or TAS-3. The operational research was conducted with Ministry of Health partners in Burkina Faso, Haiti, and Nepal and supported by the NTD-SC, Task Force for Global Health, Decatur, GA.

V. Research Questions

The two main research questions are:

1. Are households in closer proximity to TAS-case households at greater or lesser odds of harboring another LF-positive individual?
2. Do the following characteristics of a household member (work in agriculture; travel in the past 12 months; participation in MDA; use of bed net last night) or GPELF status of the household (in a community represented by a TAS-case (index-communities) versus other communities; in an EU post TAS-2 versus TAS-3) predict that the household harbors at least one LF-positive individual?

These questions will be addressed in Burkina Faso, Haiti and Nepal.

VI. Significance Statement

The knowledge acquired from this analysis will help guide TAS follow-up surveys by providing evidence to support preferentially sampling households within a certain distance from TAS-case homes. The results would support that TAS-2/3 cases represent micro-foci of LF cases in their community. If the probability of finding a positive individual is highest closer to a TAS-identified child, then the positive predictive value of follow-up survey would be higher than random sampling, especially in geographically large communities. Because of years of MDA, prevalence is low in most communities, so increasing positive predictive value (PPV) would save resources and time. Aside from proximity to the TAS-household, other predictive factors identified in this analysis would help further prioritize the sampling criteria during follow-up.

VII. Definition of Terms

Terms are defined within the text. When appropriate, abbreviations are introduced at the first mention of the term in each chapter. Key terms are highlighted below.

- Cluster sampling: Sampling strategy using one of many groups of children at different primary sampling unit locations, i.e. school or community, to represent the target population of the EU.
- Community: A distinct group of people or area defined by societal structure and geographic boundaries, such a village or town.
- Evaluation unit: A geographically defined region in a country, usually composed of at least one governmental province or health district, made up of 1 or many distinct communities, that undergoes TAS together. TAS results are applicable to the evaluation unit as a single entity.
- TAS follow-up: Subsequent investigation of individuals in the same home community as a TAS-case ("index-community"), which may be different from where TAS took place if sampling was done by school cluster. Investigation may entail administering a questionnaire and assessing LF status of community members in index-communities.

- Household: A domicile consisting of individuals that primarily reside there and consented to the operational research study.
- Household parameter: A characteristic of the domicile or of at least one member of the household.
- Index-community: A geographically distinct community in which a TAS-case (index-case) resides, which may be geographically separate from the school community if TAS was performed by school cluster sampling.
- Index-case: An LF-positive child identified during TAS, a TAS-case, that is the basis for follow-up research.
- micro-foci: A small area relative to a community or an EU with elevated LF prevalence than the broader region.
- Neighbor community: A geographically distinct community in an evaluation unit from where zero TAS-cases reside, either because the schools that these children attend were not tested in TAS, or because none of the LF-positive children identified during TAS live in these communities.
- TAS-case: an LF-positive child (6-7 years old) diagnosed during TAS.

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CHAPTER 2: REVIEW OF LITERATURE

Public health campaigns require robust monitoring and evaluation **(M&E)** strategies that include effective surveillance with high positive predictive value and sensitive and specific diagnostic tools to assess the progress of the intervention.

I. Intervention

Under the Global Program to Eliminate Lymphatic Filariasis **(GPELF)**, mass drug administration **(MDA)** targets at-risk individuals in endemic countries to reduce microfilaremia in the population and thereby interrupt vector-borne transmission. MDA is usually performed annually, for a minimum of 5 years, with 65% coverage of the target population. The WHO recommends "triple therapy", a combination of Diethylcarbamizine citrate, ivermectin and albendazole (**DEC+IVM+ALB**), where appropriate, as this regimen has the highest effectiveness in clearing the infection (C. L. King et al., 2018). Prior to 2018, the WHO recommended DEC+ALB for regions non-endemic with other filarial worms, namely *Onchoccerca volvulus* and *Loa loa* (J. King & Toubali, 2017). Regions co-endemic with *O. volvulus* were to administer IVM+ALB. Ivermectin is active against the microfilariae (**Mf**) form of both parasites, but not adult worms. Regions co-endemic with *L. loa* are discouraged from using either DEC or IVM. As a result, albendazole monotherapy is recommended for MDA, twice a year. Unfortunately, there is conflicting evidence that albendazole monotherapy is very effective (Critchley et al., 2005; Horton, 2009; Macfarlane, Budhathoki, Johnson, Richardson, & Garner, 2019).

II. Diagnostics

The most definitive manner to confirm that an individual is infected and infectious is through visualization of Mf through blood smear microscopy. However, this method has low sensitivity and is relatively resource intensive. Microfilariae density is often low and due to the nocturnal periodicity of most lymphatic filarial worms, requires nighttime blood collection. Additionally, microscopy requires technical training and is laborious for a national surveillance program.

Assay-based diagnostics are more sensitive than microscopy. Some assays can be performed at the point-of-collection, anytime, and interpreted without further equipment. ELISA-based assays, however, require laboratory processing. Commonly used assays that detect *W. bancrofti* adult worm circulating filarial antigens (CFA) in human blood are:

- ICT (Immunichromatography card test, BinaxNow®, Alere, Inc., Scarborough, ME). Recommended by the GPELF in 2000, but now discontinued.
- FTS (Filarial test strip, Alere Inc., Abbott). Introduced in 2013 and recommended by GPELF.
- Og4C3 (Tropical Biotechnology, Cellabs). ELISA-based assay.

Assays that detect the presence of specific *W. bancrofti* anti-filarial antibodies in human blood include:

- Wb123 ELISA (InBios Inc.)
- Bm14 ELISA. Cross reacts with *B. malayi.*

Both assay-targets have limitations (Helmy et al., 2006; Ravindran, Satapathy, Sahoo, & Babu Geddam, 2000; Won et al., 2018). CFA may persist when worms are no longer producing Mf or the infection has been cleared. Therefore, individuals who are no longer perpetuating transmission may still be CFA-positive. Similarly, the dynamics of antibody-mediated immunity doesn't allow for discriminating between prior and current infections, as CFA results do not always match LF-specific antibody results. However, antibody detection is more sensitive than antigen detection. Several studies have compared and contrasted various diagnostic methods for surveillance purposes. In general, prevalence by Mf is lowest, followed by antigenemia, then serology (Lau et al., 2017; Lau et al., 2014; Riches, Badia-Rius, Mzilahowa, & Kelly-Hope, 2020). The WHO recommends M&E by CFA (antigenemia), but as prevalence decreases, diagnostic accuracy is crucial to detect on-going transmission in potential microfoci.

III. Transmission Assessment Survey (TAS)

Throughout MDA, LF surveillance entails individuals $\geq$5-years-old at sentinel sites and spot check sites. WHO guidelines state that after the 5th round of MDA, implementation units (**IU**) are eligible to determine if MDA can be stopped. In most regions, prevalence must be below 2% antigenemia or 1% microfilaremia in sentinel sites and spot check sites to proceed.

The Transmission Assessment Survey (**TAS**) is the decision tool to determine if MDA is no longer required to maintain LF prevalence below a certain threshold, and thereby interrupt transmission in the IU. The results of the first TAS, "TAS-1", determines if MDA can be stopped.

TAS is subsequently used to surveille sustained low prevalence in IUs, 2 more times, 2-3 years apart, until elimination of LF in the country is validated by the WHO.

TAS is conducted in distinct evaluation units (**EU**). The TAS manual for Programme Mannagers provides explicit details on M&E (WHO, 2011). An EU may encompass multiple IUs with similar baseline prevalence, MDA coverage, pre-TAS prevalence, causative parasite and vector, but may not exceed 2 million individuals. The target population for surveillance is 6–7-year-old children because they have lived the majority of their lives under MDA intervention. LF-positive children of that age indicate on-going transmission despite MDA. The prevalence threshold for the sample population must fall below 1% in regions where *Aedes spp.* transmit the parasite, or below 2% for *Culex spp.* or *Anopheles spp.* vectors.

The Neglected Tropical Disease-Support Center (**NTD-SC**) at the Task Force for Global Health developed a Survey Sample Builder (**SSB**) algorithm that determines optimal sampling methodology and sample size based on EU characteristics (TFGH, 2009). TAS can be performed through cluster or systematic sampling per EU. The primary sampling unit is either schools (1^{st} & 2^{nd} grade) or households throughout the community/ enumeration area (**EA**). Systematic sampling is recommended if an EU has less than 40 schools or 40 communities/EAs. Sampling at schools is recommended if primary school enrollment is at least 75%. TAS is designed to give an EU ≥75% chance of passing TAS if the number of cases is half of the cut-off, and ≤ 5% chance of falsely passing TAS if the number of cases is or exceeds the cut-off.

In cluster sampling, each school or community/EA constitutes a cluster. SSB randomly selects the clusters. Cluster sampling requires fewer sites, but more children. Systematic sampling entails sampling from every school or every community/EA in the EU. Systematic sampling requires fewer children but usually requires a lot of resources based on the geographical distribution of the EU. Alternatively, TAS can be performed by census sampling and entails surveying every eligible 6–7-year-old child in the EU. This is appropriate only for small EUs where target population is small (400-1,000 depending on the vector).

SSB provides a critical cutoff number of LF-positive children for each EU. If the number falls at or below this number, then the EU "passes" TAS. If not, the EU must undergo 2 rounds of MDA and reassess TAS-1 qualifications by pre-TAS methodology (sentinel sites and spot checks). Diagnosis for *W. bancrofti* is with FTS and for *Brugia spp.* parasite is with the Brugia Rapid™ test.

IV. LF Cases after MDA Years

One of the main threats to GPELF success is unnoticed microfoci of LF transmission despite adherence to M&E guidelines. Since children from geographically distinct communities converge at school, there is concern that a positive child identified at school during TAS represents on-going focal transmission in the specific community in which he/she lives.

Community indicators

Since failed TAS-2 and TAS-3 may be a result of undetected LF focal transmission due to the TAS cluster-survey design, we can study these instances to inform follow-up strategies and interpretations. An analysis of 65 failed TAS -1, 2, and 3 out of 746, across 39 countries, showed that TAS failure was statistically associated with co-endemicity of *W. bancrofti* and *Brugia spp*., baseline prevalence ($\geq$5%), population density ($\geq$5,000 per 5 km^2), low nighttime lights, and low elevation (< 200 m) (Goldberg et al., 2019). Another research teams also found that baseline prevalence (≥5%) and low elevation (<350 m) were associated with pre-TAS failure in 47 out of 554 *W. bancrofti* endemic IU across 13 countries (Burgert-Brucker et al., 2020). Furthermore, baseline LF prevalence in Burkina Faso communities was shown to be linked to low elevation (Stanton et al., 2013).

