

## **Distribution Agreement**

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

---

Rachel Burriss

---

Date

Assessing Prevalence and Intensity of Soil Transmitted Helminth Infections and Schistosomiasis  
Mansoni During Lymphatic Filariasis Transmission Assessment Survey  
Collection in Uganda

By

Rachel Burriss  
MPH

Global Health

---

Dr. Jorge E. Vidal  
Committee Chair

---

Dr. Katie Gass  
Committee Member

Assessing Prevalence and Intensity of Soil Transmitted Helminth Infections and Schistosomiasis  
Mansoni During Lymphatic Filariasis Transmission Assessment Survey  
Collection in Uganda

By

Rachel A. Burriss

Bachelor of Arts  
DePauw University  
2015

Thesis Committee Chair: Dr. Jorge E. Vidal

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 Global Health  
2018

## Abstract

Assessing Prevalence and Intensity of Soil Transmitted Helminth Infections and Schistosomiasis  
Mansoni During Lymphatic Filariasis Transmission Assessment Survey  
Collection in Uganda  
By Rachel A. Burriss

**Introduction:** This study estimated the prevalence and intensity of Soil Transmitted Helminth (STH) infections during integrated Lymphatic Filariasis (LF) and STH Transmission Assessment Survey (TAS) collection in Uganda. This was a cross-sectional study that sampled preschoolers through adults in multiple sites using systematic sampling. The goal of this study was to investigate the burden of STH infections in an area that had been previously treated with the anthelmintic drugs, ivermectin and albendazole, during mass drug administration (MDA) for LF. The information presented in this thesis will be meaningful for the Ugandan national and district level NTD program's decision making as they move forward with MDA cutoff determination and consider integration of STH and LF elimination programming. **Methodology:** Prevalence was evaluated for five species of parasite, *Necator americanus/Ancylostoma duodenale* (Hookworm), *Ascaris lumbricoides*, and *Trichuris trichiura*, *Schistosomiasis Mansoni*, as well as Circulating Filarial Antigen for LF (CFA). Additionally, intensity information was generated for *Necator americanus/Ancylostoma duodenale* (Hookworm), *Ascaris lumbricoides*, and *Trichuris trichiura*. **Results:** Overall, the majority of helminth infections identified in the study population did not exceed the >10% prevalence threshold recommended for yearly deworming by the World Health Organization (WHO). However, hookworm infections specifically, exceeded 10% in the overall population. In Bundibugyo district, Schistosomiasis and Hookworm were well above 10% prevalence, while in Amuria prevalence for all STH infections was below 10%. Where infections existed, intensity as a measure of eggs per gram of stool, was also calculated. Intensity was low for all species apart from some moderate and high hookworm infections in Bundibugyo and Amuria. **Discussion:** STH infections have diminished overtime presumably as a result of program interventions such as MDA. Still, high prevalence of infection in Bundibugyo persists, particularly for hookworm and schistosomiasis infections. **Recommendations:** Recommendations for program next steps for Amuria and Ntoroko should be to continue with biennial deworming and other behavioral interventions, while Bundibugyo should consider scaling up deworming annually and to a greater scope of community persons such as Women of Child Bearing Age (WBCA).

Assessing Prevalence and Intensity of Soil Transmitted Helminth Infections and Schistosomiasis  
Mansoni During Lymphatic Filariasis Transmission Assessment Survey  
Collection in Uganda

By

Rachel A. Burriss

Bachelor of Arts  
DePauw University  
2018

Thesis Committee Chair: Jorge E. Vidal, PhD.

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 Global Health  
2018

## Table of Contents

I. Introduction	1
II. Literature Review	3
III. Methodology	10
IV. Results	17
a. Additional Results	30
V. Discussion	33
VI. Recommendations	36
VII. Acknowledgments	38
VIII. References	39

## List of Figures

- Figure 1. Life Cycle of Three Soil Transmitted Helminths
- Figure 2. Life Cycle of Schistosomiasis Mansoni
- Figure 3. Life Cycle of Wuchereria Bancrofti
- Figure 4. Data Cleaning Process for STH Sampling
- Figure 5. Hookworm Cases in Bundibugyo District
- Figure 6. Mean Egg Count by Coefficient of Variation in Amuria
- Figure 7. Mean Egg Count by Coefficient of Variation in Bundibugyo

## List of Tables

- Table 1. Summary of Study Population for STH and LF Sampling by Age and Sex
- Table 2. Summary of Prevalence by Disease Species, Sex, and Age
- Table 3. Amuria District Prevalence by Disease Species, Age and Sex
- Table 4. Bundibugyo District Prevalence by Disease Species, Age and Sex
- Table 5. Ntoroko District Prevalence by Disease Species, Age and Sex
- Table 6. Hookworm Intensity in Amuria by Age and Sex
- Table 7. Ascaris Intensity in Amuria by Age and Sex
- Table 8. Trichuris Intensity in Amuria by Age and Sex
- Table 9. Hookworm Intensity in Bundibugyo by Age and Sex
- Table 10. Ascaris Intensity in Bundibugyo by Age and Sex
- Table 11. Trichuris Intensity in Bundibugyo by Age and Sex
- Table 12. Intensity of Hookworm, Ascaris, and Trichuris Infections in Ntoroko District
- Table 13. Overall Prevalence of STH Infection Restricted on Coefficient of Variation
- Table 14. WHO Prevalence Thresholds for STH Treatment Strategies
- Table 15. WHO Prevalence Thresholds for SCH Treatment Strategies

## Table of Abbreviations

Bill & Melinda Gates Foundation – BMGF

Evaluation Unit – EU

Gross Domestic Product – GDP

Implementation Units – IU

Lymphatic Filariasis – LF

Mass Drug Administration – MDA

Ministry of Health – MOH

National Program for the Elimination of Lymphatic Filariasis – NPELF

Neglected Tropical Disease – NTD

Program to Eliminate Lymphatic Filariasis – PELF

Schistosomiasis Control Initiative – SCI

Schistosomiasis and Worm Control Program – BWCP

Soil Transmitted Helminths – STH

Transmission Assessment Survey – TAS

Vector Control Division – VCD

World Health Assembly – WHA

World Health Organization – WHO



## I. Introduction

Throughout history the presence of Soil Transmitted Helminths (STH) has burdened populations. Highly infectious, and easily spread through soil and other fecal-oral routes such as contaminated water and vegetables, STH infections typically burden individuals in endemic areas from infancy to adulthood<sup>27</sup>. Currently it is estimated that over 1.5 billion individuals are affected by STH. With these infections come a host of consequences namely: stunting, cognitive impairment, anemia, and malnutrition<sup>27</sup>. In areas with higher rates of infection intensity – intensity is classified as low, moderate, or high – these morbidities are further exacerbated<sup>23</sup>. The consequences of these conditions are widespread and have impact on school attendance, economic productivity and overall wellbeing of the affected populations<sup>20</sup>.

In 2001 the World Health Assembly (WHA) established the WHA 54.19, a resolution that committed global health partners to efforts to curb the spread of STH as well as Schistosomiasis - a waterborne parasitic disease also transmitted by worms<sup>27</sup>. Since that time point, national programs have increased campaigns to alleviate the burden of STH on communities. In 2006, WHO asserted that “periodic administration of preventive chemotherapy” would be effective in the control of STH as well as other neglected tropical diseases such as Schistosomiasis and LF. As a result, MDA for each neglected disease was scaled up<sup>28</sup>.

While the success of these programs has been seen through elimination of LF and Schistosomiasis in some countries, it is yet to be understood how these programs interact with each other and what burden of STH infections remain on communities who have been receiving treatment for one or more of the neglected tropical diseases. Additionally, in areas where certain programs are scaling down, overlapping programs must assess their new role.

For example, in light of the scale-down of LF programming in Uganda characterized by TAS, there is a need to assess STH and decide if/how the STH program will pick up and resume treatment.

Historically, Uganda has been endemic for LF and STH<sup>29</sup>. However, despite treatment overlap, STH and LF programs have frequently worked in silos and have not often coordinated sampling, treatment, or surveillance efforts. In 2015, the WHO published “Assessing the Epidemiology of STH during a TAS” with guidelines for how to conduct STH sampling and testing in coordination with LF program Transmission Assessment Surveys<sup>17</sup>. This recommendation provided a direction for integration of the programs and standard operating procedures for sampling and data collection. This study - conducted in three districts of Uganda - was the first time a STH survey was integrated with TAS II activities in Uganda. TAS II is the second iteration of TAS. It occurs 2-3 years after the original TAS and is intended to identify if disease recrudescence has occurred since discontinuation of MDA.

This paper will estimate the current disease burden of STH in Uganda by calculating prevalence and intensity of STH infections in the districts of Uganda where extensive programming to treat LF, including a previous TAS, has occurred<sup>31</sup>. The goal of this research is to elucidate the areas that can be targeted for future STH programs in the sampled districts particularly with regard for high-risk populations. This research will provide a valid and precise estimate of the prevalence and intensity of infection among the study population for the five species of STH *Necator americanus*/*Ancylostoma duodenale*(Hookworm), *Ascaris lumbricoides*, and *Trichuris trichiura*, and *Schistosomiasis Mansoni* and the prevalence of Circulating Filarial Antigen (CFA) for LF in the TAS Evaluation Unit (EU).

