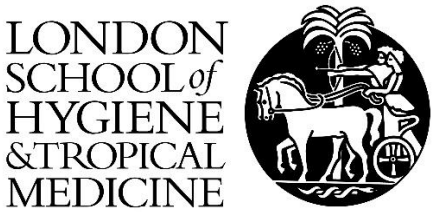


Application of multiplex bead serological assays to integrated monitoring of neglected tropical diseases

YuYen Lorenzo Chan

Thesis submitted in accordance with the requirements for the degree of

Doctor of Philosophy
of the
University of London

September 2021

Department of Infection Biology

Faculty of Infectious and Tropical Diseases

LONDON SCHOOL OF HYGIENE & TROPICAL MEDICINE

No Funding

Declaration of own work

I, YuYen Chan, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis

Signed:  Date: 23 September 2021

Abstract

Background

Neglected tropical diseases remain a significant burden to global health, despite substantial efforts among global governments and partnerships to control these diseases. In recent years, there has been considerable attention directed towards integrating control platforms across these geographically co-endemic diseases due to demonstrated resource efficacy and sustainability moving forward. As surveillance is a key component of disease control, effective surveillance tools are needed to support these integrated disease platforms. Serological multiplex bead assays are capable of monitoring numerous pathogens simultaneously by using antigens of specific pathogens. However, gaps in knowledge pertaining to the interpretation of serological data from these assays and their utility in public health surveillance necessitates further research to determine their capabilities in supporting concurrent surveillance of multiple diseases. This thesis aims to determine appropriate methods of interpreting serological data from multiplex bead assays and assess their utility in the context of public health surveillance of neglected tropical diseases.

Methods

Three datasets from Haiti (2015 Tracking Results Continuously Survey, n=4438 ; 2017 Artibonite Easy Access Group Survey, n= 6004 ; and 2017 Artibonite Community Household Survey; n=21222) and one dataset from Malaysia (2015 Sabah Household Cluster Survey, n=10100) were used in this thesis. Collectively, these datasets included antigens of twelve different pathogens that were analysed using serological multiplex bead assays .

The first objective of this thesis was to identify existing methods of characterising serological data from neglected tropical diseases using multiplex bead assays. This was done through a systematic review of literature which examined existing and potential methods of characterizing antibody responses from these assays. Several of these methods were then applied to two case studies to evaluate potential implications pertaining to method choice on public health interpretation. The second objective was to assess the utility of multiplex bead assays for multi-disease surveillance. To do this, panels of diverse tropical disease antigens (encompassing twelve different pathogens collectively) from three datasets in Haiti and one dataset from Malaysia were used to analyse concurrent monitoring of multiple diseases and to assess demographic and spatial disease risk factors for co-endemic pathogens. The third

objective of this thesis was to determine the capacity of serological multiplex bead assays to support different sampling strategies. This was done by formally comparing outputs from serological multiplex bead assays from a community household active sampling strategy and an easy-access group convenient sampling strategy in Haiti.

Results

The review of literature revealed that serological data from multiplex bead assays are typically converted to seroprevalence for programme interpretation, however there is currently an absence of a standard approach to determine serological prevalence estimates. Instead, seven different approaches were identified in the literature. Comparing three different approaches resulted in varying disease prevalence estimates in both Haiti and Malaysia, suggesting potential impacts of classification approaches on postliminary programmatic interpretation in both case studies. Multiplex bead assays provided concurrent estimates of exposure to various pathogens simultaneously at the national and subnational levels of surveillance within Haitian and Malaysian study populations. Demographic and spatial data collected alongside serologic surveys determined several consistent risk factors across antigens assessed, including age, wealth, gender, and also allowed for visualization of any spatial trends in disease exposure in both settings. Using multiplex bead assays in two different sampling approaches demonstrated its capacity to support multi-disease surveillance in different sampling approaches. In comparing prevalence estimates between surveys, observed differences may be attributable to inherent biases in sampling populations and design.

Conclusions

The research in this thesis contributes to the understanding of the utility of serological multiplex bead assays to support multi-disease surveillance and provides a foundation in the broader study of applying these platforms to neglected tropical disease control. This thesis also highlights the need to develop standardized approaches in sampling, laboratory protocols, and analysis for these platforms to ensure consistent and confident disease estimates and data reporting.

Acknowledgements

I am deeply thankful for all the support I have received during my PhD and would like to offer my sincerest appreciation to many individuals.

I would like to express my gratitude to my supervisors who provided invaluable advice and attentive academic mentoring throughout my research progression. They have helped me develop numerous research skills and provided vital opportunities to broaden my understanding of the fields of immunology, epidemiology, and neglected tropical diseases.

Many thanks to my advisory committee from the London School of Hygiene and Tropical Medicine and The Centers for Disease Control and Prevention in Atlanta for their insightful guidance and assistance with my research projects. During my PhD, I have had the privilege to work with numerous individuals connected to LSHTM, CDC, and other institutions, and I am grateful for all their support.

Lastly, I am very thankful for my mother, family, and close friends for providing the financial and emotional support throughout my studies. Thank you all very much.

Supervisor and Advisory Committee members

Supervisors:

Professor Chris Drakeley
Head of Department of Infection Biology
Faculty of Infectious and Tropical Diseases
London School of Hygiene and Tropical Medicine

Dr Gillian Stresman
Department of Infection Biology
Faculty of Infectious and Tropical Diseases
London School of Hygiene and Tropical Medicine

Advisory Committee members:

Dr Kimberly Fornace
Department of Disease Control
Faculty of Infectious and Tropical Diseases
London School of Hygiene and Tropical Medicine

Dr Lindsey Wu
Department of Infection Biology
Faculty of Infectious and Tropical Diseases
London School of Hygiene and Tropical Medicine

Dr Eric Rogier
Department of Parasitic Diseases and Malaria
Center for Global Health
United States Center for Disease Control and Prevention

Dr Diana L Martin
Department of Parasitic Diseases and Malaria
Center for Global Health
United States Center for Disease Control and Prevention

Table of Contents

List of Figures

Chapter 1

Chapter 3

Chapter 4

Chapter 5

Chapter 6

List of Tables

Chapter 1

Chapter 3

Chapter 4

Chapter 5

Chapter 6

Abbreviations

CBA	Cytometric Bead Array
DNA	Deoxyribonucleic Acid
EAG	Easy Access Groups
ELISA	Enzyme-linked immunosorbent assays
HH	Household Survey
LF	Lymphatic Filariasis
IDRS	Integrated Disease Surveillance and Response
IVM	Integrated Vector Management
HIV	Human Immunodeficiency Virus
IGG	Immunoglobulin G (IgG)
IGM	Immunoglobulin M (IgM)
MBA	Multiplex Bead Assay
MDA	Mass Drug Administration
MFI	Mean Fluorescence Intensity
MFI-BG	MFI minus Background
MM	Mixture Model
NTD	Neglected Tropical Diseases
PCR	Polymerase Chain Reaction
PN	Presumed Negative
PU	Presumed Unexposed Endemic Population
SS	*Strongyloides stercoralis*
STH	Soil Transmitted Helminths
TAS	Transmission Assessment Survey
Q	Quantiles
VPD	Vaccine Preventable Diseases
WASH	Water, Sanitation, and Hygiene
WHO	World Health Organization

CHAPTER 1: INTRODUCTION

1.1 Background and Rationale

1.1.1 Neglected Tropical Diseases: A Persistent Public Health Problem

Neglected Tropical Diseases (NTDs) are a diverse group of pathogens that comprises of helminths, protozoa, bacteria, viruses, and fungi. NTDs remain a substantial global health burden to many communities worldwide and can cause a spectrum of morbidities ranging from severe illness to chronic disability (Panel 1) (1). Example morbidities include oedema caused by lymphatic filariasis that leads to disfigurement and impairs movement; chronic diarrhoea as a result of intestinal protozoan infections such as giardiasis and amoebiasis that leads to dehydration and malabsorption; and blindness as result of ocular and papillary inflammation due to onchocerciasis and trachoma (2). Morbidities caused by NTDs can last chronically for extended years, and over 500,000 deaths are caused by these pathogens annually. As a result, approximately 57 million disability-adjusted life years are lost each year (3, 4).

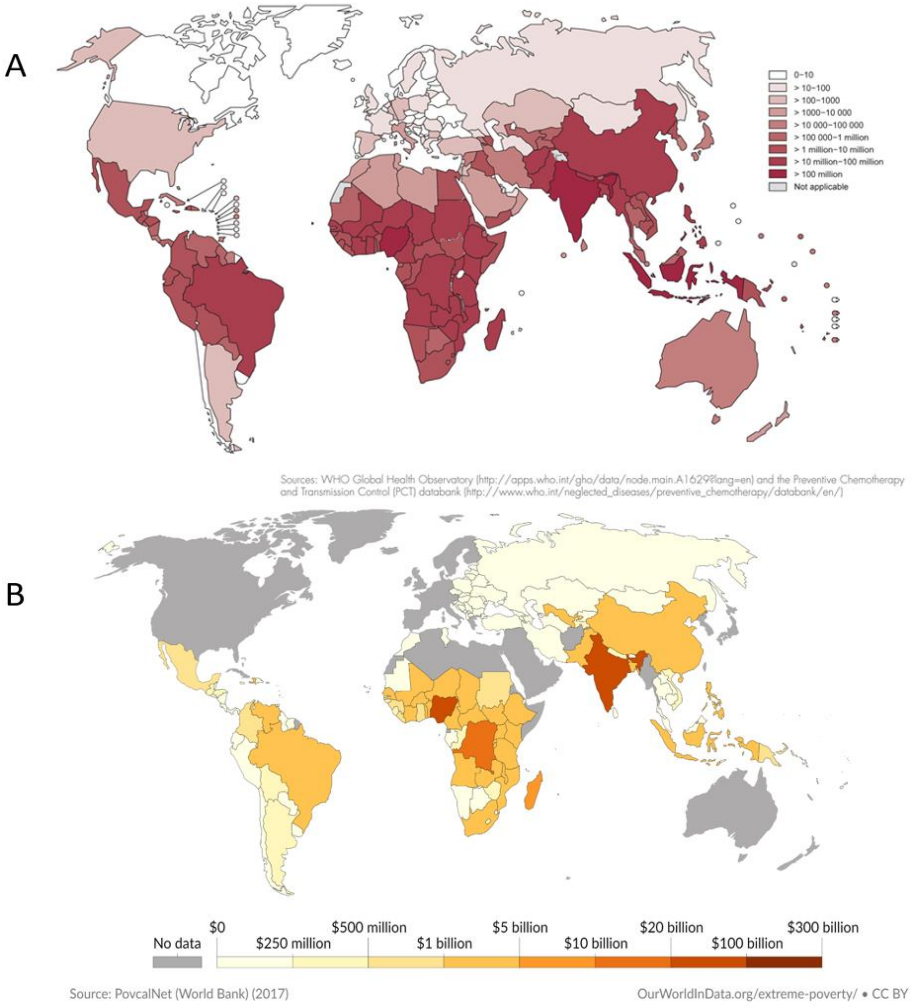
Infections	**Common Associated Morbidities**	**Example Economic Impacts**
Helminth *Ascariasis; * trichuriasis; * hookworm infection; *strongyloidiasis; toxocariasis; lymphatic filariasis; * onchocerciasis; * loiasis; dracunculiasis; *schistosomiasis; * food-borne trematodiases; taeniasis cysticercosis; echinococcosis* **Protozoan** *Leishmaniasis; * Chagas disease; * human African trypanosomiasis; * amoebiasis; giardiasis; balantidiasis* **Bacterial** *Bartonellosis; bovine tuberculosis; buruli ulcer; * leprosy; *leptospirosis; relapsing fever; rheumatic fever; trachoma; *treponematoses* **Viral** *Dengue fever; yellow fever; Japanese encephalitis; rabies; haemorrhagic fevers* **Fungal infections** *Mycetoma; paracoccidiomycosis* *Ectoparasitic infections* *Scabies; myiasis; tungiasis*	• Anemia • Lymphoedema • Hydrocele • Elephantiasis • Fever • Malnutrition • Diarrhea • Visceral tissue damage • Haemorrhagic fevers • Inflammation • Vision impairment • Skin lesions • Organ failure • Pain • Death • Impacts to mental well being • Dehydration	**Agricultural** • Reduced activity due to imparied productivity from illness • Migration from agriculturally fertile areas as a result of disease presence. **Educational** • Pediatric infections and malnutrition reduce educational performance and attendance **Financial** • Cost of tertiary health care may be expensive and deplete family assets.

*13 core neglected tropical diseases.		

T1. 1 **Panel 1. Neglected Tropical Diseases with example associated diseases and economic impacts.**

13 core neglected tropical diseases as defined by the World Health Organization (WHO), and expanded NTDs of public health significance determined by journal PloS NTD staff and WHO (1).

Neglected tropical diseases are considered 'neglected' for many reasons. Primarily, NTDs are widespread and coexist endemically in 56 countries where a billion people who are considered the poorest in the world reside (Figure 1) (5). Infection is often exacerbated by poverty that leads to factors such as malnutrition, limited medical attention, and inadequate sanitation that perpetuates transmission and disease (6, 7). In turn, these infections compounds poverty in a detrimental cycle. For example, high costs for treatments may drain financial assets that lead to inappropriate or ineffective health care seeking behaviour (2).



F1. 1 **Figure 1. Overlapping burden of NTDs with world poverty gap by country.**

Panel A shows the number of individuals affected by NTDs. Panel B shows the poverty gap representing the amount of money required to lift the income of all individuals up to the international poverty line of $1.90 a day. When comparing the two figures A and B, countries with high poverty gap tend overlap countries with high burden of NTDs.

Chronic and debilitating infections are another important, defining characteristic of NTDs with considerable global health concern (1). Occupational productivity among household members and workers may be impaired substantially by disability due to prolonged diseases. Education of children may be delayed because of ongoing paediatric infections or malnutrition that can lead into adulthood (Supplemental Table 1) (2, 8). Additionally, disfigurement as a result of enduring infections can lead to social stigmatization and negative impacts to mental health (9).

NTDs are also considered neglected due to the limiting funding and attention they receive. Within NTD endemic countries, resources are typically prioritized towards other diseases with higher public health awareness, global attention, and mortality, such as malaria and human immunodeficiency virus (HIV) (10). Additionally, developing tools to control NTDs previously fell to local governments and philanthropic non-government organizations, as there was almost no profit incentives for pharmaceutical companies to invest in developing innovations, given that these diseases are associated with the poor (11).

In recent years, international attention towards NTDs was a result of three recognized important factors: 1) their association with lasting poverty, 2) their control would lead to sustainable effects on the reduction of poverty, and 3) their control or elimination is attainable through low-cost, highly effective approaches and widely available medicines (2, 12). Relentless commitment among certain country governments and partnerships have led to substantial progress in the public health control and elimination of one or more NTDs within some countries. For example, China and Republic of Korea eliminated lymphatic filariasis (LF) as a public health problem in 2007 and 2008, respectively, while 18 other countries previously listed as LF endemic by the World Health Organization (WHO) are now conducting surveillance to validate elimination (13). Death from rabies and leprosy have seen significant decreases globally, while mass drug administration (MDA) has helped to greatly reduce prevalence in helminth diseases such as schistosomiasis, ascariasis, and onchocerciasis in Africa, Asia, and South America (14).

However, many endemic countries continue to struggle to achieve elimination targets set previously by WHO due to many factors that impede control progress (15). For example, destabilization of public

health systems can delay coordination of public health interventions and treatment for NTDs. Challenges to receiving treatment include ease of access to health care, subclinical manifestations leading reduced care-seeking behaviour, stigmatization or discrimination as a result of physical deformities, and cost of treatments (lack of universal health care) that can lead to prolonged suffering of the afflicted, sustained transmission (e.g., shedding of schistosomes), and under-reporting of diseases (16). Reduced public health priorities of NTDs when compared to other prominent diseases (e.g. malaria, HIV) due to limited financial resources may also result in infrequent NTD surveillance, which undermines the current disease burden of NTDs and dampens interest in developing reliable diagnostic innovations for monitoring (5). Lack of adequate surveillance (detailed in Section 2, 2.1) is particularly harmful to NTD control progress, as disease recrudescence or inception attributable to climate change or population migration needs to be captured promptly for effective control management, outbreak prevention, and financial support. Moreover, during interventions (e.g., mass drug administration (MDA) or vector control campaigns), routine surveillance is needed to inform programs of progress and advise cessation or scaling up of interventions. In post-elimination settings, surveillance is needed to sustain elimination certification. Public health efforts will need to address these challenges in order to effectively reduce the vast burden of NTDs (15).

1.1.2 Integrated Diseases Management: Current Initiatives and Priorities to Control NTDs

It is currently estimated that over a billion people require interventions against NTDs (2, 14). The World Health Organization (WHO) NTD Roadmap for 2021-2030 has a current objective to end suffering from these disease by 2030. Achieving this goal requires an overall reduction in the number of people requiring interventions against NTDs by 90%, increased number of countries to control, interrupt transmission, eliminate as a public health problem, or eradicate NTD incrementally (at least 100 countries to have eliminated one NTD at minimum by 2030), and to reduce the total disability adjusted life years caused by NTDs by 75% (Supplemental Table 1, (17)). Essentially, programs to need to communicate and coordinate if they want to accelerate the control and elimination as public health problem of these diseases. Key terminology according to the WHO is defined as follows: **Control** is the reduction of disease including incidence, prevalence, morbidity, and/or mortality to an acceptable level (specific to each location) as a result of deliberate public health interventions. Continued intervention measures are required to maintain the reduction of disease metrics, and control targets may or may not be related to global targets set by WHO. **Elimination of transmission** (also referred to as interruption of transmission) means reduction to zero incidences of infection caused by a specific pathogen in a defined geographical area due to deliberate public health efforts, with minimal risk of reintroduction of disease.

The process of documenting elimination of transmission is called verification, and continued actions to prevent re-establishment of transmission may be required post elimination. **Elimination as a public health problem** is a term defined by achieving measurable global targets set by WHO in relation to a specific disease. When reached, continued actions are obligatory to maintain the targets and/or to advance the interruption of transmission. Similar to elimination, the process of documenting elimination as a public health problem is called validation. **Eradication** is defined as a permanent reduction of infection to zero for a specific pathogen, globally, with no risk of reintroduction. The process of documenting eradication is called certification. Extinction means that the specific pathogen so that it no longer exists in nature or the laboratory, which may occur with or without deliberate efforts.

Currently, dracunculiasis and yaws are targeted for public health eradication. Human African trypanosomiasis *Gambiense*, leprosy, and onchocerciasis are aimed for elimination (18). Another eight NTDs are targeted for elimination as a public health problem (according to WHO global targets), and six more diseases targeted for control, which requires a reduction of incidence, prevalence, morbidity and mortality (19).

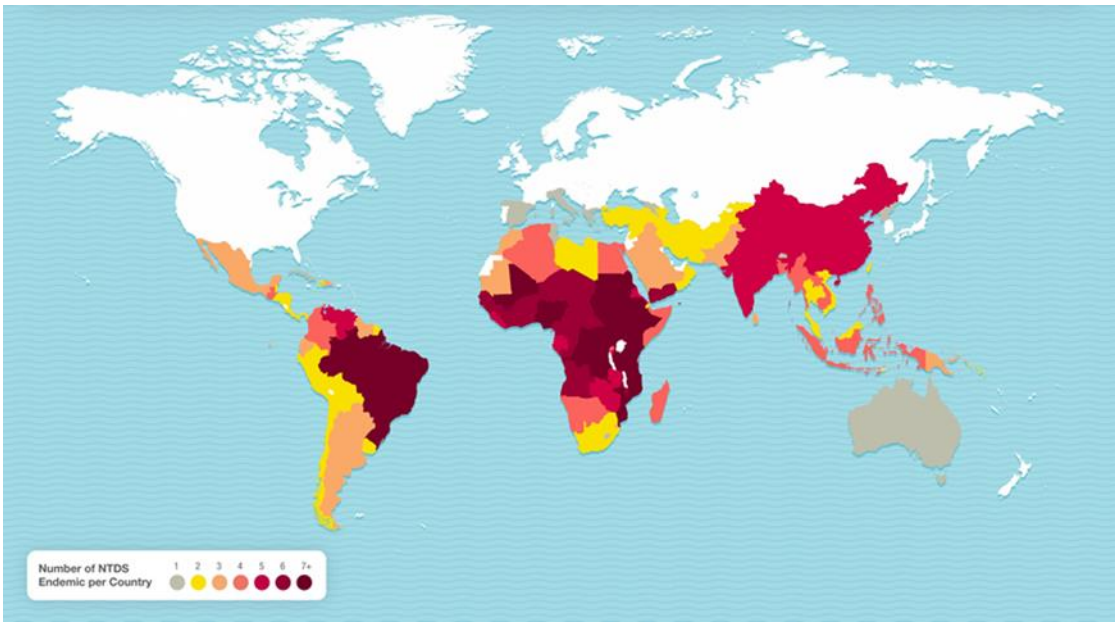
T1. 2 **Table 1. NTD control and elimination goals per disease by 2030, with current disease burden, preventative chemotherapy, and transmission pathways.**

Disease Target	WHO Roadmap Goals 2030	Number of Countries Targeted	Current Disease Burden	Preventative Chemotherapy	Transmission Pathways
Dracunculiasis	Eradication	194 *Global*	54 cases/ 4 countries	None	Contaminated water
Yaws	Eradication	194 *Global*	80472 cases/ 15 countries	Azithromycin, Benzathine penicillin	Direct skin contact with infected person
Human African Trypanosomiasis Gambiense/ Rhodesiense	Elimination/ Elimination as public health problem	15/8 *Africa*	997 cases/ 36 countries	1st stage: Pentamidine, Suramin 2nd stage: Melarsoprol, Eflornithine, Nifurtimox Both stages: Fexinidazole	Tsetse fly bites
Leprosy	Elimination	120 *Global*	184 212 cases /159 countries	Dapsone, Rifampicin, Clofazimine	Mucosal secretions
Onchocerciasis	Elimination	12 *Africa*	20.9 million/ 31 countries	Ivermectin	Blackfly bite

Chagas disease American trypanosomiasis	Elimination as a public health problem	15 *Latin America*	7 million cases / 21 countries	Benznidazole, Nifurtimox	Insect faeces Congenital Transfusions Contaminated food or water
Leishmaniasis-visceral	Elimination as a public health problem	64 *Global*	1 million cases / 10 countries	Liposomal amphotericin, Paromomycin, Miltefosine, Pentamidine	Sandfly bite
Rabies	Elimination as a public health problem	155 *Global*	30 million cases/ 150 countries	None	Direct contact with saliva or nervous tissue of infected animal
Schistosomiasis	Elimination as a public health problem	78 Global	290.8 million cases / 78 countries	Praziquantel	Direct skin contact in infested water
Soil-transmitted helminthiases	Eliminate as a public health problem	96 Global	1.5 billion cases	Albendazole, Mebendazole, Ivermectin	Direct skin contact with infested soil
Trachoma	Elimination as a public health problem	64 Global	95.2 million/ 44 countries	Azithromycin	Direct skin contact with infected person
Lymphatic filariasis	Elimination as a public health problem	58 Tropical	36 million cases / 49 countries	Albendazole, Ivermectin, Diethylcarbamazine citrate	Mosquito bite
Buruli ulcer	Control	N/A Tropical	261 cases / 33 countries	Rifampicin, Clarithromycin, Moxifloxacin	Unknown
Dengue	Control	N/A *Asia* *Latin America*	100-400 million cases	None	Mosquito bite
Taeniasis/ cysticercosis/ echinococcosis/ hydatidosis	Control	17 Africa Asia Latin America	50 million cases/	Praziquantel, Niclosamide, Albendazole	Ingestion of contaminated food
Foodborne trematodiasis	Control	11 East Asia South America	2 million cases/ 70 countries	Praziquantel, Triclabendazole	Ingestion of contaminated food
Leishmaniasis-cutaneous	Control	87 Global	1 million cases/ 10 countries	Liposomal amphotericin, Paromomycin, Miltefosine, Pentamidine	Sandfly bite
Snakebite envenoming	Control	132 Global	2.7 million	Antivenom	Snake bite

Global organizations, governments and partnerships have recognized that new, cross-cutting approaches to disease control are needed to achieve these targets. In addition to the 2013 WHO Roadmap NTDs management strategies (e.g. preventive chemotherapy at regular intervals, intensive case and vector management, and improvements in water, sanitation, and hygiene (20)), one of the fundamental changes of the 2021-2030 Roadmap is to move away from siloed, disease specific programs to implement control strategies that target multiple NTDs concurrently (17).

Detailed mapping of NTDs have revealed that endemic countries are often simultaneously afflicted with multiple NTDs due to shared climatological and socio-economic factors (21) (Figure 2). For example, several countries in sub-Saharan Africa have high incidences of NTDs such as soil transmitted helminths (STH) and schistosomiasis, in addition to other disease including malaria and HIV (22). The geographic region of South East Asia has been observed to have the highest burden of leprosy, lymphatic filariasis (LF), visceral leishmaniasis and yaws (23). In Latin America and the Caribbean, these are widespread co-endemicities of STH infections, dengue, and chagas disease, while geographically restricted hotspots of endemicity exist for onchocerciasis, LF, and schistosomiasis in these regions (24). The strategy of integrated approach to combatting NTDs brings NTD programmes into a single NTD platform (17), allowing for collaboration among different partnerships and organizations. Importantly, an integrated programmatic NTD platform can provide support to NTDs that are the most neglected or overlooked, thus ensuring systematic action towards these diseases.



F1. 2 **Figure 2. Intensity of number of NTDs per country.**
Endemic countries are often impacted by multiple NTDs, with some countries being afflicted by more than seven NTDs. Source: *Uniting to Combat NTDs* (25).

Integrated disease control has many benefits to accelerate NTD control. For example, while MDA has improved control and led to elimination of diseases in certain countries, Hotez et al. argue that the efficacy of MDAs can be enhanced through integration of MDA campaigns of different diseases (2, 5). As seen previously with a low-cost combination drug therapy of albendazole, praziquantel, ivermectin, and azithromycin that was developed in 2006 to concurrently treat the seven major tropical diseases (26), coordination among public health groups in delivering these "rapid effect packages" had an estimated cost savings of 26-47% when compared to non-integrated disease programs (5). This is because multiple NTDs such as hookworm infection, lymphatic filariasis, and onchocerciasis, can be treated with the same preventative chemotherapy, and may share geographic overlap, thus reducing the time and resources spent when compared to treating disease individually (Table 1). Examples of documented improved health outcomes because of integrated disease control includes improved childhood growth, increased school attendance among children, and reduced duration and severity of disease morbidities in children and adults (4).

Another example of integrated disease control is the application of integrated vector management (IVM), which optimises existing resources toward combined entomological vector control (27). For instance, certain mosquitoes and flies that can transmit multiple disease including anopheles mosquitoes (e.g., malaria and lymphatic filariasis), culex mosquitoes (e.g., lymphatic filariasis and west Nile virus), aedes mosquitoes (e.g., lymphatic filariasis, dengue, and chikungunya), blackflies (e.g., onchocerciasis and mansonellosis) and sandflies (e.g., leishmaniasis and bartonellosis). In these co-endemic environments, integrated vector management has been shown to be effective in controlling transmission of the associated diseases efficiently (28, 29).

A final example of integrated disease control is the application of water, sanitation, and hygiene (WASH) to the control and prevention of numerous neglected tropical diseases. Provision of safe water with adequate sanitation in combination with MDA has played a vital role in helping to concurrently manage the spread of NTDs such STH, guinea worm, and schistosomiasis (30). Collaboration between the WASH and NTD communities have demonstrated the importance of cross-sector approaches to achieving common public health goals more efficiently compared to single-disease programs. Integrated control can be extremely beneficial to help achieve the goals of the 2021-2030 WHO NTD Roadmap (17, 31).

However, action toward any type of control is based on disease indicators such as prevalence or incidence. Because many of these diseases present asymptomatically, have long incubation periods, or can be newly introduced to non-endemic regions due to migration, their global disease burden and public health severity are often underestimated, leading to delayed interventions (32).

1.2 Monitoring Burden of Disease and Prevalence

1.2.1 Surveillance: A Critical Role in Disease Management

Strategies of disease control and averting potential epidemics due to emerging or re-emerging diseases are based on effective surveillance that leads to early detection and rapid mapping of disease transmission hotspots (33) . This information is needed to rally financial and political support, and to guide decision making, such as prioritizing the mobilization of interventions and treatments (1). In low transmission, post MDA, and post elimination settings, regular surveillance of key health indicators is crucial to ensuring that countries are on track toward their elimination certification and control goals. However, without systematic and routine surveillance, it would be difficult to establish baseline prevalence and measure any progress of (integrated) control efforts.

Surveillance strategies are typically built around active and passive frameworks (33). As an intervention tool aimed to reduce transmission through monitoring and evaluation, NTD surveillance approaches tend to shift from measuring morbidity and mortality to measuring infection, exposure, and detecting transmission within the population through a variety of diagnostic tools and approaches (detailed in Section 2.2 NTD Diagnostics) (34).

Active Surveillance

In active surveillance, health and community workers contact providers, laboratories, communities, and families to obtain information regarding specific health outcomes (33). Active surveillance provides the most accurate and timely information; however, it is not cost-effective as it often requires an intensive number of resources (e.g., time, labour, sampling tools, laboratory equipment, etc.). Moreover, in low transmission or post elimination settings, a sufficiently large number of samples are necessary to generate accurate population-level prevalence estimates for each respective method.

Passive Surveillance

Passive surveillance uses case reports submitted by health facilities and public units based on individuals who seek care due to illness. It is cost-effective and can offer vital information regarding community

health with a high temporal and spatial resolution (33). However, passive surveillance has several limitations. Sub-clinical or asymptomatic infections may lead to low treatment-seeking behaviour, resulting in under-reporting of cases. For example, dengue clinical manifestations can include subclinical or mild febrile illness, which often goes undetected if patients do not seek care and are tested (35). Moreover, symptoms associated with many NTDs are typically not sufficiently specific to discriminate the aetiology, which could cause misdiagnosis (36). Additionally, certain health facilities may not have proper diagnostics tools, inefficient case-tracking, or ineffective reporting systems for passive surveillance (33).

Other surveillance strategies such as sentinel, syndromic, and integrated surveillance incorporates aspects of both active and passive surveillance (33). In sentinel surveillance, a structure reporting system is based upon selected individuals, clinics, or institutions to provide regular information regarding disease within a specific catchment. In syndromic surveillance, case detection relies strictly on clinical presentations without laboratory confirmation.

Case Detection

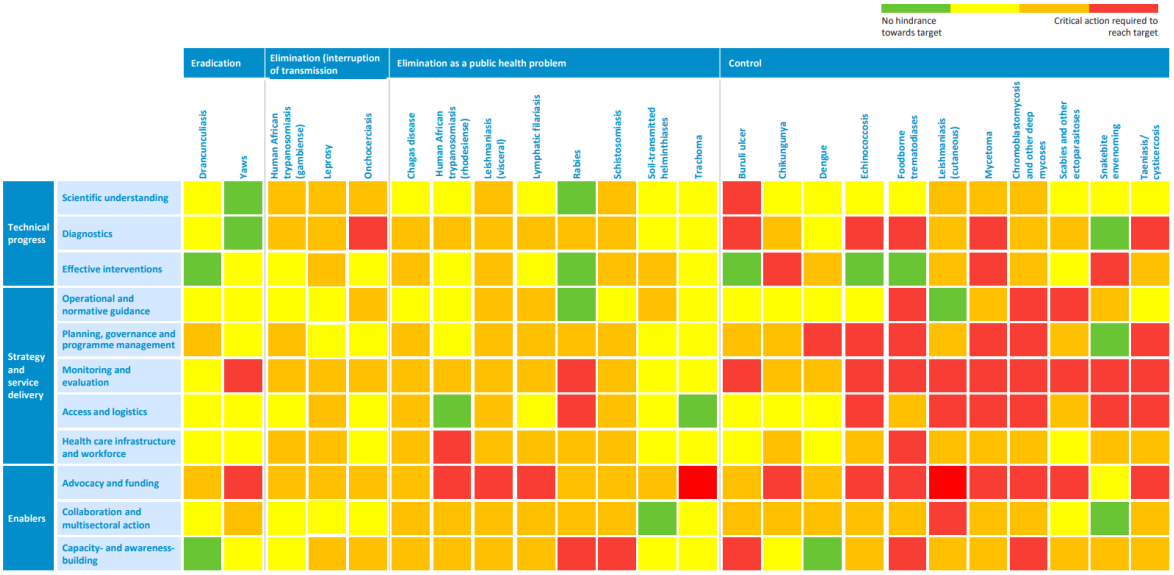
Current case detection of many NTDs primarily relies on passive surveillance (see following section (33, 37)). However, due to the limitations of passive surveillance, incomplete data on disease transmission has hindered past progression toward control and elimination both in terms of estimating disease burden and rallying financial support (38). For example, lack of basic data on endemicity and epidemiology has led to almost no control action for strongyloidiasis in many endemic countries (39). In Ghana, limited and inaccurate data in relation to MDA treatment coverage was reported to have delayed LF control progress (40). Elimination actions are often not initiated until effective, validated surveillance systems are employed to obtain what is described as the minimum essential data (e.g., provides adequate, critical information with the minimum amount of information required) needed to inform diseases control progress based on specific program objectives (41). As a result, another key priority of the WHO current initiatives is to improve surveillance and disease reporting both nationally and internationally (33).

Integrated surveillance

An integrated surveillance system utilizing both passive and active surveillance, with additional mass screening can greatly help to accurately estimate disease burdens. For example, implementing active surveillance in addition to passive surveillance in West Bengal has helped to identify a sub-clinical,

hidden burden of leishmaniasis (42). In Ecuador, passive and active surveillance captured distinct demographic subpopulations with cases of dengue fever that were difficult to sample due to access to health care and asymptomatic infections missed previously, with active surveillance accounting for more laboratory confirmed cases than passive surveillance (35) .

Moving forward, integrated surveillance is needed to strengthen health information systems and to support elimination targets in the 2030 NTD Roadmap for all targeted diseases (Figure 3), (31). This requires establishing concerted approaches and partnerships to coordinate surveillance activities such as implementing surveillance guidelines, sample collection, data collation, and real-time analysis to drive evidence-based knowledge of disease burden estimates and transmission activity into policymaking and response (33, 43). For example, passive surveillance data from both health facilities and community sampling using active surveillance can be aggregated and analysed to obtain an improved understanding of disease epidemiology. To support WHO initiatives of integrated disease control, integrated surveillance and response should incorporate coordinating surveillance of multiple diseases simultaneously that will be able to inform collaborations concurrently.



F1. 3 **Figure 3. Assessment gaps per NTD.**
Among the different public targets for the 2021 NTD Roadmap, monitoring and evaluation requires critical action for 21/23 NTDs listed to not hinder progress toward Roadmap targets (17).

Yet, monitoring numerous diseases simultaneously can be challenging. First, NTDs may have optimal, disease-specific surveillance strategies that are driven by different transmission pathways and biology

(Table 1)(44). For example, surveillance of lymphatic filariasis may target known areas with high vector (i.e., mosquito) concentrations leading to suspected transmission, while schistosomiasis surveillance may need to scale up monitoring in areas based on proximity to bodies of freshwater with known Schistosoma intermediate-host snails.

Second, as NTDs have their own range of diagnostic and surveillance tools (detailed in Section 2.2), monitoring different pathogens simultaneously may be logistically complicated (45). Diseases also have specific periods where certain techniques may not accurately capture disease presence. For example, giardia cysts and trophozoites may not be consistently present in stool of infected individuals and requires multiple stool samples over different days. Microscopic detection of microfilariae depends on appropriately timed and collected thick blood film, when microfilariae are circulating in serum. Additionally, running multiple tests using different types of samples (e.g., blood, stool, skin snips, etc.), particularly if the patient is not presenting with symptoms suggestive of most of the targeted diseases, is not easily justified in clinical settings, or not possible in resource limited settings. For example, Saleh et al. evaluated integrated disease surveillance and response (IDRS) system in 45 health facilities in Zanzibar, Tanzania and found a critical challenge to IDSR was the capacity for case confirmation of different diseases due to the lack of appropriate diagnostic equipment needed for testing and confirmation of the different diseases. (43), They found this challenge consistently reported among numerous other countries with implemented IDSR (43).

Despite these challenges, integrated surveillance of multiple diseases is possible. Developing effective and validated diagnostic tools that can monitor multiple disease simultaneously are needed, especially in areas of geographic disease overlap, and will tremendously reduce costs of individual disease surveillance while creating sustainable options in long term monitoring to support integrated surveillance of multiple diseases (20, 46).

1.2.2 NTD Diagnostics: A Need for Innovative Diagnostic Tools to Support Integrated Frameworks

A unified framework of diagnosis and surveillance is needed for all NTDs to support integrated disease surveillance. Current diagnostic tools applied to confirm NTD infections include a variety of different methods, such as clinical examination, microscopy, molecular methods, and serological methods (see pathogen specific examples, Table 2). For each method, there are different aspects of limitations when used to monitor diseases. For example, at different stages of control, different types of diagnostics are needed to inform policy making (20). In post-MDA or elimination settings, highly sensitive and specific (i.e. testing for true positives and true negatives) diagnostic tools are required to estimate residual

infections in the population. For instance, using microscopy to identify eggs in stool for schistosomiasis is not sufficiently sensitive to detect transmission in the community in low transmission settings without extremely large amounts of stool samples from the entire sample population (47). Serological Card Agglutination Trypanosomiasis Tests, used for population screening of human African trypanosomiasis in endemic areas have demonstrated low sensitivity, leading to false positives (34).

Another type of limitation pertains to the resource intensity of certain diagnostic tools. Diagnostic resources include costs, time, laboratory equipment, and trained personnel. For example, molecular methods can be expensive, especially when large quantities of specimens are required. Certain techniques also require skilled technical staff to perform, with the appropriate laboratory equipment (e.g., functioning microscopes) (20, 34). Point-of-care nucleic acid amplification tests, which measures pathogen deoxyribonucleic acid (DNA) protein fragments, while efficient, have been shown to be unaffordable in low-income settings (34). Enzyme-linked immunosorbent assays (ELISA), which measures antibodies, which can be extremely laborious and time-consuming.

Moreover, some NTDs may not have established diagnostics tools, such as leprosy and trachoma (caused by *chlamydia trachomitis* that can also cause venereal chlamydia in adults), that currently rely on clinical diagnosis for skin lesions and trachomatous inflammation, respectively. This requires accurate diagnosis based on disease symptoms, although asymptomatic infections may be overlooked without any laboratory confirmation. Additionally, disease reporting using clinical symptoms may also be limited to the patient's ability or desire to access health care.

T1. 3 **Table 2. NTD diagnostic tools recommended by the World Health Organization and Centers for Disease Control and Prevention for selected NTDs**

Example NTDs	Diagnostic Methods
Yaws WHO	-Serological tests treponema pallidum particle agglutination, rapid plasma reagin assays. -Polymerase chain reaction (PCR)
Soil-transmitted helminthiases WHO	-Microscopy of helminth eggs and Kato-Katz technique
Trachoma WHO	-Clinical examination of eyes
Lymphatic filariasis WHO	-Microscopy (Circulating microfilariae detected by examining thick smear) -Alere Filariasis Test Strip (rapid diagnostic test recommended for mapping, monitoring and transmission assessment surveys (TAS)). -The Brugia Rapid point-of-care cassette test (use during TAS) -Microfilariae DNA can be detected using PCR.

Dengue	- Rapid Test–PCR assays - ELISA used to detect of IgM and IgG anti-dengue antibodies.

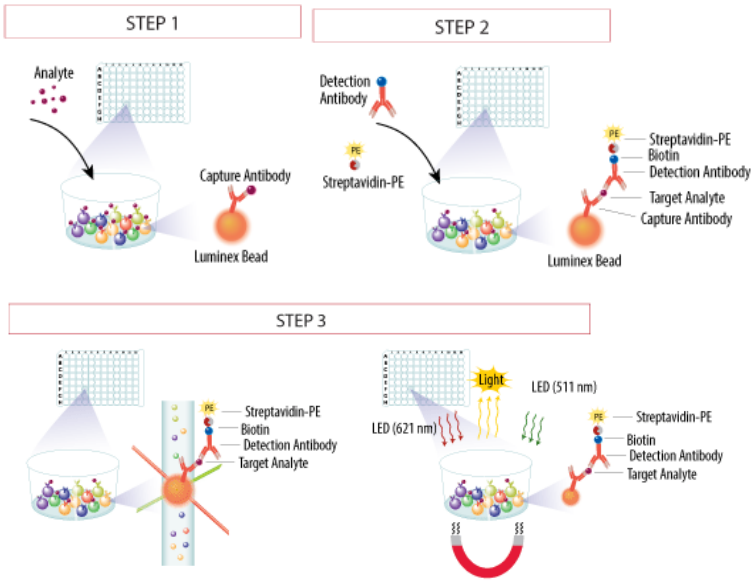
Given the various limitations of each method, the variety of diagnostic tools among the different pathogens complicates the implementation of integrated disease monitoring. Furthermore, integrated disease monitoring requires obtaining technical expertise per diagnostic method, adequate laboratory equipment and reagents needed for the different techniques and gathering ample test-specific specimens per pathogen. In countries with limited laboratory infrastructure and resources, high sample volume through-put and appropriate tools with the capacity to simultaneously evaluate multiple diseases, such as multiplexing, will help to support routine integrated disease management and research (20, 45).