Individual indicators

Various studies were conducted to determine what individual-level factors are associated with LF-positive individuals despite living in EUs that have undergone years of MDA and passed the TAS-1 benchmark. In American Samoa, individuals that were LF-positive upon follow-up of a TAS-3 failure were more likely to be male, adults (≥18 years old), living in communities with higher prevalence than surrounding communities, did not work indoors, and hadn't traveled in the last year (Lau et al., 2020). Interestingly, this same study found that a greater proportion of index household members of the TAS-3 cases were LF-positive compared to the rest of the community members, indicating a clustering of positive cases within index households. Additionally, the same study found no significant difference between LF prevalence in index communities compared to other communities in the EU by randomly surveying households. This could suggest that when an EU fails TAS, LF prevalence from which an identified TAS case lives is a fair representation of other communities in the same EU.

Proximity to TAS-case households

Follow-up surveillance of TAS cases in EUs that passed TAS can be optimized by understanding the geographical distribution of LF-positive individuals in index communities. It is important to acknowledge that TAS cases identified in school-based cluster surveys may not represent the epicenter of LF infections in their respective communities. However, previous studies show that LF-positive individuals form microfoci within the community, beyond just their households (as mentioned above). For example, a study of 6 Haitian villages after 7 years of MDA found that households with ICT-positive members cluster significantly in groups of 2, 3, 4, and 5 for *W. bancrofti* infections (Boyd et al., 2010). This could not be explained by shared attitudes towards MDA participation, as non-compliance clustering at the household level was not observed. Another study in Haiti found that even in non-endemic communities, as defined by the GPELF, households within 20 meters of an LF-positive child were 5.41 times more likely to have another ICT-positive person compared to $\geq$100 meters away (Drexler et al., 2012). Meanwhile, studies in American Samoa found that Og4C3 positive individuals are within 1.2-1.5 Km clusters (Lau et al., 2014). The different size of proposed foci may be due to the different vectors in Haiti (*Culex spp.*) compared to American Samoa (*Aedes spp.*).

Harris and Wiegand simulated various models to conduct follow-up on LF-positive individuals as a way to find foci (Harris & Wiegand, 2017). Their models simulate various approaches such as follow-up by cluster sampling versus simple random sampling, and different age groups, diagnostic test, and definitions of micro-foci. Their analysis showed that the optimal approach in identifying a 1-Km LF foci in a 30 Km^2 region with 3x background prevalence is by simple random sampling of 20-100 nearest adults or women of child-bearing age by ICT. This would be equivalent to surveying 2% of the target population and finding 1-6 LF-positive individuals to declare a micro-focus. This sampling strategy based on 20-100 nearest adults was 80% sensitive in finding micro-foci.

Overall, several lines of investigation suggest that LF cases are geographically bound in micro-foci within households or within communities. Targeting individuals who represents on-going transmission, such as a TAS-case, may be a way to increase positive predictive value of follow-up surveys and discovering micro-foci in communities.

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CHAPTER 3: METHODS

I. Introduction

This thesis entails secondary data-analysis of three datasets concerning a multi-country operational research study funded by the Neglected Topical Disease-Support Center (**NTD-SC**) at The Task Force for Global Health, Decatur Georgia. The datasets are the result of operational research regarding communities that have *passed* TAS-2 or TAS-3 but detected at least 1 circulating filarial antigen (**CFA**) positive child in the process. The primary goal of the TAS-2/3 follow-up operational research was to determine which sampling method (purposive or random) is most efficient at understanding the scope of Lymphatic Filariasis (**LF**) prevalence in communities with CFA-positive children detected in the previous TAS. The datasets were obtained after the field studies were closed and this thesis did not require IRB approval for secondary analysis.

II. Population and Sample

The population concerning this analysis are household members located in evaluations units (**EU**) that have passed TAS-2/3 while identifying at least 1 TAS-case. The sample is household members from such EUs in Burkina Faso, Haiti, and Nepal.

Burkina Faso

Burkina Faso is a West African country with a population of approximately 20.8 million (ESPEN, 2021). Neglected tropical diseases (**NTD**) and malaria were the 2nd largest cause of disability-adjusted lost years (**DALY**) in 2019 (IHME, 2020). Life expectancy at birth is estimated to be 61.6 years, while the youth ($\leq$ 14 years old) make up ~45% of the population (World Bank Group, 2021). In this region, *W. bancrofti* is endemic and primarily transmitted by *Anopheles gambiae*. National baseline prevalence was estimated at 29.2%, and up to 74% in one health district (Stanton et al., 2013).

NTD programs are administered in 70 distinct implementation units (**IU**). Mass drug administration (**MDA**) began in 2001 and entails yearly ivermectin + albendazole (**IVM+ALB**). MDA reached full national coverage of targeted health districts in 2006 (Stanton et al., 2013). As of 2019, 9 IUs (~2.1 million people) were still actively undergoing MDA against LF (WHO, 2020). The rest of the country is under post-MDA surveillance.

This thesis analyzes work done in 3 Burkina Faso EUs during April-May 2019. Léo-Sapouy and Boromo-Dédougou underwent 10 and 11 rounds of MDA, respectively, and performed TAS-3 in 2018. Central Plateau underwent 10 rounds of MDA and underwent TAS-2 in 2017. Two TAS-cases were detected in each EU.

<u>Haiti</u>

Haiti is a country in the Caribbean with a population of approximately 11.2 million (WHO, 2021). NTDs and malaria rank as the 21st cause of disease burden (IHME, 2020). Life expectancy at birth is estimated at 64 years, while the youth ($\leq$ 14 years old) make up ~33% of the population (World Bank Group, 2021).

In the Americas, LF is caused by *W. bancrofti* and transmitted by *Culex quinquefasciatus* (Fontes, da Rocha, Scholte, & Nicholls, 2020). Ninety percent of the 12.6 million individuals at risk in the 4 endemic South American countries are in Haiti (WHO, 2020). Average baseline prevalence in Haitian children was estimated to be 7.3% antigenemia (Beau de Rochars et al., 2004). There were 117 endemic communes out of 133. Haiti began MDA in 2002 with DEC+ALB, and reached full geographical coverage by 2012 (Fontes et al., 2020). In 2019, 5.9 million individuals lived in 21 IUs still requiring MDA (WHO, 2020).

This thesis analyzes data from a follow-up study to 8 TAS-2 identified LF+ children in their communities across a single EU during July-August, 2019. The EU of Nippes conducted 4 rounds of MDA. More cases were identified during TAS-2, in 2017, compared to TAS-1, in 2015, despite passing both assessments.

Nepal

Nepal is a Southeast Asian country with a population of 28.6 million as of 2019 (World Bank Group, 2021). NTDs and malaria ranked 21st as causes of diseases burden, causing ~ 331 per 100,000 DALYs (IHME, 2020). Life expectancy at birth was ~ 70.8 years (World Bank Group, 2021). The youth ($\leq$14 years old) made up 29.6% of the population.

Measurements of baseline prevalence in 7 health districts showed antigenemia prevalence of 1.06-20% (Ojha et al., 2017). Nepal has 75 health districts of which 61 were deemed endemic for LF, resulting in 65 million people were at risk. In 2003, the government of Nepal initiated MDA in Parsa district and reached all endemic districts by 2013. As of 2019, over 7.8 million people in 15 IU were still actively undergoing MDA with DEC+ALB. These IU have undergone at least 8 rounds of MDA, and most have failed pre-TAS and TAS multiple times (WHO, 2020).

This thesis analyzes TAS-3 follow-up research conducted during October-November 2019 in 2 EUs (Provinces Bagmati and Lumbini). Both passed TAS-3 in 2018, while identifying 8 LF cases. Two of those TAS-cases were identified in one school cluster in Chitwan health district in Bagmati Province. Follow-up occurred in the two index-communities for the cases (Chitraban/Chitrawan and Nawadurga/Navadurga) (**Supplemental Table 4**).

III. Research Design

The research teams collected blood samples and answers to a questionnaire in communities that were identified during TAS-2/3 as having at least 1 CFA-positive child, termed "**index-communities**". The household of TAS-identified LF-positive children were termed "**index-households**". The survey was administered to all household members who consented or assented and were $\geq$ 2-years-old. Individuals from index-households, the 50 nearest households to the index-household, and 20 randomly selected households were also surveyed in the community. Random sampling did not occur in Nepal. Household member's CFA status was determined via the Filariasis test strip (**FTS**, Alere Inc.), a rapid point of care test. In Burkina Faso and Nepal, the research team extended random sampling to adjacent communities, termed "**neighbor-communities**" if a CFA-positive person was detected in the index-community. In the final dataset for this analysis, only 2 index-communities had no CFA-positive individuals, Silmi Mossi, Burkina Faso and Maina Bagar, Nepal (**Supplemental Table 2 and 4**). In neighbor-communities, household members from 20 randomly selected households were surveyed. All CFA-positive individuals were offered anti-filarial medication.

Data collection took place in Burkina Faso, Haiti and Nepal. In Burkina Faso, 16 communities were surveyed across 3 evaluation units (**EU**). Central Plateau passed TAS-2 with 2 TAS-cases in 2017. Boromo-Dédougou and Léo-Sapouy passed TAS-3 with 2 TAS-cases each in 2018. In Nepal, 25 communities across 2 EUs that passed TAS-3 in 2018 were surveyed. Province 3 had 3 TAS-case. Province 5 had 5 TAS-cases. In Haiti, 5 communities were sampled from 1 EU. The department of Nippes passed TAS-2 in 2017 with 8 TAS-cases. Overall, 46 communities were surveyed, including 19 index-communities (**Table 1** and **Table 11**).

IV. Instruments

Three datasets were imported into SAS Studio statistical software, version 9.04 of the SAS OnDemand for Academics. Copyright © 2012-2018 SAS Institute Inc. SAS and all other SAS Institute Inc. product or service names are registered trademarks or trademarks of SAS Institute Inc., Cary, NC, USA.

V. Procedures

Individual-level dataset preparation

Non-consenting and non-assenting individuals were removed from the dataset.

Erroneous data entry was corrected based on spelling and logical inference and sequence of the questionnaire. Missing household-level values were filled-in based on answers from other household members (i.e. community name, GPS coordinates, index/neighbor community). If answers for household-level questions deviated, then the majority answer was adopted to establish a consensus per household (i.e. GPS coordinates). Individual-level questions pertaining to occupation were cleaned up based on spelling errors and categorized into agricultural work (**Agricultural worker**= y/n), which includes gardener, farmer/farming, fisherman/fishing, and shepherd.