## II. Literature Review

### *a. Demographics*

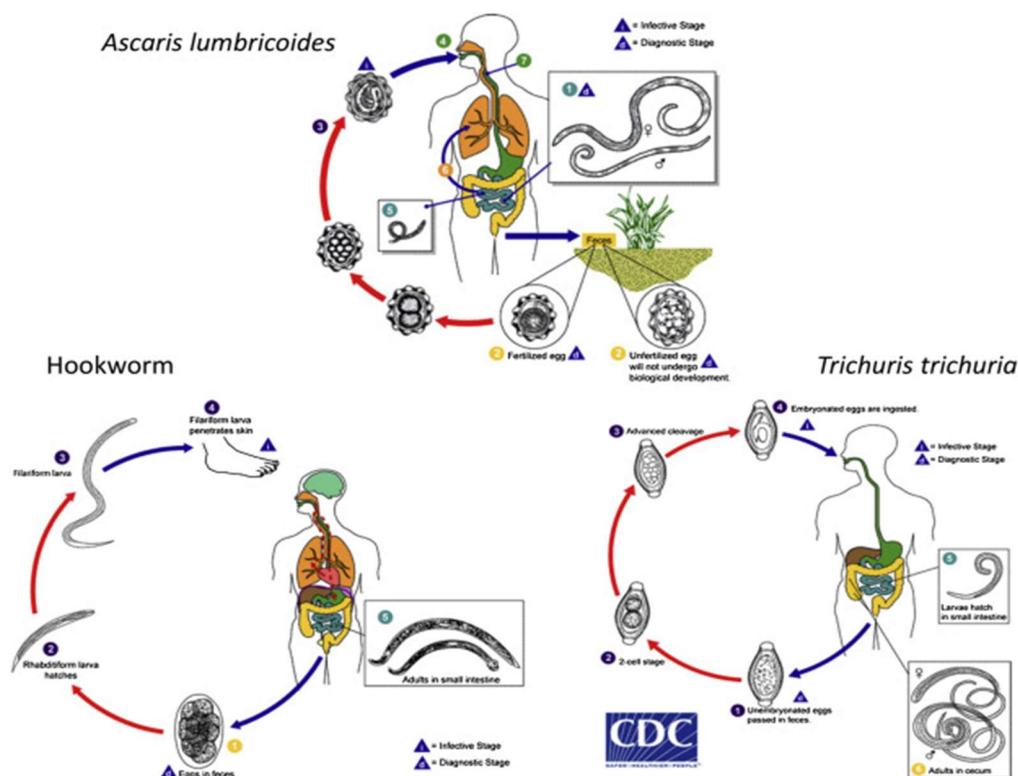
Uganda is a landlocked nation in East-Central Africa. It is bordered by Rwanda to the South, Sudan to the North, the Democratic Republic of Congo to the West and Kenya to the East. Approximately 41.49 million people live in Uganda across the 4 major regions (North, South, East and West) and the population is growing at a rate of ~3.3% annually<sup>30</sup>. The 2016 Gross Domestic Product (GDP) was \$24 billion USD. The GDP per capita is ~\$2,400, which places Uganda economically in the bottom ¼ of all countries in the world<sup>25</sup>. The current life expectancy is 59.5 years, compared to ~80 years in the United States<sup>29</sup>. Historically, Uganda was one of the most highly affected countries by Neglected Tropical Diseases (NTDs), a group of tropical infections that are frequently found in low-income populations throughout Africa, Asia, and the Americas<sup>10</sup>. These NTDs include Soil Transmitted Helminths, Schistosomiasis, and Lymphatic Filariasis<sup>10</sup>.

### *b. Soil Transmitted Helminth (STH) infection*

The three STH infections most commonly found in Uganda, *Necator americanus*/*Ancylostoma duodenale*(Hookworm), *Ascaris lumbricoides*, and *Trichuris trichiura* can all be found in the surrounding soil. *Ascaris* and *trichuris* eggs are fertilized in the human body and deposited into soil by human feces in areas where modern sanitation does not exist. STH eggs can survive for many years in the soil of the tropics, where moisture and shade are common<sup>15</sup>. Once in the soil, *ascaris* and *trichuris* eggs are subsequently consumed by human hosts through contaminated water and food. Hookworm ova are also deposited into the soil through human feces but mature to become worms while in the earth. Once mature, these

worms can burrow through human skin, typically through the foot where they multiply in the human host. Hookworm infections are not transmitted through fecal-oral routes<sup>3</sup>. Due to the nature of transmission and the parallel environments, it is not uncommon to find more than one STH species infecting a human host at a given point in time<sup>15</sup>.

**Figure 1.** Life Cycle of Three Soil Transmitted Helminths

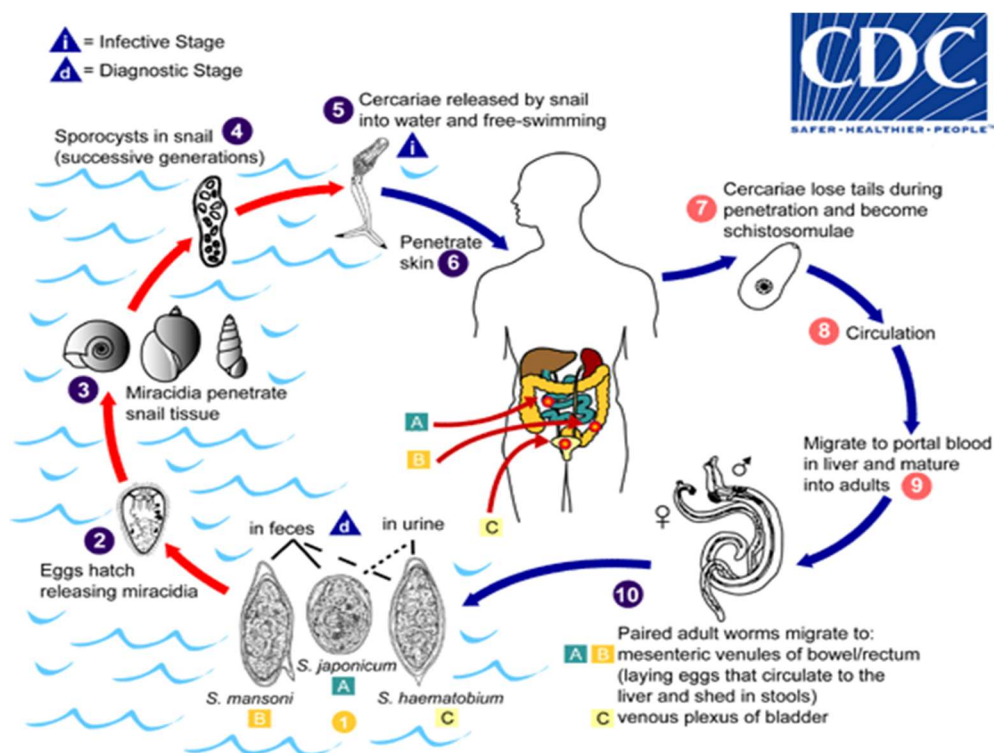


### *c. Schistosomiasis (Bilharzia)*

Schistosomiasis, also known as Bilharzia is a waterborne parasitic disease. Its life cycle is unique in that it requires not only a human host but fresh-water snails to produce successive generations of parasite. Similar to hookworm, schistosomiasis cercariae (parasitic flukes) can penetrate human skin while they wade/bathe in water. Once the cercariae

penetrate the human host, they mutate and circulate through the bloodstream and into the liver for final maturity. These mature worms mate in the bowel and then migrate once more to the liver where eggs are laid and excreted through stool<sup>2</sup>. Without treatment, schistosomiasis infection can persist for years, causing a host of complications largely affecting the liver and bladder<sup>2</sup>.

**Figure 2.** Life Cycle of Schistosomiasis Mansoni

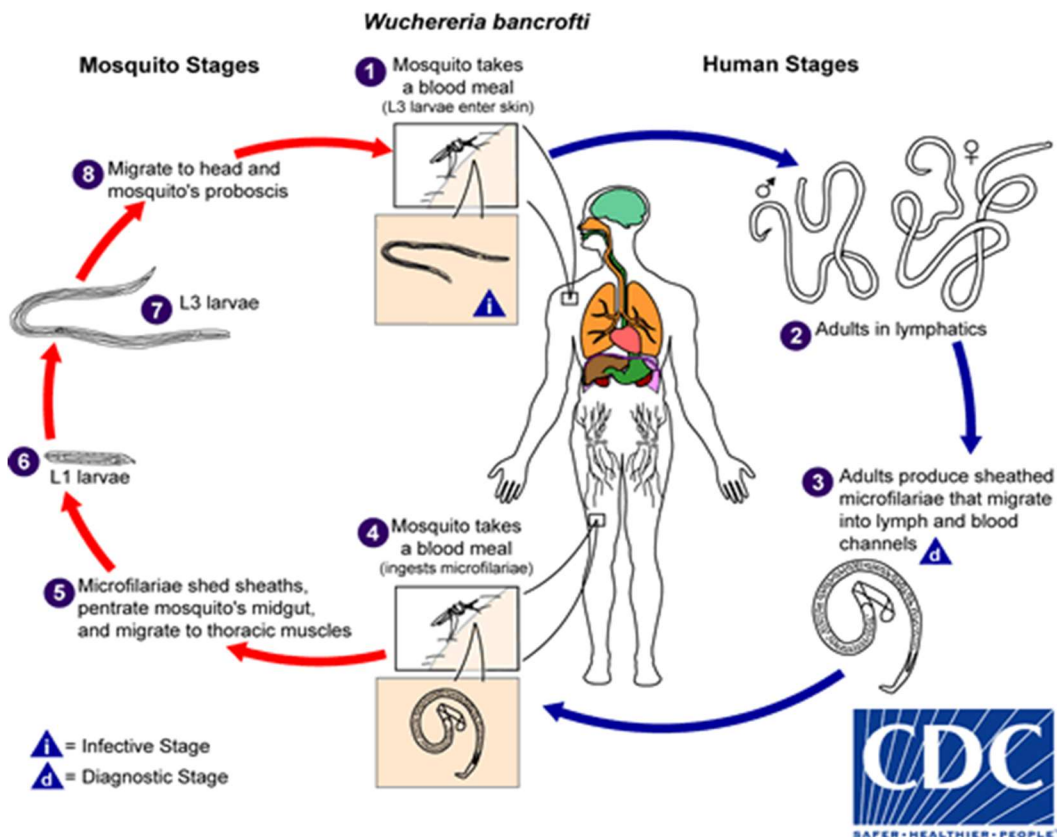


#### *d. Lymphatic Filariasis*

Lymphatic Filariasis is a neglected tropical disease transmitted by anopheles mosquitoes<sup>3</sup>. When a mosquito has a blood meal, larvae are transmitted to the human where they mature into adult worms. These adult worms then produce microfilariae that travel to the bloodstream and lymphatic system where they cause severe comorbidities due to

swelling. Subsequently, infected humans can transmit microfilariae from the bloodstream back to a mosquito during its blood meal. These microfilariae produce larvae that continue the life cycle in a succeeding human host.