Currently, there are several multiplex platforms that have the potential to be used in surveillance for multiple NTDs from a single sample. TaqMan array cards using multiplex polymerase chain reaction (PCR) can detect numerous pathogens through the presence of pathogen nucleic fragment concentrations (48). However, TaqMan array cards have been shown to have reduced sensitivity compared to traditional singleplex PCR. This may be due to the design of TaqMan array cards where the volume of sample per well (i.e. 1 µL reaction chamber) may not be consistently detected by PCR assay. Furthermore, DNA sequences at low concentrations are known to be distributed stochastically in general master mixes, where certain aliquots may not contain sample or the number of targets present is below the reproducible limit of detection for PCR (48). Additionally, serologic multiplex bead assays (MBA), microfluidic immunoassays, and cytometric bead arrays (CBA) can simultaneously quantify different human antibodies created after exposure to various pathogens with the availability of well characterized antigens (49, 50). Compared to CBAs, MBAs have been found to have less non-specific protein or antigen binding using magnetic beads. MBAs also have the advantage of a higher throughput with capacity to test more samples concurrently (51). Serological MBAs currently offer the most cost-effective method of monitoring the largest among of different diseases or antigens (46). Bead-based assays can also measure antibody responses with a high degree of sensitivity and specificity (52, 53). Using multiplex technology could reduce the need for different diagnostic tools, thus minimizing the associated labour and costs traditionally associated with integrated disease surveillance (45).

1.3 Serology and Multiplex Bead Assays in Multi-Disease Integrated Surveillance

1.3.1 Serological MBAs: A Platform Capable of Integrated Disease Surveillance

MBAs have the capacity to evaluate biomarker response to numerous pathogens concurrently (54). Platforms such as Luminex (Luminex Corporation, Austin, TX, USA) or BioRad (Bio-Rad, Hercules, CA, USA), assess multiple antigens using a mixture of color-coded beads that are coated with specific analyte capture antibodies to the pathogen(s) of interest (Figure 4). The pre-coated beads are then read using lasers and the magnitude of antigen response (reflective of antibody concentrations in sera) is recorded in mean fluorescent intensities (MFI). Depending on the platform, MBAs can analyse anywhere between 50-200 analytes for any number of pathogens, with sensitivities comparable to conventional immunoassays (50). MBAs typically analyses eluted serum samples collected using dried bloodspots on filter paper from either finger or heel pricks but can also be done with antibodies of mucosal fluid, plasma, and cell culture (55).



F1. 4 **Figure 4. Luminex Assay Protocol.**
Step 1 shows samples being added to color-coded beads. These beads coated with analyte-specific capture antibodies. Step 2 shows biotinylated detection antibodies specific to analytes being added. Next, phycoerythrin (PE)-conjugated streptavidin is also added and binds to the biotinylated antibody-antigen sandwich. Step 3 shows beads being read using dual-laser flow-based detection platforms. One laser classifies the bead and determines which analyte is used. The second laser determines the magnitude of the PE signal, which is proportional to the concentration of bound analytes. (56).

The quality of the data generated depends on several important factors (57). First, organized data management is necessary in sample collection to ensure samples are correctly labelled for MBA analysis,

given the large through-put capacity of MBAs. Second, careful laboratory practices, such as following standard operating procedures (which are needed), sufficient incubation of beads and analytes to allow binding to corresponding capture antibodies, and washing techniques to reduce non-specific binding, need to be implemented to produce good quality data. For example, low bead counts or issues with analyte or antibody binding due to improper technique may result in data loss or inaccurate results. Additionally, non-specific binding or cross-reactivity between antigens (typically with biologically similar diseases, such as certain lymphatic filariasis antigens with other filarial species) is a concern when using multiplex platforms, which can lead to spurious signals during plate readings (58). Furthermore, plate to plate consistency also needs to be accounted for and can be assessed using Levey-Jennings charts on population samples for each analyte (57). Distance (usually measured in standard deviations) from plates' mean can provide a visual indication of inter-plate variation. Plate outliers of the expected range may indicate technical issues (e.g. antigen binding or contamination).

Prior to analysis, inherent assay variability and quantitate analyte concentrations should be calibrated either using serial dilutions of standard samples or known positives and negatives to define thresholds (59). These standards can come from serum control pools or biological reference standards. MFIs from these wells are then used to generate standard curves, to which the MFIs of the sample is then converted to appropriate concentration based on these curves. Poor fitting curves can result in failure to correctly quantitate samples, as concentrations may be outside limits of quantification (59). Poor fitting curves may also be a result of excessive background noise. To estimate background noise, blanks (e.g. wells without analytes) are typically included in each assay plate, and subtracted from the MFI of standard samples (MFI-bg)(59). Additionally, assay performance can be assessed using specific antigens with known properties. For example, glutathione-S-transferase proteins has been used to test for non-specific binding, while antigens known to be present within the sample population, such as tetanus toxin with known previous vaccination campaigns has be used as a positive control as well as a more general quality control benchmark (57). A standard and validated procedure for managing the assay and data is prerequisite to ensuring quality data and using serology as a surveillance tool.

1.3.2 Serology: Advantages

Serological tools are currently a promising method being studied for integrated NTD surveillance. Exposure to a pathogen typically stimulates immune responses and the creation of antibodies that can be used to detect historical or asymptomatic exposure. Historical exposure may represent infection that has been cleared naturally or due to direct interventions, while detecting asymptomatic exposure or

infection can be helpful for capturing sub-clinical disease transmission. Additionally, as antibodies can circulate years after initial infection, depending on antibody stability and half-life, specific antibodies or concentrations may indicate active infection or historical exposure (55). These concentrations can be detected in dried blood spots or serum using pathogen-specific antigens even after extended periods of proper storage (52, 55). Another advantage is that serological tools can also be less technically challenging and more efficient to implement compared to techniques such as viral or cell culturing (60).

Using serological tools to monitor diseases can be highly informative to understanding disease transmission. Analysing antigen markers with statistical models such as sero-catalytic and antibody acquisition can provide insight toward transmission history. For instance, sero-catalytic models were used with age-specific sero prevalence data to describe a decline in trachoma transmission over time and demonstrated utility in providing post-validation estimations of follicular inflammation comparable to clinical examination (61). With geolocated data, serology can be used to identify areas of high risk for intervention targeting and spatial epidemiology of disease (46). For example, a study of lymphatic filariasis in post-elimination surveillance in American Samoa used serological data with geospatial databases to identify spatial clusters of disease that may not have been captured by routine TAS surveys (62).

Within the field of public health, programmatic applications where serological surveillance has already been applied include assessing immunization gaps of vaccine preventable diseases such as measles and rubella on multi-disease panels within population surveys (53, 60). Serological tools have also been used to estimate infection and exposure to malaria and NTDs (63, 64). For example, serological techniques such as ELISA and MBA were used to assess humoral immune responses to enteric protozoans (e.g. giardia, entamoeba, cryptosporidia) to monitor the effectiveness of implementation of a chlorination and water filtration intervention in Haiti (63). Recently, serologic multiplex assays been used to monitor patterns of changes in antigens over time of SARS-CoV-2 and has been applied to other viral diseases such as HIV and Chikungunya (65-67).

1.3.3 Serological MBAs: Application to NTDs and Global Health

When applied to integrated disease monitoring, serological MBAs can be a potent, resource-efficient tool to measure exposure to multiple diseases simultaneously in areas of overlapping geographic endemicity (20, 46, 55, 68). MBAs have been used for various integrated, multi-disease panels that include antigens of malaria, chikungunya, rubella, lymphatic filariasis, strongyloidiasis, and onchocerciasis in research settings. For example, MBAs were applied to a study in Haiti to identify

exposure to endemic dengue virus from newly introduced chikungunya virus. Furthermore, bead assays in this study helped to indicate disease inception of chikungunya in 2014 and its rapid transmission within the Haitian naïve population (69). Also in Haiti, antibody responses to three lymphatic filariasis antigens were measured using MBAs to describe exposure in children. Certain antibodies within the study were identified to be markers of infection, as they appear before circulating filarial antigens that are used to make decisions regarding the program endpoints (70). Additionally, MBAs have been used to seroprotection of vaccine preventable diseases. For example, in Kenya, Tanzania and Mozambique, assessing tetanus seroprotection was included in panel of 30 bacterial, viral, and parasitic antigens, and investigator found unexpected immunity gaps for tetanus among men and children (68). In elimination-settings, MBAs have been implemented to data from Uganda, Laos, and The Gambia that was used to determine seropositivity elimination thresholds for trachoma antigens with appropriate methods of determining these thresholds (71). The utility of serological MBAs in describing burden of co-endemicity of diseases can enable programs to strategize coordinated control measures rather than individual disease interventions (72). For example, if trachoma and yaws were found to be the same geographic location, albendazole can be administered thoroughly to treat both infections. MBAs can then be used the coverage progress of treatment for both diseases.

Furthermore, MBAs offer the unique opportunities to test for diseases that may not be included within routine surveillance in resource-limited settings, thus enhancing the understanding of global disease prevalence, recrudescence, and capture disease (re-)emergence (55). As blood samples are already collected in different programs including demographic and health surveys (DHS), NTD transmission assessment surveys (TAS), and indicator surveys for malaria and HIV, these samples have the potential to be implemented into routine serological testing for surveillance, with adjustment to protocols accordingly (55). The collected blood samples can then be used to test for numerous NTDs to further maximize existing resources to provide routine monitoring of disease transmission. For example, research activities such as the administration of malaria sero-surveys can include NTDs within their panel, where precise data on prevalence of low-profile diseases such as strongyloidiasis or yaws are unknown in many endemic countries. In another example, as countries progress towards elimination certification of lymphatic filariasis, routine surveillance activities such as TAS for LF of school aged children can incorporate MBAs with appropriate antigens to test for other NTDs such as toxoplasmosis or soil-transmitted helminths, based on known endemicity or as a screening measure. Two examples of this application in the literature include identifying tetanus immunity gaps in Kenya, Tanzania, and Mozambique using an integrated sero-survey of parasitic diseases with MBA platforms (68, 73) and the

surveillance of chikungunya epidemic in Haiti was included in a survey designed to evaluate malaria prevalence (67).

1.3.4 Serological MBAs: Challenges

There are several challenges using serology and multiplex bead assay for integrated surveillance (55).

Technical Challenges

Technical challenges include sampling approaches and standard laboratory protocols. Individual diseases may have ideal populations for monitoring, which is regulated by factors such as transmission dynamics, antigen dynamics, antigen age effects, and program targets. Therefore, coordinating optimal sampling approaches across all antigens will be challenging. For example, Arnold et al. have discussed differences in sampling populations: In certain settings, children have been shown to be reflective of population exposure for NTDs, while adults may be more suitable for understanding antibody waning in seroprotection of VPDs over time, or occupational-based pathogen exposure (74). Additionally, active sampling is often powered based on the number of individuals needed to sufficiently estimate burden of disease for specific pathogen, based on program goals or international guidelines. With integrated monitoring, this number should ideally be inclusive of all pathogens within the sample (e.g., will need to consider the rarest diseases of interest) (75). Nonetheless, having information on diseases while accounting for any limitations in sampling design will aid in understanding transmission intensity and disease burden.

Furthermore, different platforms or laboratory protocols (e.g. antibody coupling, regents used, etc.) may impact serological output (76). For example, bead-antibody coupling methods and degradation of beads over time may cause potential variability in MFI values in different settings (23) and commercial antigen-coupled beads are needed to enable standardization across laboratories. Having commercial antigens or antigens that reflect geographical or species variation, standard laboratory protocols, and reference standards approved by the WHO are necessary for consistent reporting and consequent public health decision making (76). However, despite the absence of these standards, studies have demonstrated appropriate means of interpreting serologic output.

Analytical Challenges

Analytical challenges pertain to the interpretation and analysis of MFI values. In order to inform public health programs, MFI values need to be interpretable, either in the form of categorized MFI values or converted into a public health metric. Estimating population seropositivity is a common approach (i.e.,

positive antigen test results to determine exposure or infection to/ or protection against a pathogen) but translating MFI of data without serologic reference standards to determine cut-offs may make interpretation of serological data challenging and inconsistent between settings (77) . Currently, studies must rely on different statistical approaches to identify seropositives, however, these approaches may not be universally applicable or appropriate across settings or pathogens due to factors such as transmission dynamics or pathogen-specific biology. For example, within malaria, LF, strongyloidiasis, and trachoma multiple methods of determining seropositivity have been applied (71, 73, 78). These approaches may result in different seroprevalence estimates that could impact decision making (as explored further in Chapter 3). In addition, interpretation of MFI data should also consider the kinetics of specific antigen markers as indicators of previous exposure or active infection.

1.4 Bridging the knowledge gap

Serological MBAs and their capacity in epidemiological or programmatic settings has not been thoroughly examined given their relative novelty. Consequently, determining their capabilities applied in public settings will assist programs to implement serological MBAs to integrated disease surveillance. This thesis will focus on the analytical challenges of applying serological MBAs. Determining their utility in integrated disease surveillance will require an initial understanding of appropriate methods to characterize MFI responses for analysis, given the absence of reference standard serum for many NTD antigens. Next, analysing MFI data using antigens of different pathogens in distinct epidemiological settings with appropriate statistical methods will help to assess MBA capabilities and limitations when applied to integrated disease surveillance. Lastly, interpretating analysis results will be needed to evaluate any programmatic inferences from using MFI data.

1.5 Brief overview of NTD Initiatives in Study Sites

The research in this thesis investigates data from four serological surveys- three from Haiti and one from Malaysia. This section provides a brief background of impactful tropical diseases and public health initiatives in Haiti and Malaysia.

1.5.1 Background-Haiti

There are numerous tropical diseases that impact the country of Haiti and its population, including several NTDs (i.e., lymphatic filariasis, trachoma, and yaws), soil-transmitted helminths (i.e., ascariasis and strongyloides), waterborne enteric diseases (i.e., giardia and cholera), and vectorborne diseases (i.e., chikungunya and dengue) (79). Different public health programmes have been implemented over

the past two decades, which encompasses integrated vector control, WASH initiatives, and MDAs to aid in the control and elimination of these diseases (79).

Progress and success of control programs are commonly limited by government and donor resources. In Haiti, however, numerous large-scale adverse events within the past decade have also hindered progress of disease control. For example, political crises in 2003 and subsequent unrest and violence in 2004 have caused issues in the timely administration of public health interventions. In addition, natural disasters, including several hurricanes in 2008 massive earthquakes in year 2010 and have led to disease outbreaks and devastation of public health infrastructures (80). The introduction of diseases such as chikungunya in 2015 and Covid19 in 2019 has also impacted control activities for numerous diseases across the nation.

In 2010, Haiti implemented the Haiti Neglected Tropical Disease Control Program between the Ministry of Health and Population and the Ministry of Education to eliminate lymphatic filariasis and soil transmitted helminths (79). This program involved multiple partner organizations, such as USAID, the Gates Foundation, the CDC, the WHO, and the Research Triangle Institute, among others. Key activities of this collaboration targeted mobilizing MDA with the goal of achieving national geographical coverage (>75%) across all communes in Haiti, establishment of sentinel sites for continued monitoring of NTD prevalence, and successfully completion of MDA treatment in specified Haitian departments. Additionally, this program also aimed to evaluate costs involved with the implementation of programs, improved training of medical staff, post-elimination activities, and continued monitoring and evaluation.

1.5.2 Background-Malaysia

Like Haiti, Malaysia has a long history of neglected tropical diseases. Soil-transmitted helminth infections have been documented in certain communities within Malaysia since 1938 (81), and mulitparatism has also been observed from the presence of other infections including giardiasis, ameobiasis, and blastocystosis (81). Dengue is currently a leading cause of morbidity and mortality as 2019, while rabies outbreaks are still being reported. Lymphatic filariasis, which has been historically endemic in the country, is at present being treated with MDA (82). Efforts to control neglected tropical diseases broadly include the implementation of public health initiatives such as early case detection through surveillance, vector control measures, MDA, and community awareness (82).

While Malaysia lies in a geographically stable region that is not prone to disasters such as earthquakes, tropical cyclones or volcanic activities, it has been regularly impacted by natural calamities such as

tropical storms, floods, and tsunamis(83). Floods, caused by extreme climatic events (i.e., La Nina in 2011 and 2012) or by annual monsoons, account for substantial casualties, damage to properties, and disease outbreaks. They can be. These natural calamities can cause populations to disperse or relocate to overcrowded relief centers, which poses challenges to disease prevention (84), cause a lack of clean water which propagate waterborne diseases and spreads breading grounds for vector borne diseases such as lymphatic filariasis and dengue.

In 2021, a press release by the Ministry of Health in Malaysia confirmed the government's commitment to intensify efforts to control NTDs, which is needed as the country pushes for elimination of several neglected tropical diseases in the near future (82).

1.6 NTDs in Haiti and Malaysia pertinent to Thesis

The research in this thesis includes six NTDs. The description of these disease in regards to Haiti and Malaysia are discussed in the following sections.

1.6.1 Lymphatic Filariasis

Lymphatic filariasis is caused by *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori* nematode worms, transmitted by several different species of mosquitoes. The standard method for diagnosing LF is through microscopic examination of microfilariae using a blood smear (with blood collection done in the evening). Serologic techniques can also be used in diagnosis of LF, as patients with active infections have been observed to have elevated antifilarial IgG (85). Filariasis Test Strips and immunochromatographic card test (for Circulating Filarial Antigen) are currently used in Transmission Assessment Surveys (both tests for biomarkers to *Wucheria bancrofit*). According to the WHO Global Health Observatory in 2020, both Haiti and Malaysia are receiving ongoing MDA treatment (86).

Previous studies in the late 1990s and early 2000s identified widespread prevalence of lymphatic filariasis throughout Haiti, (80). LF is transmitted by *Aedes, Culex*, *Anopheles*, and *Mansonia* mosquitoes in this region. Immunochromatographic tests used to detect circulating filarial antigens showed that 90% of communes (n=133) were above the 1% prevalence threshold as defined by the WHO, requiring subsequent MDA to the spread of LF. In year 2000, the Haiti National Program to Eliminate Lymphatic Filariasis (NPELF) control began treating an initial two million individuals in 24 communes with MDA, consisting of diethylcarbamazine (DEC) and albendazole. By 2012, increased donor support enabled full geographic coverage of MDA to over 8 million individuals in Haiti.

Aside from natural disasters and civil unrest that impacted public health programs, NPELF in Haiti faced additional technical challenges in inconsistent funding, continual commitment of partners, and administration of MDA. Additionally, there is evidence of transmission in some areas despite stable administration of MDA over five years, likely due to individuals that fail to participate in MDA that represent a reservoir of infection (80). The NPELF has made great strides in public health reduce of LF and will benefit from innovative intervention strategies moving forward to eliminate the disease in the future.

In Malaysia, LF is caused by *W. bancrofti* and *B. malayi* and transmitted by *Anopheles* and *Mansonia* genus mosquitoes. LF in Malaysia is focal, occurring in Sabah, Sarawak and several states in peninsular Malaysia (87). Five rounds of MDA had been completed between 2004-2008 with greater than 80% coverage. After TAS-1, positive cases exceeded critical cut-off. Additional MDA was administered following, and TAS-2 was conducted using a Brugia rapid test (BmR1). which showed values again higher that critical cut-off (88). Between 2010-2011 two phases of transmission-assessment surveys were again performed, with the target of eliminating LF by 2015 (87).

1.6.2 Chikungunya

Chikungunya was first identified in 1952 (Tanzania) and since has spread to over 60 countries in the world, with the Americas and Asia being most affected (89). Chikungunya is transmitted through Aedes aegypti and aedes albopictus. Reverse transcription PCR is used to confirm active CHIKV infection (90), while serological tests using ELISA can be used to establish overall presence of anti-CHIKV antibodies within a region, given the lingering presence of CHIKV IgG (67). Due to difficulties in accurate diagnosis of chikungunya, global estimates of chikungunya are not available (89). There is no specific treatment for chikungunya. Vector control and personal protection is recommended to prevent spread of disease.

In Haiti, the first reported cases of chikungunya virus were reported in 2014 by the WHO, among a group of children who tested seropositive to CHIKV IgG antibodies (69). Chikungunya spread rapidly throughout the country – 78.9% of children tested positive within one year. A nationwide study assessing prevalence of chikungunya in 2015 found that seroprevalence was 57.9% (67) In Malaysia, the first reported cases of chikungunya virus were reported in 1998 during an outbreak in a small suburban area. In 2006 and 2008, another two outbreaks were reported. Following these outbreaks, a cross sectional study of chikungunya seroprevalence across four states in Malaysia found 5.9% of the study population testing positive (91). Both Haiti and Malaysia are both currently targeting control for chikungunya in accordance with WHO guidelines.

1.6.3 Dengue

Four dengue virus types (DENV 1-4) are transmitted through Aedes aegypti and aedes albopictus mosquitoes. It is the most common mosquito-borne viral disease in the world, with an estimated 390 million infections and 96 million symptomatic cases (92). Type-specific lifelong immunity is developed after infection to one of the four virus types (93). Dengue cases are diagnosed using clinical or serological confirmation. However, as dengue symptoms are broad in clinical presentations and course of symptoms can be unpredictable, diagnosis based on clinical guidelines has proved challenging (93).

A study in 2007 in Port-au-Prince reported that 65% of children greater than 5 years of age had serological biomarkers indicating prior infection to DENV (94). A later study in 2012-2013 found that in case of 885 patients with acute febrile illness, 4% tested positive for DENV infection by RDT (95). In Malaysia, the first reported cases on dengue occurred in 1902. Since then, dengue cases have continued to rise throughout the country. In 2007 the number of dengue cases were estimated to 181/100,000 persons and increased 6.5-fold in reported cases in the past decade (96). High dengue incidence have caused a substantial burden to Malaysia's national health care system. Currently, both Haiti and Malaysia are targeting control of dengue in accordance with WHO guidelines.

1.6.4 Trachoma

Trachoma is a disease caused by infection of Chlamydia trachomatis. It is usually acquired through contact with other individuals who have infection. Currently, it is a public health problem in 44 countries (97). Elimination strategy of trachoma is to implement surgery, antibiotics, facial cleanliness and environmental improvement (FACE), and trachoma infection is typically clinically diagnosed. According to the WHO GHO, both Haiti and Malaysia are classified as not thought to require interventions in the status of elimination as a public health problem, although surveillance should be conducted in these areas to confirm their status (98).

1.6.5 Yaws

Yaws is caused by bacteria of the genus Treponema, which also causes syphilis. According to the WHO, there are 15 countries known to be endemic for yaws, and 76 countries or territories that were previously endemic with current status unknown (99). Approximately 75% of individuals affected by yaws are children. As yaws can be treated with preventative chemotherapy, it is recommended by the WHO those countries with previously endemic status incorporate yaws into integrated surveillance to avoid resurgence. Clinical symptoms (papilloma) of yaws are quite straightforward initially, however, if left untreated, ulcerated papillomas often require serological confirmation. Laboratory tests included

Treponema pallidum particle agglutination (TPPA) and rapid plasma reagin (RPR) but these tests cannot distinguish between yaws and syphilis. PCR can be used to confirm yaws in skin lesions. Haiti and Malaysia previous were endemic for yaws (100), but are not currently listed as endemic countries according to the WHO. However, updated surveillance data is at present lacking for these countries regarding yaws.

1.7 Other Diseases in Haiti and Malaysia pertinent to Thesis

There are other diseases examined in this thesis beyond NTD . Several of these diseases are in the expanded list of neglected tropical diseases based on evidence that these diseases cause prolonged and debilitating conditions that impact populations of extreme poverty (1).

1.7.1 Parasitic Diseases

Four parasitic diseases (included within the expanded list of NTDs) are included in this thesis. Toxoplasmosis is globally distributed, with foci of high distribution in Latin America, parts of eastern and central Europe, the Middle East, parts of south-east Asia, and Africa (101). Current prevalences estimates of toxoplasmosis in Haiti is unclear, which is associated with the general lack of information regarding this disease in this region (102). In Malaysia, several studies have shown that antibodies to toxoplasma are common among Malaysians. The disease is apparently more prevalent among rural dwellers and those in the lower socioeconomic group (103). Seroprevalence surveys of Toxoplasma is traditionally done using a single Toxoplasma specific assay in the form of commercial or in-house ELISA or immunofluorescent antibody assay. SAG2A is an immunodominant antigen that is expressed in bacterial (102)**.** This antigen has been shown to have high sensitivity and specificity using IgG ELISAs (104).

Soil-transmitted *Strongyloides stercoralis* threadworm causes strongyloidiasis. Strongyloidiasis is endemic world-wide and is more frequently found in tropical climates or resource limited settings (105). Prevalence is higher in resource-limited settings due to inadequate sanitation conditions. Historically, strongyloidiasis has been screened using coprological or serological tests, with lot to moderate and high sensitivity, respectively (105). Strongyloidiasis can be treated with preventative chemotherapy. A meta-review of *S. stercoralis* infections found prevalence rates to be 1.0% in Haiti (39). In Malaysia A study in 2019 using fecal smears described prevalence rates of strongyloides to be above 15% in Orang Asli in Malaysia (106). Another study in 2016 using serum samples found 11% of the population in indigenous communities to be seropositive (107). According to the global health observatory, Haiti is undergoing MDA for soil transmitted helminths and Malaysia is currently not receiving MDA.

Waterborne parasitic diseases such as globally-distributed giardiasis, caused by *Giardia lamblia* and *Giardia duodenalis*, (108) and cryptosporidiosis, caused by *Cryptosporidium parvum*, (109) contribute to substantial burden of diarrhea in areas of overcrowding and inadequate sanitation. Haiti and Malaysia currently target the control of these waterborne parasitic diseases.

1.7.2 Cholera

Cholera is a waterborne disease caused by the bacterium *Vibrio cholerae*. Currently, the global strategy on control of cholera aims to reduce cholera deaths by 90% by 2030 (110). In October 2010, cholera was introduced to as a result of human migration following a large-scale earthquake (111, 112). Haiti is currently controlling and targeting elimination of cholera (113, 114). In Malaysia, the last reported outbreak of cholera was recorded in 1996 (115).

1.7.3 Vaccine Preventable Diseases

Three vaccine preventable diseases (tetanus, measles, and influenza) are included in the research of this thesis. While the focus of this thesis is on disease particular to tropical regions, another important aim of this research is to highlight the integrated capacity of MBAs to monitoring exposure to numerous pathogens concurrently in the same geographic region.

1.8 Multiplexed serological biomarkers used in thesis

19 antigens of twelve different diseases (lymphatic filariasis, strongyloidiasis, trachoma, yaws, dengue, chikungunya, cholera, cryptosporidiosis, enterotoxin, tetanus, measles, and influenza) were collectively included in this thesis. Provenance, known kinetics, and example public health applications of the use of these antigens are included in Table 3. Further discussion pertaining to antigens use is included in Chapter 6.

T1. 4 **Table 3. Antigen kinetics and public health applications of disease antigens in thesis.**

Disease/Antigen	Provenance and various kinetics	Example Public Health Applications
Chikungunya **CHIKV**	• RNA protein for the Chikungunya RNA virus that is used for detection in other methods such as PCR and RT-PCR (116) • IgM detection test is not recommended for acute-phase samples since IgM develops on or after day two of viral infection (117)	• Included in rapid tests have been (118) • Applied previous in rapid and ELISA-based antigen tests, IgM detection tests, and immunofluorescence assays (117) • Antigen or antibody-based serological tests can be reliable diagnostic tests

	• IgG detection test suggests that IgG antibodies can be detected in the convalescent-phase samples (117)	dependent on time of sample collection (117)
Dengue **DENV-2** **DENV-3**	• DENV-2 has epitopes for antibodies to dengue virus serotype 2 and 3; DENV-3 has epitopes for antibodies to serotypes 3 and 1(119) • DENV-2 and DENV-3 are unaffected by cross-reactivity(119)	• Assess associated clinical manifestations with serological biomarkers (120) • Surveillance of exposure and prevalence (121, 122)
Toxoplasmosis **SAG2A**	• SAG2A is easily expressed in bacterial cultures as GST fusion protein(102) • IgG ELISAs using recombinant SAG2A were shown to be sensitive (96% and specific 100%) compared to crude Toxoplasma antigens(102) • No evidence of cross reactivity between rSAG2 and GST(102)	• Assess age specific SAG2A antibody responses (102) • Clinical diagnosis (123)
Lymphatic Filariasis **Wb123** **BM33** **BM14** **BMR1**	• Bm antigens are recombinant brugia malayi pepsin inhibitor homologs • Bm33 antigen is immunodominant and associated with filarial pathogenesis (124) • Differences in isotype responses show that Bm14 and Bm33 antibody responses are specific(125) • No cross reactivity between Bm33 and Bm14(125) • Bm33 was the first antibody response to be detected in children and Bm33 also had the highest seroconversions(70) • Evidence suggests that Bm14 and Bm33 antibody are infection markers(70) • Bm14 and Bm33 should be used only in areas without other filarial antigens known to illicit cross-reactivity(70)	• Bm14 and Bm33 antigens are good estimations of transmission (125) • Bm14 and Bm33 studied with Transmission Assessment Surveys (1 and 2) and demonstrated an overall decline in antibody prevalence, associated with decreased exposure (126) • Bm14 tested against WB microfilaria and filarial antigen and demonstrated high sensitivity in monitoring continued transmission (127) • Bm14 tested against both Brugia Rapid Test and Filariasis Test Strip with high sensitivity (128)

	• Luciferase immunoprecipitation system has shown Wb123 to precede the appearance of antigenemia in two separate sample populations(70) • Wb123 is an infection larval antigen	
Strongyloidiasis **NIE**	• Developed for Immunodiagnostic(129) • Non-cross reactive with sera from other related filarial infections (130) • The sensitivity and specificity for Luminex was 93% and 95%, respectively(129)	• Used for routine screening and clinical diagnosis of infection (ELISA) (129, 131) • Assessing coinfections in highly endemic areas(132)
Trachoma/ Chlamydia bacteria **Pgp3** **Ct694**	• Antigens selected based on recognition by serology from trachoma positive patients in published studies. Ct694 found to be involved in pathogenesis(133) • Pgp3 is the only plasmid encoded ORF and secreted into host cell cytoplasm during infection(133). • Pgp3 function is unknown but appears to play a role in pathogenesis(133). • It also could be a potential diagnostic marker for genital chlamydial and is related to both disease and infection status(133)	• Serological surveillance and understanding transmission dynamics (134, 135)
Yaws / Syphilis bacteria **Rp17** **TmpA**	• Rp17 is marker of historical infection and TmpA is a marker of recent or active infection(136).	• Rp17 and TmpA detected with high sensitivity and specificity compared to responses with standard reference tests.(136) • Positivity correlation with RPR (previous standard of measure) with increasing TmpA levels on MBA(136)
Giardiasis **VSP 3** **VSP 5**	• VSP is a surface protein that covers entire parasite-host-immune system is exposed to many different VSP sequences/heterogeneity(137) • IgG antibodies correlate with infection status(137).	• Serological Surveillance and understanding age related exposure (55)

	• Human antibodies against VSP may remain elevated for 12 months after infection (138)	
Cryptosporidiosis **Cry17** **Cry27**	• Potential markers of historical infection in population studies (139)	• Used in ELISA, which detected IgG antibodies to Cry 17 and Cry 27with good sensitivity and specificity relative to the gold standard Western blot in both outbreak and non-outbreak pathogens(137). • Elevated Cry17 is the most recognizable feature of an outbreak.(137)
Amoebiasis **LecA**	• Recombinant LecA is shown to capture specific responses of the E. histolytica-specific IgG antigens(140). • E. histolytica-specific recombinant protein (140)	• Assessed point-of-use water filter through serologic response (141)
Tetanus **Tetanus Toxin**	• International standard for monitoring vaccine coverage • Showed high sensitivity 99% and specificity 92% (142)	• Assessing immunity gaps in diverse populations (68, 143)
Measles **MEAV**	• Measles immunity declines over a lifetime (144) • High antibody titres are a good correlate of protection against infection (145)	• Assessing seroprevalence of measles exposure(144)
Cholera **Ct b4**	• CT toxin is documented as the major toxin during cholera manifestations(146)	• Included in molecular epidemiological studies (147, 148)
Influenza **H5**	• Associated with acute clinical disease in animals (149)	• Surveillance of influenza virus (148)

1.9 References

1. Hotez PJ, Aksoy S, Brindley PJ, Kamhawi S. What constitutes a neglected tropical disease? PLoS Negl Trop Dis. 2020;14(1):e0008001.
2. Hotez PJ, Fenwick A, Savioli L, Molyneux DH. Rescuing the bottom billion through control of neglected tropical diseases. Lancet. 2009;373(9674):1570-5.
3. Rees CA, Hotez PJ, Monuteaux MC, Niescierenko M, Bourgeois FT. Neglected tropical diseases in children: An assessment of gaps in research prioritization. PLoS Negl Trop Dis. 2019;13(1):e0007111.
4. Hotez PJ, Molyneux DH, Fenwick A, Ottesen E, Ehrlich Sachs S, Sachs JD. Incorporating a rapid-impact package for neglected tropical diseases with programs for HIV/AIDS, tuberculosis, and malaria. PLoS Med. 2006;3(5):e102.
5. Hotez PJ, Molyneux DH, Fenwick A, Kumaresan J, Sachs SE, Sachs JD, et al. Control of neglected tropical diseases. N Engl J Med. 2007;357(10):1018-27.
6. Mitja O, Marks M, Bertran L, Kollie K, Argaw D, Fahal AH, et al. Integrated Control and Management of Neglected Tropical Skin Diseases. PLoS Negl Trop Dis. 2017;11(1):e0005136.
7. Solomons NW. Malnutrition and infection: an update. Br J Nutr. 2007;98 Suppl 1:S5-10.
8. Hall A, Zhang Y, Macarthur C, Baker S. The role of nutrition in integrated programs to control neglected tropical diseases. BMC Med. 2012;10:41.
9. Hotez P, Ottesen E, Fenwick A, Molyneux D. The neglected tropical diseases: the ancient afflictions of stigma and poverty and the prospects for their control and elimination. Adv Exp Med Biol. 2006;582:23-33.
10. Feasey N, Wansbrough-Jones M, Mabey DC, Solomon AW. Neglected tropical diseases. Br Med Bull. 2010;93:179-200.
11. Trouiller P, Olliaro P, Torreele E, Orbinski J, Laing R, Ford N. Drug development for neglected diseases: a deficient market and a public-health policy failure. Lancet. 2002;359(9324):2188-94.
12. Bleakley H. Disease and Development: Evidence from Hookworm Eradication in the American South. Q J Econ. 2007;122(1):73-117.
13. The 2018 update, Global Health Workforce Statistics, World Health Organization, Geneva [Available from: http://www.who.int/hrh/statistics/hwfstats/.
14. Hotez P, Aksoy S. PLOS Neglected Tropical Diseases: Ten years of progress in neglected tropical disease control and elimination ... More or less. PLoS Negl Trop Dis. 2017;11(4):e0005355.
15. Hotez PJ. Ten failings in global neglected tropical diseases control. PLoS Negl Trop Dis. 2017;11(12):e0005896.
16. Kuper H. Neglected tropical diseases and disability-what is the link? Trans R Soc Trop Med Hyg. 2019;113(12):839-44.
17. Ending the neglect to attain the Sustainable Development Goals – A road map for neglected tropical diseases 2021–2030. Geneva: World Health Organization; 2020. Licence: CC BY-NC-SA 3.0 IGO.
18. Dowdle WR. The principles of disease elimination and eradication. Bull World Health Organ. 1998;76 Suppl 2:22-5.
19. Heymann DL. Control, elimination, eradication and re-emergence of infectious diseases: getting the message right. Bull World Health Organ. 2006;84(2):82.
20. Peeling RW, Boeras DI, Nkengasong J. Re-imagining the future of diagnosis of Neglected Tropical Diseases. Comput Struct Biotechnol J. 2017;15:271-4.
21. Steinmann P, Utzinger J, Du ZW, Zhou XN. Multiparasitism a neglected reality on global, regional and local scale. Adv Parasitol. 2010;73:21-50.
22. Simon GG. Impacts of neglected tropical disease on incidence and progression of HIV/AIDS, tuberculosis, and malaria: scientific links. Int J Infect Dis. 2016;42:54-7.

23. Narain JP, Dash AP, Parnell B, Bhattacharya SK, Barua S, Bhatia R, et al. Elimination of neglected tropical diseases in the South-East Asia Region of the World Health Organization. Bull World Health Organ. 2010;88(3):206-10.
24. Hotez PJ, Bottazzi ME, Franco-Paredes C, Ault SK, Periago MR. The neglected tropical diseases of Latin America and the Caribbean: a review of disease burden and distribution and a roadmap for control and elimination. PLoS Negl Trop Dis. 2008;2(9):e300.
25. Uniting to combat Neglected Tropical Diseases [Available from: https://unitingtocombatntds.org/ntds/.
26. Brady MA, Hooper PJ, Ottesen EA. Projected benefits from integrating NTD programs in sub-Saharan Africa. Trends Parasitol. 2006;22(7):285-91.
27. Chanda E, Ameneshewa B, Mihreteab S, Berhane A, Zehaie A, Ghebrat Y, et al. Consolidating strategic planning and operational frameworks for integrated vector management in Eritrea. Malar J. 2015;14:488.
28. Chanda E, Ameneshewa B, Bagayoko M, Govere JM, Macdonald MB. Harnessing Integrated Vector Management for Enhanced Disease Prevention. Trends Parasitol. 2017;33(1):30-41.
29. Maheu-Giroux M, Castro MC. Cost-effectiveness of larviciding for urban malaria control in Tanzania. Malar J. 2014;13:477.
30. Boisson S, Engels D, Gordon BA, Medlicott KO, Neira MP, Montresor A, et al. Water, sanitation and hygiene for accelerating and sustaining progress on neglected tropical diseases: a new Global Strategy 2015-20. Int Health. 2016;8 Suppl 1:i19-21.
31. Integrated control of the neglected tropical diseases: A neglected opportunity ripe for action. [Available from: https://www.who.int/neglected_diseases/NTD_integrated_control.pdf.
32. Fenwick A. The global burden of neglected tropical diseases. Public Health. 2012;126(3):233-6.
33. Tambo E, Ai L, Zhou X, Chen JH, Hu W, Bergquist R, et al. Surveillance-response systems: the key to elimination of tropical diseases. Infect Dis Poverty. 2014;3:17.
34. Taylor EM. NTD Diagnostics for Disease Elimination: A Review. Diagnostics (Basel). 2020;10(6).
35. Vitale M, Lupone CD, Kenneson-Adams A, Ochoa RJ, Ordonez T, Beltran-Ayala E, et al. A comparison of passive surveillance and active cluster-based surveillance for dengue fever in southern coastal Ecuador. BMC Public Health. 2020;20(1):1065.
36. Wamboga C, Matovu E, Bessell PR, Picado A, Bieler S, Ndung'u JM. Enhanced passive screening and diagnosis for gambiense human African trypanosomiasis in north-western Uganda - Moving towards elimination. PLoS One. 2017;12(10):e0186429.
37. Longbottom J, Wamboga C, Bessell PR, Torr SJ, Stanton MC. Optimising passive surveillance of a neglected tropical disease in the era of elimination: A modelling study. PLoS Negl Trop Dis. 2021;15(3):e0008599.
38. Baker MC, Mathieu E, Fleming FM, Deming M, King JD, Garba A, et al. Mapping, monitoring, and surveillance of neglected tropical diseases: towards a policy framework. Lancet. 2010;375(9710):231-8.
39. Olsen A, van Lieshout L, Marti H, Polderman T, Polman K, Steinmann P, et al. Strongyloidiasis--the most neglected of the neglected tropical diseases? Trans R Soc Trop Med Hyg. 2009;103(10):967-72.
40. da-Costa Vroom FB, Aryeetey R, Boateng R, Anto F, Aikins M, Gyapong M, et al. Data reporting constraints for the lymphatic filariasis mass drug administration activities in two districts in Ghana: A qualitative study. SAGE Open Med. 2015;3:2050312115594083.
41. Zhou XN, Bergquist R, Tanner M. Elimination of tropical disease through surveillance and response. Infect Dis Poverty. 2013;2(1):1.
42. Sengupta R, Chaudhuri SJ, Moulik S, Ghosh MK, Saha B, Das NK, et al. Active surveillance identified a neglected burden of macular cases of Post Kala-azar Dermal Leishmaniasis in West Bengal. PLoS Negl Trop Dis. 2019;13(3):e0007249.