Numeric variables were created for character variables, where applicable. Individuals with missing or ambiguous answers to the following questions were excluded: Did you ever participate in MDA medication? (**MDA participation**= y/n); Did you sleep under a bed net last night? (**Slept under bed net**= y/n); Have you traveled outside of your community in the past 12 months? (**Traveled in last 12 months**= y/n); CFA status (**CFA-positive**= y/n) was defined by FTS result. Individuals with inconclusive FTS results were excluded for this data analysis. The distance to the closest TAS-index household in meters (**Distance to index-household**) was calculated in kilometers using the *geodist* function in SAS statistical software and converted to meters. Index-households were set at distance = 0 meters.

The three cleaned datasets were merged into one multi-country individual-level dataset (**Supplemental Table 1**).

Household-level dataset preparation

Household-level datasets were established from the multi-country individual-level dataset. Household parameters were created to reflect that at least one member of the household has an affirmative value for each variable of interest. Separate household-level datasets were created for each variable of interest and merged with a master household-level dataset containing the common values for each household (i.e. GPS coordinates, GPS-accuracy, distance to index, community name, index or neighbor community, TAS-level, country). To maximize GPS accuracy while retaining all index-households, only observations with GPS accuracy $\leq$ 17 meters were included in the analysis (**Supplemental Table 1**).

VI. Data Analysis Methodology

Distance to index-household analysis

The following additional exclusions were applied to the household-level dataset to analyze household CFA status and distance to TAS-index homes in post-TAS-2/3 index-communities: 1) Households in neighbor communities, and 2) index-households.
Two-proportion chi-square and Wilcoxon sum of ranks tests were performed to compare parameters of households with negative and positive CFA status. Conditional generalized linear logistic regression was conducted with stratification for country where noted, using the “strata” statement in SAS statistical software. Assessment for effect modification on the primary exposure (distance to a TAS index-household) was calculated through likelihood ratio test by “chunk” test and then individually, if appropriate (Kleinbaum, Kupper, & Morgenstern, 1982). Full models and reduced models (performed with backward elimination or score elimination for

variable selection holding the primary exposure and relevant interaction terms) are presented. Best reduced models were chosen based on improvement in Akaike information criterion (**AIC**) i.e. decreased by 2 units, to determine goodness of fit.

Exploratory analysis for predictive factor

To study parameters of all households in index-communities, including index-households, the following modifications were made to the initial household-level dataset described above. 1) Households in neighbor communities were excluded. 2) Index-households where no other member was CFA-positive aside from the TAS-case were designated CFA-negative. This was to ensure household CFA-status is not influenced by the TAS index-case. (There were 4 index-cases in 4 households that remained CFA-positive during follow-up in Burkina Faso. In 2 of those households, no other individual was CFA-positive. Therefore, those 2 index-households were designated CFA-negative.)

No modifications were made to the household-level dataset as described above for analysis of index and neighbor communities.

Two-proportion chi-square, Wilcoxon sum of ranks tests, and generalized linear logistic regression were performed as mentioned above. Pseudo-R-square values were also considered in choosing the best model for predictive variables.

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CHAPTER 4: RESULTS

I. Description of Index-Community Households

The shortest distance to the home of a TAS-case (index-household) was determined for 1,334 households in 6 communities in Burkina Faso, Haiti and Nepal (**Table 1**). Households of TAS-cases (index-households) were excluded from the analysis. Overall, 66 households (4.95%) had at least 1 CFA-positive individual. The majority of CFA-positive households were in Nepal (27 out of 342; 7.89%), followed by Burkina Faso (23 out of 370; 6.2%) and Haiti (16 out of 622; 2.57%).

The range of distances to the closest TAS index-household was smallest in Nepal (0.51-364 m, **Table 1** and **Figure 2**). The range of distances to the closest index-household in Burkina Faso and Haiti were greater (7.8 - 11,538 m and 4.0 - 11,743 m, respectively, **Table 1** and **Figure 2**). Two communities, Bassenere + Boukuy and Pettit Trou de Nippes had distribution ranges that deviated from the rest of communities (**Figure 2**). However, multivariable logistic regression performed without these 2 communities did not notably alter the results, therefore the analysis includes them.

GPS accuracy is critical to this analysis and is preferably within a margin of error $\leq$ 5 meters. To minimize errors in GPS measurements but retain all index-households in the analysis, GPS accuracies >17 were excluded from the analysis. As a result, median GPS accuracy measurements were similar between Burkina Faso, Haiti and Nepal, 5.0, 5.0, and 4.6, respectively (**Table 1**). However, Nepal had greater variation in GPS accuracy readings, 3.2 - 16.1

(**Table 1**). In Nepal, poor GPS accuracy was confined to 3 communities. GPS accuracy was not significantly different for CFA-negative and CFA-positive households.

Overall, CFA status of households was significantly associated with several parameters (**Table 2**). A greater proportion of CFA-positive households had at least one resident that worked in agriculture (39% versus 28%, p-value= 0.0474), at least one resident that slept under a bed net the previous night (55% versus 42%, p-value= 0.0411), were in post-TAS-3 EUs compared to TAS-2 (58% versus 33%, p-value <0.0001), and were within a 300-meter radius of an index-household (73% versus 56%, p-value= 0.0060). The median distance to index-households is significantly lower for CFA-positive households compared to CFA-negative households (139 m versus 241 m, p-value= 0.0024). The proportion of households with travel history outside of the EU or participation in MDA did not differ by CFA status. Similarly, the median altitude was not significantly different between household CFA status.

In Haiti, the median distance to a TAS index-household was significantly different between positive and negative CFA households (124 m versus 380 m, p-value= 0.0036, **Table 3**). In Burkina Faso and Nepal, the median distance did not differ significantly (**Table 3**).

II. Multi-Country Logistic Regression of Distance on CFA-Positivity

Univariable logistic regression was performed for various household parameters. Distance from an index-household did not affect the probability that a household is CFA-positive (p-value=0.0621, **Table 4**), nor did travel history, MDA participation or altitude. However, households that have a resident that works in agriculture, or use of a bed net, or are located in a post TAS-3 EU were at greater odds of having a CFA-positive individual (**Table 4**). Conditional univariable logistic regression, stratified by country, showed that 10-meter increases in altitude decreases the odds that the household is CFA-positive by 4% (95%CI:0.93 – 1.00, p-value= 0.0381; **Table 4**). However, no other parameter had a significant effect on the likelihood of CFA-positivity, including distance to index-households. Because of the country effect demonstrated in univariable analysis and because of the notable difference in distances from index-households per country (**Table 1** and **Figure 1**), country-adjusted multivariable analyses was conducted alongside separate analysis for each country.

Conditional multivariable logistic regression, stratified by country, was performed (**Table 5**). Effect modification was first assessed by "chunk test" for the primary exposure, distance to an index-household. No interaction terms were significant (Likelihood Ratio (LR) statistic = 5.312, df=5, p-value= 0.3790; **Table 5**) and were dropped to form a full model without interaction (Logit {P [CFA-positive= 1]} $_{country}$ = β_1 (Distance) + β_2 (Agriculture) + β_3 (Travel) + β_4 (MDA) + β_5 (Bed net) + β_6 (Altitude) + ε). Other co-variates were dropped from the model to achieve the best goodness of fit (Logit {P [CFA-positive= 1]} $_{country}$ = β_1 (Distance) + β_2 (Altitude) + ε). Distance from an index-household did not affect CFA-positivity. However, households were 4% less likely to be CFA-positive with each 10-meter increase in altitude (OR [95%CI] = 0.95 [0.92 – 1.00], p-value=0.0304, **Table 5**), controlling for distance.

III. Logistic Regression of Distance on CFA-Positivity by Country

In Burkina Faso, the odds that a household was CFA-positive increased ~3-fold (95%CI: 1.26 - 9.52, p-value 0.0164, **Table 6**) if an agricultural worker lives there, and decreased by 17% (95%CI: 0.69 - 0.99, p-value = 0.041, **Table 6**) for each 10-meter increase in altitude. No other household parameter (distance to a TAS-household, travel history, MDA participation, and TAS-level) were significant in univariable logistic regression. A full interaction model was built for the primary exposure, distance to a TAS index-household. Effect modification was assessed by likelihood ratio test. At least one interaction term was significant compared to the "no interaction" model (LR statistic= 14.472, df=7, p-value=0.0248). Sequential elimination of the least significant interaction term and LR testing was performed to identify that effect modification by TAS-level (TAS-3 versus TAS-2) is the only interaction term that contributes significantly to the model compared to the "no interaction" model (LR statistic=5.527, df=1, p-value=0.018, **Table 7**). In this model (Logit {P [CFA-positive= 1]} = $\beta_0 + \beta_1$ (Distance) + β_2 (Distance)*(TAS-3) + β_3 (TAS-3) + β_4 (Agriculture) + β_5 (Travel) + β_6 (MDA) + β_7 (Bed net) + β_8 (Altitude) + ε), the odds that a household is CFA-positive is increased 4-fold (95%CI: 1.39 - 11.95, p-value 0.0107, **Table 7**) if a member works in agriculture, and decreased by 21% with every 10-meter increase in altitude (95%CI: 0.664 - 0.946, p-value 0.011, **Table 7**), controlling for travel history, MDA participation, bed net use and distance to an index-household, post-TAS-2. However, in index-communities post-TAS-3, those odds are further decreased by 16% for each 100-meter of distance away from an index-household (95%CI: 0.71 - 1.00, **Table 7**).

In Haiti, TAS follow-up surveys were performed only in communities post-TAS-2. Univariable logistic regression for household parameters were not significant, including distance to an index-household. Analysis for agriculture was omitted because no CFA-positive households had an agriculture worker, making this analysis uninformative (**Table 6**). A multivariable analysis was performed by first assessing interaction with the primary exposure (distance to an index-household). No effect modification was detected (LR statistic = 1.472, df=3, p-value=0.6887) and therefore all interaction terms were removed from the model. The best reduced model was determined by score variable selection, retaining "distance" (Logit {P [CFA-positive= 1]} = $\beta_0 + \beta_1$ (Distance) + β_2 (Travel) + β_3 (Bed net) + β_4 (Altitude) + ε). This model indicates that none of the covariates (travel history, MDA participation, bed net use and altitude) nor distance to an index-household affect the probability that a household is CFA-positive (**Table 8**).

In Nepali index-communities, univariable logistic regression showed that none of the parameters (agriculture worker, travel history, MDA participation bed net use or altitude) were significant, including distance to an index-household (**Table 6**). Multivariable analysis and effect modification for the primary exposure (distance to index-household) showed that no effect modification was significant, and no co-variates were significant in the reduced model obtained through score variable selection (Logit {P [CFA-positive= 1]} = $\beta_0 + \beta_1$ (Distance) + β_2 (Agriculture) + β_3 (Bed net) + β_4 (Altitude) + ε; **Table 9**).