**Figure 3.** Life Cycle of Lymphatic Filariasis - *Wuchereria Bancrofti*



#### *e. NTD Control Programs*

To combat these disease in Uganda, external partners and national organizations came together to develop elimination programs. The Vector Control Division (VCD) of the Ministry of Health (MOH) in Uganda was established in 1920 primarily to address concerns with Malaria. However, in the early 1990s external partners such as WHO, Schistosomiasis Control Initiative (SCI) and the Bill & Melinda Gates Foundation (BMGF) pledged funds to

the VCD to extend their scope of disease surveillance and control to additional NTDs<sup>16</sup>. From this support the Program to Eliminate Lymphatic Filariasis (PELF) and the Schistosomiasis and Worm Control Program (BWCP) were born in 2002 and 2003, respectively.

In 2011, following almost 10 years of NTD program activities, WHO published the second edition of *Helminth Control for School Aged Children - a guide for managers of control programmes* that updated MDA delivery timelines for STH. Currently, the recommended MDA regimen for areas with 10% or greater STH infections, is a dose of deworming medication administered every year to school-aged children and high-risk adults. In areas where infection is greater than 50% in the population, three doses should be administered annually. The recommended drugs for treatment are albendazole and mebendazole, which have been largely supplied through donations from pharmaceutical companies. The Ugandan NTD programs use a combination of albendazole, ivermectin and praziquantel for MDA treatments at different time-points and in differing locations based on their own needs and capacity for combating their endemic NTDs<sup>23</sup>.

Similarly, to the STH guidelines, the WHO has also provided program recommendations for administration of MDA to progress toward elimination of LF. The WHO recommended plan of action for LF elimination in areas that are co-endemic with onchocerciasis, such as Uganda, is administration of 50 µg/kg of body weight ivermectin plus 400 mg albendazole annually for a period of 5 years with at least 65% coverage followed by TAS<sup>17</sup>. To facilitate this strategy, country or district-based programs are tasked with administering MDA to Implementation Units (IU) across their country. After an IU has completed five successful rounds of MDA and has determined that the prevalence of LF is lower than 1% in sentinel and spot check sites, it is recommended that they conduct a TAS<sup>17</sup>.

The primary goal of a TAS is to determine if disease prevalence is low enough that transmission of disease cannot be sustained beyond the secession of MDA activities<sup>17</sup>. Districts where the TAS is being conducted are referred to as Evaluation Units (EU) and can be comprised of more than one district if they meet the recognized TAS criteria. There are multiple rounds of TAS. The first TAS determines if MDA delivery will stop in the EU, this decision is based on whether prevalence is lower than 1%. If MDA is discontinued following the first TAS a TAS II should be conducted 2-3 years later to determine that prevalence remains sufficiently low for continual secession of MDA activities. Many EUs in Uganda, such as the site of this study, are in this surveillance phase of the elimination programming for LF for the first time ever, which provides a valuable opportunity to gather novel information.

*e. Integration of STH and LF Programs*

Integration of NTD programs has been a conversation for many years and poses potential positive and negative consequences. To determine the capacity for integration, from 2013 - 2016 studies were conducted in Haiti, Benin, Tonga, Sri Lanka and Burkina Faso to evaluate the feasibility of STH sampling in EUs conducting their first TAS. The studies in Benin, Tonga and Sri Lanka, found that STH integration with LF activities was not only feasible but potentially cost effective. In their conclusions the Benin and Tonga study team suggested that standardized guidelines should be developed and field-tested to identify the ideal sampling strategy, diagnostic tests, and specimen collection procedures<sup>4</sup>.

In a continuation of this effort the research conducted in Haiti by the National Program for the Elimination of Lymphatic Filariasis (NPELF) was a study of the feasibility of the WHO recommended integration strategy of STH and LF programs. The study



generated important data on the prevalence of STH and Malaria as well as Circulating Filarial Antigen for LF and determined that not only was STH integration to LF TAS programming feasible, it could be standardized through the WHO guidelines and it had benefits to cost-effectiveness, collaboration between agencies, and generating new next steps for multiple programs at one time<sup>13</sup>.

In 2016 a study conducted in Burkina Faso examined the prevalence of STH during LF TAS. The study found that successive MDAs had lowered prevalence of STH in the population in addition to effectively diminishing prevalence of LF as well<sup>5</sup>. The study highlighted once again the feasibility of the WHO integration strategy among school age children. In conclusion it was suggested that “LF TAS provides one feasible and efficient platform to assess the STH situation for post LF MDA decision making and should be further examined and implemented as a monitoring and evaluation tool”<sup>5</sup>. The 2017 assessment of STH in Uganda during a TAS II addressed in this thesis provides additional evidence to the practice of program integration as well as information about STH prevalence following successive LF MDA and MDA secession.

#### *e. High-risk populations*

While the WHO strategy for integration of STH focuses on school age children (SAC) due to the parameters around LF treatment, it is important to recognize that the high-risk populations for STH infections are more diverse than 6-7 year-old primary school students. For example, both infants and preschool aged children are at risk of STH infections leading to cognitive impairment and stunted growth<sup>17</sup>. Unfortunately, this is a group that is often excluded from treatment regimens despite WHO recommendations<sup>15</sup>. In 2012, coverage estimates for at-risk preschool age children (PSAC) receiving treatment for

STH was roughly 25% and ranged from 5%-35% based on location<sup>15</sup>. This coverage was not only insufficient for this age group, but also represented a decline from previous estimates of coverage.

Another high-risk group is Women of Childbearing Age (WCBA) who are at risk of anemia due to STH infection. Anemia at high levels during pregnancy can lead to negative mother and neonate outcomes. The consequences of which are exacerbated in low-resource settings. Despite evidence for the need of treatment in the WCBA age group, the cohort has been excluded from many treatments programs. According to a study published in February 2018, “preventive chemotherapy targeting Women of Reproductive age is not yet being implemented in many STH-endemic countries despite the recognized benefits of deworming”<sup>19</sup>.

### III. Methodology

The methodology for this study was based on the guidelines presented in the WHO manual “Assessing the epidemiology of soil-transmitted helminths during a transmission assessment survey (TAS) in the global programme for the elimination of lymphatic filariasis”<sup>17</sup>. These guidelines address the combination of STH sampling activities during blood-collection and evaluation of transmission using FTS for LF in eligible sites. This site selection criteria states that TAS sites will be within implementation units not exceeding 1,000,000 persons, with an anticipated MDA drug coverage for LF of  $\geq 65\%$  for the past 5 years<sup>17</sup>.

The study sites of Amuria, Bundibugyo, and Ntoroko, Uganda were selected for this study based on the successful completion of a first TAS (2014) and planned implementation of TAS II (2017)<sup>31</sup>. The sites also exist in a region known to be endemic for STH permitting

the assumption that STH species could be detected<sup>31</sup>. According to a projection by the Uganda Bureau of Statistics, the Amuria EU has a total population of 270,601<sup>31</sup>. The EU is made of 16 sub counties, 86 parishes, 588 villages and 135 primary schools. The Bundibugyo population is approximately 356,174 persons and is comprised of 29 sub counties, 132 parishes, 818 villages and 157 primary schools<sup>31</sup>. Ntoroko contains approximately 24,000 persons and 3 sub counties. Sample villages within each EU for the TAS II were selected through systematic selection from the Survey Sample Builder<sup>31</sup>. Following blood-collection for the TAS evaluation, a random cross-section of school children and adults from the TAS sample were enrolled for the STH survey. This strategy is a deviation from the recommendation of school-based cluster sampling for STH assessment by the WHO. This departure from the guidelines was purposeful in this study design so that data on other high-risk groups (PSAC and WCBA) could be assessed. At study enrollment 1,600 individuals were enrolled.

Following enrollment in the study, stool collection cups necessary for the Kato-Katz test were provided to each participant. Participants were instructed on how to safely sample stool in their own homes. Participants were instructed to return stool cups to the field laboratory (located in the village) immediately after sampling their stool so the sample could be read by the microscopists. Stool samples were collected from a total of 1,463 individuals. This study was cross-sectional and stool collections occurred at a single time point.

To assess prevalence of STH, schistosomiasis, and Circulating Filarial Antigen for LF, the Kato-Katz stool smear and Alere Filariasis Test Strip were used. These are the gold standard diagnostic tests for the sampled diseases. All Kato-Katz smears were made using the stool from a single day from the selected participants in accordance with Kato-Katz protocol<sup>9</sup>. Duplicate Kato-Katz slides were prepared using 41.7 mg templates for each

sample. The slides were read daily to detect STH infection and to calculate intensity. Eggs were counted and intensity of infection was enumerated as eggs per gram of feces (epg).

Zero eggs per gram of stool were classified as “zero” intensity for all species. For hookworm infection “Low” intensity was classified as 1 – 1,999 eggs per gram of stool, “Moderate” was 2,000 – 3,999 eggs per gram of stool, and “High” was classified as 4,000+ eggs per gram of stool. For ascaris, “Low” intensity was classified as 1 – 4,999 eggs per gram of stool, “Moderate” intensity was 5,000 – 49,000 and “High” intensity was classified as 50,000+ eggs per gram. *Trichuris* infections were classified as “Low” if eggs per gram were between 1- 999, “Moderate” if 1,000 – 9,999 eggs per gram of stool were detected, and “High” if 10,000+ eggs per gram of stool were detected. These classifications were based on WHO guidelines<sup>17</sup>. The Kato-Katz samples were read 2 separate times by skilled microscopists. The two slide reads were averaged to obtain one value that was the arithmetic mean of the egg counts between the two slides.