43. Saleh F, Kitau J, Konradsen F, Mboera LEG, Schioler KL. Assessment of the core and support functions of the integrated disease surveillance and response system in Zanzibar, Tanzania. BMC Public Health. 2021;21(1):748.
44. Loh EH, Zambrana-Torrelio C, Olival KJ, Bogich TL, Johnson CK, Mazet JA, et al. Targeting Transmission Pathways for Emerging Zoonotic Disease Surveillance and Control. Vector Borne Zoonotic Dis. 2015;15(7):432-7.
45. Peeling RW, Mabey D. Diagnostics for the control and elimination of neglected tropical diseases. Parasitology. 2014;141(14):1789-94.
46. Solomon AW, Engels D, Bailey RL, Blake IM, Brooker S, Chen JX, et al. A diagnostics platform for the integrated mapping, monitoring, and surveillance of neglected tropical diseases: rationale and target product profiles. PLoS Negl Trop Dis. 2012;6(7):e1746.
47. Zhou XN, Xu J, Chen HG, Wang TP, Huang XB, Lin DD, et al. Tools to support policy decisions related to treatment strategies and surveillance of Schistosomiasis japonica towards elimination. PLoS Negl Trop Dis. 2011;5(12):e1408.
48. Rachwal PA, Rose HL, Cox V, Lukaszewski RA, Murch AL, Weller SA. The potential of TaqMan Array Cards for detection of multiple biological agents by real-time PCR. PLoS One. 2012;7(4):e35971.
49. Christopher-Hennings J, Araujo KP, Souza CJ, Fang Y, Lawson S, Nelson EA, et al. Opportunities for bead-based multiplex assays in veterinary diagnostic laboratories. J Vet Diagn Invest. 2013;25(6):671-91.
50. Elshal MF, McCoy JP. Multiplex bead array assays: performance evaluation and comparison of sensitivity to ELISA. Methods. 2006;38(4):317-23.
51. Moncunill G, Aponte JJ, Nhabomba AJ, Dobano C. Performance of multiplex commercial kits to quantify cytokine and chemokine responses in culture supernatants from Plasmodium falciparum stimulations. PLoS One. 2013;8(1):e52587.
52. Exum NG, Pisanic N, Granger DA, Schwab KJ, Detrick B, Kosek M, et al. Use of Pathogen-Specific Antibody Biomarkers to Estimate Waterborne Infections in Population-Based Settings. Curr Environ Health Rep. 2016;3(3):322-34.
53. Cutts FT, Hanson M. Seroepidemiology: an underused tool for designing and monitoring vaccination programmes in low- and middle-income countries. Trop Med Int Health. 2016;21(9):1086-98.
54. Lammie PJ, Moss DM, Brook Goodhew E, Hamlin K, Krolewiecki A, West SK, et al. Development of a new platform for neglected tropical disease surveillance. Int J Parasitol. 2012;42(9):797-800.
55. Arnold BF, Scobie HM, Priest JW, Lammie PJ. Integrated Serologic Surveillance of Population Immunity and Disease Transmission. Emerg Infect Dis. 2018;24(7):1188-94.
56. Systems RD. Luminex Assay Protocol [Available from: https://www.rndsystems.com/resources/protocols/how-run-rd-systems-luminex-assay.
57. van den Hoogen LL, Presume J, Romilus I, Mondelus G, Elisme T, Sepulveda N, et al. Quality control of multiplex antibody detection in samples from large-scale surveys: the example of malaria in Haiti. Sci Rep. 2020;10(1):1135.
58. Juncker D, Bergeron S, Laforte V, Li H. Cross-reactivity in antibody microarrays and multiplexed sandwich assays: shedding light on the dark side of multiplexing. Curr Opin Chem Biol. 2014;18:29-37.
59. Sanz H, Aponte JJ, Harezlak J, Dong Y, Ayestaran A, Nhabomba A, et al. drLumi: An open-source package to manage data, calibrate, and conduct quality control of multiplex bead-based immunoassays data analysis. PLoS One. 2017;12(11):e0187901.
60. Bolotin S, Lim G, Dang V, Crowcroft N, Gubbay J, Mazzulli T, et al. The utility of measles and rubella IgM serology in an elimination setting, Ontario, Canada, 2009-2014. PLoS One. 2017;12(8):e0181172.

61. Pinsent A, Solomon AW, Bailey RL, Bid R, Cama A, Dean D, et al. The utility of serology for elimination surveillance of trachoma. Nat Commun. 2018;9(1):5444.
62. Lau CL, Won KY, Becker L, Soares Magalhaes RJ, Fuimaono S, Melrose W, et al. Seroprevalence and spatial epidemiology of Lymphatic Filariasis in American Samoa after successful mass drug administration. PLoS Negl Trop Dis. 2014;8(11):e3297.
63. Moss DM, Priest JW, Hamlin K, Derado G, Herbein J, Petri WA, Jr., et al. Longitudinal evaluation of enteric protozoa in Haitian children by stool exam and multiplex serologic assay. Am J Trop Med Hyg. 2014;90(4):653-60.
64. Drakeley C, Cook J. Chapter 5. Potential contribution of sero-epidemiological analysis for monitoring malaria control and elimination: historical and current perspectives. Adv Parasitol. 2009;69:299-352.
65. Curtis KA, Kennedy MS, Charurat M, Nasidi A, Delaney K, Spira TJ, et al. Development and characterization of a bead-based, multiplex assay for estimation of recent HIV type 1 infection. AIDS Res Hum Retroviruses. 2012;28(2):188-97.
66. Rosado J, Pelleau S, Cockram C, Merkling SH, Nekkab N, Demeret C, et al. Multiplex assays for the identification of serological signatures of SARS-CoV-2 infection: an antibody-based diagnostic and machine learning study. Lancet Microbe. 2021;2(2):e60-e9.
67. Rogier EW, Moss DM, Mace KE, Chang M, Jean SE, Bullard SM, et al. Use of Bead-Based Serologic Assay to Evaluate Chikungunya Virus Epidemic, Haiti. Emerg Infect Dis. 2018;24(6):995-1001.
68. Scobie HM, Patel M, Martin D, Mkocha H, Njenga SM, Odiere MR, et al. Tetanus Immunity Gaps in Children 5-14 Years and Men >/= 15 Years of Age Revealed by Integrated Disease Serosurveillance in Kenya, Tanzania, and Mozambique. Am J Trop Med Hyg. 2017;96(2):415-20.
69. Poirier MJ, Moss DM, Feeser KR, Streit TG, Chang GJ, Whitney M, et al. Measuring Haitian children's exposure to chikungunya, dengue and malaria. Bull World Health Organ. 2016;94(11):817-25A.
70. Hamlin KL, Moss DM, Priest JW, Roberts J, Kubofcik J, Gass K, et al. Longitudinal monitoring of the development of antifilarial antibodies and acquisition of Wuchereria bancrofti in a highly endemic area of Haiti. PLoS Negl Trop Dis. 2012;6(12):e1941.
71. Migchelsen SJ, Martin DL, Southisombath K, Turyaguma P, Heggen A, Rubangakene PP, et al. Defining Seropositivity Thresholds for Use in Trachoma Elimination Studies. PLoS Negl Trop Dis. 2017;11(1):e0005230.
72. Arnold BF, Martin DL, Juma J, Mkocha H, Ochieng JB, Cooley GM, et al. Enteropathogen antibody dynamics and force of infection among children in low-resource settings. Elife. 2019;8.
73. Njenga SM, Kanyi HM, Arnold BF, Matendechero SH, Onsongo JK, Won KY, et al. Integrated Cross-Sectional Multiplex Serosurveillance of IgG Antibody Responses to Parasitic Diseases and Vaccines in Coastal Kenya. Am J Trop Med Hyg. 2020;102(1):164-76.
74. Arnold BF, van der Laan MJ, Hubbard AE, Steel C, Kubofcik J, Hamlin KL, et al. Measuring changes in transmission of neglected tropical diseases, malaria, and enteric pathogens from quantitative antibody levels. PLoS Negl Trop Dis. 2017;11(5):e0005616.
75. Harding-Esch EM, Brady MA, Angeles CAC, Fleming FM, Martin DL, McPherson S, et al. Lessons from the Field: Integrated survey methodologies for neglected tropical diseases. Trans R Soc Trop Med Hyg. 2021;115(2):124-6.
76. Khan SS, Smith MS, Reda D, Suffredini AF, McCoy JP, Jr. Multiplex bead array assays for detection of soluble cytokines: comparisons of sensitivity and quantitative values among kits from multiple manufacturers. Cytometry B Clin Cytom. 2004;61(1):35-9.
77. Saez-Alquezar A, Junqueira ACV, Durans ADM, Guimaraes AV, Correa JA, Provance DW, Jr., et al. Application of WHO International Biological Reference Standards to evaluate commercial serological tests for chronic Chagas disease. Mem Inst Oswaldo Cruz. 2020;115:e200214.

78. Priest JW, Jenks MH, Moss DM, Mao B, Buth S, Wannemuehler K, et al. Integration of Multiplex Bead Assays for Parasitic Diseases into a National, Population-Based Serosurvey of Women 15-39 Years of Age in Cambodia. PLoS Negl Trop Dis. 2016;10(5):e0004699.
79. The Haiti Neglected Tropical Diseases (NTDs) Control Program 2010 [Available from: https://biostat.wustl.edu/dolf/wp-content/uploads/Workplan_Year_5_Haiti.pdf#:~:text=The%20Haiti%20Neglected%20Tropical%20Disease%20%28NTD%29%20Control%20Program,as%20among%20partners%20in%20the%20US%20and%20Haiti.
80. Oscar R, Lemoine JF, Direny AN, Desir L, Beau de Rochars VE, Poirier MJ, et al. Haiti National Program for the elimination of lymphatic filariasis--a model of success in the face of adversity. PLoS Negl Trop Dis. 2014;8(7):e2915.
81. Lee SC, Ngui R, Tan TK, Muhammad Aidil R, Lim YA. Neglected tropical diseases among two indigenous subtribes in peninsular Malaysia: highlighting differences and co-infection of helminthiasis and sarcocystosis. PLoS One. 2014;9(9):e107980.
82. Novakovic D. It's time to act to beat neglected tropical diseases in Malaysia

2021 [Available from: https://www.who.int/malaysia/news/detail/30-01-2021-it-s-time-to-act-to-beat-neglected-tropical-diseases-in-malaysia.
83. Chan NW. Impacts of Disasters and Disasters Risk Management in Malaysia: The Case of Floods [Available from: https://www.eria.org/uploads/media/Research-Project-Report/RPR_FY2011_8_Chapter_14.pdf.
84. Ng YJ, Samy AL, Tan SL, Ramesh P, Hung WP, Ahmadi A, et al. Floods Amidst COVID-19 in Malaysia: Implications on the Pandemic Responses. Disaster Med Public Health Prep. 2021:1-2.
85. Won KY, Sambou S, Barry A, Robinson K, Jaye M, Sanneh B, et al. Use of Antibody Tools to Provide Serologic Evidence of Elimination of Lymphatic Filariasis in The Gambia. Am J Trop Med Hyg. 2018;98(1):15-20.
86. Organization TWH. Lymphatic Filariasis [cited 2022. Available from: https://apps.who.int/neglected_diseases/ntddata/lf/lf.html.
87. Noordin R. Lymphatic filariasis and the global elimination program. Malays J Med Sci. 2007;14(1):1-3.
88. Al-Abd NM, Nor ZM, Ahmed A, Al-Adhroey AH, Mansor M, Kassim M. Lymphatic filariasis in Peninsular Malaysia: a cross-sectional survey of the knowledge, attitudes, and practices of residents. Parasit Vectors. 2014;7:545.
89. Organization TWH. Chikungunya [cited 2022. Available from: https://www.who.int/news-room/fact-sheets/detail/chikungunya.
90. Johnson AJ, Martin DA, Karabatsos N, Roehrig JT. Detection of anti-arboviral immunoglobulin G by using a monoclonal antibody-based capture enzyme-linked immunosorbent assay. J Clin Microbiol. 2000;38(5):1827-31.
91. Azami NA, Salleh SA, Shah SA, Neoh HM, Othman Z, Zakaria SZ, et al. Emergence of chikungunya seropositivity in healthy Malaysian adults residing in outbreak-free locations: chikungunya seroprevalence results from the Malaysian Cohort. BMC Infect Dis. 2013;13:67.
92. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, et al. The global distribution and burden of dengue. Nature. 2013;496(7446):504-7.
93. Simmons CP, Farrar JJ, Nguyen v V, Wills B. Dengue. N Engl J Med. 2012;366(15):1423-32.
94. Rioth M, Beauharnais CA, Noel F, Ikizler MR, Mehta S, Zhu Y, et al. Serologic imprint of dengue virus in urban Haiti: characterization of humoral immunity to dengue in infants and young children. Am J Trop Med Hyg. 2011;84(4):630-6.

95. Salyer SJ, Ellis EM, Salomon C, Bron C, Juin S, Hemme RR, et al. Dengue virus infections among Haitian and expatriate non-governmental organization workers--Leogane and Port-au-Prince, Haiti, 2012. PLoS Negl Trop Dis. 2014;8(10):e3269.
96. Liew SM, Khoo EM, Ho BK, Lee YK, Omar M, Ayadurai V, et al. Dengue in Malaysia: Factors Associated with Dengue Mortality from a National Registry. PLoS One. 2016;11(6):e0157631.
97. Organization TWH. Trachoma [Available from: https://www.who.int/news-room/fact-sheets/detail/trachoma.
98. Organization TWH. Status of elimination of trachoma as a public health problem: Data by country [Available from: https://apps.who.int/gho/data/node.main.A1645T?lang=en.
99. Organization TWH. Yaws [Available from: https://www.who.int/news-room/fact-sheets/detail/yaws.
100. Kazadi WM, Asiedu KB, Agana N, Mitja O. Epidemiology of yaws: an update. Clin Epidemiol. 2014;6:119-28.
101. Pappas G, Roussos N, Falagas ME. Toxoplasmosis snapshots: global status of Toxoplasma gondii seroprevalence and implications for pregnancy and congenital toxoplasmosis. Int J Parasitol. 2009;39(12):1385-94.
102. Priest JW, Moss DM, Arnold BF, Hamlin K, Jones CC, Lammie PJ. Seroepidemiology of Toxoplasma in a coastal region of Haiti: multiplex bead assay detection of immunoglobulin G antibodies that recognize the SAG2A antigen. Epidemiol Infect. 2015;143(3):618-30.
103. Yahaya N. Review of toxoplasmosis in Malaysia. Southeast Asian J Trop Med Public Health. 1991;22 Suppl:102-6.
104. Bela SR, Oliveira Silva DA, Cunha-Junior JP, Pirovani CP, Chaves-Borges FA, Reis de Carvalho F, et al. Use of SAG2A recombinant Toxoplasma gondii surface antigen as a diagnostic marker for human acute toxoplasmosis: analysis of titers and avidity of IgG and IgG1 antibodies. Diagn Microbiol Infect Dis. 2008;62(3):245-54.
105. Schar F, Trostdorf U, Giardina F, Khieu V, Muth S, Marti H, et al. Strongyloides stercoralis: Global Distribution and Risk Factors. PLoS Negl Trop Dis. 2013;7(7):e2288.
106. Al-Mekhlafi HM, Nasr NA, Lim YAL, Elyana FN, Sady H, Atroosh WM, et al. Prevalence and risk factors of Strongyloides stercoralis infection among Orang Asli schoolchildren: new insights into the epidemiology, transmission and diagnosis of strongyloidiasis in Malaysia. Parasitology. 2019;146(12):1602-14.
107. Ngui R, Halim NA, Rajoo Y, Lim YA, Ambu S, Rajoo K, et al. Epidemiological Characteristics of Strongyloidiasis in Inhabitants of Indigenous Communities in Borneo Island, Malaysia. Korean J Parasitol. 2016;54(5):673-8.
108. Hooshyar H, Rostamkhani P, Arbabi M, Delavari M. Giardia lamblia infection: review of current diagnostic strategies. Gastroenterol Hepatol Bed Bench. 2019;12(1):3-12.
109. Putignani L, Menichella D. Global distribution, public health and clinical impact of the protozoan pathogen cryptosporidium. Interdiscip Perspect Infect Dis. 2010;2010.
110. Organization TWH. Cholera [Available from: https://www.who.int/news-room/fact-sheets/detail/cholera.
111. Lantagne D, Balakrish Nair G, Lanata CF, Cravioto A. The cholera outbreak in Haiti: where and how did it begin? Curr Top Microbiol Immunol. 2014;379:145-64.
112. Dolstad HA, Franke MF, Vissieres K, Jerome JG, Ternier R, Ivers LC. Factors associated with diarrheal disease among children aged 1-5 years in a cholera epidemic in rural Haiti. PLoS Negl Trop Dis. 2021;15(10):e0009726.
113. Organization PAH. Haiti reaches one-year free of Cholera [Available from: https://www3.paho.org/hq/index.php?option=com_content&view=article&id=15684:haiti-reaches-one-year-free-of-cholera&Itemid=1926&lang=en.

114. Koski-Karell V, Farmer PE, Isaac B, Campa EM, Viaud L, Namphy PC, et al. Haiti's progress in achieving its 10-year plan to eliminate cholera: hidden sickness cannot be cured. Risk Manag Healthc Policy. 2016;9:87-100.
115. Organization TWH. 1996 - Cholera in Malaysia [Available from: https://www.who.int/emergencies/disease-outbreak-news/item/1996_05_20-en.
116. Waggoner JJ, Gresh L, Mohamed-Hadley A, Ballesteros G, Davila MJ, Tellez Y, et al. Single-Reaction Multiplex Reverse Transcription PCR for Detection of Zika, Chikungunya, and Dengue Viruses. Emerg Infect Dis. 2016;22(7):1295-7.
117. Andrew A, Navien TN, Yeoh TS, Citartan M, Mangantig E, Sum MSH, et al. Diagnostic accuracy of serological tests for the diagnosis of Chikungunya virus infection: A systematic review and meta-analysis. PLoS Negl Trop Dis. 2022;16(2):e0010152.
118. Johnson BW, Russell BJ, Goodman CH. Laboratory Diagnosis of Chikungunya Virus Infections and Commercial Sources for Diagnostic Assays. J Infect Dis. 2016;214(suppl 5):S471-S4.
119. Poirier MJP, Moss DM, Feeser KR, Streit TG, Chang GJJ, Whitney M, et al. Measuring Haitian children's exposure to chikungunya, dengue and malaria. B World Health Organ. 2016;94(11):817-25.
120. Yung CF, Lee KS, Thein TL, Tan LK, Gan VC, Wong JGX, et al. Dengue serotype-specific differences in clinical manifestation, laboratory parameters and risk of severe disease in adults, singapore. Am J Trop Med Hyg. 2015;92(5):999-1005.
121. Budodo RM, Horumpende PG, Mkumbaye SI, Mmbaga BT, Mwakapuja RS, Chilongola JO. Serological evidence of exposure to Rift Valley, Dengue and Chikungunya Viruses among agropastoral communities in Manyara and Morogoro regions in Tanzania: A community survey. PLoS Negl Trop Dis. 2020;14(7):e0008061.
122. Carabali M, Lim JK, Velez DC, Trujillo A, Egurrola J, Lee KS, et al. Dengue virus serological prevalence and seroconversion rates in children and adults in Medellin, Colombia: implications for vaccine introduction. Int J Infect Dis. 2017;58:27-36.
123. Liu Q, Wang ZD, Huang SY, Zhu XQ. Diagnosis of toxoplasmosis and typing of Toxoplasma gondii. Parasit Vectors. 2015;8:292.
124. Krushna NS, Shiny C, Dharanya S, Sindhu A, Aishwarya S, Narayanan RB. Immunolocalization and serum antibody responses to Brugia malayi pepsin inhibitor homolog (Bm-33). Microbiol Immunol. 2009;53(3):173-83.
125. Moss DM, Priest JW, Boyd A, Weinkopff T, Kucerova Z, Beach MJ, et al. Multiplex bead assay for serum samples from children in Haiti enrolled in a drug study for the treatment of lymphatic filariasis. Am J Trop Med Hyg. 2011;85(2):229-37.
126. Won KY, Robinson K, Hamlin KL, Tufa J, Seespesara M, Wiegand RE, et al. Comparison of antigen and antibody responses in repeat lymphatic filariasis transmission assessment surveys in American Samoa. PLoS Negl Trop Dis. 2018;12(3):e0006347.
127. Tisch DJ, Bockarie MJ, Dimber Z, Kiniboro B, Tarongka N, Hazlett FE, et al. Mass drug administration trial to eliminate lymphatic filariasis in Papua New Guinea: changes in microfilaremia, filarial antigen, and Bm14 antibody after cessation. Am J Trop Med Hyg. 2008;78(2):289-93.
128. Djuardi Y, Jannah IF, Supali T. IgG4 antibodies against Bm14 as an evaluation tool of mass drug administration in a co-endemic area of Brugia timori and Wuchereria bancrofti. Acta Trop. 2021;227:106278.
129. Rascoe LN, Price C, Shin SH, McAuliffe I, Priest JW, Handali S. Development of Ss-NIE-1 recombinant antigen based assays for immunodiagnosis of strongyloidiasis. PLoS Negl Trop Dis. 2015;9(4):e0003694.
130. Health NIo. Recombinant NIE Antigen from Strongyloides stercoralis | Technology Transfer (nih.gov) [Available from: https://www.ott.nih.gov/bundle/tab-2669.

131. Balachandra D, Rahumatullah A, Lim TS, Mustafa FH, Ahmad H, Anuar NS, et al. A new antigen detection ELISA for the diagnosis of Strongyloides infection. Acta Trop. 2021;221:105986.
132. Fleitas PE, Floridia-Yapur N, Nieves EE, Echazu A, Vargas PA, Caro NR, et al. Strongyloides stercoralis and Trypanosoma cruzi coinfections in a highly endemic area in Argentina. PLoS Negl Trop Dis. 2022;16(2):e0010179.
133. Goodhew EB, Priest JW, Moss DM, Zhong G, Munoz B, Mkocha H, et al. CT694 and pgp3 as serological tools for monitoring trachoma programs. PLoS Negl Trop Dis. 2012;6(11):e1873.
134. Martin DL, Saboya-Diaz MI, Abashawl A, Alemayeh W, Gwyn S, Hooper PJ, et al. The use of serology for trachoma surveillance: Current status and priorities for future investigation. PLoS Negl Trop Dis. 2020;14(9):e0008316.
135. Martin DL, Bid R, Sandi F, Goodhew EB, Massae PA, Lasway A, et al. Serology for trachoma surveillance after cessation of mass drug administration. PLoS Negl Trop Dis. 2015;9(2):e0003555.
136. Cooley GM, Mitja O, Goodhew B, Pillay A, Lammie PJ, Castro A, et al. Evaluation of Multiplex-Based Antibody Testing for Use in Large-Scale Surveillance for Yaws: a Comparative Study. J Clin Microbiol. 2016;54(5):1321-5.
137. Priest JW, Moss DM, Visvesvara GS, Jones CC, Li A, Isaac-Renton JL. Multiplex assay detection of immunoglobulin G antibodies that recognize Giardia intestinalis and Cryptosporidium parvum antigens. Clin Vaccine Immunol. 2010;17(11):1695-707.
138. Hjollo T, Bratland E, Steinsland H, Radunovic M, Langeland N, Hanevik K. Longitudinal cohort study of serum antibody responses towards Giardia lamblia variant-specific surface proteins in a non-endemic area. Exp Parasitol. 2018;191:66-72.
139. Priest JW, Kwon JP, Arrowood MJ, Lammie PJ. Cloning of the immunodominant 17-kDa antigen from Cryptosporidium parvum. Mol Biochem Parasitol. 2000;106(2):261-71.
140. Leo M, Haque R, Kabir M, Roy S, Lahlou RM, Mondal D, et al. Evaluation of Entamoeba histolytica antigen and antibody point-of-care tests for the rapid diagnosis of amebiasis. J Clin Microbiol. 2006;44(12):4569-71.
141. Zambrano LD, Priest JW, Ivan E, Rusine J, Nagel C, Kirby M, et al. Use of Serologic Responses against Enteropathogens to Assess the Impact of a Point-of-Use Water Filter: A Randomized Controlled Trial in Western Province, Rwanda. Am J Trop Med Hyg. 2017;97(3):876-87.
142. Scobie HM, Patel M, Martin D, Mkocha H, Njenga SM, Odiere MR, et al. Tetanus Immunity Gaps in Children 5-14 Years and Men >= 15 Years of Age Revealed by Integrated Disease Serosurveillance in Kenya, Tanzania, and Mozambique. Am J Trop Med Hyg. 2017;96(2):415-20.
143. Scobie HM, Mao B, Buth S, Wannemuehler KA, Sorensen C, Kannarath C, et al. Tetanus Immunity among Women Aged 15 to 39 Years in Cambodia: a National Population-Based Serosurvey, 2012. Clin Vaccine Immunol. 2016;23(7):546-54.
144. Anichini G, Gandolfo C, Fabrizi S, Miceli GB, Terrosi C, Gori Savellini G, et al. Seroprevalence to Measles Virus after Vaccination or Natural Infection in an Adult Population, in Italy. Vaccines (Basel). 2020;8(1).
145. Yeung LF, Lurie P, Dayan G, Eduardo E, Britz PH, Redd SB, et al. A limited measles outbreak in a highly vaccinated US boarding school. Pediatrics. 2005;116(6):1287-91.
146. Kumar P, Jain M, Goel AK, Bhadauria S, Sharma SK, Kamboj DV, et al. A large cholera outbreak due to a new cholera toxin variant of the Vibrio cholerae O1 El Tor biotype in Orissa, Eastern India. J Med Microbiol. 2009;58(Pt 2):234-8.
147. Mishra A, Taneja N, Sharma M. Environmental and epidemiological surveillance of Vibrio cholerae in a cholera-endemic region in India with freshwater environs. J Appl Microbiol. 2012;112(1):225-37.

148. Yang P, Ma C, Shi W, Cui S, Lu G, Peng X, et al. A serological survey of antibodies to H5, H7 and H9 avian influenza viruses amongst the duck-related workers in Beijing, China. PLoS One. 2012;7(11):e50770.
149. Mon HH, Hadrill D, Brioudes A, Mon CCS, Sims L, Win HH, et al. Longitudinal Analysis of Influenza A(H5) Sero-Surveillance in Myanmar Ducks, 2006-2019. Microorganisms. 2021;9(10).

CHAPTER 2. RESEARCH AIMS AND OBJECTIVES

2.1 Research Rationale

Serological multiplex bead assays are a promising tool for integrated NTD control but their capacity in epidemiology and programmatic settings has not been thoroughly examined given their relative novelty. This research will further our knowledge of how serological MBAs could be applied to support integrated disease surveillance as part of the WHO 2021 NTD Roadmap initiates.

2.1.1 Overall Aim and Specific Objectives

The overall aim of this project is to investigate the public health utility of serological MBAs regarding integrated disease surveillance within Haiti and Malaysia using a panel of diverse antigens from 11 expanded NTDs (toxoplasmosis, LF, strongyloidiasis, chikungunya, dengue, trachoma, yaws, cholera, giardiasis, cryptosporidiosis, and amoebiasis, as described in the introduction) and 2 VPDs (tetanus and measles).

Objective 1: Investigate methods used for characterizing seropositivity according to the MFI antibody responses, and any associated implications on the corresponding estimates of seroprevalence in Haiti and Malaysia.

Measure outcome: Examine literature for existing methods and their application for different antigens and the associated seroprevalence estimated according to different approaches.

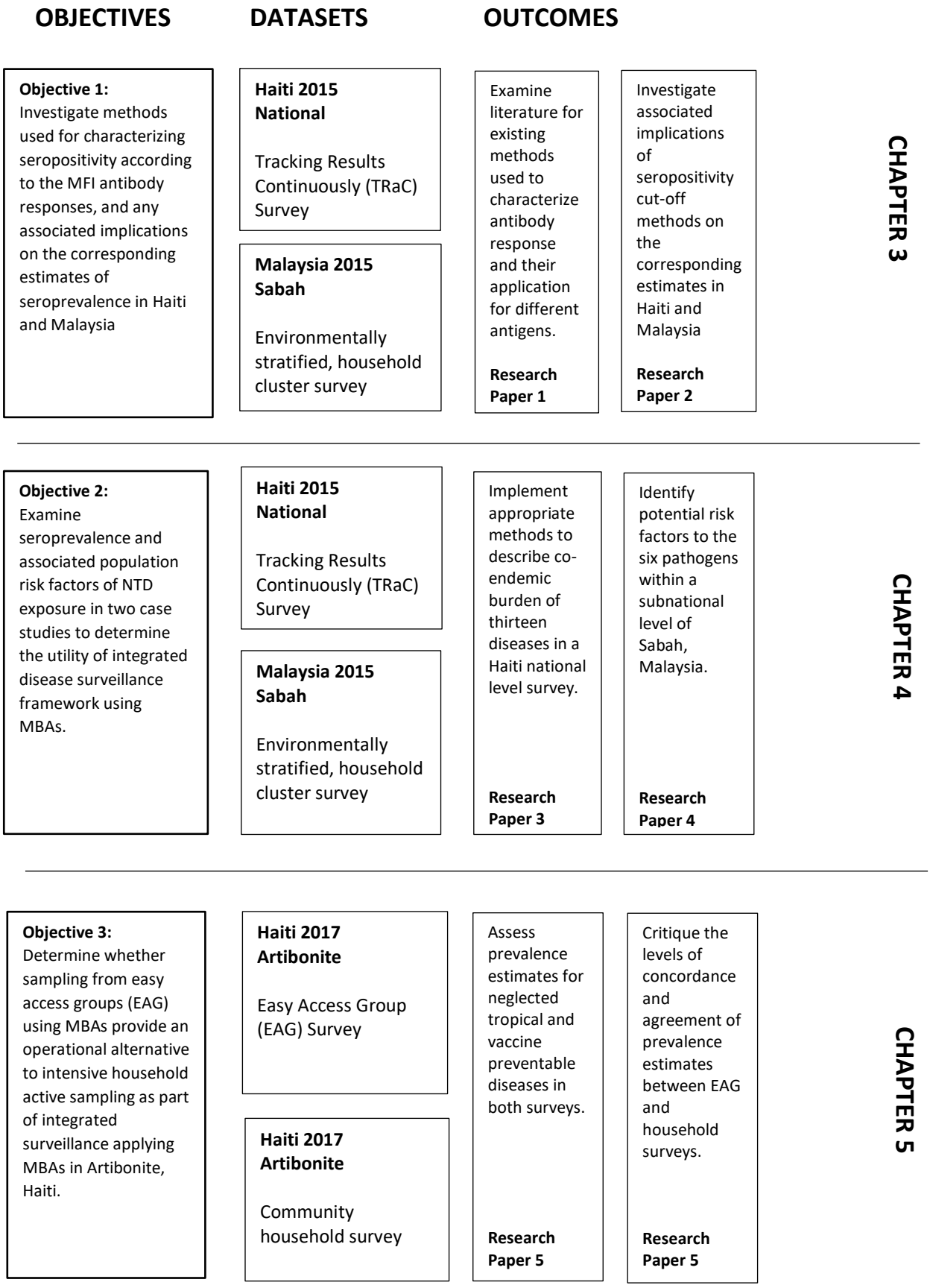
Objective 2: Examine seroprevalence and associated population risk factors of NTD exposure in two case studies to determine the utility of an integrated disease surveillance framework using MBAs.

Measured outcome: Identify potential, associated risk factors to the different diseases within two distinct surveys in Haiti and Malaysia.

Objective 3: Determine whether sampling from easy access groups (EAG) using MBAs provide an operational alternative to intensive household active sampling as part of integrated surveillance applying MBAs in Artibonite, Haiti.

Measured outcome: Assess prevalence estimates concordance between EAG and household surveys.

2.2 Research Roadmap with objectives, datasets, and targeted outcomes



2.3 Thesis Structure and Statement of Contributions

This thesis follows a Research Paper thesis guidelines, with one of the papers published, two of the papers under peer review, and two other papers prepared to a state ready for submission.

Chapter 1 provides a background on NTDs, including their global health importance, current public health targets set forth by the WHO, and existing challenges to NTD control. Specific emphasis is placed on the importance of surveillance in NTDs control and current obstacles to achieving public targets. The use of serological tools and MBAs in NTD surveillance are included in the latter portion of this chapter. I wrote all contents for this chapter.

Chapter 2 describes rationale, research aims and objectives of the thesis. A research roadmap detailing the datasets and methods applied to achieve these objectives are included, with an indication of which objective outcomes are included in which research paper. This was conceptualized together with my supervisors.

Chapter 3 addresses **Objective 1** and comprises of two research papers. The overall theme of this chapter is to determine appropriate methods of characterizing MFI response to aid program interpretation. The first paper comprehensively examines the literature to describe the current public health applications of serological MBAs to neglected tropical diseases and the methods used to characterize MFI responses (Paper 1, published). I reviewed the literature, examined the methods, and wrote the manuscript. The second paper applies three different cut-off approaches identified in literature to two retrospective datasets from Haiti (2015) and Malaysia (2015) to evaluate any public health implications based on cut-off approach choice (Paper 2, peer-reviewed). I performed the analysis and wrote the manuscript.

Chapter 4 addresses **Objective 2** and comprises of two research papers**.** The theme of this chapter is to assess the utility of integrated diseases surveillance using MBAs. The first paper assesses seroprevlance and associated age risk factors from a multi-disease panel for thirteen pathogens with MBAs in Haiti at a national level (Paper 3, prepared for journal submission). I performed the analysis for determining seropositivity, generated descriptive statistics, assessed correlation and wrote the manuscript. Eric Rogier performed the modeling analysis of seroprevalence with age. The second paper employs demongraphic and georgraphical data collected during a serological survey to describe population risk

factor to five different pathogens (Sabah, Malaysia), simultaneously. (Paper 4, prepared for journal submission). I determined seropositivity, assessed potential risk factors, and wrote the manuscript. Kim Fornace analysed the spatial components of this work.

Chapter 5 address **Objective 3.** This chapter examines the operational potential of MBAs to support EAG sampling and integrated disease monitoring of NTDs and VPDs compared to an intensive, active houshold sampling using MBAs (Paper 5, prepared for journal submission). I performed the analysis of concordance and wrote the manuscript.

Chapter 6 is the final chapter and provides an overall summary of the work and includes recommendations for implementation, practice, and research.

CHAPTER 3. INTEPRETATING SEROLOGICAL DATA FROM MULTIPLEX BEAD ASSAYS

3.1 Background and Rationale

Research Rationale

Serological MBAs can support integrated disease surveillance (1), but a current challenge remains in the interpretation of numerical output (in MFI) from MBAs, which are proportional to antibody concentrations to target antigens within mucosal or serum samples (2). While immunological methods such as ELISAs, LFTs, and Agglutination PCRs may have established standard methods to interpreting antibody output data for surveillance (3-6), MBAs at present lack concrete guidelines to programmatically interpret MFI values, as they have only recently been introduced to the field of NTD surveillance. MFI values may be used to advise program decision-making when properly analysed (7), however, different options to interpreting MFI may lead to inconsistent interpretation and subsequent decision-making.

A frequent approach to interpreting MFI values involves dichotomizing MFI values into different seropositive and seronegative states, which reflects exposure to infection, a value above which can be associated with recent infection, or lack thereof. While there are drawbacks to converting continuous data into a binary outcome, seroprevalence helps to facilitate easier program interpretation of antibody data (7, 8). However, the diversity of statistical methods to determine seroprevalence can complicate a standard approach to interpreting MFI values. Additionally, specific methods may be more appropriate for certain antigens and organisms, given their transmission biology. For serological tools to provide actionable information for programmatic decision-making, the first step is to determine suitable methods of how to translate MFI values into seroprevalence, which takes into consideration program goals, biological processes, and transmission intensity of diseases. For example, trachoma programs

using serology in elimination settings to determine interruption of transmission set appropriate thresholds to identify positivity (9), while vaccine may use seropositive thresholds to define seroprotection (10).

Overview

This chapter focuses on reviewing methods of characterizing MFI responses to establish standard approaches to determine seroprevalence and targets (**Objective 1)**. It comprises of two research papers. Research Paper 1 examines the literature to assess current public health applications of serological MBAs. Specific goals of this review were to 1) identify the different methods of characterising MFI values, 2) describe the public health settings where they were applied), and 3) conclude with a discussion of the advantages and limitations of each method with context to programmatic utility.

Research Paper 2 investigates potential implications on public health decision making given the variety of different methods currently employed in literature for categorizing MFI data. Selected methods applied in literature are concurrently evaluated using two datasets from Haiti and Malaysia. Specific goals of this analysis were to 1) evaluate any similarities and differences in prevalence estimates between cut-off methods in both settings and 2) assess any potential programmatic implications based on method choice.

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT

T: +44 (0)20 7299 4646
F: +44 (0)20 7299 4656
www.lshtm.ac.uk

RESEARCH PAPER COVER SHEET

Please note that a cover sheet must be completed for each research paper included within a thesis.

SECTION A – Student Details

Student ID Number	1603698	**Title**	Mr
First Name(s)	YuYen		
Surname/Family Name	Chan		
Thesis Title	Application of multiplex bead serological assays to integrated monitoring of neglected tropical disease		
Primary Supervisor	Chris Drakeley		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?	PLoS NTD		
When was the work published?	2021		
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion	N/A		
Have you retained the copyright for the work?*	Choose an item.	Was the work subject to academic peer review?	**Yes**

*If yes, please attach evidence of retention. If no, or if the work is being included in its published format, please attach evidence of permission from the copyright holder (publisher or other author) to include this work.

SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	
Please list the paper's authors in the intended authorship order:	
Stage of publication	Choose an item.

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	Writing and drafting paper, review of methods

SECTION E

Student Signature	YuYen Chan
Date	16/09/2021

Supervisor Signature	Chris Drakeley
Date	20/09/2021

3.2 Cut-off approaches to determining seroprevalence using median fluorescence intensities

Determining Seropositivity - A Review of Approaches to Define Population Seroprevalence when using Multiplex Bead Assays to Assess Burden of Tropical Diseases.

Authors and affiliations: YuYen Chan[1]*, Kimberly Fornace[2], Lindsey Wu[1], Ben F. Arnold[3], Jeffrey W. Priest[4], Diana L. Martin[5], Michelle A. Chang[5], Jackie Cook[6], Gillian Stresman[1], Chris Drakeley [1]

1. Department of Infection Biology, London School of Hygiene and Tropical Medicine, London, United Kingdom
2. Department of Disease Control, London School of Hygiene and Tropical Medicine, London, United Kingdom
3. Francis I. Proctor Foundation, University of California, San Francisco, San Francisco, California, United States of America
4. Division of Foodborne, Waterborne, and Environmental Diseases, United States Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America
5. Division of Parasitic Diseases and Malaria, United States Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America
6. MRC International Statistics and Epidemiology Group, Department of Infectious Disease Epidemiology, London School of Hygiene and Tropical Medicine, London, United Kingdom

yuyen.chan@lshtm.ac.uk

Abstract

Background: Serological surveys with multiplex bead assays can be used to assess seroprevalence to multiple pathogens simultaneously. However, multiple methods have been used to generate cut-off values for seropositivity and these may lead to inconsistent interpretation of results. A literature review was conducted to describe the methods used to determine cut-off values for data generated by multiplex bead assays.

Methodology/Principal Findings: A search was conducted in PubMed that that included articles published from January 2010 to January 2020, and 308 relevant articles were identified that included

the terms “serology”, “cut-offs”, and “multiplex bead assays”. After application of exclusion of articles not relevant to neglected tropical diseases (NTD), vaccine preventable diseases (VPD), or malaria, 55 articles were examined based on their relevance to NTD or VPD. The most frequently applied approaches to determine seropositivity included the use of presumed unexposed populations, mixture models, receiver operating curves (ROC), and international standards. Other methods included the use of quantiles, pre-exposed endemic cohorts, and visual inflection points.

Conclusions/Significance: For disease control programmes, seropositivity is a practical and easily interpretable health metric but determining appropriate cut-offs for positivity can be challenging. Considerations for optimal cut-off approaches should include factors such as methods recommended by previous research, transmission dynamics, and the immunological backgrounds of the population. In the absence of international standards for estimating seropositivity in a population, the use of consistent methods that align with individual disease epidemiological data will improve comparability between settings and enable the assessment of changes over time.

Author Summary

Serological surveys can provide information regarding population-level disease exposure by assessing immune responses created during infection. Multiplex bead assays (MBAs) allow for an integrated serological platform to monitor antibody responses to multiple pathogens concurrently. As programs adopt integrated disease control strategies, MBAs are especially advantageous since many of these diseases may be present in the same population and antibodies against all pathogens of interest can be detected simultaneously from a single blood sample. Interpreting serological data in a programmatic context typically involves classifying individuals as seronegative or seropositive using a ‘cut-off’, whereby anyone with a response above the defined threshold is considered to be seropositive. Although studies increasingly test blood samples with MBAs, published studies have applied different methods of determining seropositivity cut-offs, making results difficult to compare across settings and over time. The lack of harmonized methods for defining seropositivity is due to the absence of international standards, pathogen biology, or assay-specific methods that may impact resulting data. This review highlights the need for a standardized approach for which cut-off methods to use per pathogen when applied to integrated disease surveillance using platforms such as MBAs.