IV. Exploratory Analysis Including Index-Households

Index-households were not included in the analysis on the effect of distance on household CFA-status. Hence, the following analysis seeks to identify predictive factors of CFA-positive households in index-communities, including index-households. There were 21 index-households across 19 index communities. Four index-cases, from 4 different Burkina Faso communities, were still CFA-positive during follow-up (2 post-TAS-2 and 2 post-TAS-3). Two of these index-households, both in post-TAS-2 communities, do not have another CFA-positive household member. In order to study household CFA-status and avoid confounding with lingering positive TAS-cases, the CFA status of the household was not based on TAS-cases.

Conditional logistic regression analysis, stratified by country, of all index-community households indicate that the probability that a household is CFA-positive increased 5.31-fold for index-households (95%CI: 1.79 – 15.74, p-value = 0.0026) and decreased 4% (95%CI: 0.93 - 1.00, p-value = 0.0344) for each 10-meter increase in altitude (Logit {P [CFA-positive= 1]}$_{country}$ = β_1 (Index-household) + β_2 (Agriculture) + β_3 (Travel) + β_4 (MDA) + β_5 (Bed net) + β_6 (Altitude) + ε; **Table 10**).

In Burkina Faso index-communities, the likelihood that a household is CFA-positive increased 8.16-fold for index-households (95%CI: 1.23 – 54.32, p-value = 0.0300) and 3.35-fold if post-TAS-3 (95%CI: 1.37 – 8.18, p-value = 0.0081), (Logit {P [CFA-positive= 1]} = β_0 + β_1 (Index-household) + β_2 (Agriculture) + β_3 (Travel) + β_4 (MDA) + β_5 (Bed net) + β_6 (TAS-3) + β_7 (Altitude) + ε; **Table 10**).

None of the CFA-positive households were index-households or had agricultural workers in Haiti. Additionally, only post-TAS-2 communities were included in the survey. Therefore, "index-household", "agriculture" and "TAS-3" co-variates were omitted for multivariable analysis. None of the remaining co-variates were significant to predict CFA-positive households in index communities of Haiti (Logit {P [CFA-positive= 1]} = $\beta_0 + \beta_1$ (Travel) + β_2 (MDA) + β_3 (Bed net) + β_4 (Altitude) + ε; **Table 10**).

In Nepal, all households were in post-TAS-3 communities. Index-households were 6.84-fold (95% CI: 1.49 - 31.51, p-value = 0.0136) more likely to have at least one individual be CFA-positive, not including the TAS-case, in a model (Logit {P [CFA-positive= 1]} = $\beta_0 + \beta_1$ (Index-household) + β_2 (Agriculture) + β_3 (Travel) + β_4 (MDA) + β_5 (Bed net) + β_6 (Altitude) + ε; **Table 10**).

V. Exploratory Analysis of Households in Index and Neighbor Communities

In Burkina Faso and Nepal, neighboring communities in the same EU were surveyed if additional CFA-positive individuals were identified in the index community. The following section describes an exploratory analysis of all households surveyed in index and neighbor communities. **Table 11** describes household parameters for both countries. A total of 1,241 households in 41 communities are included in this multi-country analysis, where 84 (6.8%) were CFA-positive.

A greater proportion of CFA-positive households had an agricultural worker compared to CFA-negative households (45% versus 39%, p-value=0.0097, **Table 12**). Interestingly, the proportion of CFA-positive households was not significantly different in index-communities compared to neighbor-communities (7.86% versus 5.23%, p-value=0.6471, **Table 12**).

A multivariable model, stratified by country, indicates that the odds that a household was CFA-positive increased ~2-fold (95%CI: 1.21 – 3.01, p-value = 0.0058) if a member works in agriculture and decreased 3% for each 10-meter increase in altitude (95%CI: 0.95 - 0.99, p-value = 0.0043) (Logit {P [CFA-positive= 1] $_{country}$} = β_1 (Agriculture) + β_2 (Travel) + β_3 (MDA) + β_4 (Bed net) + β_5 (Altitude) + β_6 (Index) + ε; **Table 13**).

In Burkina Faso, neighbor and index communities combined, the likelihood that a household is CFA-positive increased ~2-fold (95%CI: 1.17 - 5.07, p-value= 0.0170) if a household member works in agriculture, ~3-fold (95%CI: 1.75 - 6.68, p-value= 0.003) if in a post-TAS-3 EU, and decreased 22% (95%CI: 0.68 - 0.89, p-value= 0.0002) with each 10-meter increase in altitude (Logit {P [CFA-positive= 1]} = β_0 + β_1 (Agriculture) + β_2 (Travel) + β_3 (MDA) + β_4 (Bed net) + β_5 (TAS-3) + β_6 (Altitude) + β_7 (Index) + ε; **Table 13**).

In Nepal, the model does not include TAS-level since all communities were in post-TAS-3 EUs. The likelihood of a CFA-positive households increases ~2-fold in index-communities (95%CI: 1.15 - 4.85, p-value= 0.0196) and decreases 3% (95%CI: 0.94 - 1.00, p-value= 0.0428) for each 10-meter increase in altitude (Logit {P [CFA-positive= 1]} = β_0 + β_1 (Agriculture) + β_2 (Travel) + β_3 (MDA) + β_4 (Bed net) + β_5 (Altitude) + β_6 (Index) + ε; **Table 13**).

Table 1: Description of households in index-community households, excluding index-households.

	Overall	Burkina Faso	Haiti	Nepal
Individuals	3509	1489	1077	943
Households	1334	370	622	342
Communities	19	6	5	8
household parameters				
CFA-positive	66 (4.95%)	23 (6.2%)	16 (2.57%)	27 (7.89%)
Distance to index-household, average (s.d.)	1,068 (2,525)	937 (1,844)	1,667 (3,286)	120 (75)
Distance to index-household, median (min-max)	232 (0.5 - 22,743)	431 (7.8 - 11,538)	373 (4.0 - 11,743)	114 (0.5 - 364)
≤ 300-meter from index-household	752 (56.4%)	152 (41.1%)	273 (43.9%)	327 (95.6%)
Agricultural worker	382 (28.6%)	195 (52.7%)	101 (16.2%)	86 (25.2%)
Traveled in last 12 months	448 (33.6%)	88 (23.8%)	242 (38.9%)	118 (34.5%)
MDA participation	1,116 (83.7%)	348 (94.1%)	556 (89.4%)	212 (62.0%)
Slept under bed net last night	566 (42.4%)	81 (21.9%)	174 (30.0%)	311 (90.9%)
TAS-3 (vs TAS-2)	461 (34.6%)	119 (32.2%)	0 (0.0%)	342 (100%)
Altitude (meters), mean (s.d.)	157 (156)	326 (29)	67 (136)	136 (122)
Altitude (meters), median (min - max).)	116 (-76 - 596)	322 (265 - 421)	17 (-76 - 596)	115 (-25 - 467)
GPS accuracy, mean (s.d.)	5.0 (1.2)	4.9 (0.3)	4.8 (0.4)	5.5 (2.3)
GPS accuracy, median (min-max)	5.0 (3.0 - 16.1)	5.0 (3.0 - 5.0)	5.0 (3.0 - 7.0)	4.6 (3.2 - 16.1)

Figure 2: Distribution of distance to TAS-index households in index-communities (**2a**) by country and per community in Burkina Faso (**2b**), Haiti (**2c**) and Nepal (**2d**).

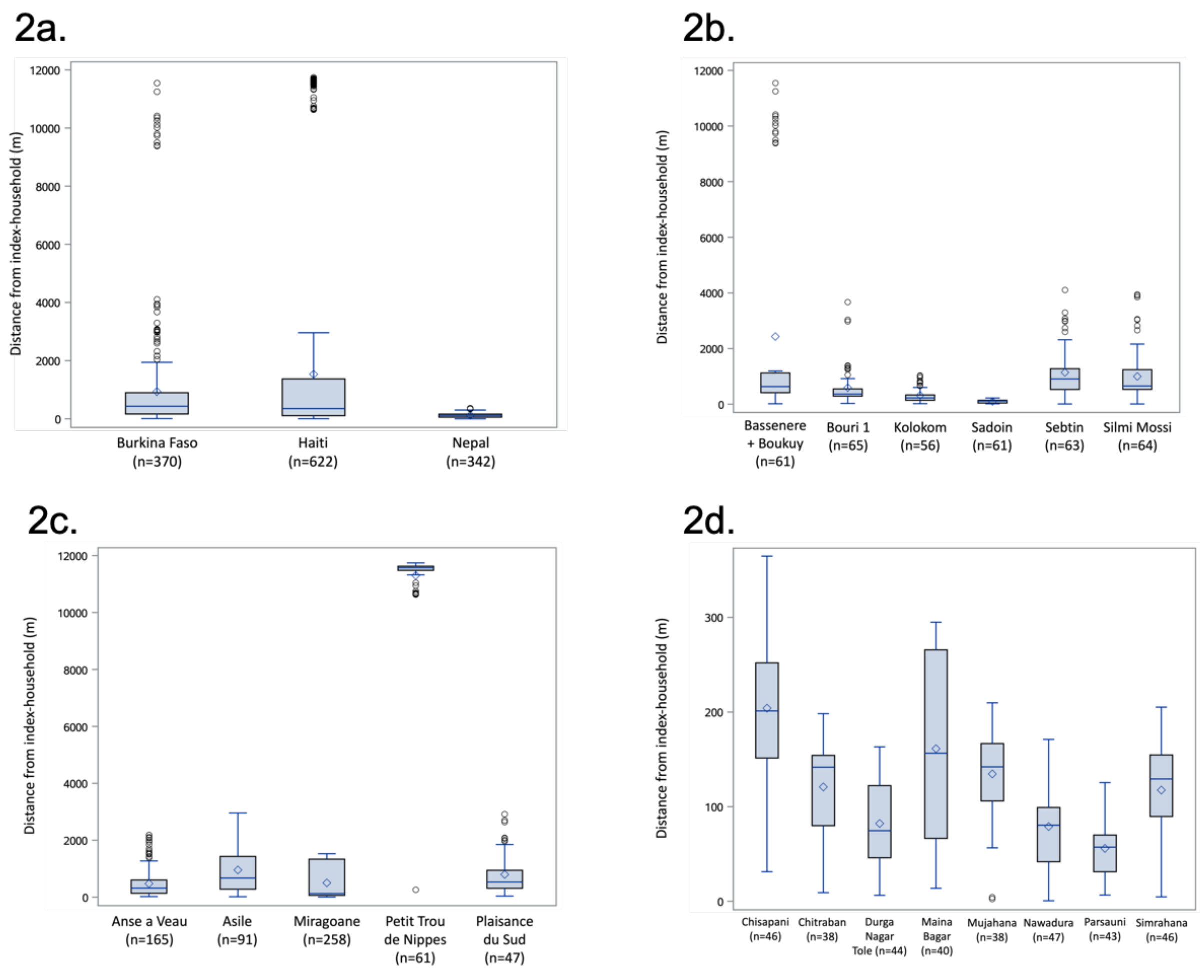

Table 2: Comparison of CFA-positive to CFA-negative households in index-community, excluding index-households. P-values are determined through chi-square analysis unless otherwise noted.