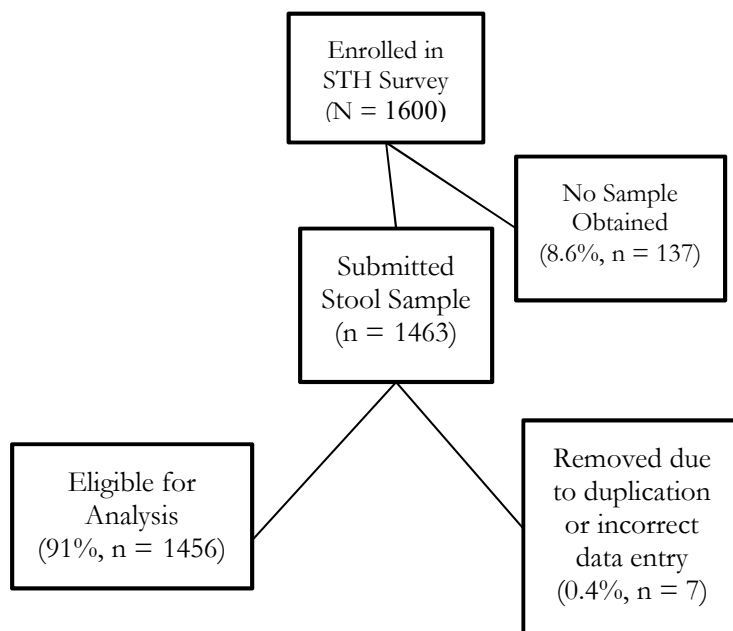
Parasitic infection status of each individual was determined by examinations of stool samples using the Kato-Katz techniques<sup>9</sup>. The stool samples were examined microscopically for eggs of *Hookworm-Necator americanus* / *Ancylostoma duodenale*, *Ascaris lumbricoides*, *Trichuris trichiura* and *Schistosoma Mansoni*. Due to the timeliness needed for the detection of hookworm all samples for Kato-Katz were read within 30 minutes, WHO guidelines recommend within 4-6 hours<sup>17</sup>. Stool data was recorded in the field on data sheets and a duplicate entry was done later in Kampala, Uganda. Prior to data analysis the STH and CFA data was cleaned in Microsoft Excel. Of the 1600 individuals, 144 were removed during data cleaning due to duplication, missing values, or inconsistent data entry. This left us with a sample size of 1456 individuals. During data cleaning a Coefficient of Variation (CV) was also calculated to

address if any samples should be thrown out based on a percent difference between the two Kato-Katz readings of >20%.<sup>1</sup>

$$\text{Coefficient of Variation} = \frac{S}{\bar{X}} \times 100$$

The CV results demonstrated a large subset of data whose percent difference between microscopist reads exceeded 20%. After reviewing this subset, it was determined that due to low egg counts the differences greater than 20% were not particularly meaningful in the context of the study and would not be discarded (e.g. 1 egg vs. 0 eggs. read).

**Figure 4.** Data Cleaning Process for STH Sampling



<sup>1</sup> S = Standard Deviation of Sample and  $\bar{x}$  = Sample mean

Following data cleaning, an estimate of prevalence was calculated for each species of parasite tested with the Kato-Katz stool smear. A positive infection was classified as a mean egg count of  $>0$  eggs identified during microscopic examination of the stool smear<sup>3</sup>. If no eggs were detected, then that stool smear was classified as having no infection and “zero” intensity of infection. If the first or second microscopist identified eggs but the other did not the participant was considered to have infection. For example, a read of 1 egg versus 0 eggs. This method was determined in light of knowledge of decay of hookworm eggs and an overall lack of sensitivity of Kato Katz. Prevalence of CFA in blood samples was also tested to identify the presence of LF transmission in the community. Filariasis Test Strips were used to identify positive and negative individuals.

Prevalence for the overall study population was calculated for each disease by dividing the number of positively identified individuals by the entire study population.

$$\text{Prevalence} = \frac{\text{\# of individuals with positive diagnosis}}{\text{Total \# number of individuals sampled}}$$

Following calculation of overall prevalence by disease the data was stratified into groups of interest. Particularly, by location (district level), age (preschool aged children, school-aged children, women of childbearing age) and sex (male, female). A chi-square analysis was conducted to determine p-values between the female and male groups as well as age groups using Microsoft Excel.

In addition to calculating prevalence, intensity was also calculated by averaging the egg count between microscopists reads multiplied by 24<sup>31</sup>. This calculation provides the eggs

per gram of stool value that can then be categorized by WHO standardized intensity thresholds.

$$\text{Eggs per gram (epg)} = \left( \frac{\# \text{ eggs 1st read} + \# \text{ eggs 2nd read}}{2} \right) \times 24$$

Before STH sampling occurred, the study team contacted technical and administrative department leaders in the prospective districts over the phone and explained the purpose and procedure of the STH survey<sup>31</sup>. This information was also relayed during study start up and enrollment of the target population. During enrollment informed consent was received from all participants<sup>31</sup>. In the case of children <18, consent was received via a parent or guardian and assent was received from the child in the form of providing stool. This study was determined to meet the criteria for approval by the Uganda National Council for Science and Technology and the Ministry of Health's Vector Control Division. The survey was fielded in October 2017 by trained field staff employed by the National Bilharzia and Worm Control Programme in Uganda<sup>31</sup>.

Limitations to this methodology are both technical and operational. First, it is likely that some information bias was generated. In particular, egg counts with a Coefficient of Variation greater than 20% were still included in the overall analysis. While this is a potential limitation, the inclusion of these individuals was intentional. Most of the Coefficients of Variation that exceeded 20% had mean egg counts <20. Thus, the difference in egg counts while potentially high mathematically, was not meaningfully different for analysis purposes.

An additional limitation of this study is the Kato-Katz stool test. The Kato-Katz test has been recognized to be a less than ideal test for schistosomiasis infections and there is speculation that this deficit is exacerbated in low intensity areas<sup>12</sup>. Additionally, the Kato-Katz requires that hookworm slides be read extremely quickly because hookworm ova tend to become undetectable within 4-6 hours of sample collection. Fortunately, the use of a field laboratory by the study team in Uganda made it possible to read all slides within 30 minutes of collection<sup>31</sup>. Still, scientifically, it is possible that some hookworm ova were lost during this timeframe. This issue may have contributed to some measurement bias in this study. Unfortunately, there are very few alternative diagnostic tests for the parasitic species identified in this study, particularly in field laboratories in lower resourced settings such as the study location in Uganda. It is worth noting that during stool sample collection in the field, some of the stool was aliquoted for testing with a new PCR tool, this data is anticipated in late 2018.

Another limitation to this study was the prohibitively low number of PSAC individuals sampled (n=4) This may have been an issue of the randomization conducted to generate the study sample or perhaps an issue with the study protocol that stipulated an inclusion criteria of >3 years of age. Given the small sample, prevalence and intensity estimates for the PSAC group are not generalizable. Additionally, there was a low number of individuals in the Ntoroko District (n=52) compared to that of Amuria and Bundibugyo, which prohibits generating a conclusive measure of prevalence in that area. This is potentially due to randomization error as well.

An additional limitation to this study methodology is the risk of selection bias. Many participants (n=137) did not return stool samples to the field lab. Their lack of participation may belie some inherent difference among those individuals that are not accounted for in



this study. It is possible that these individuals were also non-compliant during MDA. With reference to prevalence, data on these individuals could be a valuable perspective that this data is missing.

#### IV. Results

Following the testing of 3135 individuals with FTS for the LF TAS II, individuals were enrolled and sampled for the STH assessment. Of the 1600 individuals enrolled for STH, 1456 participants stool samples were analyzed. Of these persons, 610 resided in Amuria district, 794 in Bundibugyo district, and 52 in Ntoroko district. There were largely more female participants than males in the study sample, with women comprising approximately 61% of the study population. This trend remained consistent across the Amuria and Bundibugyo districts, with the exception of Ntoroko where the percentage of women and men was more or less equivalent. The SAC age group comprised the greatest percentage of the study sample, ~65%, while adults were approximately ~36% of the sample. This trend was also consistent across all districts. The PSAC age group was the smallest and was unable to generate any generalizable data. Similarly, the Ntoroko district was substantially smaller than the other two districts sampled, prohibiting a realistic estimation of prevalence in the overall district population.

**Table 1.** Summary of Study Population for STH and LF Sampling by Age and Sex

	<b>Amuria</b>	<b>Bundibugyo</b>	<b>Ntoroko</b>	<b>Total</b>
<b># Sampled:</b>				
STH Stool Sample	610	794	52	<b>1456</b>
LF Blood Sample	1553	1253	329	<b>3135</b>
<b>Sex (STH Sample):</b>				
Male, N (%)	249 (40.8)	285 (35.9)	25 (48.1)	<b>559 (38.4)</b>
Female, N (%)	361 (59.2)	509 (64.1)	27 (51.9)	<b>897 (61.6)</b>
<b>Age (STH Sample):</b>				
PSAC, N (%)	3 (0.49)	1 (0.13)	0 (0.00)	<b>4 (0.27)</b>
SAC, N (%)	416 (68.2)	483 (60.8)	40 (76.9)	<b>939 (64.5)</b>
Adult, N (%)	191 (31.3)	310 (39.0)	12 (23.1)	<b>517 (35.5)</b>

During STH evaluation prevalence estimates were generated for 5 disease species: *Necator americanus*/*Ancylostoma duodenale* (hookworm), *Ascaris lumbricoides*, *Trichuris trichiura*, *Schistosoma mansoni* and LF via Circulating Filariasis Antigen. Overall prevalence was highest for hookworm (10.71%) which exceeded the WHO threshold for annual treatment. *Schistosoma mansoni* also had a relatively high prevalence in the overall population with 8.52% of the study population infected with parasite. *Ascaris* and *trichuris* prevalence was relatively low in the population, although still present. All FTS tests for LF were negative for presence of CFA, indicating zero transmission in the sampled population. The data presented in Table 2 provides a summary of prevalence by disease and grouped by sex and age. Age groups were defined according to WHO as PSAC 1-4 years, SAC 5-14 years, and WCBA 14-49 years<sup>17</sup>.

The chi-square statistical test did not reveal significant differences between prevalence according to sex for any of the species of STH based on a p-value of 0.05. However, prevalence of hookworm and trichuris were significantly different between age groups in the overall population with WCBA more burdened compared to PSAC and SAC.

**Table 2.** Summary of Prevalence by Disease Species, Sex, and Age

Characteristics	Disease N (prevalence %)					
	Sm	Hw	Asc	Tt	CFA	
<b>Overall</b> (n=1456)	124 (8.52)	156 (10.71)	19 (1.30)	16 (1.10)	0 (0.00)	
<b>Sex</b>						
Female (n=897)	71 (7.92)	100 (11.15)	14 (1.56)	12 (1.34)	0 (0.00)	
	<b>0.30</b>	<b>0.50</b>	<b>0.28</b>	<b>0.27</b>		
Male (n=559)	53 (9.48)	56 (10.02)	5 (0.89)	4 (0.72)	0 (0.00)	
<b>Age</b>						
PSAC (n=4)**	1 (25.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	
SAC (n=939)	81 (8.63)	<b>0.40</b>	86 (9.16)	<b>0.07</b>	<b>0.96</b>	5 (0.53)
				<b>0.02*</b>		
WCBA (n=395)	30 (7.59)	52 (13.16)	5 (1.27)	9 (2.28)	0 (0.00)	

\*Values significant with respect to a p-value of .05

\*\* Expected Chi-Square value of <5

**Sm**-Schistosomiasis Mansoni, **Hw**-Hookworm, **Asc**-Ascaris, **Tt**-Trichuris, **CFA**-Circulating Filarial Antigen

**Bold font** denotes p-values as determined through Chi-Square analysis

Following calculations of prevalence in the overall study population, data was stratified based on location (district). Stratification based on district is appropriate in the context of this study because decisions to treat are made by the Ugandan STH program at

the district level<sup>10</sup>. The following tables summarize prevalence for the Amuria, Bundibugyo and Ntoroko districts by disease species as well as by age group and sex.