Introduction

Neglected tropical diseases (NTDs) and vaccine preventable diseases (VPDs) cause a significant burden on populations in developing countries, and effective surveillance plays an important role in the control and elimination of these diseases. Despite the geographical overlap of co-endemic tropical infections in many regions of the world, surveillance efforts have often focused on separate diseases [1]. Integrated approaches to controlling tropical diseases have been implemented in some programmatic settings. However, asymptomatic infections, poor health seeking behaviour, long latency periods, and inconsistent reporting of cases make effective monitoring difficult when relying on passive case detection alone [2].

Serological surveys can be highly informative when assessing the prevalence of diseases or vaccine coverage within a population [3], since antibodies can be used to detect asymptomatic infection and historical exposure to natural infection or a vaccine [1, 4]. As integrated approaches to the management of NTDs are being adopted, multiplex bead assays (MBAs) provide a platform to monitor exposure to multiple pathogens from a single blood sample [5]. MBAs typically measure antibody response in median fluorescence intensity (MFI), which is proportional to the levels of antigen-specific antibodies (most commonly IgG) in the blood [6]. Cross-sectional and longitudinal serological data have been used in various public health settings, including evaluating mass drug administration (MDA) campaigns[7, 8], assessing changes in population level exposure [9], monitoring transmission patterns [10, 11], assessing the impact of vaccine program coverage [12], and determining prevalence thresholds for confirming disease elimination [7, 13].

While serological surveys using MBA provide efficient and cost-effective benefits to integrated pathogen monitoring, a challenge remains in data interpretation. In analysis, the MFI values are often used to estimate the seroprevalence to a particular antigen through calculated or arbitrary cut-off values to define seronegative and seropositive populations. In some cases, higher MFI values are assumed likely to represent more recent or repeated exposure [14, 15]. Prior knowledge of specific antibody titres and associated kinetics would be helpful in more accurately interpreting the data. For this review we consider 'seropositive' as a general term that could represent either current or previous infection or vaccination, without interpretation pertaining to specific antibody kinetics or longevity.

The use of a binary seropositivity endpoint allows translation of continuous assay-specific MFI values into a common epidemiologic metric: seroprevalence. Different approaches have been used to define a

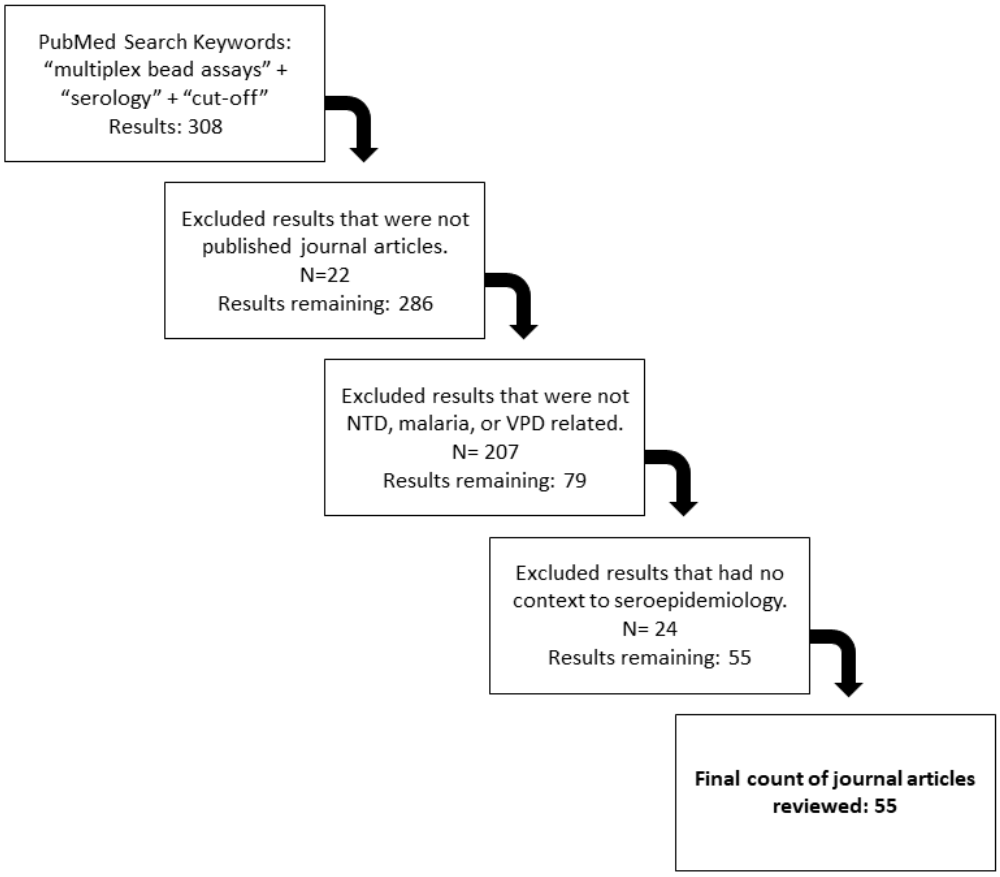
seropositive response, though the rationale or implication of the method choice is rarely made clear. The choice of approaches used are likely the result of a standard laboratory approach, adopting methods applied in previous studies, or simply ease of use. Antibody responses to different pathogens are intrinsically diverse, making it plausible that specific cut-off methods are better suited for specific antigens or situations. Understanding current approaches used for determining seropositivity is a crucial step in developing standardised methods, ensuring appropriate interpretation of the data to support more robust programmatic decision-making. To address this evidence gap, a literature review was performed of existing methods for determining cut-off values for the assessment of seroprevalence for NTDs and VPDs with MBA.

Methods

Review of literature

We conducted a literature review on PubMed for articles published between January 1, 2010 and January 31, 2020. Search terms included “multiplex bead assays” + “serology” + “cut-off”. Studies were excluded if they were not in a published journal (e.g. clinical case reports or conference abstracts), published prior to 2010, did not include serological targets for NTDs, malaria or VPDs, or used serological tools specifically for clinical diagnosis. In total, the initial search identified 308 articles of which 253 articles were not included based on title and abstract. Fifty-five articles met the inclusion criteria for full screening which included serology, NTDs (as defined by the World Health Organization and/or PloS NTD lists)[16], malaria, VPDs in tropical regions, and the use of MBA (Fig 1). Articles were then selected if they described cut-off methods using data based on quantitative antibody levels from MBA platform for

the application to seroepidemiology.



F3. 1 **Fig 1. Flowchart of article selection for inclusion in literature review.**

The PubMed search identified 308 articles with 253 being excluded because they did not meet the inclusion criteria. After review, 55 articles were retained for analysis.

Results

Literature Review –Applied Methods of Determining Seropositivity

Eight cut-off approaches were identified based on literature reviewed, with seven methods being applied that provided valid cut-off values (Table 1). A list of all the articles reviewed using each method, antigens within the study, and population origins are listed in S1 Table. Examples of applied methods in different public health settings (Table 1) and the advantages and disadvantages of the different methods (Table 2) are described below.

T3. 1 **Table 1. Examples of several applied cut-off approaches using MBAs in various settings for NTDs and VPDs.**

MBA used, location, cut-off approach, and the goal of the program are provided in this table to demonstrate the application of cut-off methods in different settings. Where, N refers to the number of studies employing the method and SD refers to standard deviations.

Disease	***Location***	***Additional cut-off details***	***Goal (study Ref)***
Presumed unexposed (N=23)			
Dengue	Haiti	United States/mean +3SD	Disease surveillance (69)
Lymphatic Filariasis	Mali Haiti		Disease recrudescence (97) Disease monitoring after MDA (70)
Trachoma	Haiti		Examining MBA as a monitoring tool (85)
Amoebiasis	Haiti		Disease dissemination (63)
Leishmania	Kenya	Japan/ mean +3SD	Application of multiplex assays (81)
Receiver Operating Curves (N=15)			
Yaws	Ghana	Clinically confirmed negatives and positives.	Evaluate antibody response in MBA (88)
Measles	Kenya	Gold standard lab technique confirmation.	Assess schistosomiasis impact on vaccine preventable diseases (98)
Strongyloidiasis	Cambodia	Presumed unexposed population used for ROC curve	Application of an integrated, multiple disease survey (99)
Mixture Models (N=14)			
Lymphatic Filariasis	Kenya	Mean of negative component + 3SD	Validation of MBA to lymphatic filariasis (81)
Chikungunya	Haiti	Mean of negative component + 2SD	Estimate exposure (100)
International Standards (N=6)			
Tetanus	Cambodia Tanzania	>100 MFI units = 0.01 IU/ml = seroprotective	Monitor progress of elimination (94) Assess immunity gaps (68)
Quantile (N=1)			
Influenza	Vietnam	No distinct cut-off, use of antibody titres	Estimating population-level antibodies (101)
Visual Inflection Point (N=1)			
Trachoma	Laos Uganda Gambia	Impartial (independent) individuals to determine cut-off	Defining seropositivity thresholds for elimination programs (71)
Pre-exposed Endemic Cohort (N=1)			
Giardiasis Cryptosporidiosis Amoebiasis Salmonellosis E. coli	Haiti Kenya Tanzania	Longitudinal cohort	Understanding force of transmission among children through seroconversion rates (102)

Norovirus Cholera Campylobacteriosis			

Presumed unexposed. Cut-off values can be determined by a population that has no expected exposure to the pathogen of interest. Depending on the pathogen, these populations are typically selected based on self-reported claims of no travel or no recent travel history to endemic countries. For NTDs, seronegative populations have been chosen from non-endemic regions, including the United States, Sweden, and Japan [6, 21, 23, 29]. When applying presumed unexposed populations to define cut-offs, a pre-specified number of standard deviations (usually three) above the mean of the MFI values with background subtracted (MFI-bg) in the presumed unexposed population are used. Any result above that MFI-bg value is considered as seropositive, and the number of standard deviations used may depend on stringency of identifying seropositives. Use of presumed unexposed populations to determine cut-off values provides a viable option where a large majority of the study population is exposed due to high transmission. In such settings, an endemic seronegative population, as required by other commonly used approaches described below, may be difficult to identify due to small numbers.

However, there are several potential sources of bias to using a presumed unexposed population to derive seropositivity. Cut-off values from presumed unexposed populations run the risk of bias as the immunological exposure of the populations being compared may not accurately represent the immunological history of the sample population. This could be due to factors such as genetic differences affecting immune responses, age differences between presumed unexposed and study population, nutritional status, and/or co-infections of multiple diseases [30-32]. As a result, cut-off values defined by presumed unexposed populations may be artificially low, leading to inflated prevalence estimates. Moreover, differentiating between active and historical infections may not be captured by an overly sensitive cut-off [21]. Conversely, while presumed unexposed populations by definition have no exposure to the infection being monitored, some individuals may have had unknown contact with the pathogen of interest or cross-reactive pathogens that may generate elevated cut-off values if not excluded.

Receiver Operating Characteristic (ROC) Curve. ROC curves can be used to generate cut-offs by plotting the true positive rate (sensitivity) against the false positive rate (specificity) [33]. The optimal

cut-off is considered the value that provides the best discrimination between the true seronegative and seropositive populations, or a cut-off that gives equal weight to sensitivity and specificity [34]. Studies have considered presumed unexposed populations, as defined above, as the true negative population, while true positive populations have been considered as those being either a clinically confirmed case or according to established laboratory gold standards for that pathogen [4, 8].

A method with perfect discrimination creates an ideal cut-off between the two populations with no overlap [35] , however, it can be rare to observe such separation in the general population (Table 2). Accurate ROC curves to define cut-offs rely on the availability of true negative and true positive reference-populations which are seldom available in practice. Additionally, the reference population used to delineate true positive/negative individuals may also bias results, similar to the disadvantages mentioned for presumed unexposed populations [36].

Finite Mixture Model. A mixture model is a probabilistic model that assumes the presence of at least two normally distributed subpopulations, or components, within the sample population [37]. These components represent underlying populations of varying antibody responses [21, 38, 39]. The negative population are assumed those within the lowest distribution of MFI values. The cut-off value can be determined using estimated parameters (i.e. mean and standard deviation) of the lowest component specified by the mixture model [40]. Commonly, the cut-off value is then calculated similar to the presumed unexposed approach, using the mean plus a pre-defined number of standard deviations [21, 36, 41]. Alternatively, mixture models can use joint probabilities of classifying individuals with specific antibody levels as either seropositive or seronegative to specify appropriate cut-off values [39] .

Mixture models can, theoretically, provide cut-off values that more closely resemble the target population immunity with a distribution of MFI values representing seronegative individuals within the target population. This is advantageous because baseline seronegative antibody concentrations have been shown to differ between populations due to transmission history of the pathogen of interest, circulating co-infections, and any population-specific genetic factors [21]. Additionally, multiple component mixture models have the potential to identify exposure history. For example, in a mixture model with three components, the lowest component could be considered as seronegative, the middle component as an “indefinite/borderline” or past exposure history group, and the highest component as seropositive or recent exposure group [21, 42]. However, the choice of how many components is also a practical challenge for different pathogens and may rely on understanding antigen-specific immunological response. Mixture models can also be fitted to different distributions depending on the

pattern of responses of the pathogen of interest, such as in the case of VPDs and distinguishing between stronger antibody responses in naturally infected compared to vaccinated individuals [43, 44].

Mixture models may not be appropriate in areas of high transmission or very low transmission [11]. When only one component is observed (e.g. everyone is exposed or unexposed) or when components have significant overlap (e.g. population with large portion of historical exposure or have received treatment), it becomes difficult to identify a reliable cut-off and classify individuals as seronegative or seropositive based on probabilities [21]. Moreover, choice of distribution for fitting mixture model and resulting cut-offs may be rejected if they do not agree with components upon visual inspection and investigator judgment. Co-circulating pathogens that may result in cross-reactivity of antibody response to the antigens being assayed can also be difficult to separate using mixture models [45].

International Standards and Units. International Standards or International Reference Materials of the World Health Organization (WHO) are used as a simple method for a uniform classification system. This allows comparison of biological targets, such as vaccine induced antibodies, across populations using pre-set cut-off values [46]. This approach requires standard reagents to generate an assay-specific standard cut-off for each of the different antigenic targets which can then be applied consistently across all settings. The main advantage includes the facilitation of between-setting comparisons. However, occasional pre-set international standard cut-off values have been found to overestimate the size of the seronegative population [47] or to classify individuals to incorrect serostatus groups [48]. This could be related to the fact that international standards are decided *a priori* and without context to the populations of interest. Therefore, any potential biases when applying the standard due to population specific genetics are not accounted for, unless they were developed using populations from all endemic countries. Moreover, the international standards may have been developed for specific applications, such as providing a clinical endpoint, and may be less suitable in a seroepidemiological context [37]. For example, international standards for rubella have been found occasionally to overlook potential immunity, due to high cut-offs set by manufacturer assays to avoid false negatives [37] .

Quantiles. Cut-offs can be determined through rank statistics that partition MFI values into quantiles of equal probabilities. Quantiles have been used outside the context of NTDs, such as understanding viral loads in influenza [26]. Theoretically, higher quantiles could be interpreted as seropositive, while lower quantiles would be interpreted as seronegative. The partitions of quantiles may furthermore represent different levels of seropositivity, such as populations of non-exposure, of historical exposure, repeated exposure resulting in 'boosting' of antibodies, or populations of active or recent infections. Quantiles require the analyst to subjectively, or based on biological and/or clinical knowledge, choose the number of quantiles for the analysis and then to specify which quantiles are seropositive or seronegative. Additionally, exceptionally high or low outlier antibody responses may create biased cut-offs that lead to misclassification [49].

Visual Inflection Point (VIP). A single study looked at using crude cut-offs determined by visually examining inflection points within MFI distributions in graphs. Migchelsen et al., in exploring options for determining trachoma cut-offs, did a convenience sample of impartial individuals to visually inspect data curves to determine an inflection point [27]. The final cut-off was considered to be the average of values reported by the participants. The mean reported cut-off values were similar to cut-offs from the mixtures models as applied to the same dataset [27]. Moreover, the process is more straightforward and intuitive compared to the mixture model approach.

Use of VIP relies on pattern recognition to subjectively generate cut off values, and inflection points may be biased based on groups of individuals asked. In addition, VIP should ideally use impartial participants and mask antigens to reduce bias. Sampling more individuals to determine the inflection point may improve the precision of the estimates of VIP, but recruiting a large number of participants can be time-consuming and challenging in certain situations. With this method there are problems with reproducibility, accuracy is likely associated with the degree of separation between the negative and positive distributions.

Pre-exposed endemic cohort. While serological assays are frequently cross-sectional, longitudinal surveys that have obtained serological data before and after infection can create a cut-off based on the change of MFI values before compared to after exposure. Arnold et al. have explored this cut-off method (termed "presumed unexposed" within their study) for enteric pathogen antibody responses

among children from Kenya and Haiti [28]. The resulting cut-offs were comparable to both mixture models and presumed unexposed referent populations, but this method also enabled estimation of cut-offs for particularly high-transmission pathogens where other methods failed. In high transmission settings, fitting mixture models can be challenging in the case where distinct components are not present (see mixture model section above), while cut-offs of presumed unexposed may not reflect immunological background of study population (see presumed unexposed section). A negative population to use for cut-off determination was generated from MFI values of <1-year-olds who later seroconverted (based on a conservative +2 increase on a $\log_{10}$ scale or a 100-fold increase in MFI). The cut-off was determined by taking the mean of the distribution of measurements before these <1-year-old children seroconverted and then adding three standard deviations.

Identifying a pre-exposed endemic cohort population using measurements from individuals who subsequently seroconvert may be useful for longitudinal studies that have collected data on individuals prior to a point change to seroconversion or infection status. However, using MFI values of unexposed infants may not represent the true seronegative MFI values in the adult population due to inherent differences in the immature and mature immune systems. Maternal antibodies may also be present in infants, leading to potentially higher responses in infants that reflects the exposure history of the mother not of the child. The choice of antibody level increase required to identify "pre-exposed endemic cohort" is a qualitative decision, and so accompanying sensitivity analyses of alternate increases could prove useful [28]. Additionally, longitudinal monitoring may not be logistically feasible for many surveillance programs administering cross-sectional surveys.

T3. 2 **Table 2. Summary of Advantages and Disadvantages of different seropositivity cut-off methods.**

Cut-off Method	Advantages	Disadvantages
Unexposed or presumed unexposed population	- Known seronegative population - Can be used with other classification methods that require a true seronegative population	- Cut-offs may not reflect true immunity of target population, leading to potential misclassification - Requires obtaining a presumed unexposed population - Only appropriate for certain diseases which are absent in the population from where negatives are selected - Potential for cross reactivity
Mixture Model	-Generates cut-off using statistical modelling without external samples needed	- May not be appropriate in very high or very low transmission settings

	-Determines an endemic, seronegative population within sample	- Possibility of an indeterminate range of overlapping seronegative and seropositive individuals
ROC Curve	- Robust cut-off generated from true positives and true negatives	- Often requires "gold standard" confirmation of positive and negatives
International Standards	- Provided by WHO - Universal method of categorizing seropositivity to enable standardization across assays and laboratories	-Fixed cut-off values may not accurately capture differences in natural and vaccinated responses due to its diagnostic purpose. -Not available for many NTDs.
Quantiles	-Visual distribution of MFI intensities and allows for comparison of means	-Choice of which quantiles to use that accurately reflects serostatus must be determined by investigator
Visual Inflection Point	-Simple method	-Arbitrarily decided by investigator -May need a statistical method to confirm - Potential for poor reproducibility across settings
Pre-exposed endemic cohort	-Provides a presumed seronegative population from the population of interest	-Requires longitudinal data and following individuals who were disease free and later developed disease. -Using MFI values of children may not accurately represent MFI values in adults

Discussion

As programs implement integrated approaches to controlling infectious diseases, effective monitoring is crucial. Serological MBAs provide a convenient method for understanding the population-level burden for multiple diseases simultaneously [50]. This is particularly relevant for those pathogens with long latency periods or with symptoms not sufficiently acute to prompt care-seeking. MBAs can also generate data at a comparatively low cost [1], making it an efficient tool for integrated surveillance of tropical and vaccine preventable diseases. Assessing disease burden through seropositivity is valuable and a more programmatically interpretable metric compared to the continuous MFI values. Additionally, assay and differences in bead coupling concentrations or methods between studies will lead to variability in overall magnitude of antibody levels measured, making the direct comparisons of MFI values almost impossible without appropriate assay standards or a standard metric, such as seropositivity. However, use of seropositivity requires careful consideration of how to define appropriate cut-off values that can meaningfully identify exposed individuals and those with disease burden according to public health programmatic guidelines.

This review highlights several approaches for determining seropositivity cut-offs. The most frequently used approaches were presumed-negative populations, ROC curves, mixture models, and international standards. Other approaches included quantiles, pre-exposed endemic cohort, and visual inflection points. Each method has its respective advantages and disadvantages. For all methods that rely on external samples, such as presumed unexposed population or ROC curve, it is important to acknowledge that antigen-bead coupling efficacy may differ between bead batches and, if not run on the same bead set, potential differences in cut-off values may be observed. In addition, instrumentation differences may impact the stability of the cut-off values. Under these circumstances, additional adjustments to the MFI values may be required for appropriate comparisons. Additional factors important to consider in identifying the most appropriate method for any given context include: the availability of confirmed seronegative and seropositive populations that are necessary for methods such as ROC and presumed unexposed; use-case scenarios based on program targets or goals; transmission intensity factors that impact the seronegative and seropositive distributions for methods that assume sub-populations; methods previously used in similar settings and diseases; and complexities in certain pathogen-host immunobiology that queries the suitability of strict cut-offs (Box 1).

As more programs implement serological surveillance strategies for neglected tropical disease monitoring, it is possible that new cut-off methods will be developed and applied. Alternatively, other classification methods without a distinct cut-off, such as K-means clustering, aims to separate high dimensional data (i.e. multiple antigenic targets for the same pathogen) into different clusters of MFI values to represent seronegative and seropositive states could be implemented [51, 52]. Use of multiple target antigens will increase the likelihood of detecting previous exposure to infection as well as reducing the likelihood of non-reactivity due to sequence variation in single antigenic targets and differential immunogenicity. However, in multi-disease panels, antigens need to be well-defined in order to avoid potential cross-reactivity that could lead to issues of inaccurate or false results due non-specific binding [53]. Furthermore, heterogeneity of individual responses that influence antibody levels apart from pathogen exposure, e.g. nutrition or health conditions, can cause increased immunoglobulin in sera, such as hypergammaglobulinemia [54]. Refining statistical techniques that allow assessment of multiple and/or combinations to generate seroprevalence will also be of benefit and aid in interpretation of data [55, 56].

Within our review, there are several limitations. Our search criteria targeted serological cut-offs according to WHO and PLOS definition of NTDs and VPDs, specifically in PubMed and in English. However, there may have other methods to determine serological cut-offs for diseases were not included in this review from other databases and also outside the specific timeframe we examined. Additionally, the search criteria focused only on the term “cut-off”, which may have overlooked similar terminology, such as “threshold” or “inflection point” that could have provided additional cut-off approaches. Our study also reviewed cut-offs primarily from MBA and enzyme-linked immunosorbent assay (ELISA) platforms due to our search criteria and did not include other serological or commercial immunoassays that may have used other approaches. However, any additional methods that we could have identified are unlikely to change the conclusions of this work.

International standards based on a large sample of reference standard sera from individuals in known elimination settings will be needed to define universal cut-offs and make program decisions based on specific levels of seropositivity. This would require procuring sera from clinically confirmed individuals with infection and those without infection from a geographically representative number of endemic countries to ensure sufficient diversity of immunological responses, as were recently done for human African trypanosomiasis [57, 58]. Sera from these candidates would then be characterized by different immunological tools to determine consistent measurements of immunological activity (with context to programmatic use) across all platforms in the form of international units. In the absence of these metrics for NTDS, ROC curves with confirmed positives and negatives from the study population are recommended as they would likely generate the most representative cut-offs that consider immunological and genetic backgrounds of the population. Without control sera mixture models are recommended as they may provide statistically robust cut-offs when adjusting for transmission intensity by using appropriate distributions and number of components to identify seropositives. In the context of integrated disease surveillance, the recommendation for an appropriate cut-off method to determine seroprevalence should additionally consider the antigen being assessed, the optimal data that is the closest reflection of true population prevalence, and other important factors and complexities that could impact decision of cut-off method listed in Box 1.

Box 1: Summary of factors to consider and complexities in choosing cut-off methods

Availability of Seropositive and Seronegative Populations

The availability of expected true seropositive and seronegative populations through screening, clinical confirmation, populations from countries without transmission, or gold standard laboratory techniques justifies the use of presumed unexposed approach, ROC curves, and other supervised classification methods. Additionally, if there are large differences in endemicity within a country, populations from low or non-transmission areas could serve as a seronegative population. Having the presence of seropositive and seronegative populations does not the exclude using other methods, however. Additionally, precision, quality, and interpretation of cut-off values are impacted by a variety factors that should be taken into consideration along with the method of determining cut-offs.

Large sample sizes

As with many statistical methods, larger sample sizes allow for a better estimation of the target population, improving both sensitivity and specificity. Additionally, certain cut-off methods, such as mixture models, can be achieved with larger sample sizes. Smaller sample sizes may require fitting different distributions [56].

Use-case scenarios

Cut off methods can be chosen depending on the goal and design of the study or the program. For example, cut-off methods such as ROC with high sensitivity or specificity may be preferred in the case of assessing program coverage [11, 19]. Cut-off methods such as quantiles or mixture models with several components that can identify multiple levels of seropositivity may be chosen when trying to understand geographic transmission patterns.

Literature past precedent or international guidelines

Decision to use a certain method could be influenced by or borrowed from other studies that focus on biologically similar diseases. This also includes international guidelines that provide cut-offs for vaccine preventable diseases. This consideration offers a simple and convenient rationale to choosing a certain cut-off method given that the cut-off has already been established. Making comparisons between studies using similar antigens may also determine the use of a certain cut-off method.

Transmission Dynamics

The justification of using certain cut-off methods may depend on the level of transmission of the pathogens. Mixture models and quantiles are more appropriate in transmission areas where the seropositive and seronegative components have some separation evident in the MFI distributions.

Complexity of the immunology of host-pathogens interactions

The use of statistical methods is an attempt to reflect a biological process in terms of exposure or lack of exposure. While statistical methods for cut-offs are important in determining seropositivity, weight should also be placed on understanding the complex immunology of the NTD of interest and the immunological background of the population. Incomplete understanding of serologic response and other immune mechanisms against pathogens of interest may impact interpretation of prevalence estimates generated from cut-offs [47]. For example, population level antibodies due to partial or waning immunity could make it difficult to define a strict cut-off value for seropositive and seronegative groups [11]. It may also be unclear whether responses observed during a chronic infection ever revert to a seronegative state [35]. Therefore, using an indeterminate range or comparing the mean MFIs in these circumstances maybe more appropriate than enforcing a strict cut-off value [59, 60].

Antigen and antibody dynamics

Antibody responses are inherently noisy and imposing a strict cut-off may lead to misclassification [61]. Furthermore, antibody longevity may impact seropositivity classification [60, 62]. Coinfections can also be difficult to detect and separate, as certain pathogens with high titres can dominate detection assays [63].

In addition, antibody dynamics in terms of boosting and decay rates post infection should be taken into consideration. For example, as control programs lead to less disease exposure in populations, lower amounts of infection-specific antibodies circulate in the population and are replaced by residual antibody responses [64]. Roscoe et al. noted *S. stercoralis* antibodies decreased over time but remained above cut-off values a year and a half after successful treatment [65]. When determining prevalence estimates with cut-off values, some of these responses may actually be the result of cleared infections with residual antibodies.

Moreover, the dynamics of antibody-antigen interactions within age groups such as children and adults should be considered when interpreting cut-off values as they have been shown to differ [66]. For instance, cut-off values determined from a population of children may not be appropriate for the entire population age range when assessing prevalence of certain pathogens as children's immune systems are predominantly short-lived B-cells, while antigen presentation and helper T-cell function are more developed in the immune systems of adults[11, 67, 68]. Lastly, the inherent nature of antibody classes, such as IgG vs IgM, may be interpreted differently regardless of cut-off method [69].

Laboratory technique and design

Although not a focus of this paper, laboratory techniques impact the quality of MFI values. Thus, the generation of good quality cut-off values and resulting prevalence estimates require appropriate assay validations with sufficient quality control protocols [70]. Additionally, cut-off thresholds are dependent on specific coupling conditions [71], and bead consistency is an absolute requirement for the generation of precise cut-off values, regardless of the cut-off determination method.

Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention. Use of trade names is for identification only and does not imply endorsement by the Public Health Service or by the U.S. Department of Health and Human Services.

References

1. Arnold BF, Scobie HM, Priest JW, Lammie PJ. Integrated Serologic Surveillance of Population Immunity and Disease Transmission. Emerg Infect Dis. 2018;24(7):1188-94.
2. Marchal B, Van Dormael M, Pirard M, Cavalli A, Kegels G, Polman K. Neglected tropical disease (NTD) control in health systems: the interface between programmes and general health services. Acta Trop. 2011;120 Suppl 1:S177-85.
3. Metcalf CJ, Farrar J, Cutts FT, Basta NE, Graham AL, Lessler J, et al. Use of serological surveys to generate key insights into the changing global landscape of infectious disease. Lancet. 2016;388(10045):728-30.
4. Cooley GM, Mitja O, Goodhew B, Pillay A, Lammie PJ, Castro A, et al. Evaluation of Multiplex-Based Antibody Testing for Use in Large-Scale Surveillance for Yaws: a Comparative Study. J Clin Microbiol. 2016;54(5):1321-5.
5. FIscher T. Luminex 2018 [cited 2018 October 26]. Available from: https://www.thermofisher.com/uk/en/home/life-science/protein-biology/protein-assays-analysis/luminex-multiplex-assays.html.
6. Moss DM, Priest JW, Boyd A, Weinkopff T, Kucerova Z, Beach MJ, et al. Multiplex bead assay for serum samples from children in Haiti enrolled in a drug study for the treatment of lymphatic filariasis. Am J Trop Med Hyg. 2011;85(2):229-37.
7. Chu BK, Deming M, Biritwum NK, Bougma WR, Dorkenoo AM, El-Setouhy M, et al. Transmission assessment surveys (TAS) to define endpoints for lymphatic filariasis mass drug administration: a multicenter evaluation. PLoS Negl Trop Dis. 2013;7(12):e2584.
8. Goodhew EB, Morgan SM, Switzer AJ, Munoz B, Dize L, Gaydos C, et al. Longitudinal analysis of antibody responses to trachoma antigens before and after mass drug administration. BMC Infect Dis. 2014;14:216.
9. Poirier MJ, Moss DM, Feeser KR, Streit TG, Chang GJ, Whitney M, et al. Measuring Haitian children's exposure to chikungunya, dengue and malaria. Bull World Health Organ. 2016;94(11):817-25A.
10. Kucharski AJ, Kama M, Watson CH, Aubry M, Funk S, Henderson AD, et al. Using paired serology and surveillance data to quantify dengue transmission and control during a large outbreak in Fiji. Elife. 2018;7.
11. Arnold BF, van der Laan MJ, Hubbard AE, Steel C, Kubofcik J, Hamlin KL, et al. Measuring changes in transmission of neglected tropical diseases, malaria, and enteric pathogens from quantitative antibody levels. PLoS Negl Trop Dis. 2017;11(5):e0005616.
12. Scobie HM, Mao B, Buth S, Wannemuehler KA, Sorensen C, Kannarath C, et al. Tetanus Immunity among Women Aged 15 to 39 Years in Cambodia: a National Population-Based Serosurvey, 2012. Clin Vaccine Immunol. 2016;23(7):546-54.
13. Pinsent A, Solomon AW, Bailey RL, Bid R, Cama A, Dean D, et al. The utility of serology for elimination surveillance of trachoma. Nat Commun. 2018;9(1):5444.
14. Wong J, Hamel MJ, Drakeley CJ, Kariuki S, Shi YP, Lal AA, et al. Serological markers for monitoring historical changes in malaria transmission intensity in a highly endemic region of Western Kenya, 1994-2009. Malar J. 2014;13:451.
15. Drakeley CJ, Corran PH, Coleman PG, Tongren JE, McDonald SL, Carneiro I, et al. Estimating medium- and long-term trends in malaria transmission by using serological markers of malaria exposure. Proc Natl Acad Sci U S A. 2005;102(14):5108-13.
16. Hotez PJ, Aksoy S, Brindley PJ, Kamhawi S. What constitutes a neglected tropical disease? PLoS Negl Trop Dis. 2020;14(1):e0008001.

17. Moss DM, Chard AN, Trinies V, Doumbia S, Freeman MC, Lammie PJ. Serological Responses to Filarial Antigens in Malian Children Attending Elementary Schools. Am J Trop Med Hyg. 2017;96(1):229-32.
18. Hamlin KL, Moss DM, Priest JW, Roberts J, Kubofcik J, Gass K, et al. Longitudinal monitoring of the development of antifilarial antibodies and acquisition of Wuchereria bancrofti in a highly endemic area of Haiti. PLoS Negl Trop Dis. 2012;6(12):e1941.
19. Goodhew EB, Priest JW, Moss DM, Zhong G, Munoz B, Mkocha H, et al. CT694 and pgp3 as serological tools for monitoring trachoma programs. PLoS Negl Trop Dis. 2012;6(11):e1873.
20. Moss DM, Priest JW, Hamlin K, Derado G, Herbein J, Petri WA, Jr., et al. Longitudinal evaluation of enteric protozoa in Haitian children by stool exam and multiplex serologic assay. Am J Trop Med Hyg. 2014;90(4):653-60.
21. Fujii Y, Kaneko S, Nzou SM, Mwau M, Njenga SM, Tanigawa C, et al. Serological surveillance development for tropical infectious diseases using simultaneous microsphere-based multiplex assays and finite mixture models. PLoS Negl Trop Dis. 2014;8(7):e3040.
22. Ondigo BN, Muok EMO, Oguso JK, Njenga SM, Kanyi HM, Ndombi EM, et al. Impact of Mothers' Schistosomiasis Status During Gestation on Children's IgG Antibody Responses to Routine Vaccines 2 Years Later and Anti-Schistosome and Anti-Malarial Responses by Neonates in Western Kenya. Front Immunol. 2018;9:1402.
23. Priest JW, Jenks MH, Moss DM, Mao B, Buth S, Wannemuehler K, et al. Integration of Multiplex Bead Assays for Parasitic Diseases into a National, Population-Based Serosurvey of Women 1539 Years of Age in Cambodia. Plos Neglect Trop D. 2016;10(5).
24. Rogier EW, Moss DM, Mace KE, Chang M, Jean SE, Bullard SM, et al. Use of Bead-Based Serologic Assay to Evaluate Chikungunya Virus Epidemic, Haiti. Emerging Infectious Diseases. 2018;24(6):995-1001.
25. Scobie HM, Patel M, Martin D, Mkocha H, Njenga SM, Odiere MR, et al. Tetanus Immunity Gaps in Children 5-14 Years and Men >/= 15 Years of Age Revealed by Integrated Disease Serosurveillance in Kenya, Tanzania, and Mozambique. Am J Trop Med Hyg. 2017;96(2):415-20.
26. Boni MF, Chau NV, Dong N, Todd S, Nhat NT, de Bruin E, et al. Population-level antibody estimates to novel influenza A/H7N9. J Infect Dis. 2013;208(4):554-8.
27. Migchelsen SJ, Martin DL, Southisombath K, Turyaguma P, Heggen A, Rubangakene PP, et al. Defining Seropositivity Thresholds for Use in Trachoma Elimination Studies. PLoS Negl Trop Dis. 2017;11(1):e0005230.
28. Arnold BF, Martin DL, Juma J, Mkocha H, Ochieng JB, Cooley GM, et al. Enteropathogen antibody dynamics and force of infection among children in low-resource settings. 2019:522920.
29. Ronnberg B, Gustafsson A, Vapalahti O, Emmerich P, Lundkvist A, Schmidt-Chanasit J, et al. Compensating for cross-reactions using avidity and computation in a suspension multiplex immunoassay for serotyping of Zika versus other flavivirus infections. Med Microbiol Immunol. 2017;206(5):383-401.
30. Sanchez-Mazas A, Cerny V, Di D, Buhler S, Podgorna E, Chevallier E, et al. The HLA-B landscape of Africa: Signatures of pathogen-driven selection and molecular identification of candidate alleles to malaria protection. Mol Ecol. 2017;26(22):6238-52.
31. Schaible UE, Kaufmann SH. Malnutrition and infection: complex mechanisms and global impacts. PLoS Med. 2007;4(5):e115.
32. Hartgers FC, Yazdanbakhsh M. Co-infection of helminths and malaria: modulation of the immune responses to malaria. Parasite Immunol. 2006;28(10):497-506.
33. Zweig MH, Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. Clin Chem. 1993;39(4):561-77.
34. Perkins NJ, Schisterman EF. The inconsistency of "optimal" cutpoints obtained using two criteria based on the receiver operating characteristic curve. Am J Epidemiol. 2006;163(7):670-5.

35. Priest JW, Moss DM, Arnold BF, Hamlin K, Jones CC, Lammie PJ. Seroepidemiology of Toxoplasma in a coastal region of Haiti: multiplex bead assay detection of immunoglobulin G antibodies that recognize the SAG2A antigen. Epidemiol Infect. 2015;143(3):618-30.
36. Gwyn S, Cooley G, Goodhew B, Kohlhoff S, Banniettis N, Wiegand R, et al. Comparison of Platforms for Testing Antibody Responses against the Chlamydia trachomatis Antigen Pgp3. Am J Trop Med Hyg. 2017;97(6):1662-8.
37. Hardelid P, Williams D, Dezateux C, Tookey PA, Peckham CS, Cubitt WD, et al. Analysis of rubella antibody distribution from newborn dried blood spots using finite mixture models. Epidemiol Infect. 2008;136(12):1698-706.
38. Everitt BS. An introduction to finite mixture distributions. Stat Methods Med Res. 1996;5(2):107-27.
39. Sepulveda N, Stresman G, White MT, Drakeley CJ. Current Mathematical Models for Analyzing Anti-Malarial Antibody Data with an Eye to Malaria Elimination and Eradication. J Immunol Res. 2015;2015:738030.
40. Vitamin A and malnutrition/infection complex in developing countries. Lancet. 1990;336(8727):1349-51.
41. Zambrano LD, Priest JW, Ivan E, Rusine J, Nagel C, Kirby M, et al. Use of Serologic Responses against Enteropathogens to Assess the Impact of a Point-of-Use Water Filter: A Randomized Controlled Trial in Western Province, Rwanda. American Journal of Tropical Medicine and Hygiene. 2017;97(3):876-87.
42. Filomena A, Pessler F, Akmatov MK, Krause G, Duffy D, Gartner B, et al. Development of a Bead-Based Multiplex Assay for the Analysis of the Serological Response against the Six Pathogens HAV, HBV, HCV, CMV, T. gondii, and H. pylori. High Throughput. 2017;6(4).
43. Vyse AJ, Gay NJ, Hesketh LM, Pebody R, Morgan-Capner P, Miller E. Interpreting serological surveys using mixture models: the seroepidemiology of measles, mumps and rubella in England and Wales at the beginning of the 21st century. Epidemiol Infect. 2006;134(6):1303-12.
44. Ohuma EO, Okiro EA, Bett A, Abwao J, Were S, Samuel D, et al. Evaluation of a measles vaccine campaign by oral-fluid surveys in a rural Kenyan district: interpretation of antibody prevalence data using mixture models. Epidemiol Infect. 2009;137(2):227-33.
45. Arnold BF, Martin DL, Juma J, Mkocha H, Ochieng JB, Cooley GM, et al. Enteropathogen antibody dynamics and force of infection among children in low-resource settings. Elife. 2019;8.
46. Organization WH. International Reference Materials 2018 [cited 2018 October 26]. Available from: http://www.who.int/bloodproducts/ref_materials/en/.
47. Kaaijk P, Gouma S, Hulscher HI, Han WG, Kleijne DE, van Binnendijk RS, et al. Dynamics of the serologic response in vaccinated and unvaccinated mumps cases during an epidemic. Hum Vaccin Immunother. 2015;11(7):1754-61.
48. Del Fava E, Rimseliene G, Flem E, Freiesleben de Blasio B, Scalia Tomba G, Manfredi P. Estimating Age-Specific Immunity and Force of Infection of Varicella Zoster Virus in Norway Using Mixture Models. PLoS One. 2016;11(9):e0163636.
49. Bennette C, Vickers A. Against quantiles: categorization of continuous variables in epidemiologic research, and its discontents. Bmc Med Res Methodol. 2012;12.
50. Fritzell C, Rousset D, Adde A, Kazanji M, Van Kerkhove MD, Flamand C. Current challenges and implications for dengue, chikungunya and Zika seroprevalence studies worldwide: A scoping review. PLoS Negl Trop Dis. 2018;12(7):e0006533.
51. Shakib JH, Ralston S, Raissy HH, Stoddard GJ, Edwards KM, Byington CL. Pertussis antibodies in postpartum women and their newborns. J Perinatol. 2010;30(2):93-7.