Household parameters	CFA-positive (n=66)	CFA-negative (n=1,268)	Odd Ratio [95%CI]	p-value
≤ 300-meter from index-household			2.14 [1.23 - 3.71]	0.0060
yes	48 (73%)	704 (56%)		
no	18 (27%)	564 (44%)		
Agricultural worker			1.67 [1.00 - 2.77]	0.0474
yes	26 (39%)	356 (28%)		
no	40 (61%)	912 (72%)		
Traveled in last 12 months			1.39 [0.84 - 2.31]	0.1962
yes	27 (41%)	421 (33%)		
no	39 (59%)	847 (67%)		
MDA participation			0.79 [0.42 - 1.47]	0.4496
yes	53 (80%)	1063 (84%)		
no	13 (20%)	205 (16%)		
Slept under bed net last night			1.67 [1.02 - 2.75]	0.0411
yes	36 (55%)	530 (42%)		
no	30 (45%)	738 (58%)		
TAS-3 (vs TAS-2)			2.71 [1.64 - 4.48]	<0.0001
yes	38 (58%)	423 (33%)		
no	28 (42%)	845 (67%)		
Altitude, median (min - max)	132 (-22 - 379)	115 (-76 - 596)	.	0.3298*

*p-value determined by Wilcoxon rank sums test.

Table 3: Distance to the index-household (meters) in index-communities by country. P-values are calculated by Wilcoxon-rank sums test.

	CFA-positive	CFA-negative	p-value
Overall			
Distance to index-household, mean (s.d.)	363 (624)	1,104 (2,580)	0.0024*
Distance to index-household, median (min - max)	137 (12 - 3,665)	241 (0.5 - 11,743)	
GPS accuracy, mean (s.d.)	5.01 (1.12)	4.98 (1.12)	0.7743*
GPS accuracy, median (min - max)	5.0 (3.2 - 10.7)	5.0 (3.0 - 16.1)	
Burkina Faso			
Distance to index-household, mean (s.d.)	703 (908)	953 (1,890)	0.9055
Distance to index-household, median (min - max)	484 (14 - 3,665)	430 (8 - 11,538)	
GPS accuracy, mean (s.d.)	4.9 (0.2)	4.9 (0.3)	0.4079
GPS accuracy, median (min - max)	4.0 (4.0-5.0)	5.0 (3.0 -5.0)	
Haiti			
Distance to index-household, mean (s.d.)	301 (429)	1,703 (3,321)	0.0036
Distance to index-household, median (min - max)	124 (12 - 1,415)	380 (4.0 - 1,1743)	
GPS accuracy, mean (s.d.)	4.7 (0.4)	4.8 (0.4)	0.3806
GPS accuracy, median (min - max)	5.0 (3.0 - 5.0)	5.0 (3.0 - 7.0)	
Nepal			
Distance to index-household, mean (s.d.)	111 (41)	121 (78)	0.8712
Distance to index-household, median (min - max)	128 (32 - 167)	113 (0.5 - 365)	
GPS accuracy, mean (s.d.)	5.3 (1.7)	5.5 (2.3)	0.7544
GPS accuracy, median (min - max)	4.5 (3.2 - 10.7)	4.6 (3.2 - 16.1)	

Table 4: Univariate logistic regression of households in Index-communities, excluding index-households, unadjusted and stratified by country, with Wald p-values.

	Unadjusted		Stratified by country	
Household parameters	Odd ratio [95% CI]	p-value	Odd ratio [95% CI]	p-value
Distance to index-household †	0.96 [0.92 - 1.00]	0.0621	0.97 [0.93 - 1.01]	0.1348
Agricultural worker	1.67 [1.00 - 2.77]	0.0495	1.42 [0.83 - 2.45]	0.2001
Traveled in last 12 months	1.39 [0.84 - 2.31]	0.1977	1.53 [0.92 - 2.56]	0.1023
MDA participation	0.79 [0.42 - 1.47]	0.4503	1.04 [0.53 - 2.04]	0.9107
Slept under bed net last night	1.67 [1.02 - 2.75]	0.0430	1.24 [0.65- 2.36]	0.5165
Household altitude ††	1.00 [0.99 - 1.01]	0.9124	0.96 [0.93 -1.00]	0.0381

† Odds ratio estimates are presented for 100-meter increments of "distance to index-household".
†† Odds ratio estimates are presented for 10-meter increments of "household altitude".

Table 5: Multivariable logistic regression of households in Index-communities, excluding index-households. Stratified by country. Full model and reduced model through backward elimination.

	Full model		Reduced model	
Household parameters	Odd ratio [95% CI]	p-value	Odd ratio [95% CI]	p-value
Distance to index-household †	0.97[0.93 - 1.00]	0.1039	0.97 [0.94 - 1.01]	0.1181
Agricultural worker	1.59 [0.91 - 2.78]	0.1043	.	.
Traveled in last 12 months	1.57 [0.93 - 2.64]	0.0890	.	.
MDA participation	1.11 [0.56 - 2.21]	0.7691	.	.
Slept under bed net last night	1.15 [0.59 - 2.22]	0.6818	.	.
Household altitude ††	0.96 [0.92 - 0.99]	0.0216	0.96 [0.92 - 1.00]	0.0304
AIC	489.587		487.516	
pseudo-R-square	0.0427		0.0286	

† Odds ratio estimates are presented for 100-meter increments of "distance to index-household".
†† Odds ratio estimates are presented for 10-meter increments of "household altitude".

Table 6: Univariable logistic regression of households in index-communities, excluding index-households, per country.

	Burkina Faso		Haiti		Nepal	
Household parameters	Odd ratio [95% CI]	p-value	Odd ratio [95% CI]	p-value	Odd ratio [95% CI]	p-value
Distance to index-household †	0.99 [0.96- 1.02]	0.5378	0.89 [0.78 - 1.01]	0.0705	0.830 [0.480- 1.433]	0.5035
Agricultural worker	3.46 [1.26 - 9.52]	0.0164	*	*	1.28 [0.54- 3.04]	0.5765
Traveled in last 12 months	1.14 [0.44 - 2.99]	0.7889	2.69 [0.96 - 7.49]	0.0588	1.34 [0.60 - 2.98]	0.4785
MDA participation	1.42 [0.18 - 11.01]	0.7396	0.83 [0.18 - 3.72]	0.8039	1.05 [0.46 - 2.36]	0.9134
Slept under bed net last night	0.99 [0.36 - 2.76]	0.9854	2.65 [0.98 - 7.18]	0.0551	0.54 [0.17 - 1.67]	0.2850
Household altitude ††	0.83 [0.69 - 0.99]	0.0401	0.96 [0.90 -1.03]	0.2302	0.972 [0.932 - 1.014]	0.1925
TAS-3 (vs TAS-2)	2.03 [0.87 - 4.74]	0.1025	**	**	**	**

† Odds ratio estimates are presented for 100-meter increments of "distance to index-household".
†† Odds ratio estimates are presented for 10-meter increments of "household altitude".
* "Agricultural worker" variable was excluded from the model due to complete separation of data points for the Haiti dataset. Zero CFA-positive households had a member who worked in agriculture in the Haiti dataset.
** "TAS-3" variable was excluded from the model for Haiti and Nepal. All households surveyed in Haiti were in post-TAS-2 communities. All households surveyed in Nepal were post-TAS-3 communities.

Table 7: Burkina Faso index-communities, excluding index-households, multivariate logistic regression. TAS-level is an effect modifier on the association between distance to an index-household and household CFA-status. Full model and reduced model by backward elimination.

	Full model with interaction *		Reduced model with interaction	
Household parameters	Odd ratio [95% CI]	p-value	Odd ratio [95% CI]	p-value
Distance to index-household †	.	0.8053	.	0.8013
Distance to index-household*TAS-3	.	0.0527**	.	0.0539
Distance to index-household, TAS3=0†	1.00 [0.97 - 1.03]	.	1.00 [0.96 - 1.03]	.
Distance to index-household, TAS3=1†	0.84 [0.71 - 1.00]	.	0.85 [0.73 - 1.00]	.
Agricultural worker	4.07 [1.39 - 11.95]	0.0107	4.02 [1.38 - 11.69]	0.0106
Traveled in last 12 months	0.68 [0.23 - 2.04]	0.4874	.	.
MDA participation	1.47 [0.17 - 13.14]	0.7295	.	.
Slept under bed net last night	0.78 [0.25 - 2.48]	0.6744	.	.
TAS-3 (vs TAS-2)	.	0.0016	.	0.0025
Household altitude ††	0.792 [0.664 - 0.946]	0.0100	0.80 [0.68 - 0.95]	0.0098
AIC	168.166		163.115	
pseudo-R-square	0.1561		0.1496	

*Assessment of full model with all interaction terms for primary exposure "distance to index-household" and full model with no interaction terms by likelihood ratio "chunk" test: LR statistic= 14.472, df=7, p-value=0.0248.
** Interaction term "distance*TAS-3" is significant. Assessed by likelihood ratio test compared to no interaction full model: LR statistic=5.527, df=1, p-value=0.018.
† Odds ratio estimates are presented for 100-meter increments of "meters to index-household".
†† Odds ratio estimates are presented for 10-meter increments of "household altitude".

Table 8: Haiti post-TAS-3 index-communities, excluding index-households, multivariate logistic regression. Full model and Reduced model by Score selection.

	Full model *		Reduced model	
Household parameters	Odd ratio [95% CI]	p-value	Odd ratio [95% CI]	p-value
Distance to index-household †	0.92 [0.81 - 1.04]	0.1972	0.92 [0.81 - 1.04]	0.1972
Traveled in last 12 months	2.10 [0.73 - 5.99]	0.1669	2.10 [0.73 - 5.99]	0.1669
MDA participation	0.85 [0.18 - 3.93]	0.8351	.	.
Slept under bed net last night	2.34 [0.84 - 6.51]	0.1022	2.34 [0.84 - 6.51]	0.1022
Household altitude ††	0.95 [0.86 - 1.04]	0.2632	0.95 [0.87 - 1.04]	0.2632
AIC	145.082		143.124	
pseudo-R-square	0.1167		0.1164	

*Assessment of full model with all interaction terms for primary exposure "distance to index-household" and full model with no interaction terms by likelihood ratio "chunk" test: LR statistic= 1.472, df=3, p-value=0.6887.
† Odds ratio estimates are presented for 100-meter increments of "meters to index-household".
†† Odds ratio estimates are presented for 10-meter increments of "household altitude".