The data presented in Table 3 are from the Amuria district. There were 610 stool samples analyzed from the Amuria district. Of these samples 361 were female and 249 were male. There were 3 individuals in the PSAC age group, 416 in the SAC age group, and 143 in the WCBA age group. Overall prevalence in the Amuria district for each STH disease species was relatively low compared to WHO thresholds for STH annual treatment (>10%). Schistosomiasis was most prevalent in the population at 3.44%. Hookworm, ascariis and trichuris were also prevalent in the Amuria district to a lesser extent, at 1.80%, 1.48% and 0.16%, respectively. The Circulating Filarial Antigen for LF was 0%, indicating no transmission of LF in the Amuria district. Prevalence of schistosomiasis and hookworm were significantly different between males and females in Amuria based on a p-value of 0.05. Males were more burdened than females. Similarly, prevalence among age groups was significantly different for schistosomiasis, with WCBA more greatly affected.

**Table 3.** Amuria District Prevalence by Disease Species, Age and Sex

Characteristics	Disease, N (prevalence %)								
	Sm		Hw		Asc		Tt		CFA
<b>Overall</b> (n=610)	21 (3.44)		11 (1.80)		9 (1.48)		1 (0.16)		0 (0.00)
<b>Sex</b>									
Female (n=361)	7 (1.94)	<b>0.01*</b>	2 (0.55)	<b>0.01*</b>	7 (1.94)	<b>0.25</b>	0 (0.00)	<b>0.23</b>	0 (0.00)
Male (n=249)	14 (5.62)		9 (3.61)		2 (0.80)		1 (0.40)		0 (0.00)
<b>Age</b>									
PSAC (n=3)**	1 (33.33)		0 (0.00)		0 (0.00)		0 (0.00)		0 (0.00)
SAC (n=416)	10 (2.40)	<b>0.01*</b>	8 (1.92)	<b>0.24</b>	5 (1.20)	<b>0.41</b>	0 (0.00)	<b>n/a</b>	0 (0.00)
WCBA (n=143)	5 (3.50)		0 (0.00)		4 (2.80)		0 (0.00)		0 (0.00)

\*Values significant with respect to a p-value of .05

\*\* Expected Chi-Square value of &lt;5

Sm-Schistosomiasis Mansoni, Hw-Hookworm, Asc-Ascaris, Tt-Trichuris, CFA-Circulating Filarial Antigen

**Bold font** denotes p-values as determined through Chi-Square analysis

The data presented in Table 4 are from the Bundibugyo district. There were 794 stool samples analyzed from the Bundibugyo district. Of these samples 509 were female and 285 were male. There was 1 individual in the PSAC age group, 483 in the SAC age group, and 244 in the WCBA age group. Overall prevalence in the Bundibugyo district for hookworm was relatively high compared to WHO thresholds for annual STH treatment (>10%) at 18.26% respectively. Schistosomiasis was similarly high at 12.85%. Ascaris and trichuris were also prevalent in the Bundibugyo district to a lesser extent, 1.26% and 1.89%, respectively. The Circulating Filarial Antigen for LF was 0%, indicating no transmission of

LF in the Bundibugyo district. There was no significant difference between male and female prevalence for any of the diseases in Bundibugyo based on a p-value of 0.05. However, for age, trichuris prevalence was significantly different among the groups with WCBA more greatly impacted.

**Table 4.** Bundibugyo District Prevalence by Disease Species, Age and Sex

Characteristics	Disease N (prevalence %)				
	Sm	Hw	Asc	Tt	CFA
<b>Overall</b> (n=794)	102 (12.85)	145 (18.26)	10 (1.26)	15 (1.89)	0 (0.00)
<b>Sex</b>					
Female (n=509)	63 (12.38)	98 (19.25)	7 (1.38)	12 (2.36)	0 (0.00)
	<b>0.60</b>	<b>0.34</b>	<b>0.70</b>	<b>0.20</b>	
Male (n=285)	39 (13.68)	47 (16.49)	3 (1.05)	3 (1.05)	0 (0.00)
<b>Age</b>					
PSAC (n=1)**	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
SAC (n=483)	70 (14.49)	78 (16.15)	8 (1.66)	5 (1.04)	0 (0.00)
	<b>0.26</b>	<b>0.21</b>	<b>0.35</b>	<b>0.05*</b>	
WCBA (n=244)	25 (10.25)	52 (21.31)	1 (0.41)	9 (3.69)	0 (0.00)

\*Values significant with respect to a p-value of .05

\*\* Expected Chi-Square value of <5

**Sm**-Schistosomiasis Mansoni, **Hw**-Hookworm, **Asc**-Ascaris, **Tt**-Trichuris, **CFA**-Circulating Filarial Antigen  
**Bold font** denotes p-values as determined through Chi-Square analysis

The data presented in Table 5 are from the Ntoroko district. There were 52 stool samples analyzed from the Ntoroko district. Of these samples 27 were from female participants and 25 were from male participants. There were no individuals in the PSAC age group, 40 in the SAC age group, and 8 in the WCBA age group. Only schistosomiasis was

prevalent in the Ntoroko district. The Circulating Filarial Antigen for LF was 0%, indicating no transmission of LF in the Ntoroko district. For the Chi-squared test, there was no significant difference for any of the age groups or sexes.

**Table 5.** Ntoroko District Prevalence by Disease Species, Age and Sex

Characteristics	Disease N (prevalence %)				
	Sm	Hw	Asc	Tt	CFA
Overall (n=52)	1 (1.92)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
<b>Sex</b>					
Female (n=27)	1 (3.70)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Male (n=25)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
	<b>0.33</b>	<b>n/a</b>	<b>n/a</b>	<b>n/a</b>	<b>n/a</b>
<b>Age</b>					
PSAC (n=0)**	n/a	n/a	n/a	n/a	n/a
SAC (n=40)	1 (2.50)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
WCBA (n=8)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
	<b>n/a</b>	<b>n/a</b>	<b>n/a</b>	<b>n/a</b>	<b>n/a</b>

\*\* Expected Chi-Square value of <5

**Sm**-Schistosomiasis Mansoni, **Hw**-Hookworm, **Asc**-Ascaris, **Tt**-Trichuris, **CFA**-Circulating Filarial Antigen  
**Bold font** denotes p-values as determined through Chi-Square analysis

In addition to prevalence, intensity of infections was calculated and reported as a value of eggs per gram for hookworm, ascaris, and trichuris. Infection intensity was grouped by disease into low, moderate and high-intensity based on WHO delineated thresholds<sup>18</sup>. Intensity of infection is an important indicator in this dataset because the severity of STH infection is associated with the burden of worms in the system of infected individuals<sup>23</sup>. A

low intensity infection is less likely to cause severe comorbidities compared to a high intensity infection. Intensity data was also stratified by location.

Tables 6, 7 and 8 contain the results of the intensity assessment for three species of STH in Amuria district. Of the hookworm infections identified in Amuria overall, only 1.80% were low-intensity. There were no moderate or high intensity infections. When grouped by age, only the SAC age group had infection at low-intensity (n=8). Likewise, between male and female groups, the males had a greater number of low-intensity infections (n=9). Males presented the largest percentage of low-intensity infection at 3.61%.

**Table 6.** Hookworm Intensity in Amuria by Age and Sex

	<b>Intensity (eggs per gram)</b>			
	<b>Zero*</b> N (%)	<b>Low**</b> N (%)	<b>Moderate†</b> N (%)	<b>High‡</b> N (%)
Overall (n=610)	599 (98.20)	11 (1.80)	0 (0.00)	0 (0.00)
PSAC (n=3)	3 (100.00)	0 (0.00)	0 (0.00)	0 (0.00)
SAC (n=416)	408 (98.08)	8 (1.92)	0 (0.00)	0 (0.00)
WCBA (n=143)	143 (100.00)	0 (0.00)	0 (0.00)	0 (0.00)
Male (n=249)	240 (96.39)	9 (3.61)	0 (0.00)	0 (0.00)
Female (n=361)	359 (99.45)	2 (0.55)	0 (0.00)	0 (0.00)

\*0 epg; \*\*1-1,999 epg; † 2,000-3,999 epg; ‡ 4,000+ epg

Overall, only 1.31% of the ascaris infections identified in Amuria were classified as low-intensity infections. One individual had a moderate infection (0.16%). When grouped by age, both the SAC age group and the WCBA group had low-intensity infections. However,



low-intensity infections made up a larger percentage of the WBCA group compared to the SAC group. Between male and female groups, both had low-intensity infections, equating to 0.80% and 1.66% respectively.

**Table 7.** Ascaris Intensity in Amuria by Age and Sex

	<b>Intensity (eggs per gram)</b>			
	Zero* N (%)	Low** N (%)	Moderate† N (%)	High‡ N (%)
Overall (n=610)	601 (98.52)	8 (1.31)	1 (0.16)	0 (0.00)
PSAC (n=3)	3 (100.00)	0 (0.00)	0 (0.00)	0 (0.00)
SAC (n=416)	411 (98.79)	4 (0.96)	1 (0.24)	0 (0.00)
WCBA (n=143)	139 (97.20)	4 (2.79)	0 (0.00)	0 (0.00)
Male (n=249)	247(99.19)	2 (0.80)	0 (0.00)	0 (0.00)
Female (n=361)	354 (98.06)	6 (1.66)	1 (0.28)	0 (0.00)

\*0 epg; \*\*1-4,999 epg; †5,000-49,999 epg; ‡ 50,000+ epg

Of the trichuris infections identified in Amuria overall, only one individual in the sample had a low-intensity infection. Of the 610 individuals sampled, 609 had zero eggs per gram of stool. The singular individual identified with low-intensity trichuris infection was an adult male who did not belong to one of the specific age groups stratified on in the study.