52. West SK, Munoz B, Kaur H, Dize L, Mkocha H, Gaydos CA, et al. Longitudinal change in the serology of antibodies to Chlamydia trachomatis pgp3 in children residing in a trachoma area. Sci Rep. 2018;8(1):3520.
53. Juncker D, Bergeron S, Laforte V, Li H. Cross-reactivity in antibody microarrays and multiplexed sandwich assays: shedding light on the dark side of multiplexing. Curr Opin Chem Biol. 2014;18:29-37.
54. Acevedo GR, Girard MC, Gomez KA. The Unsolved Jigsaw Puzzle of the Immune Response in Chagas Disease. Front Immunol. 2018;9:1929.
55. Longley RJ, White MT, Takashima E, Brewster J, Morita M, Harbers M, et al. Development and validation of serological markers for detecting recent Plasmodium vivax infection. Nat Med. 2020;26(5):741-9.
56. Sepulveda N, Drakeley C. Sample size determination for estimating antibody seroconversion rate under stable malaria transmission intensity. Malar J. 2015;14:141.
57. Franco JR, Simarro PP, Diarra A, Ruiz-Postigo JA, Jannin JG. The Human African trypanosomiasis specimen biobank: a necessary tool to support research of new diagnostics. PLoS Negl Trop Dis. 2012;6(6):e1571.
58. Metcalf CJ, Mina MJ, Winter AK, Grenfell BT. Opportunities and challenges of a World Serum Bank - Authors' reply. Lancet. 2017;389(10066):252.
59. Wiegand RE, Cooley G, Goodhew B, Banniettis N, Kohlhoff S, Gwyn S, et al. Latent class modeling to compare testing platforms for detection of antibodies against the Chlamydia trachomatis antigen Pgp3. Sci Rep. 2018;8(1):4232.
60. Gwyn SE, Xiang L, Kandel RP, Dean D, Gambhir M, Martin DL. Prevalence of Chlamydia trachomatis-Specific Antibodies before and after Mass Drug Administration for Trachoma in Community-Wide Surveys of Four Communities in Nepal. Am J Trop Med Hyg. 2018;98(1):216-20.
61. Arnold BF, Priest JW, Hamlin KL, Moss DM, Colford JM, Jr., Lammie PJ. Serological measures of malaria transmission in Haiti: comparison of longitudinal and cross-sectional methods. PLoS One. 2014;9(4):e93684.
62. Wilson NO, Badara Ly A, Cama VA, Cantey PT, Cohn D, Diawara L, et al. Evaluation of Lymphatic Filariasis and Onchocerciasis in Three Senegalese Districts Treated for Onchocerciasis with Ivermectin. PLoS Negl Trop Dis. 2016;10(12):e0005198.
63. Liu Y, Xu ZQ, Zhang Q, Jin M, Yu JM, Li JS, et al. Simultaneous detection of seven enteric viruses associated with acute gastroenteritis by a multiplexed Luminex-based assay. J Clin Microbiol. 2012;50(7):2384-9.
64. Won KY, Sambou S, Barry A, Robinson K, Jaye M, Sanneh B, et al. Use of Antibody Tools to Provide Serologic Evidence of Elimination of Lymphatic Filariasis in The Gambia. American Journal of Tropical Medicine and Hygiene. 2018;98(1):15-20.
65. Rascoe LN, Price C, Shin SH, McAuliffe I, Priest JW, Handali S. Development of Ss-NIE-1 recombinant antigen based assays for immunodiagnosis of strongyloidiasis. PLoS Negl Trop Dis. 2015;9(4):e0003694.
66. Won KY, Kanyi HM, Mwende FM, Wiegand RE, Goodhew EB, Priest JW, et al. Multiplex Serologic Assessment of Schistosomiasis in Western Kenya: Antibody Responses in Preschool Aged Children as a Measure of Reduced Transmission. Am J Trop Med Hyg. 2017;96(6):1460-7.
67. Chard AN, Trinies V, Moss DM, Chang HH, Doumbia S, Lammie PJ, et al. The impact of school water, sanitation, and hygiene improvements on infectious disease using serum antibody detection. PLoS Negl Trop Dis. 2018;12(4):e0006418.
68. McDonald M. Neglected Tropical AND Zoonotic Diseases and Their Impact on Women's and Children's Health. In: Institute of Medicine (US) Forum on Microbial Threats. The Causes and Impacts of Neglected Tropical and Zoonotic Diseases: Opportunities for Integrated Intervention Strategies.

Washington (DC): National Academies Press (US); 2011. A15. Available from: https://www.ncbi.nlm.nih.gov/books/NBK62515/.
69. Mohamed Ismail NA, Wan Abd Rahim WE, Salleh SA, Neoh HM, Jamal R, Jamil MA. Seropositivity of dengue antibodies during pregnancy. ScientificWorldJournal. 2014;2014:436975.
70. Planatscher H, Rimmele S, Michel G, Potz O, Joos T, Schneiderhan-Marra N. Systematic reference sample generation for multiplexed serological assays. Sci Rep. 2013;3:3259.
71. Feeser KR, Cama V, Priest JW, Thiele EA, Wiegand RE, Lakwo T, et al. Characterizing Reactivity to Onchocerca volvulus Antigens in Multiplex Bead Assays. Am J Trop Med Hyg. 2017;97(3):666-72.

S1 Table. List of Articles Reviewed

T3. 3 **Supplementary Table 1. List of articles reviewed**.

Reviewed articles with antigens within the study, study populations, and applied methods (indicated with X). ROC= receiver operating characteristic curve, PN= Presumed Negative, MM= Mixture Model, IS= International Standards, VIP= Visual Inflection Point, Q= Quantile, PEC= Pre-exposed Endemic Cohort, CPR=Cut-off previously reported (not included as a cut-off approach). The total number for each method is included in the bottom row.

Article	Antigen(s)	Population	ROC	PN	MM	IS	VIP	Q	PEC	CPR
ARNOLD et al. 2019 (72)	*Giardia* VSP3 + VSP5, *Cryptosporidium* Cp17 + Cp23, *E. histolytica* LecA, *Salmonella* LPS grp B + grp D, ETEC LT B, *Norovirus* GI.4 + GII. 4.NO, Cholera toxin B, *Campylobacter* p18, p39	Haiti Kenya Tanzania	X	X					X	
ARNOLD et al. 2014 (133)	*P. falciparum* $MSP1_{19}$	Haiti		X						
ARNOLD et al. 2017 (74)	Wb123 antigen *P. falciparum* antibody response measured with the IgG indirect fluorescent antibody (IFA) test, *Cryptosporidium parvum* Cp17 and Cp23, *Escherichia coli* (ETEC) heat-labile toxin β subunit (EtxB), *E. histolytica* Gal/GalNAc lectin heavy chain subunit (LecA), lipopolysaccharide (LPS) from Salmonella enterica serotype Typhimurium (Group B), Purified recombinant norovirus GI.4 and GII.4	Cook Islands Nigeria Haiti United States			X					
ASSEFA et al. 2019 (144)	six *Plasmodium* antigens: four human malaria species-specific merozoite surface protein-1 19kD antigens (MSP-1) and Apical Membrane Antigen-1 (AMA-	Ethiopia			X					

	1) for *Plasmodium falciparum* and *Plasmodium vivax*.									
AUGUSTINE et al. 2017 (145)	*Campylobacter jejuni* Heat-killed whole bacterial cells, *Helicobacter pylori* Bacterial cell lysate, *Toxoplasma gondii* SAG1, *Hepatitis A virus* Cell culture concentrate, Norovirus GI.1, Norovirus GII.4.	Puerto Rico		X						
BONI et al. 2013 (101)	Influenza HA 1	Vietnam						X		
CHARD et al. 2018 (139)	*Campylobacter jejuni* p18, p39 *Cryptosporidium parvum* 17 kda 27 kda, dengue 2, dengue 3, *Entamoeba histolytica, Escherichia coli, Giardia intestinalis (VSP 3, VSP 5) Norovirus (Sydney strain), Plasmodium falciparum (MSP19, 42, AMA1) Salmonella enteritidis, Salmonella typhimurium, Schistosoma mansoni, Chlamydia trachomatis CT-694, Pgp, Vibrio cholerae*	Mali								X
CHU et al. 2013 (91)	ICT test for filarial antigens, BmR1	American Samoa, Burkina Faso, Dominican Republic, Ghana, Indonesia, Malaysia, Philippines, Sri Lanka, Tanzania,				X				

		Togo, and Vanuatu								
COOLEY et al. 2016 (88)	*Treponema pallidum* recombinant rp 1 and treponemal membrane antibody TmpA	Ghana Papua New Guinea	X							
DEL FAVA et al. 2016 (121)	VZ Virus	Norway			X					
FEESER et al. 2017 (143)	*Onchocerca volvulus* OV-16, OV-17, and OV-33, other filarial antigens	Uganda Ethiopia	X							
FILOMENA et al. 2017 (116)	HAV- VP4-VP2, VP3, and VP1, HBV- HBcAg HBsAg ad and HBsAg ay, HCV - Core g4a, Core g1b, NS3 g1a, and NS3 g1b, CMV- whole cell lysate of strain AD169, *T. gondii* whole tachyzoites, *H. pylori* soluble protein extract of strain 49503	European		X						
FUJII et al. 2014 (81)	*Entamoeba histolytica* (C-IgL), *Leishmania donovani* (KRP42), *Toxoplasma gondii* (SAG1), *Wuchereria bancrofti* (SXP1), HIV (gag, gp120 and gp41), and *Vibrio cholerae* (cholera toxin)	Kenya		X	X					
GOODHEW et al. 2014 (92)	*Chlamydia trachomatis* Pgp3 and CT694	Tanzania	X							
GOODHEW et al. 2012 (85)	*Chlamydia trachomatis* Pgp3 and CT694	Tanzania		X						
GWYN et al. 2017 (110)	*Chlamydia trachomatis* Pgp3	Tanzania	X							
HAMLIN et al. 2012 (70)	W. bancrofti Wb123, Brugia malayi Bm33 and Bm14, WSP from Wolbachia	Haiti		X						
HARDELID et al. 2008 (111)	Rubella IgG	United Kingdom			X					

KAAIJK et al. 2015 (120)	Anti-mumps nucleoprotein antibody	Netherlands								X
LIU et al. 2012 (135)	rotavirus A (RVA), noroviruses (NoVs) (including genogroups GI and GII), sapoviruses (SaV), human astrovirus (HAstV), enteric adenoviruses (EAds), and human bocavirus 2 (HBoV2)	China		X						
MIGCHELSEN et al. 2017 (71)	*Chlamydia trachomatis* Pgp3	Laos Uganda Gambia		X			X			
MOSS et al. 2017 (97)	*W. bancrofti* Wb123, *Brugia malayi* Bm14	Mali		X						
MOSS et al. 2011 (90)	*Brugia malayi* Bm33 and Bm14	Haiti		X						
MOSS et al. 2014 (63)	*E. histolytica* LecA , *C.parvum*, Cry17, and Cry27, *G. intestinalis* assemblage A, VSP1–VSP3, and two from assemblage B, VSP4 and VSP5	Haiti		X						
NJENGA et al. 2020 (73)	*Brugia malayi* Bm33 and Bm14, *Strongyloides stercoralis* NIE, *P. falciparum* $MSP1_{19}$, *W. bancrofti* Wb123, *Schistosoma mansoni* SEA + GST, Tetantus toxoid, diphtheria toxoid, measles virus neutralizing antibody	Kenya	X	X	X	X				
ONDIGO et al. 2018 (98)	*P. falciparum* $MSP1_{19}$, *Schistosoma mansoni* SEA, *Strongyloides stercoralis* NIE, diphtheria toxoid, Tetanus toxoid, Measles nucleoprotein, *Ascaris suum* AsHb	Kenya	X		X	X				

PLUCINSKI et al. 2018 (146)	*P. falciparum* $MSP1_{19}$ + CSP + LSA, *W. bancrofti* Wb123, *B. malayi Bm33, Strongyloides stercoralis* NIE	Mozambique	X	X						
POIRIER et al 2016 (69)	Recombinant chikungunya virus antigen, dengue DENV-2 and DENV-3, *P. falciparum* antigens 19-kDa fragment, from clone 3D7, linked to glutathione-*S*-transferase, 42-kDa fragment from clone 3D7, 42-kDa fragment from clone FVO	Haiti		X						
PRIEST et al. 2016 (99)	NIE for *Strongyloides stercoralis,* SAG2A for *Toxoplasma gondii*, T24H for cysticercosis, PfMSP-1_{19} (3D7 strain) and PfMSP-1_{42} (3D7 strain and FVO strain) for *P. falciparum* malaria, PvMSP-1_{19} (Belem strain) for *P. vivax* malaria, For lymphatic filariasis, *Brugia malayi* Bm14 and *W. bancrofti* Wb12	Cambodia		X						
PRIEST et al. 2015 (109)	Toxoplasma SAG2A	Haiti	X							
RASCOE et al. 2015 (137)	NIE for *Strongyloides stercoralis*	Haiti		X						
REDER et al. 2008 (80)	tetanus toxin, diphtheria toxin, and pertussis toxin	Germany				X				
ROGIER et al. 2015 (147)	42kD fragment of MSP-1: MSP-1p42(D) and MSP-1p42(F) from the 3D7 and FVO strains, respectively, 19 kD fragment (MSP-1p19) fused to glutathione S-	Haiti		X	X					

	transferase (GST) cloned from *P. falciparum* isolate 3D7.									
ROGIER et al. 2018 (100)	*Chikungunya* Anti-CHIKV IgG	Haiti		X						
RONNBERG et al. 2017 (103)	whole virus antigens (WV), recombinant glycosylated E proteins (E), and non-structural protein 1 (NS1)	European Travelers to endemic countries		X						
SCOBIE et al. 2016 (94)	Tetanus toxoid	Cambodia				X				
SCOBIE et al. 2017 (68)	Tetanus toxoid	Kenya Tanzania Mozambique				X				
SEPULVEDA et al. 2015 (128)	*P. falciparum* MSP 1 and AMA	African/ Non African			X					
SEPULVEDA et al. 2015 (113)	*P. falciparum* MSP 1 and AMA	Equatorial Guinea			X					
VYSE et al. 2006 (117)	Rubella. measles and mumps-specific IgG	England Wales			X					
WEST et al. 2018 (125)	*Chlamydia trachomatis* Pgp3	Tanzania	X							
WIEGAND et al. 2018 (131)	*Chlamydia trachomatis* Pgp3	Bolivia Nepal United States	X		X					
WILSON et al. 2016 (134)	*O. volvulus* Ov16 *W. bancrofti* Wb123	Senegal								X
WON et al. 2017 (138)	*Schistosoma mansoni* SEA and Sm25	Kenya	X	X						
WON et al. 2018 (148)	*W. bancrofti* Wb123, *Brugia malayi* Bm33 and Bm14	American Samoa	X	X						
WON et al. 2018 (136)	*W. bancrofti* Wb123, *Brugia malayi* Bm14	Gambia	X	X						
ZAMBRANO et al. 2017 (115)	*Cryptosporidium parvum* Cp17 and Cp23, *Escherichia coli* (ETEC) heat-labile toxin β	Rwanda	X	X	X					

	subunit (EtxB), *E. histolytica* Gal/GalNAc lectin heavy chain subunit (LecA), *T. gondii* SAG2A									
Total number			15	23	13	6	1	1	1	3

S1 Table References

1. Arnold BF, Martin DL, Juma J, Mkocha H, Ochieng JB, Cooley GM, et al. Enteropathogen antibody dynamics and force of infection among children in low-resource settings. Elife. 2019;8.
2. Arnold BF, Priest JW, Hamlin KL, Moss DM, Colford JM, Jr., Lammie PJ. Serological measures of malaria transmission in Haiti: comparison of longitudinal and cross-sectional methods. PLoS One. 2014;9(4):e93684.
3. Arnold BF, van der Laan MJ, Hubbard AE, Steel C, Kubofcik J, Hamlin KL, et al. Measuring changes in transmission of neglected tropical diseases, malaria, and enteric pathogens from quantitative antibody levels. PLoS Negl Trop Dis. 2017;11(5):e0005616.
4. Assefa A, Ali Ahmed A, Deressa W, Sime H, Mohammed H, Kebede A, et al. Multiplex serology demonstrate cumulative prevalence and spatial distribution of malaria in Ethiopia. Malar J. 2019;18(1):246.
5. Augustine SAJ, Simmons KJ, Eason TN, Curioso CL, Griffin SM, Wade TJ, et al. Immunoprevalence to Six Waterborne Pathogens in Beachgoers at Boqueron Beach, Puerto Rico: Application of a Microsphere-Based Salivary Antibody Multiplex Immunoassay. Front Public Health. 2017;5:84.
6. Boni MF, Chau NV, Dong N, Todd S, Nhat NT, de Bruin E, et al. Population-level antibody estimates to novel influenza A/H7N9. J Infect Dis. 2013;208(4):554-8.
7. Chard AN, Trinies V, Moss DM, Chang HH, Doumbia S, Lammie PJ, et al. The impact of school water, sanitation, and hygiene improvements on infectious disease using serum antibody detection. PLoS Negl Trop Dis. 2018;12(4):e0006418.
8. Chu BK, Deming M, Biritwum NK, Bougma WR, Dorkenoo AM, El-Setouhy M, et al. Transmission assessment surveys (TAS) to define endpoints for lymphatic filariasis mass drug administration: a multicenter evaluation. PLoS Negl Trop Dis. 2013;7(12):e2584.
9. Cooley GM, Mitja O, Goodhew B, Pillay A, Lammie PJ, Castro A, et al. Evaluation of Multiplex-Based Antibody Testing for Use in Large-Scale Surveillance for Yaws: a Comparative Study. J Clin Microbiol. 2016;54(5):1321-5.
10. Del Fava E, Rimseliene G, Flem E, Freiesleben de Blasio B, Scalia Tomba G, Manfredi P. Estimating Age-Specific Immunity and Force of Infection of Varicella Zoster Virus in Norway Using Mixture Models. PLoS One. 2016;11(9):e0163636.
11. Feeser KR, Cama V, Priest JW, Thiele EA, Wiegand RE, Lakwo T, et al. Characterizing Reactivity to Onchocerca volvulus Antigens in Multiplex Bead Assays. Am J Trop Med Hyg. 2017;97(3):666-72.
12. Filomena A, Pessler F, Akmatov MK, Krause G, Duffy D, Gartner B, et al. Development of a Bead-Based Multiplex Assay for the Analysis of the Serological Response against the Six Pathogens HAV, HBV, HCV, CMV, T. gondii, and H. pylori. High Throughput. 2017;6(4).
13. Fujii Y, Kaneko S, Nzou SM, Mwau M, Njenga SM, Tanigawa C, et al. Serological surveillance development for tropical infectious diseases using simultaneous microsphere-based multiplex assays and finite mixture models. PLoS Negl Trop Dis. 2014;8(7):e3040.
14. Goodhew EB, Morgan SM, Switzer AJ, Munoz B, Dize L, Gaydos C, et al. Longitudinal analysis of antibody responses to trachoma antigens before and after mass drug administration. BMC Infect Dis. 2014;14:216.
15. Goodhew EB, Priest JW, Moss DM, Zhong G, Munoz B, Mkocha H, et al. CT694 and pgp3 as serological tools for monitoring trachoma programs. PLoS Negl Trop Dis. 2012;6(11):e1873.
16. Gwyn S, Cooley G, Goodhew B, Kohlhoff S, Banniettis N, Wiegand R, et al. Comparison of Platforms for Testing Antibody Responses against the Chlamydia trachomatis Antigen Pgp3. Am J Trop Med Hyg. 2017;97(6):1662-8.

17. Hamlin KL, Moss DM, Priest JW, Roberts J, Kubofcik J, Gass K, et al. Longitudinal monitoring of the development of antifilarial antibodies and acquisition of Wuchereria bancrofti in a highly endemic area of Haiti. PLoS Negl Trop Dis. 2012;6(12):e1941.
18. Hardelid P, Williams D, Dezateux C, Tookey PA, Peckham CS, Cubitt WD, et al. Analysis of rubella antibody distribution from newborn dried blood spots using finite mixture models. Epidemiol Infect. 2008;136(12):1698-706.
19. Kaaijk P, Gouma S, Hulscher HI, Han WG, Kleijne DE, van Binnendijk RS, et al. Dynamics of the serologic response in vaccinated and unvaccinated mumps cases during an epidemic. Hum Vaccin Immunother. 2015;11(7):1754-61.
20. Liu Y, Xu ZQ, Zhang Q, Jin M, Yu JM, Li JS, et al. Simultaneous detection of seven enteric viruses associated with acute gastroenteritis by a multiplexed Luminex-based assay. J Clin Microbiol. 2012;50(7):2384-9.
21. Migchelsen SJ, Martin DL, Southisombath K, Turyaguma P, Heggen A, Rubangakene PP, et al. Defining Seropositivity Thresholds for Use in Trachoma Elimination Studies. PLoS Negl Trop Dis. 2017;11(1):e0005230.
22. Moss DM, Chard AN, Trinies V, Doumbia S, Freeman MC, Lammie PJ. Serological Responses to Filarial Antigens in Malian Children Attending Elementary Schools. Am J Trop Med Hyg. 2017;96(1):229-32.
23. Moss DM, Priest JW, Boyd A, Weinkopff T, Kucerova Z, Beach MJ, et al. Multiplex bead assay for serum samples from children in Haiti enrolled in a drug study for the treatment of lymphatic filariasis. Am J Trop Med Hyg. 2011;85(2):229-37.
24. Moss DM, Priest JW, Hamlin K, Derado G, Herbein J, Petri WA, Jr., et al. Longitudinal evaluation of enteric protozoa in Haitian children by stool exam and multiplex serologic assay. Am J Trop Med Hyg. 2014;90(4):653-60.
25. Njenga SM, Kanyi HM, Arnold BF, Matendechero SH, Onsongo JK, Won KY, et al. Integrated Cross-Sectional Multiplex Serosurveillance of IgG Antibody Responses to Parasitic Diseases and Vaccines in Coastal Kenya. Am J Trop Med Hyg. 2020;102(1):164-76.
26. Ondigo BN, Muok EMO, Oguso JK, Njenga SM, Kanyi HM, Ndombi EM, et al. Impact of Mothers' Schistosomiasis Status During Gestation on Children's IgG Antibody Responses to Routine Vaccines 2 Years Later and Anti-Schistosome and Anti-Malarial Responses by Neonates in Western Kenya. Front Immunol. 2018;9:1402.
27. Plucinski MM, Candrinho B, Chambe G, Muchanga J, Muguande O, Matsinhe G, et al. Multiplex serology for impact evaluation of bed net distribution on burden of lymphatic filariasis and four species of human malaria in northern Mozambique. PLoS Negl Trop Dis. 2018;12(2):e0006278.
28. Poirier MJ, Moss DM, Feeser KR, Streit TG, Chang GJ, Whitney M, et al. Measuring Haitian children's exposure to chikungunya, dengue and malaria. Bull World Health Organ. 2016;94(11):817-25A.
29. Priest JW, Jenks MH, Moss DM, Mao B, Buth S, Wannemuehler K, et al. Integration of Multiplex Bead Assays for Parasitic Diseases into a National, Population-Based Serosurvey of Women 1539 Years of Age in Cambodia. Plos Neglect Trop D. 2016;10(5).
30. Priest JW, Moss DM, Arnold BF, Hamlin K, Jones CC, Lammie PJ. Seroepidemiology of Toxoplasma in a coastal region of Haiti: multiplex bead assay detection of immunoglobulin G antibodies that recognize the SAG2A antigen. Epidemiol Infect. 2015;143(3):618-30.
31. Rascoe LN, Price C, Shin SH, McAuliffe I, Priest JW, Handali S. Development of Ss-NIE-1 recombinant antigen based assays for immunodiagnosis of strongyloidiasis. PLoS Negl Trop Dis. 2015;9(4):e0003694.

32. Reder S, Riffelmann M, Becker C, Wirsing von Konig CH. Measuring immunoglobulin g antibodies to tetanus toxin, diphtheria toxin, and pertussis toxin with single-antigen enzyme-linked immunosorbent assays and a bead-based multiplex assay. Clin Vaccine Immunol. 2008;15(5):744-9.
33. Rogier E, Wiegand R, Moss D, Priest J, Angov E, Dutta S, et al. Multiple comparisons analysis of serological data from an area of low Plasmodium falciparum transmission. Malar J. 2015;14:436.
34. Rogier EW, Moss DM, Mace KE, Chang M, Jean SE, Bullard SM, et al. Use of Bead-Based Serologic Assay to Evaluate Chikungunya Virus Epidemic, Haiti. Emerging Infectious Diseases. 2018;24(6):995-1001.
35. Ronnberg B, Gustafsson A, Vapalahti O, Emmerich P, Lundkvist A, Schmidt-Chanasit J, et al. Compensating for cross-reactions using avidity and computation in a suspension multiplex immunoassay for serotyping of Zika versus other flavivirus infections. Med Microbiol Immunol. 2017;206(5):383-401.
36. Scobie HM, Mao B, Buth S, Wannemuehler KA, Sorensen C, Kannarath C, et al. Tetanus Immunity among Women Aged 15 to 39 Years in Cambodia: a National Population-Based Serosurvey, 2012. Clin Vaccine Immunol. 2016;23(7):546-54.
37. Scobie HM, Patel M, Martin D, Mkocha H, Njenga SM, Odiere MR, et al. Tetanus Immunity Gaps in Children 5-14 Years and Men >/= 15 Years of Age Revealed by Integrated Disease Serosurveillance in Kenya, Tanzania, and Mozambique. Am J Trop Med Hyg. 2017;96(2):415-20.
38. Sepulveda N, Drakeley C. Sample size determination for estimating antibody seroconversion rate under stable malaria transmission intensity. Malar J. 2015;14:141.
39. Sepulveda N, Stresman G, White MT, Drakeley CJ. Current Mathematical Models for Analyzing Anti-Malarial Antibody Data with an Eye to Malaria Elimination and Eradication. J Immunol Res. 2015;2015:738030.
40. Vyse AJ, Gay NJ, Hesketh LM, Pebody R, Morgan-Capner P, Miller E. Interpreting serological surveys using mixture models: the seroepidemiology of measles, mumps and rubella in England and Wales at the beginning of the 21st century. Epidemiol Infect. 2006;134(6):1303-12.
41. West SK, Munoz B, Kaur H, Dize L, Mkocha H, Gaydos CA, et al. Longitudinal change in the serology of antibodies to Chlamydia trachomatis pgp3 in children residing in a trachoma area. Sci Rep. 2018;8(1):3520.
42. Wiegand RE, Cooley G, Goodhew B, Banniettis N, Kohlhoff S, Gwyn S, et al. Latent class modeling to compare testing platforms for detection of antibodies against the Chlamydia trachomatis antigen Pgp3. Sci Rep. 2018;8(1):4232.
43. Wilson NO, Badara Ly A, Cama VA, Cantey PT, Cohn D, Diawara L, et al. Evaluation of Lymphatic Filariasis and Onchocerciasis in Three Senegalese Districts Treated for Onchocerciasis with Ivermectin. PLoS Negl Trop Dis. 2016;10(12):e0005198.
44. Won KY, Kanyi HM, Mwende FM, Wiegand RE, Goodhew EB, Priest JW, et al. Multiplex Serologic Assessment of Schistosomiasis in Western Kenya: Antibody Responses in Preschool Aged Children as a Measure of Reduced Transmission. Am J Trop Med Hyg. 2017;96(6):1460-7.
45. Won KY, Robinson K, Hamlin KL, Tufa J, Seespesara M, Wiegand RE, et al. Comparison of antigen and antibody responses in repeat lymphatic filariasis transmission assessment surveys in American Samoa. Plos Neglect Trop D. 2018;12(3).
46. Won KY, Sambou S, Barry A, Robinson K, Jaye M, Sanneh B, et al. Use of Antibody Tools to Provide Serologic Evidence of Elimination of Lymphatic Filariasis in The Gambia. American Journal of Tropical Medicine and Hygiene. 2018;98(1):15-20.
47. Zambrano LD, Priest JW, Ivan E, Rusine J, Nagel C, Kirby M, et al. Use of Serologic Responses against Enteropathogens to Assess the Impact of a Point-of-Use Water Filter: A Randomized Controlled Trial in Western Province, Rwanda. American Journal of Tropical Medicine and Hygiene. 2017;97(3):876-87.

3.2.1 Research Conclusions

This research addressed the first outcome of **Objective 1**, which is to examine literature for existing methods used to characterize MFI antibody responses into seroprevalence. To do this, I reviewed the literature for currently applied methods of determining seroprevalence when serologic MBAs were applied in numerous research settings. This work identified seven different approaches to identify cut-off values applied in literature. There was no standard method per antigen or organism, and across different public health and research study settings. As seroprevalence is a metric consistently applied throughout the rest of this thesis, this work was needed to ensure appropriate analysis of the data and lend confidence to conclusions drawn in my research.

In lieu of a standard approach, determining which cut-off approaches are suitable for each antigen under investigation and identifying factors to guide the decision of choice of cut-off method is an important initial step to assess the programmatic public health utility of MBAs. Given that cut-off approaches are currently applied inconsistently between settings, there is the potential that choice of cut-off methods could result in different prevalence estimates and impact downstream program action. For example, if a presumed negative approach generates a high cut-off value but mixture models generate a low cut-off value, the prevalence estimates would be higher using the latter models, which may result in program action (depending on the actual estimates). The next research aims to investigate this directly through the potential impact on programmatic decision-making based on choice of cut-off approach.

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT

T: +44 (0)20 7299 4646
F: +44 (0)20 7299 4656
www.lshtm.ac.uk

RESEARCH PAPER COVER SHEET

Please note that a cover sheet must be completed for each research paper included within a thesis.

SECTION A – Student Details

Student ID Number	1603698	**Title**	Mr
First Name(s)	YuYen		
Surname/Family Name	Chan		
Thesis Title	Application of multiplex bead serological assays to integrated monitoring of neglected tropical disease		
Primary Supervisor	Chris Drakeley		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?			
When was the work published?			
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion			
Have you retained the copyright for the work?*	Choose an item.	Was the work subject to academic peer review?	**No**

*If yes, please attach evidence of retention. If no, or if the work is being included in its published format, please attach evidence of permission from the copyright holder (publisher or other author) to include this work.

SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	PLoS NTD
Please list the paper's authors in the intended authorship order:	YuYen L. Chan, Kimberly Fornace, Eric. Rogier, Samuel E. Jean , Lindsey Wu, Ben F. Arnold, Jeffrey W. Priest, Diana L. Martin, Michelle E. Chang, Jackie Cook, Chris Drakeley, Gillian Stresman
Stage of publication	**Undergoing revision**

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	Writing and drafting paper, review of literature, comparatory analysis of cut-off approaches

SECTION E

Student Signature	YuYen Chan
Date	16/09/2021

Supervisor Signature	Chris Drakeley
Date	20/09/2021

3.3 Programmatic implications of seroprevalence by cut-off method choice

Programmatic implications of approach applied for deriving seropositivity on prevalence estimates for lymphatic filariasis, strongyloidiasis, and trachoma/chlamydia in Haiti and Malaysia as part of integrated serological surveillance.

Authors and affiliations: YuYen L. Chan[1], Kimberly Fornace[2], Eric. Rogier[3], Samuel E. Jean[6] , Lindsey Wu[1], Ben F. Arnold[4], Jeffrey W. Priest[5], Diana L. Martin[3], Michelle E. Chang[3], Jackie Cook[7], Chris Drakeley[1], Gillian Stresman[1]

1. Department of Infection Biology, London School of Hygiene and Tropical Medicine, London, United Kingdom
2. Department of Disease Control, London School of Hygiene and Tropical Medicine, London, United Kingdom
3. Division of Parasitic Diseases and Malaria, United States Centers for Disease Control and Prevention, Atlanta, GA, USA
4. Francis I. Proctor Foundation, University of California, San Francisco, San Francisco, CA, USA
5. Division of Foodborne, Waterborne, and Environmental Diseases, United States Centers for Disease Control and Prevention, Atlanta, GA, USA
6. Population Services International/Organisation Haïtienne de Marketing Social pour la Santé, Port-au-Prince, Haiti
7. MRC International Statistics and Epidemiology Group, Department of Infectious Disease Epidemiology, London School of Hygiene and Tropical Medicine, London, United Kingdom

Abstract

Background: Integrated serological surveys can be used to assess seroprevalence to multiple pathogens simultaneously. There are several statistical methods typically applied to generate cut-off values to dichotomize the continuous data generated into a measure of seropositivity. There is currently little evidence as to whether choice of cut-off method influences programmatic decisions based on seroprevalence. The consistency in seroprevalence estimates obtained using three cut-off approaches was assessed using serological data from Haiti and Malaysia.

Methodology/Principal Findings: Presumed unexposed (PU), mixture models (MM), and quartile (Q) cut-off methods were applied to antibody responses to antigens of lymphatic filariasis (LF, *Brugia malayi* [Bm] and *Wuchereria bancrofti* [Wb]), strongyloidiasis (*Strongyloides stercoralis*, [Ss]), and trachoma/chlamydia (*Chlamydia trachomatis*, [Ct]) in a national survey in Haiti and a study in Sabah, Malaysia. Seroprevalence estimates ranged by cut-off method for each antigen target in Haiti. For LF Bm14 the range was 5.7% to 31.0%, for Wb 123 the range was 5.2% to 25.4%, for Ss NIE the range was 3.0% to 25.1%, and for Ct Pgp3 the range was 2.3% to 25.1%. Similarly, in Sabah, Malaysia, prevalence estimates also differed per cut-off method: LF Bm14 ranged from 3.5% to 25.0% and LF Wb 123 ranged from 5.2% to 25.1%, Ss NIE ranged from 3.0% to 25.1%, and Ct Pgp3 ranged from 2.3% to 25.1%. District seroprevalence rankings in Haiti were different based on both cut-off method and the specific antigen, with some consistency in classification observed for LF Wb123 and Ct Pgp3 antigens when comparing MM and Q to PU (spearman's rank p-value <0.05). PU often generated more conservative seroprevalence estimates, while Q method resulted in the highest estimates among all antigens.

Conclusions/Significance: Seroprevalence estimates varied based on cut-off method for all pathogens studied here. In a programmatic context, these differences make it difficult to compare results across settings. This could be particularly important if the different methods result in disparate classifications of disease burden which may influence targeting of interventions. While modelling approaches may generate the least bias cut-offs in the appropriate settings, a standard approach is needed for defining seropositivity per antigen to ensure consistent programmatic conclusions.

Introduction

Many countries suffer from co-endemic transmission of neglected tropical diseases (NTDs). Whilst previous control strategies have often focused on individual diseases (1), current advocacy among global stakeholders has rallied substantial support to transition to an integrated approach to surveillance and control of diseases that are co-endemic (2, 3). Fundamental to control efforts is the capacity to maintain frequent and reliable surveillance data for all the diseases transmitted in the target population so key indicators can be produced in a timely manner (4, 5). However, the burden of NTDs is often underestimated as a result of asymptomatic infections, infrequent surveillance due to limited resources, and limited availability for reliable diagnostic tools (6, 7).

Using serological surveillance to simultaneously monitor exposure to multiple NTD antigens provides a powerful tool on which to support an integrated disease surveillance platform. Serological tests have the

advantage of detecting asymptomatic or subclinical exposure to the pathogen of interest (1). Integrating serological tests adds the potential logistical convenience of combining sample collection, costs, and implementation compared to other resource-intensive diagnostic methods such as cell culturing (8). As a surveillance tool, serological multiplex bead assays (MBAs) can assess multiple pathogens simultaneously from a single blood sample (9, 10). The serological MBA output is median fluorescence intensity (MFI), a continuous measure which reflects levels of antigen-specific antibodies in the blood (11). MBAs has potential as an integrated diseases serosurveillance tool, as has been demonstrated, but interpreting the MFI data and converting it to programmatically meaningful outputs needs to be carefully considered, particularly given the lack of standardized tools (12-15).

Serological surveys using MBAs typically employ methods to determine seropositive and seronegative status to estimate the population seroprevalence (16). However, different approaches have been used to determine these cut-offs for NTDs, as has been recently reviewed (17). While the rationale behind the cut-off approach employed is seldomly made explicit, possible explanations regarding method choices could be past precedents, accessibility of methods, pragmatism given availability of data, and empirical antibody distributions. Given the different cut-off approaches, it is possible that the resulting prevalence estimates will vary and therefore impact programmatic interpretation and decision-making.

To our knowledge, no studies to date have directly considered the programmatic implications of different approaches for determining cut-off values and the corresponding seroprevalence estimates. In this study, we aim to investigate these potential programmatic implications by assessing three cut-off approaches applied to data measuring serological responses to three different pathogens in Haitian and Malaysian populations.

Methods

Three different statistical cut-off approaches which consistently appeared in the literature (17) were applied to data from two case studies that employed MBAs and included antigens specific to pathogens that cause lymphatic filariasis (LF, *Brugia malayi* [Bm] and *Wuchereria bancrofti* [Wb]), strongyloidiasis (*Strongyloides stercoralis*, Ss), and trachoma/chlamydia (*Chlamydia trachomatis*, CT) to investigate any implications on the corresponding seroprevalence estimates.

Cut-off methods used

Approach 1 – Presumed unexposed (PU): The presumed unexposed (or presumed negative) cut-off method uses selected populations with self-reported claims of either no recent travel or known travel history to endemic countries. Typically, presumed unexposed populations have been chosen from non-endemic regions, such as the United States and Europe. Cut-off values are determined using a pre-determined number of standard deviations above the mean MFI values minus the background levels of the assay (MFI-bg). A possible caveat to using presumed unexposed populations is that immunological background of individuals from non-endemic countries may not accurately reflect the immunity of the sample population, as suggested by Fujii et al (18). This method has been used to determine seropositivity cut-offs in public health applications including disease surveillance (12), understanding the force of infection (19), and monitoring transmission after a mass drug administration (20).

Approach 2 – Mixture model (MM): The mixture model approach assumes the presence of at least two subpopulations or components, within the sample population. The lowest distribution is considered to represent those within the population unexposed to the pathogen, or the seronegative population, whereas the second distribution reflects those exposed, or seropositive to the pathogen of interest. The cut-off is derived based on a pre-determined number of estimated standard deviations above the estimated mean MFI-bg in the seronegative population (21). Mixture models work best when multiple components are visually discernible. Multiple components may not be observed in very high or low transmission settings, since everyone is either exposed or not and therefore any derived cut-off value may not be meaningful. Similarly, with the mixture model approach, depending on the degree of separation of the distributions, the obtained cut-off values may misclassify individuals if the indeterminant range is too wide. An alternative approach uses classification probabilities to determine appropriate cut-off values (22). Mixture models have been applied in numerous public health settings such as estimating exposure for chikungunya (23), assessing seroprotection to vaccine preventable diseases, understand spatial distribution of malaria (24), and defining programmatic seropositivity thresholds for trachoma (25).

Approach 3 – Quantiles (Q): The quantiles method determines the cut-off value by employing rank statistics to partition MFI values into equal probabilities, where higher quantiles may be interpreted as seropositive and lower quantiles as seronegative. For example, quantiles have been used to interpret viral loads, where higher quantiles correspond with seropositive individuals (26). However, quantiles necessitate investigators to determine the appropriate number of breaks based and specify which

quantiles are considered to be seropositive and seronegative. Assumptions of homogeneity within exposure groups determined by quantiles may also lead to inaccurate estimations (27).

Data collection

Data previously collected as part of cross-sectional surveys in Haiti and Malaysia were used to demonstrate the impact of the choice of cut-off method on subsequent seroprevalence estimates using multiplex technology (23, 28).

Briefly, in Haiti, blood samples were collected from 4438 individuals (ages 1-99 years) using a two-stage cluster random sampling in 117 enumeration areas (EA) across the country in 2015. 15 households were sampled randomly per EA and all family members were tested (23). Samples were assayed using the BioPlex-200 (Bio-Rad, Hercules, CA, USA). In Malaysia, a non-self-weighting two stage sampling design was used to sample 180 villages in four districts of northern Sabah in 2015. Twenty households were selected randomly from each village, and blood samples were collected from 10,100 people (age 3 months-105 years). Samples were assayed using the MAGPIX (Luminex Corporation, Austin, TX, USA) (23). Both surveys were designed based on malaria risk profiles in the target populations.

Here, we investigated IgG antibody responses to four antigens from three pathogens in samples from both settings. Antigens in both screening panels included LF Wb123 and Bm14, Ss NIE and Ct Pgp3. The use of these antigens has previously been described (29-31). Presumed seronegative populations comprising of United States adults (32) and United Kingdom residents (28) were assayed concurrently with the Haitian and Malaysian populations, respectively.