Excluded from model: agricultural worker (complete separation of data points; zero CFA-households of 16 had an agriculture worker) and TAS-3 (only TAS-2 cases were followed-up during operational research in Haiti).

Table 9: Nepal post-TAS-2 index-communities, excluding index-households, multivariate logistic regression. Full model and Reduced model by Score selection.

	Full model*		Reduced model	
Household parameters	Odd ratio [95% CI]	p-value	Odd ratio [95% CI]	p-value
Distance to index-household †	0.90 [0.49 - 1.66]	0.7407	0.90 [0.49 - 1.66]	0.7453
Agricultural worker	1.38 [0.58 - 3.33]	0.4683	1.38 [0.58 - 3.13]	0.4675
Traveled in last 12 months	1.40 [0.62 - 3.19]	0.4207	.	.
MDA participation	1.10 [0.48 - 2.55]	0.8223	.	.
Slept under bed net last night	0.45 [0.14 - 1.5]	0.1816	0.43 [0.13 - 1.40]	0.1626
Household altitude ††	0.97 [0.92 - 1.01]	0.1491	0.97 [0.93 - 1.01]	0.1776
AIC	197.963		194.690	
pseudo-R-square	0.0339		0.0289	

*Assessment of full model with all interaction terms for primary exposure "distance to index-household" and full model with no interaction terms by likelihood ratio "chunk" test: LR statistic= 2.748 with DF=5, p-value=0.097.
† Odds ratio estimates are presented for 100-meter increments of "meters to index-household".
†† Odds ratio estimates are presented for 10-meter increments of "household altitude".

Excluded from model: TAS-3 (Only TAS-3 cases were followed-up during operational research).

Table 10. Multivariable logistic regression for index-communities, including 19 index-households (n=1,352 HH). Stratified by country and separately.

Household parameters	Odd ratio [95%CI] p-value			
	Stratified model	Burkina Faso	Haiti	Nepal
Index-household	5.31 [1.79 - 15.74] 0.0026	8.16 [1.23 - 54.32] 0.0300	*	6.84 [1.49 - 31.51] 0.0136
Agricultural worker	1.44 [0.84 - 2.47] 0.1897	2.63 [0.99 - 7.00] 0.0530	*	1.44 [0.62 - 3.36] 0.3960
Traveled in last 12 months	1.61 [0.97 - 2.65] 0.0638	0.89 [0.33 - 2.40] 0.8179	2.60 [0.93 - 7.29] 0.0688	1.25 [0.56 - 2.77] 0.5830
MDA participation	0.966 [0.50-1.87] 0.9189	1.53 [0.18 - 13.24] 0.6983	0.75 [0.16 - 3.44] 0.7103	0.95 [0.43 - 2.11] 0.8986
Slept under bed net last night	1.07 [0.56 - 2.02] 0.8449	1.18 [0.43 - 3.28] 0.7515	2.40 [0.88 - 6.57] 0.0892	0.39 [0.132 - 1.16] 0.091
TAS-3 (versus TAS-2)	**	3.35 [1.37 - 8.18] 0.0081	**	**
Household altitude ††	0.96 [0.93 - 1.00] 0.0344	0.85 [0.72 - 1.00] 0.0516	0.96 [0.90 - 1.03] 0.2830	0.97 [0.93 - 1.01] 0.1395
AIC	516.452	180.158	150.292	208.111
pseudo-R-square	0.0419	0.1318	0.0657	0.0666

* "Index-household and "agricultural worker" variables were excluded from the model due to complete separation of data points in the Haiti dataset. Zero CFA-positive households were also index-households or had a member who worked in agriculture in the Haiti dataset.
** "TAS-3" variable was excluded from the model for Haiti and Nepal. All households surveyed in Haiti were in post-TAS-2 communities. All households surveyed in Nepal were post-TAS-3 communities.
†† Odds ratio estimates are presented for 10-meter increments of "household altitude".

Table 11: Description of households in neighbor and index communities, combined.

	Overall	Burkina Faso	Nepal
Individuals	4,175	2,405	1,770
Households	1,241	582	659
Communities	41	16	25
Household parameters			
CFA-positive	84 (6.8%)	43 (7.4%)	41 (6. 2%)
Agricultural worker	499 (40.2%)	321 (55.2%)	178 (27.0%)
Traveled in last 12 months	339 (27.3%)	142 (24.4%)	197 (29.9%)
MDA participation	978 (78.8%)	547 (94.0%)	431 (65.4%)
Slept under bed net last night	759 (61.2%)	154 (26.5%)	605 (91.8%)
TAS-3 (versus TAS-2)	868 (69.9%)	209 (36%)	659 (100%)
Index-community	725 (58.4%)	376 (64.6%)	349 (53.0%)
Neighbor-community	516 (41.6%)	206 (35.4%)	310 (47%)
Household altitude, median (min-max)	296 (-25 - 572)	320 (263 - 421)	117 (-25 - 572)
Household altitude, mean (s.d.)	234 (135)	325 (30)	154 (140)
GPS accuracy, median (min-max)	5.0 (3.0 - 16.1)	5.0 (3.0 - 5.0)	4.6 (3.2 - 16.1)

Table 12: Comparison of CFA-positive and CFA-negative households in neighbor and index-communities. P-values are determined through chi-square analysis unless otherwise noted.

Household parameters	CFA-positive (n=84)	CFA-negative (n=1,157)	Odds Ratio [95%CI]	p-value
Agricultural worker			1.79 [1.15 - 2.79]	0.0097
yes	45 (54%)	454 (39%)		
no	39 (46%)	703 (61%)		
Traveled in last 12 months			1.14 [0.70 - 1.89]	0.6024
yes	25 (30%)	314 (27%)		
no	59 (70%)	843 (73%)		
MDA participation			1.15 [0.66 - 2.02]	0.6183
yes	68 (81%)	910 (79%)		
no	16 (19%)	247 (21%)		
Slept under bed net last night			0.75 [0.48 - 1.18]	0.2127
yes	46 (55%)	713 (62%)		
no	38 (45%)	444 (38%)		
TAS-3 (versus TAS-2)			1.41 [0.84 - 2.36]	0.1959
yes	64 (76%)	804 (69%)		
no	20 (24%)	353 (31%)		
Index community (versus neighbor)			0.6471 [0.40 - 1.04]	0.0692
yes	57 (68%)	668 (58%)		
no	27 (32%)	489 (42%)		
Household altitude, median (min - max)	269 (21 - 379)	299 (-25 - 572)		0.0280*
GPS accuracy, median (min - max)	5.0 (3.2 - 16.1)	5.0 (3.0 - 16.1)		0.9784*

*P-value by Wilcoxon rank sum

Table 13: Multivariate logistic regression of households in neighbor and index-communities.

Household parameters	Stratified by country	Burkina Faso	Nepal
Agricultural worker	1.92 [1.21 - 3.01] 0.0058	2.44 [1.17 - 5.07] 0.0170	1.41 [0.70 - 2.83] 0.3348
Traveled in last 12 months	1.15 [0.70 - 1.88] 0.5745	0.81 [0.38 - 1.73] 0.5806	1.19 [0.60 - 2.37] 0.6230
MDA participation	1.23 [0.67 - 2.27] 0.5019	1.99 [0.42 - 9.40] 0.3861	1.20 [0.61 - 2.38] 0.6019
Slept under bed net last night	0.59 [0.34 -1.04] 0.0668	0.78 [0.36 - 1.71] 0.5409	0.56 [0.21 - 1.54] 0.2617
TAS-3 (versus TAS-2)	*	3.42 [1.75 - 6.68] 0.0003	*
Household altitude †	0.97 [0.95 - 0.99] 0.0043	0.78 [0.68 - 0.89] 0.0002	0.97 [0.94 - 1.00] 0.0428
Index community (versus neighbor)	1.49 [0.92 - 2.41] 0.1021	1.07 [0.54 - 2.13] 0.8408	2.36 [1.15 - 4.85] 0.0196
AIC	616.581	289.243	306.985
pseudo-R-square	0.0396	0.1367	0.0570

* "TAS-3" variable was excluded from the model for Haiti and Nepal. All households surveyed in Haiti were in post-TAS-2 communities. All households surveyed in Nepal were post-TAS-3 communities.
† Odds ratio estimates are presented for 10-meter increments of "household altitude".

Supplemental Table 1: Description of individual-level missing data points and observations excluded from analysis.

Individuals	Burkina Faso	Haiti	Nepal
cleaned-up dataset	2,916	1,937	2,780
consent not "yes"	441	1	156
Consenting dataset	**2,475**	**1,936**	**2,624**
Excluded individuals due to variables:			
bed net	0	0	0
MDA med	52	842	744
travel	0	0	0
agriculture	0	0	0
CFA	18	0	0
GPS coordinates	0	4	0
GPS accuracy $\geq$ 17	0	0	110
complete individual-level dataset 1	**2,405**	**1,090**	**1,770**
Excluded for distance analysis:			
index HH members	47	13	28
neighbor community	869	0	799
complete individual-level dataset 2	**1,489**	**1,077**	**943**

Exclusion from data analysis was due to answers being anything other than "yes" or "no". Individuals with *MDA med* answers "I don't know/I don't remember/ (missing)", or *CFA* answers "(missing)" or GPS answers "missing" were excluded.

Supplemental Table 2: Burkina Faso CFA prevalence by community. (Index communities are underlined.)

	Individuals			Households		
	(n)	CFA-positive	CFA prevalence	(n)	CFA-positive	CFA prevalence
TAS-3						
EU: Central Plateau	**922**	**29**	**3.1%**	**209**	**23**	**11.0%**
District: Zorgho	**468**	**22**	**4.7%**	**103**	**19**	**18.4%**
Sebtin	310	12	3.9%	64	10	15.6%
Ladè V5	75	9	12.0%	19	8	42.1%
Rapadama V4 Centre	83	1	1.2%	20	1	5.0%
District: Boussé	**454**	**7**	**1.5%**	**106**	**4**	**3.8%**
Kolokom	230	6	2.6%	57	3	5.3%
Poédogo	85	1	1.2%	21	1	4.8%
Rintigkoudou	139	0	.	28	0	.
TAS-2						
EU: Boromo - Dédougou	**770**	**2**	**0.3%**	**167**	**2**	**1.2%**
Dedougou district	**770**	**2**	**0.3%**	**167**	**2**	**1.2%**
Silmi Mossi	316	0	.	65	0	.
Bassnere + Boukuy	269	2	0.7%	62	2	3.2%
Oula + Denka + Koroby + Kelgum	100	0	.	20	0	.
Yamane + Signonghin	85	0	.	20	0	.
EU: Leo-Sapouy	**713**	**17**	**2.4%**	**204**	**18**	**8.8%**
Leo District	**713**	**17**	**2.4%**	**204**	**18**	8.8%
Bouri 1	191	8	4.2%	64	8	12.5%
Bouri 2	85	2	2.4%	19	3	15.8%
Yayou	77	0	.	20	0	.
Sadoin 1 (douyoubio)	220	4	1.8%	62	4	6.5%
Sadoin 2 (Douyoufaro)	74	1	1.4%	19	1	5.3%
Tonon (Pissy)	66	2	3.0%	20	2	10.0%

Supplemental Table 3: Haiti CFA prevalence by community. (Index communities are underlined.)