**Table 8.** Trichuris Intensity in Amuria by Age and Sex

	<b>Intensity (eggs per gram)</b>			
	<b>Zero*</b> N (%)	<b>Low**</b> N (%)	<b>Moderate<sup>†</sup></b> N (%)	<b>High <sup>‡</sup></b> N (%)
Overall (n=610)	609 (99.83)	1 (0.16)	0 (0.00)	0 (0.00)
PSAC (n=3)	3 (100.00)	0 (0.00)	0 (0.00)	0 (0.00)
SAC (n=416)	416 (100.00)	0 (0.00)	0 (0.00)	0 (0.00)
WCBA (n=143)	143 (100.00)	0 (0.00)	0 (0.00)	0 (0.00)
Male (n=249)	248 (99.59)	1 (0.40)	0 (0.00)	0 (0.00)
Female (n=361)	361 (100.00)	0 (0.00)	0 (0.00)	0 (0.00)

\*0 epg; \*\*1-999 epg; <sup>†</sup>1,000-9,999 epg; <sup>‡</sup> 10,000+ epg

Tables 9, 10 and 11 contain the results of the intensity assessment for three species of STH in Bundibugyo district. Of the hookworm infections identified in Bundibugyo overall, 17.63% were low-intensity. Additionally, 3 individuals had moderate intensity infection (0.38%) and 2 individuals had high intensity infections (0.25%). When grouped by age, the SAC age group had 15.53% of infections at low-intensity. WCBA had 21.31% of total infections at low-intensity. Between male and female groups, females presented the largest percentage of low-intensity and moderate-intensity infections at 18.47% and 0.59% , respectively. Two high intensity infections were identified, one in the SAC age group and one among an adult not included in a stratified age group.

**Table 9.** Hookworm Intensity in Bundibugyo by Age and Sex

	<b>Intensity (eggs per gram)</b>			
	Zero* N (%)	Low** N (%)	Moderate† N (%)	High‡ N (%)
Overall (n=794)	649 (81.74)	140 (17.63)	3 (0.38)	2 (0.25)
PSAC (n=1)	1 (100.00)	0 (0.00)	0 (0.00)	0 (0.00)
SAC (n=483)	405 (83.85)	75 (15.53)	2 (0.41)	1 (0.21)
WCBA (n=244)	192 (78.69)	52 (21.31)	0 (0.00)	0 (0.00)
Male (n=285)	238 (83.51)	46 (16.14)	0 (0.00)	1 (0.35)
Female (n=509)	411 (80.75)	94 (18.47)	3 (0.59)	1 (0.19)

\*0 epg; \*\*1-1,999 epg; †2,000-3,999 epg; ‡ 4,000+ epg

Of the ascaris infections identified in Bundibugyo overall, 0.88% were low-intensity. Additionally, 3 individuals had moderate intensity infections, comprising 0.38% of the overall population. There were no high-intensity infections in the population. When grouped by age, the SAC age group had 5 of the 7 low-intensity infections. This was equivalent to 1.04% of infections in the SAC age group. Between males and females, both had low-intensity infections. Males were more greatly burdened with 1.05% of the group having low-intensity infections while females had 0.79% low-intensity infections. All moderate infections were among females in the SAC age group.

**Table 10.** Ascaris Intensity in Bundibugyo by Age and Sex

	<b>Intensity (eggs per gram)</b>			
	Zero* N (%)	Low ** N (%)	Moderate <sup>†</sup> N (%)	High <sup>‡</sup> N (%)
Overall (n=794)	784 (98.74)	7 (0.88)	3 (0.38)	0 (0.00)
PSAC (n=1)	1 (100.00)	0 (0.00)	0 (0.00)	0 (0.00)
SAC (n=483)	475 (98.34)	5 (1.04)	3 (0.62)	0 (0.00)
WCBA (n=244)	243 (99.59)	1 (0.41)	0 (0.00)	0 (0.00)
Male (n=285)	282 (98.95)	3 (1.05)	0 (0.00)	0 (0.00)
Female (n=509)	502 (98.62)	4 (0.79)	3 (0.59)	0 (0.00)

\*0 epg; \*\*1-4,999 epg; <sup>†</sup>5,000-49,999 epg; <sup>‡</sup> 50,000+ epg

Of the trichuris infections identified in Bundibugyo overall, 1.89% of the sample had a low-intensity infection, there were no moderate or high infections. Of the age groupings, WCBA had the most low-intensity infections (n=9). WCBA also possessed the highest percentage of low-intensity infections in the population at 3.69%. Males and females had 1.05% low-intensity and 2.36% low-intensity infections, respectively. There were no moderate or high intensity infections.

**Table 11.** Trichuris Intensity in Bundibugyo by Age and Sex

	<b>Intensity (eggs per gram)</b>			
	Zero* N (%)	Low** N (%)	Moderate† N (%)	High‡ N (%)
Overall (n=794)	779 (98.11)	15 (1.89)	0 (0.00)	0 (0.00)
PSAC (n=1)	1 (100.00)	0 (0.00)	0 (0.00)	0 (0.00)
SAC (n=483)	478 (98.96)	5 (1.04)	0 (0.00)	0 (0.00)
WCBA (n=244)	235 (96.31)	9 (3.69)	0 (0.00)	0 (0.00)
Male (n=285)	282(98.95)	3(1.05)	0 (0.00)	0 (0.00)
Female (n=509)	497 (97.64)	12 (2.36)	0 (0.00)	0 (0.00)

\*0 epg; \*\*1-999 epg; †1,000-9,999 epg; ‡ 10,000+ epg

No STH Infections were detected in the Ntoroko district upon stratification. Thus, there are no low, moderate, or high intensity infections.

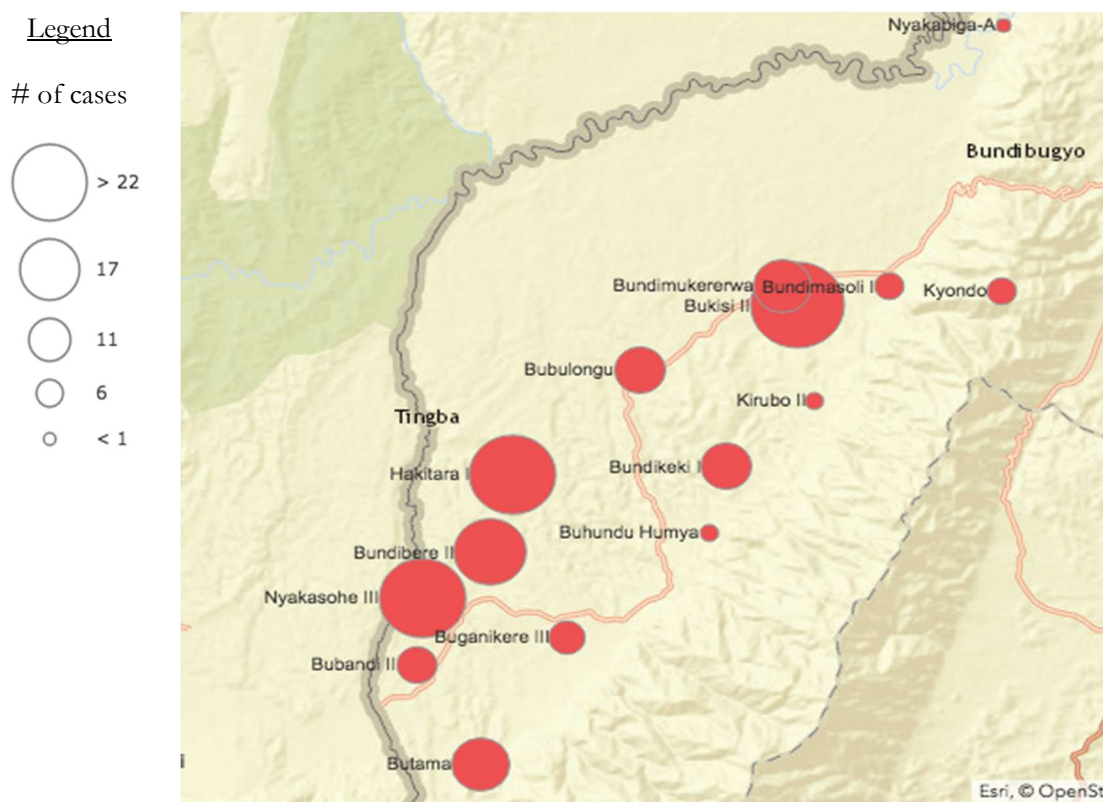
**Table 12.** Intensity of Hookworm, Ascaris, and Trichuris Infections in Ntoroko District

	<b>Intensity (eggs per gram)</b>			
	<b>0 epg</b>	<b>1-1,999 epg</b>	<b>2,000-3,999 epg</b>	<b>+4,000 epg</b>
<b>Hookworm</b>				
Overall (n=52)	52 (100.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
<b>Ascaris</b>				
Overall (n=52)	52 (100.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
<b>Trichuris</b>				
Overall (n=52)	52 (100.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)

*a. Additional Results*

Given the unanticipated high prevalence of hookworm in the Bundibugyo district, a map, shown in Figure 5, was generated using ArcGIS online software. The purpose of mapping was to investigate if clustering of hookworm infection might be occurring in specific villages within Bundibugyo district.

**Figure 5.** Hookworm Cases in Bundibugyo District by Number of Cases



While there do not appear to be any distinct clusters of infection due to geographic location, there is relatively high infectivity seen near the border with the Democratic

Republic of the Congo and high infection consistently along the river's pathway.

Additionally, certain villages are more heavily burdened when compared to others, suggesting differential treatment at the village level. For example, high rates of infection exist in Bundimbere II, Hakitara I, Nyakasohe III, and Bundimukererwa while low infection is seen in Bubandi II and Buhundu Humya.

As mentioned previously in the methodology section of this thesis, calculations of CV that resulted in greater than 20% difference between two Kato-Katz microscopy reads were not removed from analysis because they did not appear to be scientifically meaningful. Still, as this was a potential limitation to the study, overall prevalence in the study population when restricting on CV was calculated to better understand the difference between analyses. This restricted analysis is presented in Table 13.