Statistical Analysis

For each case study, cut-off values from natural log transformed MFI-background (MFI-bg) were calculated in Rstudio to generate estimates for presumed unexposed populations (mean plus three standard deviations), quartiles, and 2 component mixture models (33), respectively. Quartiles were chosen based on their frequent use in categorizing continuous data, with the highest quartile being positive. While the observed single distributions of the MFI-bg values would make the use of mixture models inappropriate for many of these antigens, mixture models were nonetheless included to demonstrate the resulting cut-offs and seroprevalence estimates when applied to this type of distribution.

Next, to determine any potential programmatic implications, with the data from Haiti, seroprevalence estimates at the administrative departmental level per antigen were calculated per cut-off method and ranked according to magnitude. Spearman's rank correlation coefficient was calculated to assess any impact on how surveillance or intervention resources may be differentially prioritized due to the choice of cut-off method. Specifically, if the resulting prevalence estimates were different, but the 'high' and 'low' burden areas consistently ranked the same, there would likely be minimal impact on which areas would receive interventions under the assumption that programmatic decisions are based on targeting the highest burden areas and not those with a specific seroprevalence threshold. Finally, to provide a visual example of the impact of cut-off values on household-level seroprevalence estimates, households in the Malaysian study site were mapped for the selected antigens using PU and MM methods. For visualization, Jenks natural breaks for PU were calculated and applied to both presumed unexposed and mixture model prevalence estimates, and mapped using ArcGIS, Version 10.5 (Redlands, CA: Environmental Systems Research Institute).

Ethics Approval

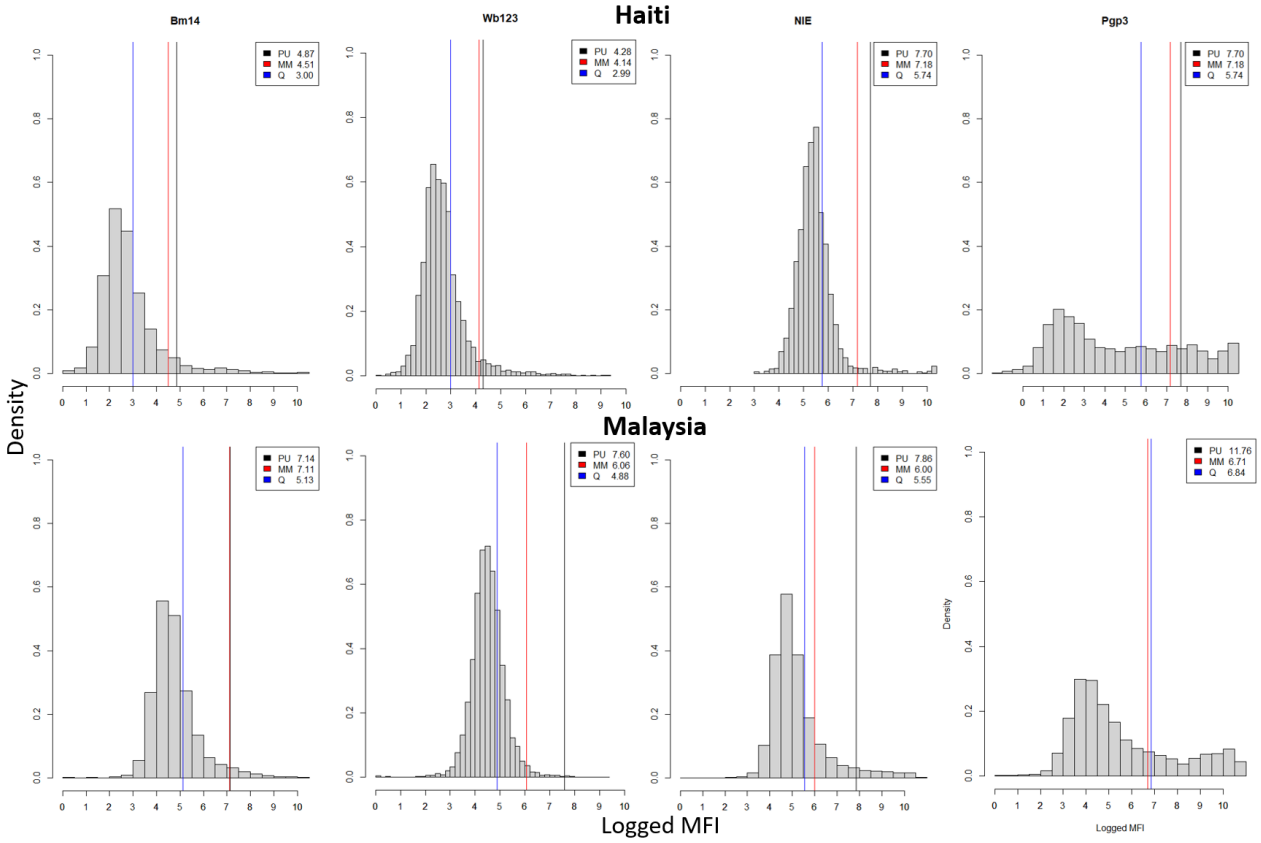
The National Ethics Committee (Comité National de Bioéthique - MSPP) from the Haitian Ministry of Health and the Internationale des Services Publics (PSI) Ethics Committee (PSI REB) based in Washington DC approved the Malaria Tracking Results Continuously (TRaC) 2015 Study. All standard research ethics policies and procedures were adhered to. The Medical Research Sub-Committee of the Malaysian Ministry of Health (NMRR-14-713-21117) and the Research Ethics Committee of the London School of Hygiene and Tropical Medicine (8340) approved the Malaysian study.

Written informed consent was obtained from all study participants prior to testing and household questionnaire administration. For both studies, Centers for Disease Control and Prevention (CDC) investigators were not considered to be engaged in human subjects research.

Results

The distribution of logged MFI-bg values and corresponding cut-offs according to the different methods tested are presented per antigen (Figure 1) with accompanying prevalence estimates (Table 1). In both settings, PU method generated the highest cut-offs resulting in the lowest number of seropositives compared to all other methods, but the magnitude of this difference was antigen specific. In Haiti, a

difference of only 1.05 logged MFI between cut-off values for the LF Bm14 antigen was observed when comparing the PU and MM methods resulting in a 14% relative difference in prevalence estimates. Similar results were observed with the Malaysian dataset, where for LF Bm14, a difference of 1.72 logged MFI in cut-off when comparing PU and MM methods also resulted in a 14% difference in prevalence. For LF Wb123 antigen, prevalence estimates in Haiti were similar for PU (5.21%) and MM (7.92%) but were significantly higher using Q (25.12%). For LF Wb123 in Malaysia, with a larger difference in cut-off values (2.30 logged MFI), prevalence estimates differed considerably when comparing the PU (5.21%) to MM (24.36%) and Q (25.06%). For Ss NIE, estimates also ranged by cut-off approach in both settings (Haiti range: 2.95%-26.18%, Malaysia range: 2.95%-25.05%, Table 2). For Ct Pgp3 antigen in Haiti, high prevalence estimates were identified for PU (44.93%), MM (50.23%), and Q (26.18%). In Malaysia, prevalence estimates for Ct Pgp3 were particularly distinct between PU (0%, cut-off value = 11.76 logged MFI), MM (43.87%, cut-off value = 5.1 logged MFI), and Q (25.02%, cut-off value = 6.84 logged MFI).



F3. 2 **Figure 1. Seropositivity cut-offs based on method with underlying histogram of logged MFI-bg values.**

Four antigens (LF Bm14 and LF Wb123 in Panel A, Ss NIE and Ct Pgp3 in Panel B) that were present in both data sets were selected to illustrate differences in cut-off values between settings on a natural log scale. The various lines mark different calculated cut-off methods in this case study and their respective cut-off value. The solid black lines are cut-offs from presumed unexposed populations, red solid lines are cut-offs from mixture model, blue solid lines represent the median, and the dotted blue lines represent upper and lower quartiles around the median of the quantile approach. For Pgp3 in Malaysia, the cut-off derived from all aged UK presumed unexposed population was 11.76, which is well above the distribution of Pgp3 presented. The differences in underlying distributions in comparing Haiti to Malaysia could be due to the different transmission intensities but most likely reflect differences in bead batches and instruments that were used in Haiti and Malaysia studies. Therefore, a direct comparison is not appropriate.

T3. 4 **Table 1. Comparison of cut-off methods for example neglected tropical diseases with corresponding prevalence estimates.**

Cut-off values in logged MFI-bg from using presumed unexposed populations, mixture models, and 75th percentile from quartiles using MBAs are presented for comparison. Cut-off values and the corresponding prevalence estimate in parentheses are presented to visualize the impact of differences between cut-off values.

Disease	Antigen	Presumed Unexposed	Mixture Model	Quartiles (75th percentile)
Haiti				
Lymphatic Filariasis	Bm14	4.87 (5.7%)	4.51 (7.6%)	3.00 (25.4%)
Lymphatic Filariasis	Wb123	4.28 (5.2%)	4.14 (5.9 %)	2.99 (25.4%)
Strongyloidiasis	*Ss*-NIE	7.70 (2.2%)	7.18 (2.9%)	5.74 (25.1%)
Chlamydia/Trachoma	Pgp3	10.52 (0%)	3.96 (50.0%)	7.09 (25.0%)
Malaysia				
Lymphatic Filariasis	Bm14	7.14 (2.4%)	7.11 (3.6%)	5.13 (25.0%)
Lymphatic Filariasis	Wb123	7.60 (0.2%)	6.06 (1.7%)	4.88 (24.9%)
Strongyloidiasis	*Ss* NIE	7.86 (5.31%)	6.00 (16.8%)	5.55 (25.0%)
Chlamydia/trachoma	Pgp3	11.76 (0%)	6.71 (25.9%)	6.84 (25.0%)

Prevalence estimates were calculated using PU, MM, and Q approaches per district in Haiti (Table 2). When ordering districts based on magnitude of disease burden, rankings were different for some antigens ($p<0.05$) but not for others when using the PU approach as a referent for comparison to MM

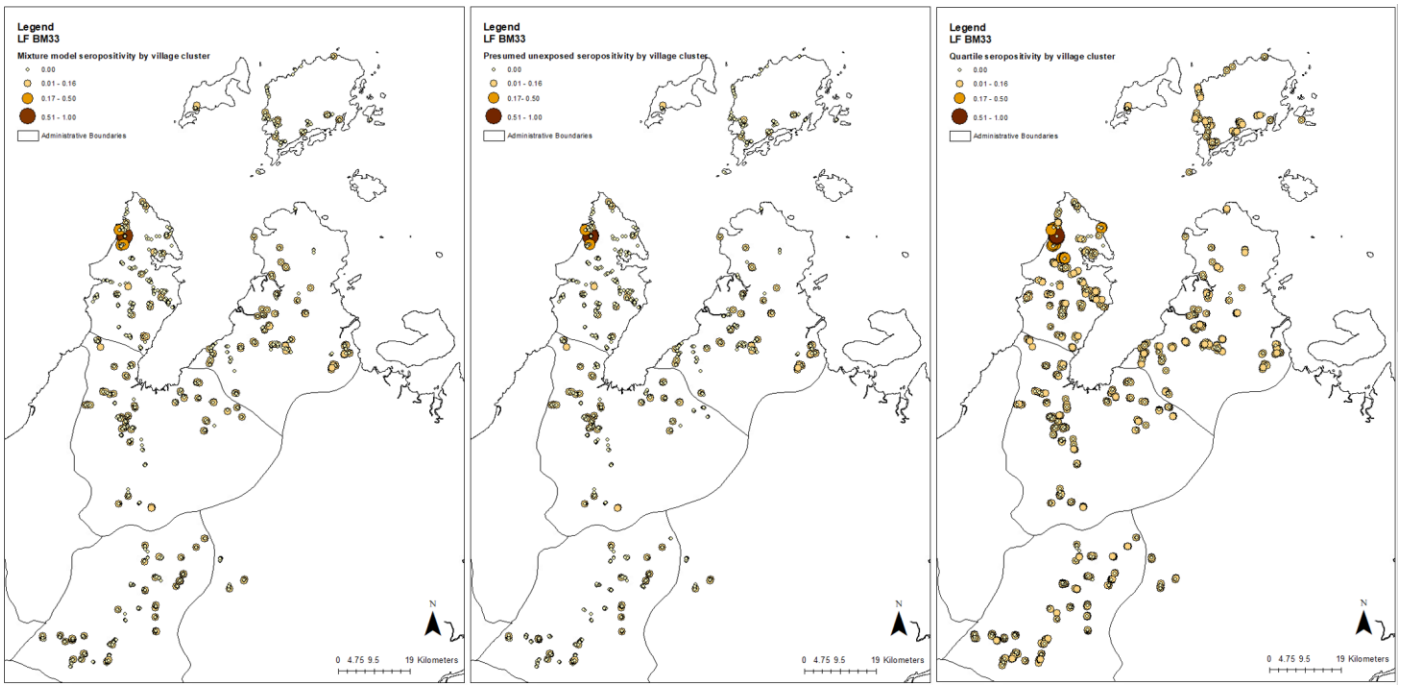
and Q. For example, in Haiti, the choice of cut-off method in ranking district prevalence estimates by district for LF Wb123, Bm14, and CT Pgp3 were generally consistent between two or all methods ($p<0.05$), respectively, but not for Ss NIE (Supplemental Table 1).

T3. 5 **Table 2. Seroprevalence estimates based on several districts in Haiti according to cut-off approach**

Prevalence estimates are presented in percentages with the corresponding confidence intervals in parentheses. The upper quartile was used for the quantile cut-off approach. Presumed unexposed population cut-off method was used as a reference cut-off method for a comparison test using Spearman's Rank Correlation, with accompanying p-values and Spearman's Rank Correlation coefficient.

Antigens by Department	Aire-Met	Arti-bonite	Centre	Grand Anse	Nippes	Nord	Nord Est	Nord Ouest	Quest Sans AM	Sud	Sud Est	Spearman's Rank Correlation Coefficient and P-values
Bm14												
Presumed Unexposed	7.7 (6.6-8.5)	6.9 (6.2-7.6)	2.1 (1.7-2.5)	5.0 (4.4-5.6)	4.8 (4.2-5.4)	7.2 (6.4-7.9)	0.7 (0.5-1.0)	6.6 (5.8-7.3)	4.6 (4.0-5.2)	3.8 (3.2-4.3)	3.6 (3.1-4.1)	Ref
Mixture Model	10.9 (9.9-11.8)	8.9 (8.1-9.7)	3.2 (2.7-3.7)	6.4 (5.7-7.1)	5.5 (4.8-6.2)	10.1 (9.2-10.9)	1.3 (1.0-1.6)	8.0 (7.2-8.8)	5.9 (5.2-6.6)	5.3 (4.6-5.9)	4.0 (3.4-4.6)	ρ = 0.99 p <0.000
Quantiles	36.9 (35.5-38.3)	25.9 (24.6-27.2)	14.9 (13.9-15.9)	17.3 (16.2-18.4)	13.1 (12.1-14.1)	29.2 (27.9-30.5)	9.8 (8.9-10.7)	25.3 (24.4-26.9)	25.7 (24.4-26.6)	21.3 (20.1-22.5)	15.2 (14.1-16.3)	ρ = 0.84 p = 0.003
Wb123												
Presumed Unexposed	4.5 (3.9-5.1)	5.5 (4.8-6.2)	3.2 (2.7-3.7)	2.3 (1.9-2.7)	3.4 (2.9-3.9)	7.9 (7.1-8.7)	1.7 (1.3-2.1)	5.5 (4.8-6.2)	9.9 (9.0-10.8)	1.5 (1.1-1.9)	1.8 (1.4-2.2)	Ref
Mixture Model	5.2 (4.5-5.9)	6.3 (5.6-7.0)	3.2 (2.7-3.7)	2.3 (1.8-2.7)	3.4 (2.9-3.9)	8.7 (7.9-9.5)	3.4 (2.9-3.9)	6.1 (5.4-6.8)	11.1 (10.2-12.0)	2.1 (1.7-2.5)	2.2 (1.8-2.6)	ρ = 0.93 p <0.000
Quantiles	25.8 (24.5-27.1)	26.3 (25.0-27.6)	29.0 (27.6-30.3)	17.7 (16.6-18.8)	13.8 (12.8-14.8)	28.7 (27.4-30.0)	14.3 (13.3-15.3)	22.2 (20.9-23.4v	34.5 (33.1-35.9)	17.9 (16.8-19.0)	22.3 (21.1-23.5)	ρ = 0.62 p = 0.040
SS NIE												
Presumed Unexposed	3.0 (2.3-3.7)	0.3 (0.1-0.5)	4.0 (3.2-4.8)	3.0 (2.3-3.7)	3.0 (2.3-3.7)	4.0 (3.2-4.8)	1.7 (1.2-2.2)	0.9 (0.5-1.3)	0.3 (0.1-0.5)	5.0 (4.1-5.9)	1.9 (1.3-2.5)	Ref
Mixture Model	3.5 (2.7-4.3)	0.3 (0.1-0.5)	4.5 (3.6-5.4)	4.5 (3.6-5.4)	3.0 (2.3-3.7)	6.0 (5.0-6.9)	2.5 (1.9-3.1)	1.8 (1.2-2.4)	1.4 (0.9-1.9)	5.0 (4.1-5.9)	3.2 (2.5-3.9)	ρ= 0.94 p <0.000
Quantiles	24.5 (22.7-26.3)	19.6 (17.9-21.2)	40.4 (38.4-42.4)	41.8 (39.8-43.8)	18.2 (16.6-19.8)	20.8 (19.9-20.2)	27.7 (25.8-29.6)	18.6 (17.0-20.2)	26.1 (24.3-27.9)	15.0 (13.5-16.5v	21.2 (19.5-22.9)	ρ= -0.09 p = 0.7979
Pgp3												
Presumed Unexposed	44.1 (42.4-45.7)	47.2 (45.4-48.9)	52.0 (50.2-53.7)	58.2 (56.5-59.8)	38.3 (36.6-39.9)	54.0 (52.3-55.6)	43.8 (42.1-45.4)	54.3 (52.6-55.9)	42.8 (41.1-44.4)	38.6 (36.9-40.2)	50.9 (49.1-52.6)	Ref
Mixture Model	47.0 (45.3-48.6)	49.3 (47.5-51.0)	56.1 (54.4-57.7)	62.7 (61.0-64.3)	41.7 (40.0-43.3)	56.8 (55.1-58.4)	43.8 (42.1-45.4)	57.1 (55.4-58.7)	45.9 (44.2-47.5)	42.6 (40.9-44.2)	52.2 (50.4-53.9)	ρ = 0.99 p <0.000
Quantiles	23.8 (22.3-25.2)	26.3 (24.8-27.7)	28.3 (26.7-29.8)	37.3 (35.6-38.9)	15.0 (13.7-16.2)	27.2 (25.6-28.7)	20.0 (18.6-21.3)	30.4 (28.8-31.9)	22.0 (20.5-23.4)	20.8 (19.4-22.1)	25.4 (23.9-26.8)	ρ = 0.95 p <0.000

To further illustrate how prevalence estimates may vary at a more granular spatial scale, prevalence by household clusters were mapped in Sabah Malaysia for two antigens, comparing two cut-off methods, PU and MM. Upon visual inspection, household clusters with seroprevalence greater than 25% were similar in both methods for LF Bm14 (Figure 2). For areas of zero versus low prevalence, levels of seropositivity were considerably different between the two cut-off methods for both antigens. For example, while the PU method classified certain household clusters of having zero seroprevalence for all antigens, the MM method instead classified these areas of ranging from low to high seropositivity (Figure 2).



F3. 3 **Figure 2. Seropositivity by two cut-off methods for LF Bm14 in Malaysian district.**

Cut-offs were generated using PU of all aged individuals from United States adults for Haiti (A), MM (B), and Q. Seroprevalence by household clusters are presented. Small dark red dots represent absence of disease, while yellow dots indicate very low prevalence. Household clusters of increasing seroprevalence were enlarged for better visualization of the influence of cut-off methods on seroprevalence.

Discussion

Serological surveys with MBAs provide an efficient method for describing population-level burden for multiple diseases simultaneously and could support an integrated NTD surveillance platform (1, 10, 34).

However, interpreting serological data for a programmatic context is not straightforward. For example, higher antibody concentrations may represent more recent or repeated exposure (35, 36), while specific antibody kinetics may reflect historical exposure or current infection. To alleviate some of these challenges, values of raw antibody concentrations converted into a standard metric such as seropositivity can be advantageous and aid interpretation. However, given the inconsistent application of various cut-off approaches, it is imperative to investigate any resulting implications for applying these approaches to the context of public health.

The results of this work demonstrate that seroprevalence at the study level, as well as when aggregating estimates to the departmental and household-level, could be influenced by choice of cut-off method applied. Overall differences in the prevalence estimates according to cut-off approach typically varied per antigen with some differences being as small as less than two percent, and other differences being larger than twenty five percent. We observed that the PU method generated the highest cut-offs, which resulted in the lowest seroprevalence estimates in both Haiti and Malaysia. This may be due to different disease exposures between PU and sample populations (18). Upper quartiles often led to the highest prevalence estimates for all antigens, which may have been impacted by the statistical methodology that arbitrarily selects approximately a quarter of the population as seropositive (27).

In control settings, program interventions often occur when prevalence estimates exceed a defined threshold, such as is the case with LF transmission assessment surveys (37). Additionally, in resource constrained settings, treatment may be prioritized based on areas of high burden. To understand whether the choice of cut-off method and the corresponding difference in estimated seroprevalence would identify similar areas of high and low prevalence, we examined district rankings in Haiti and household clusters in Malaysia. In Haiti, conserved departmental rankings of prevalence estimates were observed for LF Bm14 and Ct Pgp3 antigens when employing the PU, MM and Q methods. For LF Wb123 and Ss NIE, PU and MM methods also lead to similar rankings, but not for Q. In Sabah, Malaysia, all methods identified similar areas of high seroprevalence village clusters for LF Bm14 (>25%) but were different in areas of low or zero prevalence.

The variation of the described seropositivity due to cut-off methods becomes particularly critical if it leads to disparate decisions being made for control or elimination activities with interventions either potentially scaled up or removed erroneously. As we have shown here, some inconsistency in overall prevalence estimates, as well as estimates in the rankings of district-level and household cluster-level among different approaches suggest that identifying a cut-off approach that can be uniformly applied

per antigen will help to ensure accurate interpretation of the data and lend confidence to any resulting programmatic decisions.

Within our study there are limitations. Data from Haiti and Malaysia were presented concurrently, however, differences in laboratory techniques and epidemiological settings between the two studies may impact direct comparisons. For the comparison using crude seroprevalence rankings in Haiti, a more formative comparison would entail using prevalence thresholds defined by program targets to determine these rankings. We also lacked any international standards and did not include receiver operating characteristic curves as a gold standard to compare our cut-off values, which limits conclusions to a best approach per antigen. Additional limitations within the case study includes using all ages to determine cut-offs from presumed negative populations that may not be appropriate for Ct Pgp3 given cross-reactivity with chlamydia and applying mixture models to single antigen distributions that may bias conclusions.

Despite these limitations, our study demonstrates the potential impact of choice of cut-off approaches on seroprevalence estimates. Future studies comparing cut-off approaches should consider the aforesaid limitations of this study. They should also compare other cut-off methods not included in this study that may provide additional insight toward appropriate cut-offs per antigen, such as receiver operating characteristic curves (38). A universal approach and standard to calculating cut-offs per antigen, and ideally applicable across MBA platforms, is needed to ensure consistent reporting and support decision making. While each method has limitations, using modelling approaches may generate less bias cut-offs but may not be appropriate in highly skewed transmission settings. Programs implementing serological platforms to monitor multiple pathogens are advised to consider the kinetics of the individual antigens assessed, specific pathogen biology, site-specific transmission settings, and available resources in determining an appropriate cut-off method.

Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention. Use of trade names is for identification only and does not imply endorsement by the Public Health Service or by the U.S. Department of Health and Human Services.

Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention. Use of trade names is

for identification only and does not imply endorsement by the Public Health Service or by the U.S. Department of Health and Human Services.

References

1. Arnold BF, Scobie HM, Priest JW, Lammie PJ. Integrated Serologic Surveillance of Population Immunity and Disease Transmission. Emerg Infect Dis. 2018;24(7):1188-94.
2. Hotez PJ, Fenwick A, Savioli L, Molyneux DH. Rescuing the bottom billion through control of neglected tropical diseases. Lancet. 2009;373(9674):1570-5.
3. Hotez PJ. Ten failings in global neglected tropical diseases control. PLoS Negl Trop Dis. 2017;11(12):e0005896.
4. Gyapong JO, Gyapong M, Yellu N, Anakwah K, Amofah G, Bockarie M, et al. Integration of control of neglected tropical diseases into health-care systems: challenges and opportunities. Lancet. 2010;375(9709):160-5.
5. Zhou XN, Bergquist R, Tanner M. Elimination of tropical disease through surveillance and response. Infect Dis Poverty. 2013;2(1):1.
6. Dowdle WR, Cochi SL. The principles and feasibility of disease eradication. Vaccine. 2011;29 Suppl 4:D70-3.
7. Bergquist R, Yang GJ, Knopp S, Utzinger J, Tanner M. Surveillance and response: Tools and approaches for the elimination stage of neglected tropical diseases. Acta Trop. 2015;141(Pt B):229-34.
8. Bolotin S, Lim G, Dang V, Crowcroft N, Gubbay J, Mazzulli T, et al. The utility of measles and rubella IgM serology in an elimination setting, Ontario, Canada, 2009-2014. PLoS One. 2017;12(8):e0181172.
9. FIscher T. Luminex 2018 [cited 2018 October 26]. Available from: https://www.thermofisher.com/uk/en/home/life-science/protein-biology/protein-assays-analysis/luminex-multiplex-assays.html.
10. Lammie PJ, Moss DM, Brook Goodhew E, Hamlin K, Krolewiecki A, West SK, et al. Development of a new platform for neglected tropical disease surveillance. Int J Parasitol. 2012;42(9):797-800.
11. Moss DM, Priest JW, Boyd A, Weinkopff T, Kucerova Z, Beach MJ, et al. Multiplex bead assay for serum samples from children in Haiti enrolled in a drug study for the treatment of lymphatic filariasis. Am J Trop Med Hyg. 2011;85(2):229-37.
12. Poirier MJ, Moss DM, Feeser KR, Streit TG, Chang GJ, Whitney M, et al. Measuring Haitian children's exposure to chikungunya, dengue and malaria. Bull World Health Organ. 2016;94(11):817-25A.
13. Kucharski AJ, Kama M, Watson CH, Aubry M, Funk S, Henderson AD, et al. Using paired serology and surveillance data to quantify dengue transmission and control during a large outbreak in Fiji. Elife. 2018;7.
14. Arnold BF, van der Laan MJ, Hubbard AE, Steel C, Kubofcik J, Hamlin KL, et al. Measuring changes in transmission of neglected tropical diseases, malaria, and enteric pathogens from quantitative antibody levels. PLoS Negl Trop Dis. 2017;11(5):e0005616.
15. Scobie HM, Mao B, Buth S, Wannemuehler KA, Sorensen C, Kannarath C, et al. Tetanus Immunity among Women Aged 15 to 39 Years in Cambodia: a National Population-Based Serosurvey, 2012. Clin Vaccine Immunol. 2016;23(7):546-54.
16. Metcalf CJ, Farrar J, Cutts FT, Basta NE, Graham AL, Lessler J, et al. Use of serological surveys to generate key insights into the changing global landscape of infectious disease. Lancet. 2016;388(10045):728-30.
17. Determining Seropositivity - A Review of Approaches to Define Population Seroprevalence when using Multiplex Bead Assays to Assess Burden of Tropical Diseases. Plos Neglect Trop D. 2021.
18. Fujii Y, Kaneko S, Nzou SM, Mwau M, Njenga SM, Tanigawa C, et al. Serological surveillance development for tropical infectious diseases using simultaneous microsphere-based multiplex assays and finite mixture models. PLoS Negl Trop Dis. 2014;8(7):e3040.

19. Arnold BF, Martin DL, Juma J, Mkocha H, Ochieng JB, Cooley GM, et al. Enteropathogen antibody dynamics and force of infection among children in low-resource settings. 2019:522920.
20. Hamlin KL, Moss DM, Priest JW, Roberts J, Kubofcik J, Gass K, et al. Longitudinal monitoring of the development of antifilarial antibodies and acquisition of Wuchereria bancrofti in a highly endemic area of Haiti. PLoS Negl Trop Dis. 2012;6(12):e1941.
21. Hardelid P, Williams D, Dezateux C, Tookey PA, Peckham CS, Cubitt WD, et al. Analysis of rubella antibody distribution from newborn dried blood spots using finite mixture models. Epidemiol Infect. 2008;136(12):1698-706.
22. Sepulveda N, Stresman G, White MT, Drakeley CJ. Current Mathematical Models for Analyzing Anti-Malarial Antibody Data with an Eye to Malaria Elimination and Eradication. J Immunol Res. 2015;2015:738030.
23. Rogier EW, Moss DM, Mace KE, Chang M, Jean SE, Bullard SM, et al. Use of Bead-Based Serologic Assay to Evaluate Chikungunya Virus Epidemic, Haiti. Emerging Infectious Diseases. 2018;24(6):995-1001.
24. Assefa A, Ali Ahmed A, Deressa W, Sime H, Mohammed H, Kebede A, et al. Multiplex serology demonstrate cumulative prevalence and spatial distribution of malaria in Ethiopia. Malar J. 2019;18(1):246.
25. Migchelsen SJ, Martin DL, Southisombath K, Turyaguma P, Heggen A, Rubangakene PP, et al. Defining Seropositivity Thresholds for Use in Trachoma Elimination Studies. PLoS Negl Trop Dis. 2017;11(1):e0005230.
26. Boni MF, Chau NV, Dong N, Todd S, Nhat NT, de Bruin E, et al. Population-level antibody estimates to novel influenza A/H7N9. J Infect Dis. 2013;208(4):554-8.
27. Bennette C, Vickers A. Against quantiles: categorization of continuous variables in epidemiologic research, and its discontents. Bmc Med Res Methodol. 2012;12.
28. Fornace KM, Brock PM, Abidin TR, Grignard L, Herman LS, Chua TH, et al. Environmental risk factors and exposure to the zoonotic malaria parasite Plasmodium knowlesi across northern Sabah, Malaysia: a population-based cross-sectional survey. Lancet Planet Health. 2019;3(4):e179-e86.
29. Goodhew EB, Priest JW, Moss DM, Zhong G, Munoz B, Mkocha H, et al. CT694 and pgp3 as serological tools for monitoring trachoma programs. PLoS Negl Trop Dis. 2012;6(11):e1873.
30. Won KY, Robinson K, Hamlin KL, Tufa J, Seespesara M, Wiegand RE, et al. Comparison of antigen and antibody responses in repeat lymphatic filariasis transmission assessment surveys in American Samoa. Plos Neglect Trop D. 2018;12(3).
31. Priest JW, Jenks MH, Moss DM, Mao B, Buth S, Wannemuehler K, et al. Integration of Multiplex Bead Assays for Parasitic Diseases into a National, Population-Based Serosurvey of Women 1539 Years of Age in Cambodia. Plos Neglect Trop D. 2016;10(5).
32. Rogier E, Wiegand R, Moss D, Priest J, Angov E, Dutta S, et al. Multiple comparisons analysis of serological data from an area of low Plasmodium falciparum transmission. Malar J. 2015;14:436.
33. Benaglia T, Chauveau D, Hunter DR, Young DS. mixtools: An R Package for Analyzing Mixture Models. 2009. 2009;32(6):29.
34. Fritzell C, Rousset D, Adde A, Kazanji M, Van Kerkhove MD, Flamand C. Current challenges and implications for dengue, chikungunya and Zika seroprevalence studies worldwide: A scoping review. PLoS Negl Trop Dis. 2018;12(7):e0006533.
35. Wong J, Hamel MJ, Drakeley CJ, Kariuki S, Shi YP, Lal AA, et al. Serological markers for monitoring historical changes in malaria transmission intensity in a highly endemic region of Western Kenya, 1994-2009. Malar J. 2014;13:451.
36. Drakeley CJ, Corran PH, Coleman PG, Tongren JE, McDonald SL, Carneiro I, et al. Estimating medium- and long-term trends in malaria transmission by using serological markers of malaria exposure. Proc Natl Acad Sci U S A. 2005;102(14):5108-13.

37. Shamsuzzaman AK, Haq R, Karim MJ, Azad MB, Mahmood AS, Khair A, et al. The significant scale up and success of Transmission Assessment Surveys 'TAS' for endgame surveillance of lymphatic filariasis in Bangladesh: One step closer to the elimination goal of 2020. PLoS Negl Trop Dis. 2017;11(1):e0005340.
38. Zweig MH, Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. Clin Chem. 1993;39(4):561-77.

3.3.1 Research Conclusions

In continuation with the theme of this chapter, this work investigated potential programmatic implications based on choice of cut-off approaches identified within the literature review of the research paper 1 and addressed the second outcome of **Objective 1**. To do this, I compared three different cutoff approaches: presumed negatives, mixture models, and quantiles in Haiti and Malaysia.

The analysis of this work demonstrated the possibility of dissimilar prevalences estimates based on choice of cut-off approaches. These differences can have potential implications on susequent public health interventions. Importantly, because of this potential impact of cut-off approaches on seroprevalence, there is a need for universal approaches or international standards to determining seropostivity to avoid misinterreputation of MFI values. Despite not having current standard appraoches, assessing antibody distributions and biological factors of transmission can help to identify apporpriate cut-off approaches per antigen to produce exposure estimates using serological MBAs. For example, antibody responses showing distinct bimodial distributions may implore the application of mixure models to determine cut-offs given the indicated seronegative and seropositive populations, while high exposure resulting in a single distribution may necessitate identifying or using presumed negative populations.

3.4 Chapter Conclusions

Summary

This chapter addressed **Objective 1.** Converting MFI values into data that reflects infection and exposure within the community of interest to inform programmatic action is an important preliminary step to assessing the utility of MBAs. While seroprevalence is a popular health metric used to understand antibody concentrations, how to accurately determine seroprevalence using MFI values remains a challenge as there are different statistical approaches and uncertainty around which approach accurately reflects biological trends per antigen.

To address this knowledge gap, I investigated the literature and identified seven applied cut-off approaches, each with individual strengths and limitations. For example, ROC curves can generate highly robust cut-offs but are dependent on the availability of clinically confirmed positives and negatives that are ideally from the same geographic region of the sample population. Modelling approaches can also produce robust cut-offs but may not be appropriate in extremely high or low transmission settings due to unimodal antibody distributions in the population that can cause challenges in interpreting overlapping seronegatives and seropositives. Presumed negative approach is useful in high transmission settings, but differences in background immunity between sample and control populations may lead to inaccurate estimation of disease burden.

The strengths and limitations of the different methods can be surmised to be one of several reasons as to why cut-off approaches are observed to be applied inconsistently among different antigens and different settings. Inconsistent application of cut-off approaches may become concerning if they were to result in different prevalence estimates that influence decision making. To investigate the potential impact of cut-off approaches on seroprevalence, I applied several different methods identified in the literature review to four antigens of three pathogens within two case studies. The results demonstrated that choice of cut-off methods had some impact on prevalence estimates that could potentially lead to disparate public health action.

Given the different approaches of determining seroprevalence and their possible consequences on subsequent public health action, the research from this chapter highlights the importance of having a standard approach that would allow for consistency in data interpretation. The establishment of standard approaches would preferably take into consideration program or research goals. For example,

programs aiming to detect actives cases may need to implement ROC curves based on clinical infection data from respective endemic countries to determine reliable cut-offs, as the antibody responses reflect those with infection within a respective environment. For programs aiming to understand exposure or possible transmission, modelling approaches with appropriate age-restricted endemic populations (assumed to not be exposed) may be used as a consistent approach across NTDs. International standard units could potentially eliminate the need for cut-off approaches as there would be a universal cut-off value, however, determining such a cut-off (and for each individual antigen) would necessitate sera from enough endemic populations to be applicable to global settings, and should also include different cut-offs based on program goals. Without standard approaches, potential influencing factors have been discussed by various authors that may help discern appropriate choice of cut-off approaches. These diverse factors ranged from pathogen biology to programmatic use-case scenarios to antibody dynamics that may help to guide the use of specific methods or presumed unexposed endemic cohorts (e.g., children or infants) to determine sensible cut-offs.

Programmatic interpretation of MFI values and choice of cut-off approach may also be impacted by specific antibody marker types as exposure or infection, with some of these kinetics currently being studied. For example, 30% of the population seropositive to an exposure marker versus 30% of the population seropositive to an active infection marker will likely have very different implications for interventions. Understanding the kinetics of these antibodies, ideally using longitudinal data with continuous MFI values to capture changes in antibodies over time, will help to identify these markers as well as the interpretation of the antigen. This will be further discussed in Chapter 6.

In integrated disease surveillance platform using serological MBAs, applying a consistent cut-off approach across all antigens may streamline the process of determining seropositivity for multi-disease panels, but may overlook antigen kinetics or biological factors of individual antigens. For example, using a presumed unexposed approach across all NTDs may not be suitable, as cross reactivity in some NTDs to venereal counterparts present in presumed unexposed populations could lead to elevated cut-offs. Therefore, identifying standard cut-off methods per antigen will help to simplify some of the complexities in determining seropositivity in integrated disease surveillance using serological MBAs.

Conclusions

Chapter 3 provided insight regarding appropriate methods to characterize MFI values from MBAs. Despite not having standard cut-off approaches, MBAs can still be used to generate prevalence

estimates with ROC curves or carefully considered cut-off approaches to support integrated surveillance. This requires assessing specific population antibody responses to determine appropriate cut-offs. First, study or surveillance goals may also help to justify decision of which cut-off approach would be appropriate. Second, visualizing the data and inspecting the distribution(s) will help to discern seronegative/ seropositive populations and which cut-off approaches are suitable given the distribution(s). Third, understanding transmission biology and age patterns will help to determine ideal populations to use for chosen cut-off approach. In the subsequent chapters I will primarily utilize mixture models to determine seroprevalence through careful inspection of the data.

While seropositivity is programmatically intuitive, there are several anticipated challenges and considerations moving forward with the use of this health metric. All cut-off approaches may have some imperfections in determining appropriate cut-offs that may result in potential difficulty in classifying individuals close to the point at which seropositive cut-offs are calculated. A concern is the possibility of a type 2 statistical error, or false negative, where specificity of cut-offs fails to capture some true positives. This may be particularly important where certain programs need to correctly identify those with diseases. Conversely, antibody transmission dynamics may make it difficult to assess some false high-level antibody response, as discussed in the review of literature. Residual antibody responses, even after cleared infections, may be still circulating for extended periods of time (8). This may not be capture by a strict-off approach and can lead to potential false positives. A consideration to help address this challenge is to apply different statistical stringencies in certain cut-off approaches based on program or study goals, such as setting probability priors or an expected “indeterminate range” to account for individuals within the population where serostatus is difficult to classify (9). A programmatically conservative approach may consider this indeterminate range as seropositive. Additionally, determining uncertainty around strict cut-offs values (such as calculating a confidence interval) per cut-approach may also help to account for potential overlap in serostatus and may be a topic for future research.

It is possible in multi-disease antigen panels that differing opinions about ideal cut-off approaches pertain to the different expectations of how the data should be analysed. For example, some collaborators may prefer to focus on potential exposure, while others may be interested in assessing the potential for ongoing infection of certain pathogens. As result, in multi-disease panels, different cut-off approaches per antigen may complicate or delay initial analysis of MFI data and reiterates the need for standard cut-off approaches (per antigen, but not necessarily one method for all antigens) or

international units. Additionally, antigen specific analysis may continue until approaches become more widespread and standardised reagents become available.

For this thesis, the interpretation of results will focus on exposure, with acknowledgment of the discussed limitations and the potential of some undetectable of antibody levels immediately after exposure due to a lag in the initial immune system response. In the ensuing chapter, the next objective will utilize knowledge of different cut-off approaches, their advantages, and disadvantages, as well the limitations of using cut-offs in general, to the analysis of MFI data and to investigate MBA capacity to support public health surveillance.