	Individuals			Households		
TAS-2	(n)	CFA-positive	CFA prevalence	(n)	CFA-positive	CFA prevalence
EU: Nippes	**1091**	**18**	**1.6%**	**629**	**16**	**2.5%**
Anse a Veau	283	5	1.8%	167	4	2.4%
l asile	172	0	.	91	0	.
Miragoane	458	13	2.8%	261	12	4.6%
Petit Trou de Nippes	96	0	.	62	0	.
Plaisance Du Sud	82	0	.	48	0	.

Supplemental Table 4: Nepal CFA prevalence by community. (Index communities are underlined.)

	Individuals			Households		
TAS-3	(n)	CFA-positive	CFA-prevalence	(n)	CFA-positive	CFA-prevalence
EU: Bagmati Province 3	**745**	**25**	**3.4%**	**272**	**24**	**8.8%**
District: Makwanpur	**330**	**0**	**0**	**126**	**0**	**0**
Chisapani	123	0	.	47	0	.
Gairigaoun	63	0	.	19	0	.
Gauritar	52	0	.	20	0	.
Purbeli tole	44	0	.	20	0	.
Sipalichock	48	0	.	20	0	.
District: Chitwan	**415**	**25**	**6.0%**	**146**	**24**	**16.4%**
Chitraban/Chitrawan	91	7	7.7%	39	7	17.9%
Buddha Tol	56	3	5.4%	20	3	15.0%
Kalibhairabh (tarkarimandii)	51	2	3.9%	18	2	11.1%
Shantinagar	53	2	3.8%	22	2	9.1%
Nawadurga/Navadurga	164	11	6.7%	47	10	21.3%

EU: Lumbini Province 5	**1134**	**26**	**2.3%**	**441**	**26**	**5.9%**
District: Rupandehi	**1134**	**26**	**2.3%**	**441**	**26**	**5.9%**
Mujahana	101	2	2.0%	39	1	2.6%
Bhagatpurwa	42	0	.	14	0	.
Bhujaiya	36	0	.	19	0	.
Simrahana/Shimrahana	152	7	4.6%	47	6	12.8%
Vasihawa	49	1	2.0%	18	1	5.6%
Simrahani	60	1	1.7%	47	6	12.8%
Maina Bagar 2/Mainabagar	79	0	.	41	0	.
Manakamana 5	26	1	3.8%	13	1	7.7%
Shree ram nagar	20	0	.	11	0	.
Durga Nagar Tole	164	5	3.0%	45	4	8.9%
Top majuwa	48	0	.	20	0	.
Durgangar	164	5	3.0%	45	4	8.9%
Parsauni	97	2	2.1%	44	2	4.5%
Chhapiya	51	2	3.9%	18	1	5.6%
Krishnapur	45	0	.	20	0	.

CHAPTER 5: DISCUSSION

I. Summary

The Global Programme to Eliminate Lymphatic Filariasis (**GPELF**) has made significant progress towards its goal. As prevalence diminishes to very low levels, the success of eliminating LF requires surveillance strategies with high positive predictive value that can detect pockets of micro-focal transmission in larger geographical spaces, particularly within communities. Reliable detection of these " hotspots" is essential to proactively prevent recrudescence of LF across endemic regions.

Currently, national programs conduct LF surveillance, after years of mass drug administration (**MDA**), through the transmission assessment survey (**TAS**) in distinct evaluation units (**EU**). Children are surveyed primarily in school clusters throughout the EU but may reside in geographically distinct communities. If the number of CFA-positive children in the EU is below a threshold, then TAS is *passed.* Passing TAS signifies that on-going LF transmission is likely too low to be sustainable, even in the absence of MDA, in the EU. However, it is not clear how to interpret TAS-cases as they pertain to transmission in their respective communities in EUs that have passed TAS.

The WHO recommends that CFA-positive children identified during TAS (**index-cases**) are given medication and suggests conducting follow-up surveillance in their respective communities (**index-communities**). However, the best method to conduct follow-up investigation in the context of anticipated low prevalence and limited resources hasn't been determined.

This thesis was a secondary data analysis of a multi-country operational research project on TAS-2/3 follow-up surveillance. The primary question of this thesis was whether TAS-cases, diagnosed after many years of MDA and post-MDA surveillance, are indicative of LF micro-foci inside their respective communities. Using logistical regression, the analysis sought to determine if spatial proximity to homes of TAS-cases (**index-households**) is a predictor that another household has at least one CFA-positive resident. Additionally, the analysis assessed whether other factors were associated with CFA-positive households found after TAS-2/3.

II. Discussion of key Results

Households closest to TAS-index homes are not more likely to be CFA-positive

Previous work suggested that LF cases can be found in clusters of household members and in clusters of households within a community (Boyd et al., 2010; Drexler et al., 2012; Lau et al., 2014). In this analysis, the median distance to a TAS index-household, in all 3 combined study sites, was significantly smaller for CFA-positive households compared to CFA-negative households (137 m versus 241 m, p-value = 0.0024, **Table 3**). However, through logistic regression the odds that a household is CFA-positive was not affected by distance to index-households (**Table 3**), even when controlling for household parameters, such as agriculture work, travel history, MDA medication, bed net use, household altitude and TAS-level follow-up (T**able 4** and **Table 5**). This could be because of differences in the structural make up of communities in the different study sites. For instance, the geographical spread of households, based on sampling, is notably larger in Burkina Faso and Haiti, compared to Nepal (**Figure 2**). In Burkina

Faso and Haiti, 41% and 44%, respectively, of sampled homes were within 300-meters of an index-household. Whereas 96% of households were within a 300-meter radius of index-households in Nepal (**Table 1**).

Burkina Faso, Haiti and Nepal are geographically, culturally and ecologically different. Spatial orientation of communities and infrastructure could affect vector behavior and therefore distribution of CFA-households. LF is primarily transmitted by *Anopheles gambiae* in Burkina Faso, and by *Culex quinquefasciatus* in Haiti and Nepal. *A. gambiae* preferably breeds in clean water and is most prevalent in rural setting, partially due to anti-malarial vector control initiatives in urban settings (Simonsen & Mwakitalu, 2013). On the other hand*, C. quinquefasciatus* is associated with urban settings, and can breed in dirty organic-waste water. Because of the differences in geography and vector behavior, country-specific analyses were performed to investigate the effect of distance to TAS-index homes.

In Burkina Faso, the effect of distance was modified by the TAS-level of the community. Households in TAS-3 follow-up communities were 15% (95%CI: 0.73 – 1.00, **Table 7**) less likely to be CFA-positive for each 100-meter increment away from TAS index-households. However, in TAS-2 follow-up communities, distance did not change the probability that a household is CFA-positive. This suggests that CFA-positive households are contained to geographical micro-foci associated with TAS index-households in communities that have passed the TAS-3 benchmark. Meanwhile, CFA-positive household in TAS-2 index communities may be dispersed or at least not spatially linked to index-households.

The TAS-3 index-communities sampled in Burkina Faso for this analysis were Sebtin in Zorgho district and Kolokom in Boussé district, both in EU Central Plateau. Central Plateau underwent 10 years of MDA and passed TAS-3. The sample from this analysis indicates that 3.1% of individuals and 11% of households are CFA-positive (**Supplemental Table 2**). Although the data suggests that micro-foci are present inside the 2 index-communities in Central Plateau, there is no indication that the entire index-communities themselves are LF foci in their districts when compared to the prevalence of neighboring communities (discussed in later section; **Table 13**).

Concerning Haiti and Nepal, there was no evidence to supports that distance from TAS index-households affects the probability that another household is CFA-positive in the same community (**Table 8** and **Table 9**). This suggests that TAS-cases are not affiliated with micro-foci in their respective communities. The reason may be explained by high population density. The distribution of household sampling, as a proxy to population density, was relatively high in Nepali index-communities (**Figure 1**). Also, one of the Haitian communities, providing ~42% of the data points is a dense urban center called Miragoane. It may be that LF micro-foci are unlikely to emerge, or be detectable with this analysis, in dense urban communities, unlike in Burkina Faso. In dense urban settings, infection may therefore be observed in a seemingly random pattern since transmission is not limited by the distance covered by the vector. However, results comparing index-communities to their neighbors suggests that foci may be defined as the entire index-community in densely populated Nepal (discussed in later section; **Table 13).**

<u>Household members of TAS-cases are significantly more likely to be CFA-positive</u>

The WHO advises national programs to treat LF-positive children with antihelminth medication upon diagnosis during TAS, but does not go further to recommend treating members of the child's household (WHO, 2011). This analysis shows that index-households, excluding the index-case, are ~6-fold (95%CI: 1.7 – 15.7) more likely to be CFA-positive, overall (**Table 10**). In Burkina Faso, the probability was increased ~8-fold (95%CI: 1.23- 54.3) for index-households and further increased ~3-fold (95%CI: 1.4 – 8.2) if surveyed post-TAS-3 (**Table 10**). This indicates that a TAS-case may be indicative of a single household hotspot of LF positive individuals in the home (i.e. index-household). There may be significant on-going transmission between individuals of the same household. Due to co-endemicity with other filarial worms, chemotherapeutics for Burkina Faso is restricted to ivermectin + albendazole, which is not the most optimal treatment against adult worms. Four TAS-cases that were still positive upon follow-up were all in Burkina Faso (2 in EU Central Plateau, 1 in EU Boromo-Dédougou, and 1 in Leo-Sapouy). Nepal is eligible for regimens containing diethyl carbamazine (DEC), which is active against Mf and adult forms of the worm and may be more effective in clearing the worm. However, the data suggests that household transmission is a significant occurrence.