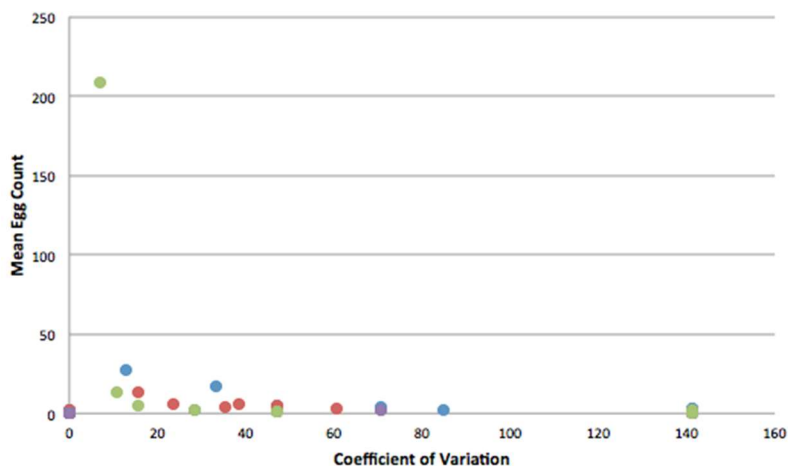
**Table 13.** Overall Prevalence of STH Infection Restricted on Coefficient of Variation by Disease Species, Sex, and Age

Characteristics	Disease, N (prevalence %)			
	Sm	Hw	Asc	Tt
<b>Overall</b>	27 (1.99)	156 (11.74)	8 (0.55)	0 (0.00)
<b>Sex:</b>				
Female	19 (2.25)	17 (2.09)	6 (0.67)	0 (0.00)
Male	8 (1.56)	13 (2.33)	2 (0.36)	0 (0.00)
<b>Age:</b>				
PSAC	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
SAC	17 (1.94)	15 (1.73)	7 (0.75)	0 (0.00)
WCBA	8 (2.14)	9 (2.56)	1 (0.26)	0 (0.00)

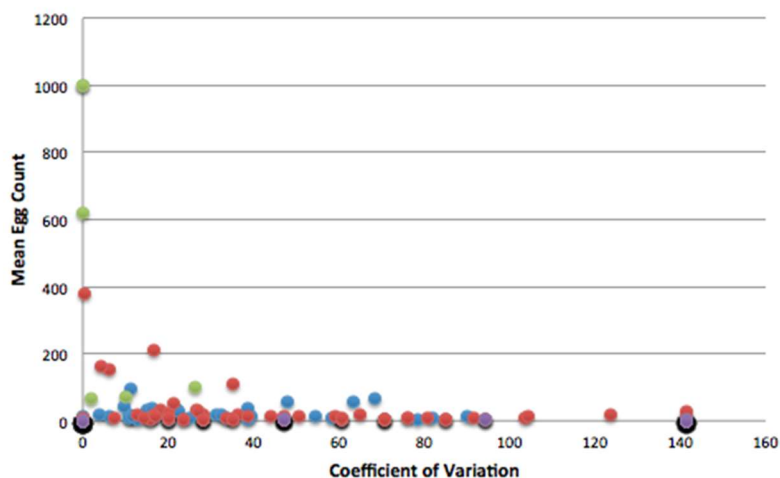
**Sm**-Schistosomiasis Mansoni, **Hw**-Hookworm, **Asc**-Ascaris, **Tt**-Trichuris

When removing the >20% difference CV values, the prevalence of all species of disease decreases substantially, except in the case of hookworm infections. This is potentially concerning; however, when looking at the mean egg counts by CV as a graphical representation by district in Figures 6 and 7, the assumption that only low mean egg counts produced CVs >20% holds true. This allows us to confidently assume that the CV values that were restricted were not particularly meaningful given that egg count means were so small and a difference of 20% was similarly small.

**Figure 6.** Mean Egg Count by Coefficient of Variation in Amuria



**Figure 7.** Mean Egg Count by Coefficient of Variation in Bundibugyo





## V. Discussion

While the LF FTS data demonstrated the effectiveness of the LF MDA program in Uganda – no detectable CFA in any of the sites, compared to 0.4–30.7% prevalence nationally in 2007<sup>10</sup> – the STH survey demonstrated that some transmission of STH still exists in the Amuria and Bundibugyo districts of Uganda.

While all STH species were detectable in the Amuria and Bundibugyo districts – small sample size did not permit a valid estimate of STH prevalence in Ntoroko – the overall prevalence was not particularly high for any of the disease species. Overall prevalence in the population was <10% for schistosomiasis, ascariis, and trichuris meaning that annual MDA is not recommended by WHO. One exception to this was the prevalence of hookworm infection in the overall study population at 10.71%, which exceeds the WHO threshold of 10% which demands annual treatment among primary school children.

**Table 14.** WHO Prevalence Thresholds for STH Treatment Strategies

Prevalence (%)	Strategy
≥2% - <10%	Preventive chemotherapy once every two years
≥10% - <20%	Preventive chemotherapy once a year
≥20% - <50%	Preventive chemotherapy once a year
≥50 %	Preventive chemotherapy three times a year

The prevalence of schistosomiasis was 8.52%, the second highest disease prevalence in the study sample. According to WHO guidelines this prevalence denotes that the sample

population is at low-risk. However, since the prevalence in this study is close to the 10% threshold for treatment it may be worth monitoring moving forward; biennial treatment should continue.

**Table 15.** WHO Prevalence Thresholds for SCH Treatment Strategies

Prevalence (%)	Strategy
<1%	Conduct serology
≥1% - <10%	Preventive chemotherapy once every two years
≥10% - <50%	Preventive chemotherapy once every two years
≥50 %	Preventive chemotherapy two times a year

A positive take away from this study data is that prevalence estimates for STH have notably declined from previous estimates. A countrywide study in 2005 estimated the burden of hookworm to be 43.5% and ascaris and trichuris infections to be 6.3% and 5.0%<sup>14</sup>. In the current study, overall prevalence of ascaris was 1.30% and trichuris was 1.10%<sup>14</sup>. This decrease in overall prevalence is an important indication that overlapping MDA for NTDs may be having positive effects on disease prevalence in the study regions.

The overall prevalence decreases for these infections varied between the disease species. For hookworm, the reduction went from 43.5% to 10.71%, representing a 4.06-fold change in prevalence. Whereas for ascaris the reduction was 4.84-fold and for trichuris 4.54-fold. Given that the same drug treats all three of these species, the difference in decrease although small may suggest differential susceptibility of these species to the treatment drug

(albendazole). As STH infections are treated over time, it is important to understand where drug treatment is efficacious and where current drug combinations may be failing.

The most distinct insight displayed in this data is the disparate results between district locations. Bundibugyo was overwhelmingly affected by schistosomiasis and hookworm infections in comparison to Amuria and Ntoroko. Overall prevalence for schistosomiasis and hookworm in Bundibugyo was >10%. While Amuria and Ntoroko had <5% for each species. This finding suggests that there may be a distinct difference between the environment or treatment strategy in Bundibugyo. Unfortunately, mapping of hookworm infections in the area did not provide much geographical insight to the issue, which suggests that it may be an issue of district program management. Similarly, given that Ntoroko borders Bundibugyo to the north, geographic reasoning for the lack of similarity is unlikely. Moving forward it would be beneficial to review the program practices and behavioral interventions such as hand washing and shoe wearing at the district level.

An additional important note about the prevalence data is that the prevalence of hookworm and schistosomiasis seemed to disproportionately affect men in the Amuria district. While prevalence levels were still below 10% in males, this may be an indication that there is a disparity between sexes regarding treatment in the Amuria district, perhaps because adult males are not classified as a high-risk group by WHO and thus they receive treatment less frequently when compared to children and WCBA.

When looking at distinct age groups, WCBA were significantly different from the other age groups in the overall study population for prevalence trichuris. Additionally, in Bundibugyo district the WCBA group was disproportionately affected with prevalence of hookworm exceeding 20% and prevalence of trichuris at 3.69%. Given the consequences of

these infections on mothers it is important that action be taken to treat this specific population in the Bundibugyo district.

As a whole, intensity data demonstrated that while infections exist in the study sites, where there is infection, intensity is low. In all three districts few moderate intensity infections and high intensity infections were identified throughout all the disease species. This is important when considering the co-morbidities caused by these diseases. Even while levels of infection persist, low intensity means that populations will be less likely to experience the negative impacts of moderate and high intensity infections such as stunting, cognitive development delay, fatigue, etc. This widely impacts the potential for children in schools as well as adults in their work, and subsequently the betterment of the economy and society writ large. Low intensity is an indication that treatment programs are doing well overall.

## VI. Recommendations

The STH assessment in this study demonstrated how certain districts, namely Bundibugyo, remain infected with Soil Transmitted Helminths. Prevalence in Bundibugyo is high enough to warrant additional MDA treatment and certain groups, such as Women of Childbearing Age need more targeted treatment. Although LF MDA has ceased in the TAS II area, yearly treatment should continue for STH based on the WHO threshold of >10% for annual deworming. In this case, treatment will target persistent hookworm infection. Bundibugyo should also be treated biennially with praziquantel for >10% prevalence of schistosomiasis infections. In addition to treatment, given the high rates of STH in WCBA in Bundibugyo, it would benefit the program to consider a larger scope of treatment to specifically include adult women, rather than majority school-based treatments.

Additionally, when applying this methodology to future work in this region and outside of Uganda it would be beneficial to ensure that sampling includes a sufficient subset of the PSAC age-group. The PSACs are a high-risk population for STH infections and it was a significant limitation for this study to only have a sample of n=4 PSAC individuals. Also, regarding sampling, this study generated substantial information on the WBCA group that is known to be at high risk for STH. Prevalence of infection among this age group in the study was largely comparable to that of the SAC group across districts. With that in mind, it would be valuable to continue including the WBCA population in future sampling, even though they are not inherent to the sampling strategy for the WHO integration of STH into TAS.

Additionally, based on past research from Haiti and Burkina Faso, as well as the field report generated by the study team in Uganda, this study reaffirms the recommendation that STH and TAS activities continue to be integrated where possible<sup>31</sup>. This is a time efficient and cost-effective way to gather data and, according to field team members, it was convenient to accomplish survey activities together.

Finally, as certain NTDs approach elimination targets it is critical to understand where other NTDs may still be thriving despite large scale overlap in MDA strategies. While the data in this study show that STH infections have lower prevalence in the population than previous estimates, there is still room for improvement and reduction of cases. Furthermore, the dissimilar status of STH prevalence across districts suggests that coverage is not universal across programs and that more investigation is needed to determine where programs should be scaling up and where they can wind down.