3.5 References

1. Arnold BF, Scobie HM, Priest JW, Lammie PJ. Integrated Serologic Surveillance of Population Immunity and Disease Transmission. Emerg Infect Dis. 2018;24(7):1188-94.
2. Chen TH, Lee F, Lin YL, Pan CH, Shih CN, Tseng CH, et al. Development of a multiplex Luminex assay for detecting swine antibodies to structural and nonstructural proteins of foot-and-mouth disease virus in Taiwan. J Microbiol Immunol Infect. 2016;49(2):196-207.
3. Reder S, Riffelmann M, Becker C, Wirsing von Konig CH. Measuring immunoglobulin g antibodies to tetanus toxin, diphtheria toxin, and pertussis toxin with single-antigen enzyme-linked immunosorbent assays and a bead-based multiplex assay. Clin Vaccine Immunol. 2008;15(5):744-9.
4. Fujii Y, Kaneko S, Nzou SM, Mwau M, Njenga SM, Tanigawa C, et al. Serological surveillance development for tropical infectious diseases using simultaneous microsphere-based multiplex assays and finite mixture models. PLoS Negl Trop Dis. 2014;8(7):e3040.
5. Sea-Liang N, Sereemaspun A, Patarakul K, Gaywee J, Rodkvamtook W, Srisawat N, et al. Development of multiplex PCR for neglected infectious diseases. PLoS Negl Trop Dis. 2019;13(7):e0007440.
6. Ty Hang V, Minh Nguyet N, The Trung D, Tricou V, Yoksan S, Minh Dung N, et al. Diagnostic Accuracy of NS1 ELISA and Lateral Flow Rapid Tests for Dengue Sensitivity, Specificity and Relationship to Viraemia and Antibody Responses. Plos Neglect Trop D. 2009;3(1):e360.
7. Metcalf CJ, Farrar J, Cutts FT, Basta NE, Graham AL, Lessler J, et al. Use of serological surveys to generate key insights into the changing global landscape of infectious disease. Lancet. 2016;388(10045):728-30.
8. Goodhew EB, Priest JW, Moss DM, Zhong G, Munoz B, Mkocha H, et al. CT694 and pgp3 as serological tools for monitoring trachoma programs. PLoS Negl Trop Dis. 2012;6(11):e1873.
9. Migchelsen SJ, Martin DL, Southisombath K, Turyaguma P, Heggen A, Rubangakene PP, et al. Defining Seropositivity Thresholds for Use in Trachoma Elimination Studies. PLoS Negl Trop Dis. 2017;11(1):e0005230.
10. Voysey M, Sadarangani M, Pollard AJ, Fanshawe TR. Computing threshold antibody levels of protection in vaccine clinical trials: An assessment of methodological bias. PLoS One. 2018;13(9):e0202517.

CHAPTER 4: ASSESSING THE UTILITY OF SEROLOGICAL MULTIPLEX BEAD ASSAYS

4.1 Background and Rationale

Research Rationale

Although universal cut-offs or standard methods of determining seroprevalence using MFIs are currently unavailable for many NTDs, serologic MBAs can still ascertain exposure estimates to numerous diseases simultaneously, using approaches discussed in the previous chapter. As MBA capacity to monitoring numerous pathogens is an important feature of these platforms, determining their utility when applied to integrated monitoring of tropical diseases is needed to support current integrated disease initiatives. Studying population exposure of tropical pathogens in the same geographic region, or co-endemicity, can provide estimates where none currently exist and aid programs in facilitating joint interventions for different disease. Additionally, assessing potential risk factors impacting disease transmission may provide valuable information regarding at-risk populations and spatial targets, and changes in them over time (2-4). For example, identifying spatial clusters of high prevalence may lead to prioritized interventions in that area, or washing habits associated with high exposure of a pathogen(s) may result in education campaigns to avoid transmission and WASH control.

Overview

Chapter 4 focuses on **Objective 2** to apply serological MBAs to understand exposure of multiple diseases**.** This chapter consists of two research papers. Research Paper 3 examines MFI values from a national serosurvey in Haiti. The objectives of Paper 3 were to 1) demonstrate the utility of MBAs to capture co-endemicity by estimating seroprevalence to eleven pathogens and 2) assess any age-associated patterns on country-wide exposure patterns exposure based on age as a risk factor of cumulative exposure over time. Paper 4 transitions to a sub national spatial scale in Malaysia to

examine co-endemicity to five pathogens using MBA data in an ecologically stratified sampling strategy at a more granular spatial scale. The objectives of Paper 4 were to 1) determine seroprevalence of four pathogens to understand burden of co-endemicity in Sabah and 2) assess demographic, socio-economic, and spatial risk factors of exposure per antigen.

RESEARCH PAPER COVER SHEET

Please note that a cover sheet must be completed for each research paper included within a thesis.

SECTION A – Student Details

Student ID Number	1603698	**Title**	Mr
First Name(s)	YuYen		
Surname/Family Name	Chan		
Thesis Title	Application of multiplex bead serological assays to integrated monitoring of neglected tropical disease		
Primary Supervisor	Chris Drakeley		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?			
When was the work published?			
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion			
Have you retained the copyright for the work?*	Choose an item.	Was the work subject to academic peer review?	Choose an item.

*If yes, please attach evidence of retention. If no, or if the work is being included in its published format, please attach evidence of permission from the copyright holder (publisher or other author) to include this work.

SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	AJTMH
Please list the paper's authors in the intended authorship order:	YuYen Chan, Gretchen Cooley, Diana Martin, Kimberley Mace, Samuel E. Jean, Gillian Stresman, Chris Drakeley, Michelle Chang, Jean F. Lemoine, Venkatachalam Udhayakymar, Patrick J. Lammie, Jeffrey W. Priest, Eric Rogier

Stage of publication	**Not yet submitted**

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	Writing and drafting manuscript, analysis included descriptive analysis and assessing seropositivity to each antigen

SECTION E

Student Signature	YuYen Chan
Date	16/09/2021

Supervisor Signature	Chris Drakeley
Date	20/09/2021

4.2 Applying serological multiplex bead assays to understand burden of co-endemic neglected tropical diseases

Multiplex serology for measurement of IgG antibodies against eleven infectious diseases in a Haitian national serosurvey

YuYen Chan[1], Gretchen Cooley[2], Diana Martin[2], Kimberley Mace[2], Samuel E. Jean[3], Gillian Stresman[1], Chris Drakeley[1], Michelle Chang[2], Jean F. Lemoine[4], Venkatachalam Udhayakymar[2], Patrick J. Lammie[2], Jeffrey W. Priest[5], Eric Rogier[3]

1) The London School of Hygiene & Tropical Medicine, London, UK
2) Division of Parasitic Diseases and Malaria, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, GA
3) Population Services International/Organisation Haïtienne de Marketing Social pour la Santé, Port-au-Prince, Haiti
4) Programme National de Contrôle de la Malaria/MSPP, Port-au-Prince, Haiti
5) Division of Foodborne, Waterborne, and Environmental Diseases, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA

Background: Integrated means of surveillance for multiple diseases can be an efficient use of resources and advantageous for national public health programs. Detection of antibodies typically indicates previous exposure to a pathogen but can potentially also serve to assess active infection status, and serological multiplex bead assays have recently been developed to evaluate exposure to multiple antigenic targets simultaneously. Haiti is an island nation in the Caribbean region with multiple endemic infectious diseases, many of which have a paucity of data for population-level prevalence or exposure.

Methodology/Principle Findings: From December 2014 to February 2015, a nationwide serosurvey occurred in Haiti. Filter paper blood samples (n=4,438) were collected from participants in 117 locations and assayed on a multiplex bead assay containing 15 different antigens from 11 pathogens: *Plasmodium falciparum*, *Toxoplasma gondii*, lymphatic filariasis roundworms, *Strongyloides stercoralis*, chikungunya virus, dengue virus, *Chlamydia trachomatis*, *Treponema palladium*, enterotoxogenic *Escherichia coli*, *Entamoeba histolytica*, and *Cryptosporidium parvum*. Assay signal

was dichotomized to indicate if persons were IgG antibody positive (seropositive) or seronegative to antigens, and kept continuous to assess IgG antibody levels. Antibodies against each antigen provided different proportions of the Haitian population considered seropositive with antigens from *T. gondii*, *C. parvum*, dengue, chikungunya, and *C. tracomatis* showing the highest rates of seroprevalence. Antibody responses to *T. palladium*, and lymphatic filariasis were the lowest, with less than 5% of all samples IgG seropositive to antigens from these pathogens. Clear trends of increasing seropositivity with age was seen for all antigens except chikungunya and *E. histolytica*. **Conclusions/Significance:** Multiplex serological assays can provide a wealth of information about population exposure to different infectious diseases. For the Haitian data presented here, some of these infectious diseases had a paucity or complete absence of published serological studies in Haiti. Clear trends of disease burden with respect to age and location in Haiti can be used by national programs and partners for follow-up studies and intervention planning.

Introduction

Tropical and other infectious diseases cause high morbidity and mortality worldwide, and many are co-endemic due to socioeconomic, environmental, climatological, and many other factors (1). Epidemiology, control, and potential elimination of these diseases benefits from continued surveillance and monitoring for acute infection or past exposure. As symptomatic surveillance alone may not be a reliable indicator of infection for many tropical diseases, serological confirmation provides an effective way of estimating exposure within a population (2-6). Additionally, as infectious disease transmission is reduced in an area, standard diagnostic methods for many pathogens tend to provide less accurate estimates of true prevalence (7, 8). Serological assays that detect antibodies against pathogen-specific antigens are used for a variety of purposes such as providing history of infection of diseases within a population (9, 10), understanding transmission patterns (5), strategizing control and elimination efforts (11, 12), and assessing host immune status (13).

Conventionally, single-analyte detection methods such as western blotting, lateral flow assays (LFAs), or enzyme-linked immunosorbent assays (ELISAs) have been used to detect human antibodies against infectious disease antigens. The bead-based multiplex platform for detection and quantitation of antibodies against multiple antigens is efficient for the concurrent analysis of an individual's serological profile to numerous infectious diseases (2, 9, 13, 14). Additional benefits include the time and reduced costs of multiplexing targets for several pathogens compared to traditional single-plex assays while still remaining relatively easy to operate in a laboratory setting

(15, 16). Thus, multiplex assays can offer a practical and more comprehensive understanding of epidemiologic patterns and co-endemic burdens of infectious diseases for an area (13, 17).

In this current study, a multiplex bead assay (MBA) was utilized to assess IgG antibody levels for 4,438 blood samples collected during a Haitian national community-based household survey that took place from December 2014 to February 2015. The MBA included 15 antigenic targets encompassing 11 infectious diseases endemic to the nation of Haiti. Data is displayed to estimate department-level and national-level seroprevalence estimates and trends by age categories.

Methods

Sample Collection. The study protocol was approved by the Haitian Ministry of Health. Participant consent (and parental assent if under 15 years) was verbal. The Haitian population was sampled from December 2014 to February 2015 as part of the Global Fund program against Malaria (Round 8) implemented by Population Services International (PSI) Haiti as Principal Recipient. Sampling sites throughout the country (*sections d'énumération*, SDE) were chosen on a proportional sampling by predicted malaria risk strata within the country as had been determined by predictive modeling (18). A target of 20 households were randomly selected by field teams within each SDE, and all members of the household were offered the opportunity to participate. Blood was collected by fingerprick on Whatman 903 Protein Saver cards (GE Healthcare), dried overnight, and individually stored in plastic bags with desiccant. Samples were assigned unique identification numbers that were not traceable to the individual. A total of 4,535 persons were enrolled in the survey, of which 4,438 (97.9%) provided DBS for serological assays. Participants in the survey were aged 1–99 years, with a median number of persons sampled per SDE of 30, and 117 total SDEs sampled throughout the country.

Samples from U.S. resident blood donors were used to represent a population of persons who would be seronegative to tropical diseases not endemic to the U.S. All blood samples were from consenting adults who had screened negative for HIV and hepatitis B viruses and had no reported history of international travel in the last 6 months, and use was approved by CDC IRB under non-engagement in human subjects research status.

Antigens Used for Multiplex Bead Assay (MBA). The 19-kDa fragment of the *P. falciparum* merozoite surface protein 1 (MSP-1p19) was cloned from *P. falciparum* isolate 3D7 and expressed as previously described (17, 19, 20). The SAG2A antigen from *T. gondii* was cloned from the RH strain and produced recombinantly as described previously (21-23). The production of *Brugia* roundworm

recombinant antigens Bm33 and Bm14 have been described previously (24-27). *Wuchereria bancrofti* antigen Wb123 was a kind gift from T. Nutman (National Institutes of Health, Bethesda, MD)(28). The *S. stercoralis* NIE antigen (Ss-NIE-1) produced by L3 parasites was recombinantly produced as described previously (29, 30). The chikungunya virus envelope glycoprotein E1 was purchased through CTK Biotech (Porway, CA). The dengue serotype 2 virus-like particle was grown and isolated from transfected eukaryotic cell culture as described previously (31). The *C. trachomatis* antigens pgp3 and CT694 were recombinantly expressed and purified as described previously (32). The recombinant *T. palladium* antigen rp17 was purchased by Chembio Diagnostic Systems (Medford, NY) and recombinant TmpA through ViroGen (Boston, MA), and dialyzed overnight before bead coupling as described previously (2). Recombinant enterotoxigenic *E. coli* heat-labile enterotoxin B subunit (LTB)produced in *Pichia pastoris* was purchased from Sigma Aldrich (St. Louis, MO)(33). The LecA recombinant antigen was kindly provided by William Petri, Jr., (University of Virginia, Charlottesville, VA) and Joel Herbein (TechLab, Blacksburg, VA) (34, 35). The recombinant 27-kDa antigen from C. parvum (Cp23), has been previously described (36, 37).

Antigen Binding to Beads. Antigens were covalently bound to polystyrene BioPlex® COOH beads (BioRad, Hercules, CA; 1715060XX) or Luminex® SeroMap beads (Luminex Corp, Austin, TX, L100-S0XX) by the commonly used EDC/Sulfo-NHS intermediate reaction. Reactive esters were formed on the carboxylated beads in the presence of 5mM EDAC (1-Ethyl-3-(3'-dimethylaminopropyl)carbodiimide)(EMD Millipore; 341006) and 5 mM Sulfo-NHS (N-hydroxysulfosuccinimide, ThermoScientific; 24510) under light agitation for 20 min. Carboxyl to primary amine crosslinking took place in buffer at pH 5 (0.85% NaCl and 0.05 M 4-morpholineethanesulfonic acid, MES) or at pH 7.2 (phosphate buffered saline, PBS, 0.1M PO_4 and 0.15M NaCl) under light agitation for 2 h. Nonspecific protein binding was blocked by BSA incubation (PBS pH 7.2, + 1% BSA) for 30 min, and beads were resuspended in blocking buffer with the addition of 0.02% NaN_3 and protease inhibitors as described previously (25). Each antigen had been previously optimized to the appropriate coupling concentration and pH: CHIK-E1 (pH 5, 17 µg/mL); Dengue 2 VLP (pH 7.2, 30 µg/mL); Bm14 (pH 7.2, 120 µg /mL); Wb123 (pH 7.2, 120 µg/mL); Bm33 (pH 6.0, 2M urea, 20 µg/mL); Enterotoxigenic *E. coli* (ETEC) heat-labile enterotoxin beta subunit (pH 5, 30 µg/mL); *Chlamydia trachomatis* Pgp3 pCT03 (pH 7.2, 120 µg/mL); *C. trachomatis* CT694 (pH 7.2, 30 µg/mL); *Treponema pallidum* TmpA (pH 5, 15 µg/mL); *T. pallidum* rp17 (pH 5, 15 µg /mL); *Toxoplasma gondii* SAG2A (pH 5, 12.5 µg/mL); *Plasmodium falciparum* MSP1p19 (pH 5, 30 µg/mL); *Stronglyloides stercoralis* NIE (pH 7.2, 2M urea, 20 µg/mL); *Cryptosporidium parvum* Cp27 (pH 5, 12.5 µg/mL); *Entamoeba histolytica* LecA (pH 5.0, 30 ug/mL). As an internal control to test for non-

specific binding or any serum IgG against GST fused to recombinant antigens, a bead was included in the panel that was coupled to GST (coupling concentration of 15 µg/mL at pH5).

Blood Spot Elution and MBA. A 6mm circular punch corresponding to approximately 10 µL whole blood was taken from the center of each blood spot for whole blood elution. Samples were shaken in 100 µL protein elution buffer overnight at room temperature (PBS pH 7.2, 0.05% Tween-20, 0.05% sodium azide), and stored at 4°C until further processing. Elution from blood spots provided an initial 1:10 dilution, and samples were further diluted 1:40 in Luminex sample diluent (PBS, 0.5% Polyvinyl alcohol (Sigma), 0.8% Polyvinylpyrrolidine (Sigma), 0.1% casein (ThermoFisher, Waltham, MA), 0.5% BSA (Millipore, Burlington, MA), 0.3% Tween-20, 0.02% sodium azide, and 3 ug/mL *E. coli* extract to prevent non-specific binding) for a final whole blood dilution of 1:400, corresponding to a serum dilution of approximately 1:800 with the assumption of 50% hematocrit in whole blood.

For the MBA, a mix was prepared for all bead regions in 5mL reagent dilutent (PBS, 0.05% Tween20, 0.5% BSA, 0.02% NaN_3). Filter bottom plates (Multiscreen 1.2 µm, Millipore) were pre-wetted with PBS-T, 50 uL bead mix (approximately 1,500 beads/analyte) added to wells and wells washed twice, and beads incubated with sample in duplicate for 1.5 h under gentle shaking. Secondary antibodies tagged with biotin (1:500 monoclonal mouse anti-human total IgG (Southern Biotech); 1:625 monoclonal mouse anti-human IgG_4 (Southern Biotech)) were incubated with the beads for 45 min, and subsequent incubation with streptavidin-phycoerythrin (1:200, Invitrogen) for 30 min. Plates had a final wash incubation with reagent diluent for 30 min and were read on a Bio-Plex 200 machine by generating the median fluorescence intensity (MFI) signal for 50 beads/analyte and then the mean fluorescence intensity between duplicate wells. Background MFI was generated from blank wells containing only sample diluent, and this value was subtracted from each antigen's raw MFI to give an MFI-bg value which was used for analysis. Due to shortage of antigen-coupled beads, not all samples had data collected for IgG against all antigens. Total number of persons with IgG antibody data collected for each antigen is summarized in Supplementary Table 1.

Determining Seropositivity or Seroprotection Thresholds. Determining the MFI-bg assay signal threshold above which an individual was determined to be IgG positive (seropositive) for each of the antigens in the study was accomplished through different approaches. The MFI-bg signal thresholds are all shown in Supplementary Table 1. No cut-off estimate was available for the the Enterotoxigenic *E. coli* LT B subunit antigen as a negative population was not available for comparison (38).

Non-exposed U.S Residents Approach. For all infectious diseases assayed in this study that were endemic only to tropical areas, the antigen panel for those diseases was assayed with blood samples

from 92 U.S. residents, who were unlikely to have been exposed to these infectious diseases. From this population of U.S. residents, the lognormal mean MFI signal plus three standard deviations was exponentiated to derive the seropositivity signal threshold (in MFI-bg units). This approach was used for the malaria, lymphatic filariasis, strongyloidiasis, chikungunya, dengue, and *E. histolytica* antigens.

Mixture Model Approach. Some pathogens are endemic in the U.S., so there is an increased probability that U.S. resident blood donors could have previously been exposed, and individuals cannot be assumed to be seronegative. To determine inherent seropositive and seronegative sub-populations in a dataset, a 2-component mixture model strategy was used (Supplementary Figure 1). From the first distribution (component) of log-transformed data, which is assumed to be the distribution of the signal of the putative seronegative population, the mean plus three standard deviations was exponentiated to derive the seropositivity signal threshold (in MFI-bg units). This approach was used for the *T. gondii*, *C. trachomatis,* and *T. palladium* antigens.

Known panel containing seropositive and seronegative specimens. For responses to the *C. parvum* Cp23 antigen, the typical approach is to use a panel of Western blot positives and negatives to establish a cut-off by Receiver operator characteristic curve analysis (39). The beads used in this study were previously determined to have a cutoff of 1870 MFI-bg by this method(40). However, since this study used only 50% of the serum concentration in each assay well as the previous work, the cutoff was adjusted to 935 MFI-bg to account for the difference.

Statistical Analysis.

Statistical procedures were performed in SAS® 9.4 software (SAS Institute, Cary, NC), at the 5% significance level (alpha: 0.05), applying both Anderson-Darling and Cramér-von Mises null hypotheses. Descriptive statistics and histograms in SAS software were summarized at the 95% confidence interval and created using the PROC FREQ, PROC UNIVARIATE, and PROC MEANS statements. Ages were categorized into eight mutually exclusive groups (0-4 years, 5-10 years, 11-15 years, 16-20 years, 21-30 years, 31-40 years, 41-50 years, >50 years) due to observed differences between antibody concentrations of younger and older populations. Unweighted, two-component finite mixture models (FMM) of log-transformed data were compared using the FMM procedure in SAS. Logistic and linear regressions were created using PROC REG and PROC GLM. Analysis of potential correlation between antigens were produced through PROC LOGIT and PROC CORR

statements. Seroprevalence estimates were not generated for the Enterotoxigenic *E. coli* LT B subunit antigen as exposure in the population is ubiquitous(38).

Results

As part of the 2014/2015 Tracking Results Continuously (TRaC) nationwide survey, 117 locations were sampled throughout Haiti and communities sampled from are shown in Figure 1.

F4. 1 **Figure 1. Sampling Locations in Haiti from the 2014/2015 Nationwide Survey.**

Each of the 117 sampling locations are indicated by a black dot. Boundaries of the ten Haitian departments are also shown.

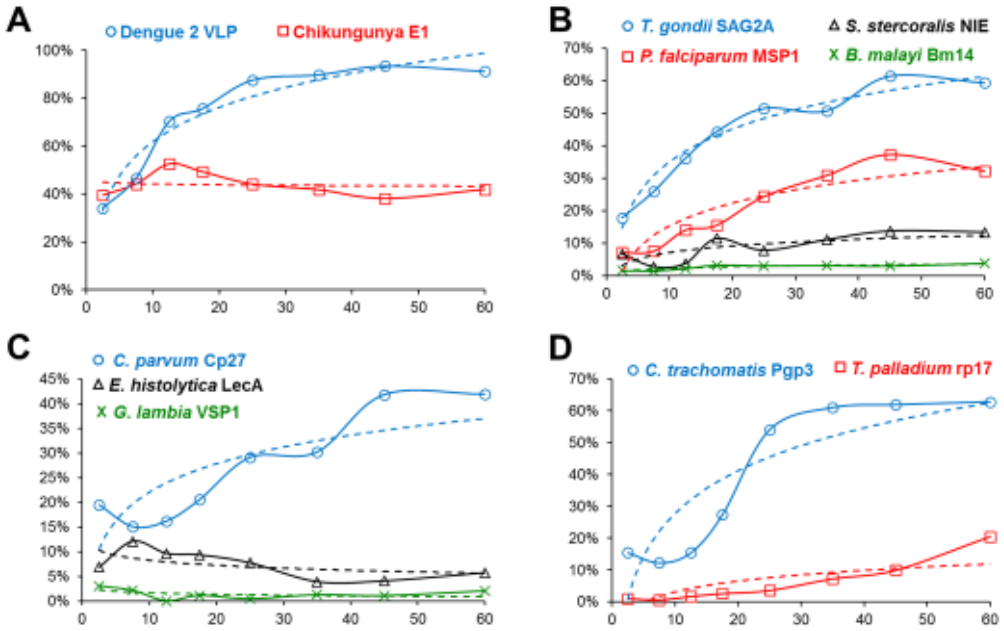
For IgG antibody data collection, 16 different antigens were multiplexed by the bead assay to collect data on various infectious diseases known to be historically endemic or recently introduced to Haiti (Table 1).

T4. 1 **Table 1. Infectious Diseases Represented and Antigens used for Multiplex Serology**

Pathogen	Disease	Antigen
Plasmodium falciparum	Malaria	PfMSP1-19
Toxoplasma gondii	Toxoplasmosis	Sag2A
Wuchereria bancrofti	Lymphatic filariasis	Wb123
Brugia malayi	Lymphatic filariasis	Bm14
Brugia malayi	Lymphatic filariasis	Bm33
Strongyloides stercoalis	Strongyloidiasis	NIE
CHIKV	Chikungunya	Chik E1
DENV2	Dengue	Dengue 2 VLP
Chlamydia spp.	Trachoma / Chlamydia	pgp3
Chlalamydia trachomatis	Trachoma / Chlamydia	Ct694
Treponema spp.	Yaws / Syphilis	rp17

Treponema pallidum	Yaws / Syphilis	TmpA
Enterotoxic *E. coli*	Diarrhea	ETEC-LTB
Entamoeba histolytica	Amoebiasis	LecA
Cryptosporidium parvum	Cryptosporidiosis	Cp23

The current study was powered to present nationwide estimates, but to gauge the relative disparities in seroprevalence among different areas of the country, IgG seropositivity to different antigens is displayed by Haitian departments in Supplementary Table 2. For the Ouest department, estimates for the city of Port-au-Prince were displayed separately from the more rural areas, as this is a broad urban area that is densely populated. Large numbers of elevated IgG responses to several infectious disease antigens were found throughout the country, indicating widespread endemicity for some pathogens. A large percentage of the population in all departments was seropositive to dengue virus serotype 2 (dengue 2 VLP) and chikungunya (Chik E1), ranging from 65.0 – 91.9% and 20.8 – 59.3%, respectively. Seropositivity to antigens for the parasitic pathogens *C. parvum* (Cp23) *and T. gondii* (SAG2A) was found to be high as well with 26.1% and 45.0% of all persons seropositive, respectively. Lymphatic filariasis, *C. trachomatis*, and *T. palladium* all had multiple antigens with which to assess population exposure history. Among the three lymphatic filariasis antigens (Wb123, Bm14, and Bm33) all three showed low seropositivity estimates for the entire population, with Wb123 and Bm14 never reaching above 3.6% for all persons within a department. The two antigens for *C. trachomatis* (pgp3 and Ct694) provided similar seropositivity estimates, as well as the two antigens for *T. palladium* (rp17 and TmpA).

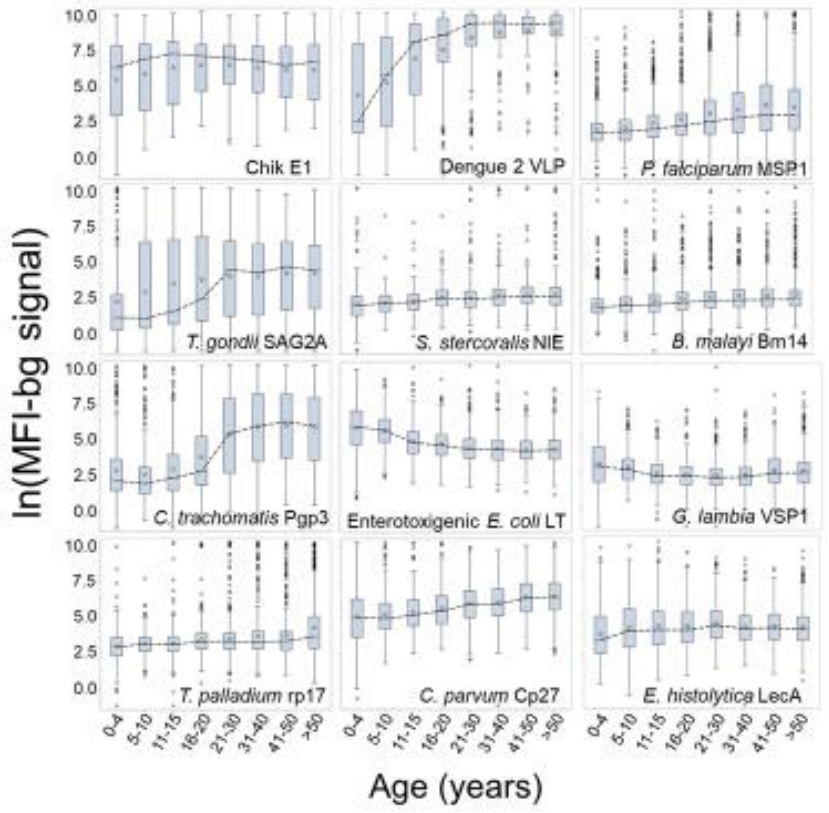


F4. 2 **Figure 2**. **Seroprevalence by Age and Regression Fitting for Selected Antigens**.

By age category, mean seroprevalence (as plotted by percent IgG positive) was plotted on y-axis and age on x-axis. Dashed regression lines were fitted to a logarithmic equation for positivity by age and grouped into similar categories of arboviruses (A), non-waterborne parasites (B), waterborne pathogens (C), and other bacterial pathogens (D).

Figure 2 depicts mean seroprevalence to a subset of antigens by age, grouped into categories of disease or pathogen similarities. In Figure 2, single antigens were included to represent lymphatic filarisis, *C. trachomatis,* and *T. palladium*. For all antigens, seropositivity data was fitted to a logarithmic equation with intercept and slope estimates displayed in Supplementary Table 3. Through the notion that IgG acquisition can only occur through pathogenic exposure, the magnitude of the slope infers the increase in the likelihood of exposure to a pathogen as a person ages. Positive trends suggest that the likelihood to find a seropositive individual in the general population increases with age, while negative trends suggests that seropositivity is more likely among younger populations, and humoral immunity (or exposure) wanes over time. Positive slope estimates were highest for dengue virus 2, *T. gondii*, and the *C. trachomatis* antigens. Two of the antigens provided negative slopes (chik E1 and *E. histoyltica* LecA), indicating by modeling estimates for all ages that IgG antibodies were predicted to be lost as persons age in the Haitian population. When modeling for seropositivity by age, logarithmic regression provided a strong goodness of fit (R^2>0.75) for PfMSP1, SAG2A, Wb123, Bm14, dengue 2 VLP, Pgp3, and CT694 (Supplementary Table 3).

The MBA provides a fluorescence signal (median fluorescence intensity, MFI) which is directly proportional to the amount of IgG in a person's serum sample specific for a particular antigen. Figure 3 presents the log-transformed MFI minus background (MFI-bg) assay signal by age category for the same selected antigens in Figure 2 with the addition of ETEC-LTB.



F4. 3 **Figure 3. Dynamics in MFI-bg IgG Signal by Age for Selected Antigens**.

The same age categories in Fig. 2 were used to create boxplots for the natural logged MFI-bg signal, with a dashed line connecting boxplot medians.

Regression estimates for the effect of age on IgG titer are shown in Supplementary Table 4. In the same way as the seroprevalence plots in Figure 2, these estimates are indicative of mean population exposures and not individual exposures, and the magnitude of a slope estimates how quickly persons in the general population would be expected to gain (or lose) IgG to certain infectious disease antigens. When modeling for acquisition or loss of MFI-bg assay signal by age, most estimates for the age parameter were found to be statistically significant within the regression model (Supplementary Table 4), with only the Wb123, Bm33, and chik E1 antigens not significant. Much in the same way as modeling for seropositivity by age, modeling for change in MFI-bg assay signal by age found some slope estimates to be positive (gain of antibody titer with age) and some estimates negative (loss of titer with age). Though the Cp23 showed clear increases in seropositivity with age, the other antigen from a waterborne pathogen (LecA) showed a consistent negative slope when modeling for seropositivity (Supplementary Table 3) or MFI-bg signal (Supplementary Table 4) by age. All other antigens had consistent positive slopes for seropositivity and IgG acquisition with

age except SAG2A (positive slope for seropositivity by age, negative slope for MFI-bg IgG response by age) and chik E1 (negative slope for seropositivity with age, positive slope for MFI-bg IgG intensity by age). In general, slopes for seropositivity and antibody acquisition by age were in positive correlation (Supplementary Figure 2). For the ETEC-LT and dengue 2 VLP antigens, regression estimates were also generated for only young children to show the rapid loss (or gain) of IgG response throughout their first years of life (Supplementary Table 4). Correlation of MFI-bg signal among all antigens is shown in Supplementary Table 5, with Spearman correlation coefficients statistically significant for many of the direct comparisons among antigens.

Discussion

In this report, we show the capacity of the MBA to investigate multiple diseases of interest from samples gathered in a single nationwide survey in Haiti. Primary analyses and estimates took into account participants' age and area of residence, but additional studies with demographic and spatial data could allow for more informative epidemiological outputs (41, 42). Haitian estimates for population-level exposure to each of the pathogens will be described below as grouped by infectious disease category and will include examples of how serological data generated by MBA can be applied.

Arboviruses. Two arboviruses were represented in this serosurvey: chikungunya and dengue serotype 2 (DENGV2). Chikungunya virus is transmitted by *Aedes* spp. mosquitoes with symptoms including headache, muscle pain, joint swelling and rash within 3-7 days, and a robust IgG response has been noted in previous studies (32, 33, 43, 44). Chikungunya virus can spread relatively quickly in a population, and serological data can help to identify new cases and outbreaks (45). Dengue is caused by four viral serotypes: DENGV 1-4, and transmission occurs through the bite of the *Aedes aegypti* and *Aedes albopictus* mosquitoes. Symptoms can include vomiting, abdominal pain, difficulty breathing, and hemorrhagic fever (46). Our study found the transmission dynamics for these two arboviruses to be quite different, with the DENGV2 VLP antigen providing a population seroprevalence curve indicating increase likelihood of lifetime exposure as persons aged. Seemingly immediately after birth, seroprevalence and IgG levels rise rapidly in the first 15 years of life. By age 30, Haitians had a greater than 80% chance of having been infected with DENGV2, and typically displayed very high IgG titers. In contrast, the seroprevalence curve by age categories for chikungunya was mostly flat, likely indicative of recent introduction of the disease into the country in 2014, and the rapid spread of this arbovirus (45).

Waterborne Pathogens. Antigens for two waterborne pathogens are included in this survey: *C. parvum* and Enterotoxigenic *Escherichia coli* (ETEC). *C. parvum* is a parasite that causes watery diarrhea, with symptoms including stomach cramps, dehydration, nausea, and vomiting, and fever(47). Similar to estimates from this current study, a previous serostudy from Haiti found a similar pattern of small, but consistent, increases in antibody titer to the Cp23 antigen with age (35). ETEC is a gram-negative bacterium found in the environment, animals, and food, and transmission is ubiquitous in regions of the world with poor water sanitation(48). The heat-labile enterotoxin secreted by ETEC causes diarrhea, abdominal cramping, and fever, and illness occurs 1-2 days after exposure and lasts 3-4 days on average. Interestingly, we found the IgG levels against the ETEC-LT antigen to be high in the youngest ages, but the responses decrease during the first few decades of life, and remain flat for older age groups. This finding may suggest immune tolerance to this *E. coli* antigen, as has been noted for another *E. coli* antigen, lipopolysaccharide (LPS) (49).

Other Parasites. Plasmodium falciparum is transmitted through *Anopheles* spp. mosquitos and is the primary causative agent of malaria in Haiti (50, 51). Symptoms, including headaches, fevers, and chills, usually appear 10-15 days after an infected mosquito bite, and high IgG titers are elicited against *P. falciparum* antigens (17, 45, 52). Our current study showed a consistent increase in seropositivity and population antibody titer was seen with age. The model of PfMSP1-19 seropositivity estimates that a person age 45 would be 3.7-fold more likely to be seropositive compared to someone who is age 5. Malaria serology data can be applied to understanding areas of ongoing transmission (17), and percentages of the population seropositive to PfMSP1 were lowest in the Port au Prince metropolitan area (12.0%) and the highest in the Centre department (37.1%).

Toxoplasmosis is a zoonotic infection caused by a single celled parasitic protozoan, *Toxoplasma gondii.* Transmission occurs when eating undercooked, contaminated meat or by oral ingestion of the oocyst stage when humans come into contact with infected cat feces (53). As *T. gondii* infection typically goes into latency in the human host (54), seropositivity would likely indicate active infection (55). Our study found reliable increases in IgG prevalence to the SAG2A antigen with age, indicating past and current stable transmission of this parasite. Our data estimated that by the time a Haitian reaches adulthood, the risk of exposure to *T. gondii* is greater than 50% (21).

Adult lymphatic filariasis (LF) worms live in the lymph system and microfilariae circulate in the blood, and this disease is found throughout the tropical and subtropical areas of the world (33, 56). In Haiti, LF is caused by the roundworm *Wucheria bancrofti*, and current targets for elimination will benefit

from continual serosurveillance efforts as the endemic range is reduced (57). In this study, we employed three filarial antigens in effort to identify seropositive persons in this low-transmission setting. A low proportion of the population was seropositive to the worm antigens, with low (but positive) slope estimates for increase of seroprevalence and IgG titer with age as has been seen in other low transmission settings (10).

The roundworm *Strongyloides stercoralis* is the causative agent of strongyloidiasis. This soil-transmitted helminth is transmitted when skin comes into contact with free-living larvae in contaminated soil, and the majority of people infected are asymptomatic (32, 58, 59). Previous studies have presented *Strongyloidiasis* seroprevalence estimates (60), and our study found an overall low seroprevalence to the *S. stercoralis* NIE antigen, with the lowest seroprevalence found in Artibonite (0.7%) and the highest in Grand'Anse (7.5%). Surveillance for roundworms through serological data could be utilized for directing mass drug administration campaigns in areas where active infection and parasite prevalence are difficult to estimate.

Other Bacterial Pathogens. Serological data was collected on two other bacterial pathogens: *Chylamidia trachomatus,* and *Treponema palladium*. Trachoma is an ocular disease caused by *C. trachomatis*, and is the world's leading infectious cause of blindness (33). Genital carriage of this bacterium is also the causative agent of the sexually-transmitted infection (STI) chlamydia, and seropositivity to IgG antibodies would be unable to differentiate between the STI and trachomatis forms. Our study found consistent increases to the *C. trachomatis* antigens pgp3 and Ct694 with age, with the most pronounced increases during the ages of sexual debut – indicative of STI exposure. A previous report utilizing serology data from multiple studies has projected that seroprevalence against these antigens in ages 1-9 years as a proxy for trachomatous inflammation – follicular (TF) burden on the population (11). In our study, we found 9.2% (52/566) children aged 1-9y were seropositive for the pgp3 antigen and 8.7% (62/716) seropositive for the Ct694 antigen in Haiti. Though these are low estimates for the juvenile population in Haiti, studies are still ongoing to determine the relationship between seropositivity rates and TF rates (11, 61, 62).

Treponema bacteria cause the skin diseases yaws (*Treponema pallidum*, sub species *pertenue*), and the STI syphilis *(T. pallidum ssp pallidum)*. Yaws affects the skin, bone, and cartilage (33), and the rp17 and TmpA antigens for *T. pallidum* would be unable to differentiate exposure to yaws or syphilis bacteria. In our Haitian study, MBA detection of IgG to rp17 and TmpA found a low prevalence in the study population, with minor yet consistent increase in seropositivity and titer with age. Unlike the *C. trachomatus* antigens, rapid increases in seroprevalence were not seen in the

teenage population, indicating a trend of more gradual risk of exposure over life rather than abrupt exposure during sexual debut. As has been suggested before for age restriction in evaluating serological data for rp17 and TmpA (2), 0.4% (2/472) of 0-5y olds were found to be seropositive to the rp17 antigen, and 1.3% (6/468) to the TmpA antigen.

Laboratory-based serological assays are dependent on the sensitivity and specificity of the assays used for IgG detection, and some diseases do not have well-defined antigens that are known to elicit strong IgG responses or have antigens with known IgG cross-reactivity issues with other pathogen antigens. Seropositivity cut-off values may also need to be refined continually, especially in elimination programs where populations have decreased exposure and the probability of finding cases are rarer (10, 17). Increasing survey sample sizes can facilitate in overcoming statistically biased estimates and to increase precision. Defining seroconversion, boosting effects after re-exposure, antibody decay, and immunocompetency of the host are all primary concerns for some infectious diseases, and continued investigation is required to correctly interpret serology data for different diseases. Among the factors listed above, another limitation to this study is that sampling design was powered for the modeled malaria prevalence in Haiti (18). Additionally, the survey was cross-sectional, and regression estimates of data representing trends over time assume consistent dynamics of endemicities and transmission intensity. Future studies in Haiti should investigate if similar findings would be observed.

This nationwide Haiti survey employed a 15-antigen MBA panel measuring IgG presence and titer to eleven infectious diseases. As some pathogens are cleared from the host within a few days or weeks, assaying for antibodies greatly augments the window of time in which to survey for exposure in a population. In addition, accurate surveillance for recurrent-type infectious diseases can be confounded by asymptomatic infections, poor access to healthcare or healthcare reporting, or poor diagnostics. Understanding the co-endemic disease burden on a national level allows for collaborative strategies of multiple stakeholders focused on combined interventions at the community level. Multiple programs, especially those targeting multiple diseases, can be monitored simultaneous through one well-designed, population-representative integrated survey (57, 63, 64).

Acknowledgements

The authors would like to acknowledge the Haitian survey teams and participants for their involvement in this study. The authors thank T. Nutman (NIH), W. Petri, Jr. (University of VA), and J.

Herbein (TechLab) for reagent contribution, and Delynn Moss (CDC) for assistance in running the multiplex immunoassay.