Higher altitude decreases the probability of CFA-positive households in TAS-index communities

Other parameters were assessed that increase the probability that a households have at least one CFA-positive individual in all communities surveyed. Across all 3 countries, households were 3.4% less likely to be CFA-positive for every 10-meter increase in altitude in neighbor and index-communities, combined (95%CI: 0.93 - 1.00, p-value= 0.034, **Table 10**). The effect of altitude on LF prevalence was reported in numerous other studies (Burgert-Brucker et al., 2020 ; Goldberg et al., 2019; Stanton et al., 2013). It is possible that higher altitude has a negative effect on transmission. Studies on *C. quinquefasciatus* distribution in Nepal found decreasing vector density at higher elevation sites (Dhimal, Gautam, Kreß, Müller, & Kuch, 2014). However, the vector was still present in the high mountains. Another study in Mali showed that *A. gambiae* fitness is not affected, as determined by oviposition and blood feed, in high altitude compared to low altitudes (Sanogo et al., 2021). It is probable that higher altitude invokes a combination of host behavioral changes (i.e. wearing more clothing in the cooler temperature, spending less time outdoors, etc.) in addition to slight changes in vector population, resulting in lower Lymphatic Filariasis prevalence.

Households are more likely to be CFA-positive in index versus neighbor communities in Nepal, but not in Burkina Faso

TAS is performed in EUs that are made up of many communities. Some communities may not be sampled during cluster sampling or “flagged” by the discovery of a TAS-case. The result of TAS is an average for the EU, and prevalence may be heterogeneous across communities in the EU. Therefore, it is not clear if the probability of CFA-positive households is similar in TAS-index communities compared to their neighbor communities in the same EU. Combining households from Nepal and Burkina Faso, households are just as likely to be CFA-positive whether located in an index community or a neighboring community (**Table 13**). The overall proportion of CFA-positive households in TAS-index communities was 7.86% (95%CI:5.9 - 9.8) and 5.23% (3.3 - 7.2) from neighbor communities and not significantly different (χ^2 p-value = 0.0600). The overall estimated prevalence for CFA-positive individuals in TAS-index communities was 2.3% [95%CI:2.0 – 3.3] and 1.7% [1.1 – 2.4] from neighbor communities and also not significantly different (χ^2 p-value = 0.16).

However, households in Nepal were twice more likely to be CFA-positive in TAS index communities compared to neighbor communities (95%CI: 1.15 - 4.85, p-value=0.0196, **Table 13**). The proportion of households with a CFA-positive individual was 8.6% (95%CI: 5.1 – 12.0) in index-communities compared to 5.49% (95%CI: 1.5 – 5.6) in neighboring communities (p-value= 0.0074) (**Supplemental Table 4**). LF prevalence in Nepali index-communities was 3.5% (95%CI: 2.4 – 4.7), compared to 1.5% (95%CI: 0.1 – 2.4) in neighbor communities (p-value= 0.0085) (**Supplemental Table 4**). In Nepal, only purposively sample households in post-TAS-3 communities were surveyed in TAS-index communities as part of the operational research project. Therefore, the interpretation of this finding may be that purposively sampled homes, which are the presumed 50 closest household to the TAS-index homes, are more likely to be CFA-positive than households in neighboring communities. Also, the spatial distribution of households in Nepali communities indicate that the communities were densely populated. There was no evidence of "hotspots" inside post-TAS-3 index-communities. However, based on this analysis, entire index-communities may be considered "hotspots" in the EU compared to neighboring communities.

In contrast, in Burkina Faso, both purposive and randomly selected households were sampled in index-communities and compared to households in neighbor-communities in both post-TAS-2 and post-TAS-3 EUs. The proportion of households with at least one CFA-positive individual did not differ between index (7.2% [95%CI: 4.6 – 9.8]) and neighbor (7.8% [95%CI: 4.1 – 11.4]) communities (p-value= 0.7960). Individual-level prevalence was also not significantly different between index-communities (2.1% [95%CI: 1.4 – 2.8]) and neighbor communities, (2.0% [95%CI: 1.0 – 2.9]; (**Supplemental Table 2**). Index compared to neighbor communities had similar LF prevalence and proportion of CFA-positive households in post-TAS-2 EU, (1.41% vs. 1.23%, p-value = 0.7855; 5.49% vs. 5.08%, p-value= 0.8716) and in post-TAS-3 EU (3.33% vs. 2.88%, p-value= 0.6974; 10.7% vs. 11.4%, p-value= 0.8876). Nevertheless, households in post-TAS-3 EUs were 3.42-fold more likely to be CFA-positive compared to TAS-2 follow-EUs, controlling for agriculture worker and altitude, regardless of community type, travel history, MDA participation, and bed net usage (95%CI: 1.75 - 6.68, p-value = 0.0003, **Table 13**). These results indicate that index-communities may be representative of the whole EU, since the percentage of CFA-positive households is not different in neighboring communities. Therefore, the data does not support that index-communities are "hotspots" in Burkina Faso. However, analysis on index-communities only suggests that "hotspots" may be present inside communities, post-TAS-3, specifically. The sampled communities in Burkina Faso are sparsely distributed compared to the Nepali sample. This could indicate that "hotspots" may emerge and be detected in sparsely populated communities, such as the post-TAS-3 communities in Burkina Faso, but in densely populated regions, "hotspots" emerge as entire communities.

Agriculture work is associated with CFA-positive households in Burkina Faso

In Burkina Faso, households were 2.44-fold more likely to be CFA-positive if they included an agricultural worker (95%CI: 1.17 - 5.07, p-value=0.0170, **Table 13**), controlling for other factors across all community types. (However, in index-communities only, the presence of an agricultural worker was not significant (p-value = 0.0530, **Table 10**)). Agriculture work was not a significant indicator in Nepal, and did not apply to Haiti for this sample (**Table 13**).

The main vector in Burkina Faso, *A. gambiae* is a dusk-night biting mosquito, similar to *C. quinquefasciatus* in Haiti and Nepal. It is not clear how agricultural work would increase the odds of CFA-positivity in the household based on differences in vector behavior. It may be that human behavior, agricultural work, increases exposure to vectors at dusk, creating more opportunities for transmission and infection. For this analysis, agricultural workers included individuals who reported being a gardener, farmer/farming, fisherman/fishing, and shepherd. The presence of these occupations in a household could serve as an indicator to better target sampling during TAS follow-up in Burkina Faso.

III. Limitations and Strengths

The generalization of the findings in this analysis are limited by the operational research design, the completeness of the collected data, and recall bias while answering the survey questionnaire.

The sampling strategy for the operational research was to survey individuals from 50 households closest to TAS index-households and 20 randomly selected households in the same community. In Nepal, no randomly selected households were sampled. As a result, the household sampling used to generate this data is not a representative distribution of households in communities (**Figure 2**).

The operational research questionnaire was designed for individual-level variables. Since, this thesis evaluated households as the unit of analysis, household parameters were defined by affirmation of at least one consenting household individual. Individuals who did not provide an answer for all parameters were omitted from the household and therefore from the analysis. This strategy can introduce bias and incomplete household information since individuals that were absent, non-consenting or did not provide answers to all relevant questions for this analysis do not contribute to the parameters of their own household. For example, in Nepal, 28% (744/2,624) of consenting participants did not provide a "yes" or "no" answer the MDA medication questions (**Supplemental Table 1**). Eight of them were CFA-positive. The effect of these individuals for their households and for the analysis is lost.

Certain survey questions are prone to recall bias, specifically "Did you travel in the past 12-months?" (travel) or "Did you participate/take MDA medication?" (MDA med). Since all EUs are post TAS-2 or TAS-3 and the operational research questionnaire was given in 2019, the last MDA would have taken place at least 4 years prior in Burkina Faso and 5 years prior in Haiti and Nepal.

Other limitations to interpretation are due to statistical procedures. For instance, the independent variable "agriculture" and the dependent variable were perfectly colinear in the Haitian dataset. None of the 16 CFA-positive households had a household member who reported working in agriculture. Therefore, this thesis cannot assess the effect of agriculture work and probability of CFA-positive households in Haiti. Comparing TAS-index communities to neighbor communities should be performed with match analysis, since they represent the same EUs. However, not all TAS-index communities had matching neighboring communities.

Finally, interpretation is limited by the assay used for diagnosis. The study uses detection of circulating filarial antigen, which may persist in individuals that no longer harbor live or fecund worms, and therefore are no longer relevant for LF transmission. Since the overarching goal is to detect micro-foci of on-going transmission in order to intervene, it is imperative that CFA-positive individuals accurately predict the potential to shed microfilaria. The target population during TAS is 6-7-year-old children who would have lived the majority of their lived under MDA. Therefore, CFA-positivity in this population is presumed to be from on-going transmission. However, the target population of this thesis were individuals $\geq$ 2-years-old. It is likely that CFA-positive results reflect newly acquired and years-old infections.

IV. Implications

Despite these limitations, the analysis provides interesting results that can guide future operational research questions and recommendations on TAS-follow-up surveys.

In general, and specifically in Haiti and Nepal, there was no evidence that households of TAS-identified children are indicative of micro-foci inside their respective communities. The analysis showed no correlation between the likelihood of a CFA-positive household and distance to TAS-index homes. This implies that TAS follow-up can be done with random sampling of households throughout the index communities. Random sampling throughout the community entails greater geographical coverage and allows for community-wide decisions that may be easier to implement.

In general, a TAS-case may be indicative of a single household hotspot of LF positive individuals in the home (i.e. index-household), since index-households were ~5 times likely to have another CFA-positive individual. This implies that significant transmission is on-going between individuals of the same household.

The current result show that index-communities may either represent a "hotspot" or background prevalence, but not an under estimation of the measurement for communities in the EU. In Burkina Faso, specifically, "hotspots" may emerge and be detected within sparsely populated post-TAS-3 communities. However, in densely populated TAS-3 Nepali communities, "hotspots" may be defined as the whole index-community in comparison to neighboring communities.

V. Recommendations

In general, follow-up surveillance of TAS-2/3 cases should prioritize **1)** TAS index-households and **2)** households at lower altitudes.

In Burkina Faso, follow-up surveillance should also prioritize households with **1)** agricultural workers and **2)** TAS-3 over TAS-2 evaluation units. Additionally, households proximal to TAS-index homes should be prioritized during TAS-3 follow-up surveillance.

In Nepal, follow-up surveillance in index-communities should be prioritized over surveillance in neighbor communities. Sampling distribution of households can be random.

This study did not find useful evidence to present recommendations specific to Haiti.

The WHO recommends treating LF-positive children, diagnosed during TAS, with medication. It may be beneficial to extend treatment to all household members of TAS-cases.

VI. Conclusion

In conclusion, this thesis hypothesized that the likelihood of households having at least one CFA-positive member is affected by the distance to the home of a TAS-identified LF-positive child. If TAS-index homes represent a cluster or a micro-foci of LF-positive individuals, then households in closest proximity should have greater odds of being CFA-positive. Despite the specific circumstances of post-TAS-3 Burkina Faso, the data does not support this hypothesis in Nepal, Haiti or overall. However, this analysis found that "hotspots" could be detected inside relatively sparsely populated post-TAS-3 Burkina Faso index-communities and that "hotspots" could be detected in evaluation units as defined by the whole index-community in densely populated Nepal.

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