## VII. Acknowledgments

Special acknowledgement should be given to my faculty and external advisors, Dr. Jorge E. Vidal, PhD. and Dr. Katherine Gass, MPH, PhD. for their contributions to this paper intellectually and technically. Additionally, acknowledgement is appropriately given to the National Bilharzia and Worm Control Programme in Uganda and the Program to Eliminate Lymphatic Filariasis for their organization and technical expertise in the field during data collection. Lastly thanks are given to the Neglected Tropical Diseases Support Center at the Task Force for Global Health and their donors, USAID, GSK, and the Bill & Melinda Gates Foundation for making this work possible.

## VIII. References

- [1] Ahmed, A., Al-Mekhlafi, H. M., Choy, S. H., Ithoi, I., Al-Adhroey, A. H., Abdulsalam, A. M., & Surin, J. (2011, December 30). The burden of moderate-to-heavy soil-transmitted helminth infections among rural malaysian aborigines: An urgent need for an integrated control programme. Retrieved April 02, 2018, from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3259102/>
- [2] CDC, Parasites - Lymphatic Filariasis. (2013, June 14). Retrieved April 04, 2018, from <https://www.cdc.gov/parasites/lymphaticfilariasis/>
- [3] CDC. (2012, November 07). Parasites - Schistosomiasis, Biology. Retrieved April 09, 2018, from <https://www.cdc.gov/parasites/schistosomiasis/biology.html>
- [4] Chu, B. K., Gass, K., Batcho, W., 'Ake, M., Dorkenoo, A. M., Adjinacou, E., . . . Addiss, D. G. (2014, February 13). Pilot Assessment of Soil-Transmitted Helminthiasis in the Context of Transmission Assessment Surveys for Lymphatic Filariasis in Benin and Tonga. Retrieved April 09, 2018, from <http://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0002708>
- [5] Drabo, F., Ouedraogo, H., Bougma, R., Bougouma, C., Zongo, D., Bagayan, M., . . . Zhang, Y. (2016, May 10). Successful Control of Soil-Transmitted Helminthiasis in School Age Children in Burkina Faso and an Example of Community-Based Assessment via Lymphatic Filariasis Transmission Assessment Survey. Retrieved April 09, 2018, from <http://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0004707#pntd.0004707.ref027>
- [6] GAHI. (2013). Status of lymphatic filariasis elimination programme in Uganda. Retrieved April 09, 2018, from <http://www.thiswormyworld.org/maps/status-of-lymphatic-filariasis-elimination-programme-in-uganda>

- [7] Gunawardena, S., Gunawardena, N. K., Kahathuduwa, G., Karunaweera, N. D., Silva, N. R., Ranasinghe, U. B., . . . Weil, G. J. (, April 02). Integrated School-Based Surveillance for Soil-Transmitted Helminth Infections and Lymphatic Filariasis in Gampaha District, Sri Lanka. Retrieved March 18, 2018, from <http://www.ajtmh.org/content/journals/10.4269/ajtmh.13-0641>
- [8] Influential Points Contributors. (n.d.). Coefficient of variation. Retrieved April 09, 2018, from [http://influentialpoints.com/Training/coefficient\\_of\\_variation-principles-properties-assumptions.htm#intr](http://influentialpoints.com/Training/coefficient_of_variation-principles-properties-assumptions.htm#intr)
- [9] Katz, N., Chaves, A., & Pellegrino, J. (1972, December 14). A simple device for quantitative stool thick-smear technique in Schistosomiasis mansoni. Retrieved April 09, 2018, from <https://www.ncbi.nlm.nih.gov/pubmed/4675644>
- [10] Kolaczinski, J. (2007, September 07). Neglected tropical diseases in Uganda: The prospect and challenge of integrated control. Retrieved April 09, 2018, from <https://www.sciencedirect.com/science/article/pii/S1471492207002206#bib14>
- [11] Kongs, A., Marks, G., Verlé, P., & Stuyft, P. V. (2008, July 07). The unreliability of the Kato-Katz technique limits its usefulness for evaluating *S. mansoni* infections. Retrieved March 07, 2018, from <http://onlinelibrary.wiley.com/doi/10.1046/j.1365-3156.2001.00687.x/full>
- [12] Knopp, S., Speich, B., Hattendorf, J., Rinaldi, L., Mohammed, K. A., Khamis, I. S., . . . Utzinger, J. (2011, April 12). Diagnostic Accuracy of Kato-Katz and FLOTAC for Assessing Anthelmintic Drug Efficacy. Retrieved February 09, 2018, from <http://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0001036>



- [13] Knipes, A. K., Lemoine, J. F., Monestime, F., Fayette, C. R., Direny, A. N., Desir, L., . . . Lammie, P. J. (2017, February 16). Partnering for impact: Integrated transmission assessment surveys for lymphatic filariasis, soil transmitted helminths and malaria in Haiti. Retrieved March 10, 2018, from <http://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0005387>
- [14] Lamberton, P. H., Kabatereine, N. B., Oguttu, D. W., Fenwick, A., & Webster, J. P. (2014, September 11). Sensitivity and Specificity of Multiple Kato-Katz Thick Smears and a Circulating Cathodic Antigen Test for *Schistosoma mansoni* Diagnosis Pre- and Post-repeated-Praziquantel Treatment. Retrieved April 09, 2018, from <http://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0003139>
- [15] Menzies, S. K., Rodriguez, A., Chico, M., Sandoval, C., Broncano, N., Guadalupe, I., & Cooper, P. J. (2014, February 8). Risk Factors for Soil-Transmitted Helminth Infections during the First 3 Years of Life in the Tropics; Findings from a Birth Cohort. Retrieved April 09, 2018, from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3937274/>
- [16] MOH, Uganda. (2016, February 28). About, Vector Control Division. Retrieved April 09, 2018, from <https://vectorcontroldivisionuganda.wordpress.com/about/>
- [17] Montresor, A. (Ed.). (2015, September). Assessing the epidemiology of soil-transmitted helminths during a transmission assessment survey (TAS). Retrieved April 09, 2018, from [http://www.who.int/lymphatic\\_filariasis/resources/9789241508384/en/](http://www.who.int/lymphatic_filariasis/resources/9789241508384/en/)
- [18] Montresor, A., Deol, A., N., Lethanh, N., & Jankovic, D. (2016, April 1). Markov Model Predicts Changes in STH Prevalence during Control Activities Even with a Reduced Amount of Baseline Information. Retrieved April 1, 2018, from <http://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0004371>

[19] Mupfasoni, D., Mikhailov, A., Mbabazi, P., King, J., Gyorkos, T. W., & Montresor, A. (2018, February). Estimation of the number of women of reproductive age in need of preventive chemotherapy for soil-transmitted helminth infections. Retrieved March 09, 2018, from <http://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0006269>

[20] Pabalan, N., Singian, E., Tabangay, L., Jarjanazi, H., Boivin, M. J., & Ezeamama, A. E. (2018, January 12). Soil-transmitted helminth infection, loss of education and cognitive impairment in school-aged children: A systematic review and meta-analysis. Retrieved February 09, 2018, from <http://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0005523>

[21] Parker, M., & Allen, T. (2011, January 06). Does mass drug administration for the integrated treatment of neglected tropical diseases really work? Assessing evidence for the control of schistosomiasis and soil-transmitted helminths in Uganda. Retrieved April 09, 2018, from <https://health-policy-systems.biomedcentral.com/articles/10.1186/1478-4505-9-3>

[22] Pilotte, N., Papaïakovou, M., Grant, J. R., Bierwert, L. A., Llewellyn, S., McCarthy, J. S., & Williams, S. A. (2016, March 30). Improved PCR-Based Detection of Soil Transmitted Helminth Infections Using a Next-Generation Sequencing Approach to Assay Design. Retrieved April 02, 2018, from <http://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0004578>

[23] RTI, Envision. (2016, October). Uganda Work Plan. Retrieved April 5, 2018, from [https://www.ntdenvision.org/sites/default/files/docs/uganda\\_fy17\\_py6\\_envision\\_wp-external\\_final.pdf](https://www.ntdenvision.org/sites/default/files/docs/uganda_fy17_py6_envision_wp-external_final.pdf)

[24] Savoli, L. (2012, January 27). Schistosomiasis: Population requiring preventive chemotherapy and number of people treated in 2010. Retrieved April 09, 2018, from [http://www.who.int/neglected\\_diseases/resources/who\\_wer8704/en/](http://www.who.int/neglected_diseases/resources/who_wer8704/en/)

- [25] The World Factbook. (2018, January). COUNTRY COMPARISON :: GDP - PER CAPITA (PPP). Retrieved April 09, 2018, from <https://www.cia.gov/library/publications/the-world-factbook/rankorder/2004rank.html>
- [26] Truscott, J. E. (2016, October 07). Soil-Transmitted Helminths: Mathematical Models of Transmission, the Impact of Mass Drug Administration and Transmission Elimination Criteria. Retrieved April 09, 2018, from <https://www.sciencedirect.com/science/article/pii/S0065308X1630077X>
- [27] WHO Contributors. (2017, September). Soil-transmitted helminth infections - Fact Sheet. Retrieved April 02, 2018, from <http://www.who.int/mediacentre/factsheets/fs366/en/>
- [28] World Health Organization. (2011). Monitoring and epidemiological assessment of mass drug administration in the global programme to eliminate lymphatic filariasis : a manual for national elimination programmes. Retrieved February 27, 2018 from <http://www.who.int/iris/handle/10665/44580>
- [29] World Health Organization. (2017, Spring). Fact sheets relating to NTD. Retrieved March 17, 2018, from [http://www.who.int/neglected\\_diseases/mediacentre/factsheet/en/](http://www.who.int/neglected_diseases/mediacentre/factsheet/en/)
- [30] World Bank Group. (2018, January). Uganda, Facts Sheet. Retrieved April 09, 2018, from <https://data.worldbank.org/country/uganda>

### Unpublished Sources:

- [31] National Bilharzia and Worm Control Programme. (October, 2017). Progress report for field evaluation of a standardized Multi-parallel PCR diagnostic test versus the Kato-Katz Stool test during transmission assessment survey collection in Uganda. Unpublished raw data.