Disclaimer

Use of trade names is for identification only and does not imply endorsement by the Public Health Service or by the U.S. Department of Health and Human Services. The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

References

1. WHO. Accelerating work to overcome the global impact of neglected tropical diseases – A roadmap for implementation. 2012.
2. Cooley GM, Mitja O, Goodhew B, Pillay A, Lammie PJ, Castro A, et al. Evaluation of Multiplex-Based Antibody Testing for Use in Large-Scale Surveillance for Yaws: a Comparative Study. J Clin Microbiol. 2016;54(5):1321-5.
3. Kurkjian KM, Vaz LE, Haque R, Cetre-Sossah C, Akhter S, Roy S, et al. Application of an improved method for the recombinant k 39 enzyme-linked immunosorbent assay to detect visceral leishmaniasis disease and infection in Bangladesh. Clin Diagn Lab Immunol. 2005;12(12):1410-5.
4. Won KY, Kanyi HM, Mwende FM, Wiegand RE, Goodhew EB, Priest JW, et al. Multiplex Serologic Assessment of Schistosomiasis in Western Kenya: Antibody Responses in Preschool Aged Children as a Measure of Reduced Transmission. The American journal of tropical medicine and hygiene. 2017;96(6):1460-7.
5. Rogier EW, Moss DM, Mace KE, Chang M, Jean SE, Bullard SM, et al. Use of Bead-Based Serologic Assay to Evaluate Chikungunya Virus Epidemic, Haiti. Emerging infectious diseases. 2018;24(6):995-1001.
6. van Hooij A, Tjon Kon Fat EM, Batista da Silva M, Carvalho Bouth R, Cunha Messias AC, Gobbo AR, et al. Evaluation of Immunodiagnostic Tests for Leprosy in Brazil, China and Ethiopia. Sci Rep. 2018;8(1):17920.
7. Smith JL, Auala J, Tambo M, Haindongo E, Katokele S, Uusiku P, et al. Spatial clustering of patent and sub-patent malaria infections in northern Namibia: Implications for surveillance and response strategies for elimination. PloS one. 2017;12(8):e0180845.
8. Arnold BF, van der Laan MJ, Hubbard AE, Steel C, Kubofcik J, Hamlin KL, et al. Measuring changes in transmission of neglected tropical diseases, malaria, and enteric pathogens from quantitative antibody levels. PLoS Negl Trop Dis. 2017;11(5):e0005616.
9. Rogier E, Moss DM, Chard AN, Trinies V, Doumbia S, Freeman MC, et al. Evaluation of Immunoglobulin G Responses to Plasmodium falciparum and Plasmodium vivax in Malian School Children Using Multiplex Bead Assay. The American journal of tropical medicine and hygiene. 2017;96(2):312-8.
10. Won KY, Robinson K, Hamlin KL, Tufa J, Seespesara M, Wiegand RE, et al. Comparison of antigen and antibody responses in repeat lymphatic filariasis transmission assessment surveys in American Samoa. PLoS neglected tropical diseases. 2018;12(3):e0006347.
11. Pinsent A, Solomon AW, Bailey RL, Bid R, Cama A, Dean D, et al. The utility of serology for elimination surveillance of trachoma. Nat Commun. 2018;9(1):5444.
12. Won KY, Sambou S, Barry A, Robinson K, Jaye M, Sanneh B, et al. Use of Antibody Tools to Provide Serologic Evidence of Elimination of Lymphatic Filariasis in The Gambia. The American journal of tropical medicine and hygiene. 2018;98(1):15-20.
13. Fujii Y, Kaneko S, Nzou SM, Mwau M, Njenga SM, Tanigawa C, et al. Serological surveillance development for tropical infectious diseases using simultaneous microsphere-based multiplex assays and finite mixture models. PLoS Negl Trop Dis. 2014;8(7):e3040.
14. Lammie PJ, Moss DM, Brook Goodhew E, Hamlin K, Krolewiecki A, West SK, et al. Development of a new platform for neglected tropical disease surveillance. Int J Parasitol. 2012;42(9):797-800.
15. Mohanty I, Dash M, Sahu S, Narasimham MV, Panda P, Padhi S. Seroprevalence of chikungunya in southern odisha. Journal of family medicine and primary care. 2013;2(1):33-6.
16. Elshal MF, McCoy JP. Multiplex bead array assays: performance evaluation and comparison of sensitivity to ELISA. Methods. 2006;38(4):317-23.
17. Rogier E, Wiegand R, Moss D, Priest J, Angov E, Dutta S, et al. Multiple comparisons analysis of serological data from an area of low Plasmodium falciparum transmission. Malar J. 2015;14:436.

18. Sutherland LJ, Cash AA, Huang YJ, Sang RC, Malhotra I, Moormann AM, et al. Serologic evidence of arboviral infections among humans in Kenya. The American journal of tropical medicine and hygiene. 2011;85(1):158-61.
19. Blackman MJ, Ling IT, Nicholls SC, Holder AA. Proteolytic processing of the Plasmodium falciparum merozoite surface protein-1 produces a membrane-bound fragment containing two epidermal growth factor-like domains. Mol Biochem Parasitol. 1991;49(1):29-33.
20. Egan A, Waterfall M, Pinder M, Holder A, Riley E. Characterization of human T- and B-cell epitopes in the C terminus of Plasmodium falciparum merozoite surface protein 1: evidence for poor T-cell recognition of polypeptides with numerous disulfide bonds. Infect Immun. 1997;65(8):3024-31.
21. Priest JW, Moss DM, Arnold BF, Hamlin K, Jones CC, Lammie PJ. Seroepidemiology of Toxoplasma in a coastal region of Haiti: multiplex bead assay detection of immunoglobulin G antibodies that recognize the SAG2A antigen. Epidemiology and infection. 2015;143(3):618-30.
22. Prince JB, Auer KL, Huskinson J, Parmley SF, Araujo FG, Remington JS. Cloning, expression, and cDNA sequence of surface antigen P22 from Toxoplasma gondii. Mol Biochem Parasitol. 1990;43(1):97-106.
23. Parmley SF, Sgarlato GD, Mark J, Prince JB, Remington JS. Expression, characterization, and serologic reactivity of recombinant surface antigen P22 of Toxoplasma gondii. Journal of clinical microbiology. 1992;30(5):1127-33.
24. Hamlin KL, Moss DM, Priest JW, Roberts J, Kubofcik J, Gass K, et al. Longitudinal monitoring of the development of antifilarial antibodies and acquisition of Wuchereria bancrofti in a highly endemic area of Haiti. PLoS Negl Trop Dis. 2012;6(12):e1941.
25. Moss DM, Priest JW, Boyd A, Weinkopff T, Kucerova Z, Beach MJ, et al. Multiplex bead assay for serum samples from children in Haiti enrolled in a drug study for the treatment of lymphatic filariasis. The American journal of tropical medicine and hygiene. 2011;85(2):229-37.
26. Chandrashekar R, Curtis KC, Li BW, Weil GJ. Molecular characterization of a Brugia malayi intermediate filament protein which is an excretory-secretory product of adult worms. Mol Biochem Parasitol. 1995;73(1-2):231-9.
27. Dissanayake S, Xu M, Nkenfou C, Piessens WF. Molecular cloning and serological characterization of a Brugia malayi pepsin inhibitor homolog. Mol Biochem Parasitol. 1993;62(1):143-6.
28. Kubofcik J, Fink DL, Nutman TB. Identification of Wb123 as an early and specific marker of Wuchereria bancrofti infection. PLoS Negl Trop Dis. 2012;6(12):e1930.
29. Rascoe LN, Price C, Shin SH, McAuliffe I, Priest JW, Handali S. Development of Ss-NIE-1 recombinant antigen based assays for immunodiagnosis of strongyloidiasis. PLoS Negl Trop Dis. 2015;9(4):e0003694.
30. Ravi V, Ramachandran S, Thompson RW, Andersen JF, Neva FA. Characterization of a recombinant immunodiagnostic antigen (NIE) from Strongyloides stercoralis L3-stage larvae. Mol Biochem Parasitol. 2002;125(1-2):73-81.
31. Chang GJ, Hunt AR, Holmes DA, Springfield T, Chiueh TS, Roehrig JT, et al. Enhancing biosynthesis and secretion of premembrane and envelope proteins by the chimeric plasmid of dengue virus type 2 and Japanese encephalitis virus. Virology. 2003;306(1):170-80.
32. Goodhew EB, Priest JW, Moss DM, Zhong G, Munoz B, Mkocha H, et al. CT694 and pgp3 as serological tools for monitoring trachoma programs. PLoS Negl Trop Dis. 2012;6(11):e1873.
33. Rezaee MA, Rezaee A, Moazzeni SM, Salmanian AH, Yasuda Y, Tochikubo K, et al. Expression of Escherichia coli heat-labile enterotoxin B subunit (LTB) in Saccharomyces cerevisiae. J Microbiol. 2005;43(4):354-60.
34. Houpt E, Barroso L, Lockhart L, Wright R, Cramer C, Lyerly D, et al. Prevention of intestinal amebiasis by vaccination with the Entamoeba histolytica Gal/GalNac lectin. Vaccine. 2004;22(5-6):611-7.

35. Moss DM, Priest JW, Hamlin K, Derado G, Herbein J, Petri WA, Jr., et al. Longitudinal evaluation of enteric protozoa in Haitian children by stool exam and multiplex serologic assay. Am J Trop Med Hyg. 2014;90(4):653-60.
36. Perryman LE, Jasmer DP, Riggs MW, Bohnet SG, McGuire TC, Arrowood MJ. A cloned gene of Cryptosporidium parvum encodes neutralization-sensitive epitopes. Mol Biochem Parasitol. 1996;80(2):137-47.
37. Priest JW, Kwon JP, Moss DM, Roberts JM, Arrowood MJ, Dworkin MS, et al. Detection by enzyme immunoassay of serum immunoglobulin G antibodies that recognize specific Cryptosporidium parvum antigens. J Clin Microbiol. 1999;37(5):1385-92.
38. Arnold BF, Martin DL, Juma J, Mkocha H, Ochieng JB, Cooley GM, et al. Enteropathogen antibody dynamics and force of infection among children in low-resource settings. Elife. 2019;8.
39. Zweig MH, Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. Clin Chem. 1993;39(4):561-77.
40. Mosites E, Miernyk K, Priest JW, Bruden D, Hurlburt D, Parkinson A, et al. Giardia and Cryptosporidium antibody prevalence and correlates of exposure among Alaska residents, 2007-2008. Epidemiology and infection. 2018;146(7):888-94.
41. Biggs J, Raman J, Cook J, Hlongwana K, Drakeley C, Morris N, et al. Serology reveals heterogeneity of Plasmodium falciparum transmission in northeastern South Africa: implications for malaria elimination. Malar J. 2017;16(1):48.
42. Kerkhof K, Sluydts V, Heng S, Kim S, Pareyn M, Willen L, et al. Geographical patterns of malaria transmission based on serological markers for falciparum and vivax malaria in Ratanakiri, Cambodia. Malar J. 2016;15(1):510.
43. Redwan el RM, Al-Awady MK. Prevalence of tetanus immunity in the Egyptian population. Human antibodies. 2002;11(1-2):55-9.
44. Durbaca S. Antitetanus and antidiphtheria immunity in newborns. Roumanian archives of microbiology and immunology. 1999;58(3-4):267-72.
45. Poirier MJ, Moss DM, Feeser KR, Streit TG, Chang GJ, Whitney M, et al. Measuring Haitian children's exposure to chikungunya, dengue and malaria. Bull World Health Organ. 2016;94(11):817-25A.
46. Flamand C, Fritzell C, Prince C, Abboud P, Ardillon V, Carvalho L, et al. Epidemiological assessment of the severity of dengue epidemics in French Guiana. PloS one. 2017;12(2):e0172267.
47. Current WL, Reese NC, Ernst JV, Bailey WS, Heyman MB, Weinstein WM. Human cryptosporidiosis in immunocompetent and immunodeficient persons. Studies of an outbreak and experimental transmission. The New England journal of medicine. 1983;308(21):1252-7.
48. Croxen MA, Law RJ, Scholz R, Keeney KM, Wlodarska M, Finlay BB. Recent advances in understanding enteric pathogenic Escherichia coli. Clin Microbiol Rev. 2013;26(4):822-80.
49. Seeley JJ, Ghosh S. Molecular mechanisms of innate memory and tolerance to LPS. J Leukoc Biol. 2017;101(1):107-19.
50. Frederick J, Saint Jean Y, Lemoine JF, Dotson EM, Mace KE, Chang M, et al. Malaria vector research and control in Haiti: a systematic review. Malar J. 2016;15(1):376.
51. Lemoine JF, Boncy J, Filler S, Kachur SP, Fitter D, Chang MA. Haiti's Commitment to Malaria Elimination: Progress in the Face of Challenges, 2010-2016. The American journal of tropical medicine and hygiene. 2017;97(4_Suppl):43-8.
52. Drakeley CJ, Corran PH, Coleman PG, Tongren JE, McDonald SL, Carneiro I, et al. Estimating medium- and long-term trends in malaria transmission by using serological markers of malaria exposure. Proc Natl Acad Sci U S A. 2005;102(14):5108-13.
53. Hill D, Dubey JP. Toxoplasma gondii: transmission, diagnosis and prevention. Clin Microbiol Infect. 2002;8(10):634-40.
54. Zainodini N, Zare-Bidaki M, Abdollahi SH, Afrooz M, Ziaali N, Ebrahimian M, et al. Molecular and serological detection of acute and latent toxoplasmosis using real-time PCR and ELISA techniques in blood donors of rafsanjan city, iran, 2013. Iran J Parasitol. 2014;9(3):336-41.

55. Bela SR, Oliveira Silva DA, Cunha-Junior JP, Pirovani CP, Chaves-Borges FA, Reis de Carvalho F, et al. Use of SAG2A recombinant Toxoplasma gondii surface antigen as a diagnostic marker for human acute toxoplasmosis: analysis of titers and avidity of IgG and IgG1 antibodies. Diagn Microbiol Infect Dis. 2008;62(3):245-54.
56. Joseph H, Maiava F, Naseri T, Silva U, Lammie P, Melrose W. Epidemiological assessment of continuing transmission of lymphatic filariasis in Samoa. Annals of tropical medicine and parasitology. 2011;105(8):567-78.
57. Knipes AK, Lemoine JF, Monestime F, Fayette CR, Direny AN, Desir L, et al. Partnering for impact: Integrated transmission assessment surveys for lymphatic filariasis, soil transmitted helminths and malaria in Haiti. PLoS neglected tropical diseases. 2017;11(2):e0005387.
58. Paradies P, Iarussi F, Sasanelli M, Capogna A, Lia RP, Zucca D, et al. Occurrence of strongyloidiasis in privately owned and sheltered dogs: clinical presentation and treatment outcome. Parasites & vectors. 2017;10(1):345.
59. Greaves D, Coggle S, Pollard C, Aliyu SH, Moore EM. Strongyloides stercoralis infection. BMJ. 2013;347:f4610.
60. Krolewiecki AJ, Lammie P, Jacobson J, Gabrielli AF, Levecke B, Socias E, et al. A public health response against Strongyloides stercoralis: time to look at soil-transmitted helminthiasis in full. PLoS neglected tropical diseases. 2013;7(5):e2165.
61. Kim JS, Oldenburg CE, Cooley G, Amza A, Kadri B, Nassirou B, et al. Community-level chlamydial serology for assessing trachoma elimination in trachoma-endemic Niger. PLoS neglected tropical diseases. 2019;13(1):e0007127.
62. Woodhall SC, Gorwitz RJ, Migchelsen SJ, Gottlieb SL, Horner PJ, Geisler WM, et al. Advancing the public health applications of Chlamydia trachomatis serology. Lancet Infect Dis. 2018;18(12):e399-e407.
63. Priest JW, Jenks MH, Moss DM, Mao B, Buth S, Wannemuehler K, et al. Integration of Multiplex Bead Assays for Parasitic Diseases into a National, Population-Based Serosurvey of Women 15-39 Years of Age in Cambodia. PLoS Negl Trop Dis. 2016;10(5):e0004699.
64. Solomon AW, Engels D, Bailey RL, Blake IM, Brooker S, Chen JX, et al. A diagnostics platform for the integrated mapping, monitoring, and surveillance of neglected tropical diseases: rationale and target product profiles. PLoS neglected tropical diseases. 2012;6(7):e1746.

Supplementary Tables and Figures

T4. 2 **Supplementary Table 1. MBA Assay Antigen Formats and Seropositivity**

Antigen	Format	MFI-bg Threshold for Seropositivity	Number of Samples With Data Collected (% of 4,438)
PfMSP1	Recombinant	61	4424 (99.7%)
SAG2A	Recombinant	40	1682 (37.9%)
Wb123	Recombinant	411	4395 (99.0%)
Bm14	Recombinant	530	4438 (100.0%)
Bm33	Recombinant	457	4309 (97.1%)
NIE	Recombinant	493	2238 (50.4%)
Chik E1	Recombinant	1353	4438 (100.0%)
Dengue 2 VLP	Virus-like particle	543	1253 (28.2%)
Pgp3	Recombinant	161	3325 (74.9%)
CT694	Recombinant	74	4118 (92.8%)
rp17	Recombinant	314	4395 (99.0%)
TmpA	Recombinant	46	4395 (99.0%)
ETEC-LT	Recombinant	NA	4411 (99.4%)
VSP1	Recombinant	611	2747 (61.9%)
LecA	Recombinant	190	2833 (63.4%)
Cp27	Recombinant	925	2790 (62.9%)

T4. 3 **Supplementary Table 2: Seropositivity of Infectious Disease Antigens by Haitian Department**

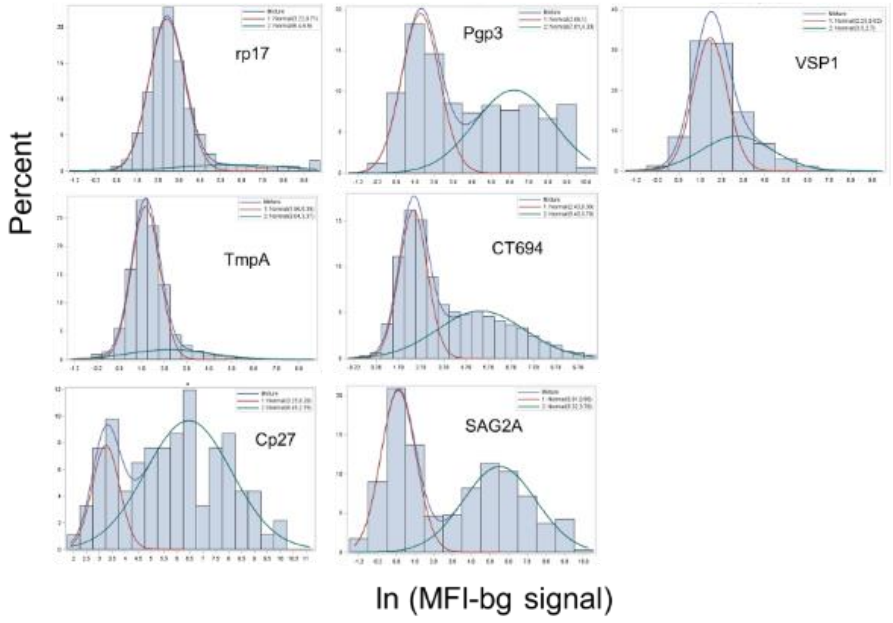
	Antigen Positivity (% of all persons sampled)														
Department	**PfMSP1**	**SAG2A**	**Wb123**	**Bm14**	**Bm33**	**NIE**	**Chik E1**	**Dengue 2 VLP**	**pgp3**	**Ct694**	**rp17**	**TmpA**	**VSP1**	**LecA**	**Cp27**
Port-au-Prince	12.0	54.3	1.0	3.1	5.5	9.1	59.3	87.3	38.7	35.1	4.1	4.1	0.9	8.2	28.4
Artibonite	24.9	30.8	1.3	3.5	8.3	4.6	46.4	68.8	41.9	40.7	6.2	5.6	1.5	7.5	28.0
Centre	37.1	37.9	0.4	0.7	6.7	12.6	21.6	67.2	46.5	35.3	6.0	3.2	2.1	10.3	29.0
Grand'Anse	16.0	56.7	0.0	3.2	5.0	14.9	25.9	91.9	55.2	39.6	8.6	5.5	1.0	2.9	41.0
Nippes	35.9	54.6	0.0	1.4	6.2	9.1	45.5	ND	28.3	27.6	11.0	7.6	2.4	4.7	27.1
Nord	20.2	58.8	2.4	3.6	9.5	11.4	35.4	74.2	48.2	35.6	7.6	5.4	0.4	10.3	18.2
Nord'Est	19.5	50.0	0.0	0.0	8.4	11.8	20.8	65.0	37.5	37.7	5.8	2.6	0.0	5.8	26.0
Nord'Ouest	20.8	24.8	2.3	3.5	9.2	6.2	42.8	ND	51.1	37.1	6.0	4.0	1.8	7.1	28.3
Ouest (Outside PAP)	21.8	43.6	2.6	2.6	7.2	9.8	47.4	75.3	36.2	28.1	7.4	6.1	0.9	3.2	26.6
Sud'Est	20.1	48.4	0.5	2.2	7.1	9.0	38.4	79.8	45.1	35.7	10.3	5.8	1.7	5.8	33.3
Sud	30.1	37.5	0.3	2.7	5.3	7.5	45.3	90.0	35.6	35.8	6.5	5.0	4.4	8.2	34.6
Nationwide	**21.8**	**45.0**	**1.3**	**2.8**	**7.2**	**4.2**	**43.5**	**75.6**	**41.7**	**35.2**	**6.6**	**5.0**	**1.4**	**7.2**	**26.1**

T4. 4 **Supplementary Table 3: Change in Antibody MFI-bg Levels to Different Antigens with Age**

Antigen	Intercept, β_0 (95% CI)	Age, β_x (95% CI)	Chi-Square Statistic	Model p value
PfMSP1	108 (-28 , 244)	13 (9, 17)	45.3	**< 0.0001**
SAG2A	2098 (1713, 2482)	-21 (-31, -10)	14.9	**0.0001**
Wb123	37 (20, 54)	0.3 (-0.2, 0.7)	1.22	0.268
Bm14	78 (16, 139)	2 (0, 3)	4.01	**0.045**
Bm33	203 (156, 250)	0.1 (-1, 1)	0.05	0.827
NIE	270 (110, 429)	7 (3, 12)	10.6	**0.001**
Chik E1	2114 (1927, 2280)	1 (-4, 6)	0.14	0.705
Dengue 2 VLP	5763 (4899, 6628)	145 (122, 168)	150.2	**< 0.0001**
Pgp3	1089 (708, 1471)	58 (47, 68)	111.9	**< 0.0001**
CT694	268 (143, 394)	13 (9, 16)	51.8	**< 0.0001**
rp17	-62 (-245, 120)	25 (20, 30)	91.3	**< 0.0001**
TmpA	20 (4, 36)	0.5 (0, 1)	3.86	**0.045**
ETEC-LT	953 (880, 1025)	-14 (-16, -12)	195.2	**< 0.0001**
VSP1	72 (39, 105)	-0.4 (-1, 0.5)	0.6	0.435
LecA	84 (73, 94)	-0.4 (-0.7, -0.1)	8.7	**0.003**
Cp27	926 (711, 1140)	13 (7, 19)	20.0	**< 0.0001**
ETEC 0-10y olds	2161 (1864, 2457)	-159 (-207, -110)	40.9	**< 0.0001**
DENGV2 0-15y olds	1594 (136, 3052)	377 (206, 547)	18.8	**< 0.0001**

T4. 5 **Supplemental Table 4. Correlation of IgG Levels Among Different Antigens**

Spearman Correlation Coefficients																
Prob > |r| under H0: Rho=0																
Number of Observations																
	PfMSP1	Chik E1	Dengue 2 VLP	ETEC-LT	SAG2A	Wb123	Bm14	Bm33	Pgp3	CT694	rp17	TmpA	NIE	VSP1	LecA	Cp27
PfMSP1	1	0.09202	0.29118	-0.0527	0.15599	0.3015	0.37721	0.2715	0.33254	0.33498	0.34405	0.36243	0.33128	0.12436	0.28561	0.24035
		<.0001	<.0001	0.0005	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
	4424	4424	1248	4424	1677	4381	4424	4296	3314	4105	4381	4381	2231	2740	2826	2783
Chik E1		1	0.35254	-0.00514	0.1619	0.18853	0.20327	0.11099	0.08343	0.12715	0.18925	0.16348	0.15059	0.02172	0.08872	0.1277
			<.0001	0.7322	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.2551	<.0001	<.0001
		4438	1253	4438	1682	4395	4438	4309	3325	4118	4395	4395	2238	2747	2833	2790
Dengue 2 VLP			1	-0.13824	0.20922	0.26575	0.28936	0.24509	0.36493	0.33291	0.24493	0.26356	0.278	-0.06386	0.12554	0.19409
				<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0291	<.0001	<.0001
			1253	1253	1253	1210	1253	1210	1210	1253	1253	1210	1167	1167	1253	1210
ETEC-LT				1	-0.1041	0.084	0.05713	0.03083	-0.17906	-0.11064	0.04429	0.0568	0.04693	0.25906	0.174	-0.029
					<.0001	<.0001	0.0001	0.043	<.0001	<.0001	0.0033	0.0002	0.0264	<.0001	<.0001	0.1258
				4438	1682	4395	4438	4309	3325	4118	4395	4395	2238	2747	2833	2790
SAG2A					1	0.20695	0.21477	0.17233	0.21134	0.19312	0.20626	0.1671	0.20932	0.03966	0.1328	0.19874
						<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.1132	<.0001	<.0001
					1682	1639	1682	1639	1639	1682	1639	1639	1596	1596	1682	1639
Wb123						1	0.59604	0.40162	0.2065	0.283	0.41905	0.48001	0.47277	0.19561	0.30085	0.22447
							<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
						4395	4395	4309	3282	4075	4352	4352	2238	2704	2790	2747
Bm14							1	0.45584	0.3018	0.36638	0.47392	0.53873	0.44399	0.15401	0.39075	0.23337
								<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
							4438	4309	3325	4118	4395	4395	2238	2747	2833	2790
Bm33								1	0.20076	0.23079	0.29275	0.32108	0.37202	0.15772	0.31021	0.15625
									<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
								4309	3196	3989	4266	4266	2152	2618	2704	2661
Pgp3									1	0.83458	0.29519	0.26712	0.25964	0.03518	0.20881	0.30246
										<.0001	<.0001	<.0001	<.0001	0.1063	<.0001	<.0001
									3325	3091	3282	3325	2024	2109	2152	2152
CT694										1	0.35538	0.3518	0.28429	0.07964	0.24062	0.29423
											<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
										4118	4075	4075	2238	2747	2833	2790
rp17											1	0.63956	0.36378	0.23641	0.31513	0.27057
												<.0001	<.0001	<.0001	<.0001	<.0001
											4395	4352	2195	2704	2790	2747
TmpA												1	0.50189	0.22075	0.32787	0.28969
													<.0001	<.0001	<.0001	<.0001
												4395	2195	2747	2790	2790
NIE													1	0.18371	0.2659	0.23936
														<.0001	<.0001	<.0001
													2238	2152	2238	2195
VSP1														1	0.20313	0.16221
															<.0001	<.0001
														2747	2747	2747
LecA															1	0.19323
																<.0001
															2833	2790
Cp27																1
																2790



F4. 4 **Supplemental Figure 1. Use of the Finite Mixture Model to Derive Seropositivity Cutoff Threshold**.

Log-transformed data from the Haiti survey was fitted to a two-component mixture model for antigens that U.S. residents have greater likelihood of exposure to. For each antigen's figure legend, estimates of mean and variance are displayed for both components. Seropositivity threshold value for each antigen was calculated adding three standard deviations to the mean of the first component and exponentiating back to linear scale.

4.2.1 Research Conclusions

This research addressed the first outcome of **Objective 2**, which was to assess the utility of serologic MBAs to describe co-endemic burden of diseases. To do this, I analyzed MFI data from a national serosurvey in Haiti to ascertain seroprevalence to eleven different pathogens. The results demonstrated that serologic MBAs were capable of capturing the disease burden of several tropical diseases across different adminstrative departments. Additionally, analysis of the data using seropositivity and continuous MFI values with age provided indications of changes in transmission over time, or force of infection, and accquision of antibodies over life time for most pathogens listed in this panel.

When applied to integrated disease surveillance of NTDs, serologic MBAs can be used to support NTD control efforts. For example, in this study I demonstrated the utility of serologoical MBAs in generating national and departmental administative estimates of co-endemic diseases in Haiti. However, given that this survey was designed to obtain broader estimates across the entire nation, sampling strategies for individual departments may need to be adjusted accordingly based factors such as representative sampling, population dispersion, and demographic information to establish more robust exposure estimates. Morever, integrating different diseases into a single survey may be challenging in the fact that each disease may have different or optimal sampling strategies. This will be further discused in Chapter 6.

Serological estimates using MBAs may also need to be valdiated against clinical incidence data or existing knowledge of disease history within the target population to ensure that estimates are sensible. For example, observing extremely high MFI values in a population with clinical data that has reported no recent or historical infections may question the reliability of the data and laboratory techinque. Addtionally, having well validated antigens, as in antigens that been vigrously tested and haved demonstrated suitability for monitoring a specific pathogen (futher discussed in Chapter 6), in multi-disease panels may lend confidence to any unusual MFI signals of non-endemic diseases captured within a population (accounting for cross-reactivity or improper laboratory technique) , and could potentially help to identify disease inception or imported exposure.

This work highlighted the potential of these platforms to monitor various pathogens from a single national survey. The next piece of research (paper) in this chapter transitions to a smaller adminstative region in the state of Sabah, Malaysia to assess serologic MBAs in supporting multi-disease monitoring. It is possible that compared to national serological surveys, state serological

surveys may be implemented more routinuely as it is less resource intensive, but depends purpose of specific surveillance. While research activitites may typically be implemented at more local spatial scales, state administrative surveys may help to indentify exposure heterogeneity to target specific areas for integrated control. Additionally, demonstrating MBA capacity at this level may help to enable more routine multi-disease surveillance. This research also focused on this dataset because a rich array of risk factors and spatial data were collected as part of the survey. This enables the analysis of potential risk factors associated with co-endemic exposure to inform integrated disease programs.

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT

T: +44 (0)20 7299 4646
F: +44 (0)20 7299 4656
www.lshtm.ac.uk

RESEARCH PAPER COVER SHEET

Please note that a cover sheet must be completed for each research paper included within a thesis.

SECTION A – Student Details

Student ID Number	1603698	**Title**	Mr
First Name(s)	YuYen		
Surname/Family Name	Chan		
Thesis Title	Application of multiplex bead serological assays to integrated monitoring of neglected tropical disease		
Primary Supervisor	Chris Drakeley		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?			
When was the work published?			
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion			
Have you retained the copyright for the work?*	Choose an item.	Was the work subject to academic peer review?	Choose an item.

*If yes, please attach evidence of retention. If no, or if the work is being included in its published format, please attach evidence of permission from the copyright holder (publisher or other author) to include this work.

SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	PLoS NTD
Please list the paper's authors in the intended authorship order:	YuYen L. Chan, Katie Patterson, Diana L. Martin, Jeffrey W. Priest, Gillian Stresman, Timothy William, Tock H. Chua, Patrick Lammie, Chris Drakeley, Kimberly M Fornace
Stage of publication	**Undergoing revision**

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	Writing and drafting paper, determination of seroprevalence, non-spatial risk factor analysis

SECTION E

Student Signature	YuYen Chan
Date	16/09/2021

Supervisor Signature	Chris Drakeley
Date	20/09/2021

4.3 Applying serological multiplex bead assays to assess risk factors of neglected tropical diseases

Assessing seroprevalence and associated risk factors to several neglected tropical diseases in Sabah, Malaysia using serological multiplex bead assays.

Authors: YuYen L. Chan[1], Katie Patterson[1], Diana L. Martin[2], Jeffrey W. Priest[3], Gillian Stresman[1], Timothy William[4], Tock H. Chua[5], Patrick Lammie[1], Chris Drakeley[1], Kimberly M Fornace[5]

1. Department of Infection Biology, London School of Hygiene and Tropical Medicine, London, United Kingdom
2. Division of Parasitic Diseases and Malaria, United States Centers for Disease Control and Prevention, Atlanta, GA, USA
3. Division of Foodborne, Waterborne, and Environmental Diseases, United States Centers for Disease Control and Prevention, Atlanta, GA, USA
4. Infectious Diseases Society Sabah-Menzies School of Health Research Clinical Research Unit, Kota Kinabalu, Malaysia
5. Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, Kota Kinabalu, Malaysia
6. Department of Disease Control, London School of Hygiene and Tropical Medicine, London, United Kingdom

Introduction

Within the last decade, disease control efforts including mass drug administration, improved sanitation, and public health awareness have helped to reduce the burden of neglected tropical (NTDs) and other infectious diseases in Malaysia. However, many of these diseases continue to persist especially among isolated, resource-constrained, and aboriginal communities in Sabah, resulting in sustained morbidity and chronic impact to quality of life (1). For example, helminth diseases in Malaysia include strongyloidiasis (2-4) and lymphatic filariasis (LF) (5) that can cause a range of illnesses leading to malnutrition and disability (6-8). Persistent protozoan diseases in Malaysia include giardiasis (9), toxoplasmosis (10), and malaria (11, 12). Giardiasis can result in malnutrition to chronic diarrhea (13, 14) while toxoplasmosis symptoms can vary between asymptomatic to severe clinical manifestations that occur typically in immunocompromised patients (15). In Malaysia, bacterial diseases include leptospirosis (16, 17), trachoma (18), and yaws (19) that can impact the skin, eyes, joints, and other parts of the body.

A practical challenge to control efforts is routine and reliable surveillance for many of these infections where current epidemiological trends are lacking (18). Especially in low-transmission and post-elimination settings, characterizing disease burden becomes particularly difficult due to sparse incidence and sub-clinical infections in this region. Assessing population prevalence can help to identify areas of transmission resurgence or introduction, yet low transmission status, mild morbidity, and limited resources may have reduced public health priority towards systematic monitoring of these diseases. Since transmission of many of these pathogens geographically overlap and can result in co-infections, integrated multi-diseases monitoring would provide resource efficient alternatives compared to single disease surveillance (20). While diverse biological targets of tropical infections often require different laboratory methods to capture disease burden (e.g. stool microscopy, polymerase chain reaction, or antibody testing), a unified platform monitoring exposure to diverse pathogens may help to overcome some of these logistical challenges towards concurrent NTD monitoring.

Integrated monitoring may be attainable using serological multiplex bead assays (MBA). MBAs can quantify immune responses to multiple pathogens from a single blood spot (21). Serology can be effective in capturing asymptomatic infections and revealing any historical pathogen exposure by measuring pathogen-specific antibody responses (22). The use of serology in monitoring NTDs and vaccine preventable diseases (VPDs) has been applied in numerous settings (21-24). Furthermore,

demographic, and environmental data collected in population-based surveys provide key opportunities to assess potential and shared risk factors of the different diseases that may enhance controls strategies and community awareness. While certain socio-economic risk factors have been studied for several NTDs in Malaysia (4, 13, 25, 26), spatial and other risk factors are not well characterized for many of these diseases.

To our knowledge, multiplex bead assays have yet to be applied to assessing NTD seroprevalence and associated risk factors in Malaysia. In this study, we used MBA on samples collected during a 2015 cross-sectional survey in Northern Sabah, Malaysia to estimate population exposure to multiple pathogens. We aimed to 1) describe population level exposure to six NTDs, 2) assess pathogen-specific individual risk factors for exposure; and 3) determine spatial and environmental risk factors and predict population-level exposure probabilities.

Methods

Study site and sampling

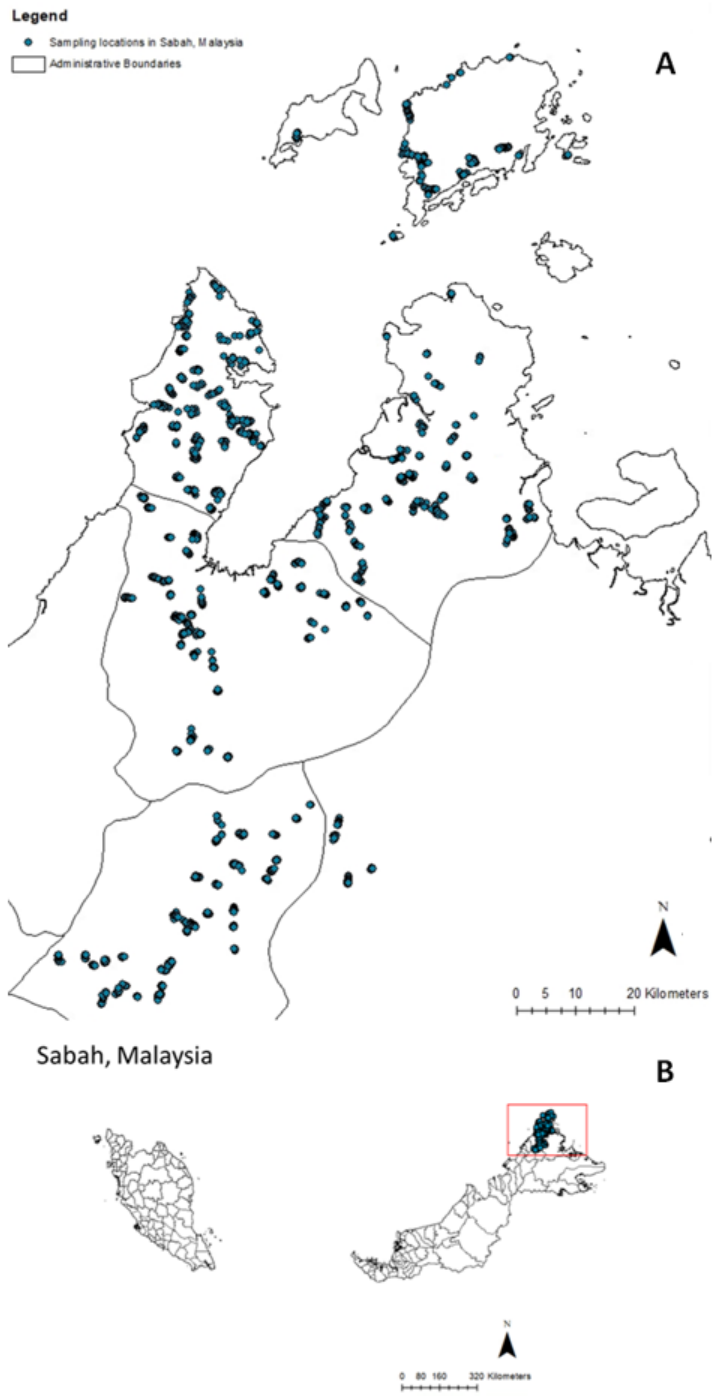


Figure 1. Sampling sites in Sabah, Malaysia.

Sampling sites (A) and Sabah state in Malaysia (B).

This study was conducted in four districts of Northern Sabah in Malaysian Borneo (Figure 1). This area is tropical with elevations ranging from sea-level to over 4000 meters above sea-level (MSL). The population is predominantly rural, and most occupations are associated with agricultural or

plantation activities. To determine risk factors for malaria, an environmentally stratified, population-based cross-sectional survey was conducted from September 17, 2015 to December 12, 2015, as described by Fornace et al. (27). Briefly, seroprevalence was estimated using a non-self-weighting two-stage sampling design of 919 villages stratified by forest cover, with a target sample size of 2650 households and 36 households sampled per village (powered for plasmodium knowlesi seroprevalence). All individuals residing in selected households were asked to participate (ages 3 months -105 years). Finger prick blood sampling was used to prepare blood spots of filter paper (3MM, Whatman, Maidstone, UK).

Ethics Approval

The Medical Research Sub-Committee of the Malaysian Ministry of Health (NMRR-14-713-21117) and the Research Ethics Committee of the London School of Hygiene and Tropical Medicine (8340) approved the Malaysian study and written informed consent was obtained from all study participants.

Multiplex IgG detection assay

The IgG responses to 12 NTD disease antigens were assayed for six pathogens (Table 1). Merozoite surface protein 1-19 (MSP1-19) and apical membrane antigen-1 (AMA-1) antigens from *Plasmodium falciparum* and *P. vivax* were also included with appropriate control sera as internal positive controls. Excluding the malaria proteins, all antigen-coupled microspheres were provided by the Centers for Disease Control and Prevention (Atlanta, GA, USA) and coupled according to standard Luminex protocols to minimize the signal-to-noise ratio (27). Malaria antigen coupling was optimized in-house as described previously (27, 28) (Luminex Corporation, Austin, TX, USA). Test samples were eluted from a 3-mm dried blood spot (DBS) punch, corresponding to 2.1 µl of whole blood, and shaken overnight at room temperature in 200 µl of elution buffer (1xPBS, 0.05% sodium azide and 0.05% Tween-20), resulting in a 1:200 pre-dilution, assuming 50% hematocrit. At least 1 day prior to testing, samples were diluted to a final 1:400 dilution using Luminex buffer B (1xPBS, 0.05% Tween, 0.5% BSA, 0.02% sodium azide, 0.1% casein, 0.5% polyvinyl alcohol (PVA), 0.5% polyvinyl pyrrolidone (PVP) and 15.25ug/ml *E. coli* extract) to prevent non-specific binding. Negative and positive controls were also incubated in buffer B at least one day before testing, with negative controls prepared at 1:400, a pooled *P. falciparum* positive prepared at 1:400 and 1:4000, and a pooled *P. vivax* positive control prepared in a 6-point 2-fold serial dilution (1:400 – 1:12,800). 50 ul of the samples were co-incubated with antigen-coupled beads in a one-day multiplex serological assay described previously (Wu et al., 2020). Using a Luminex MAGPIX

bioanalyzer and xPONENT software (version 4.2), the background-adjusted median fluorescent intensity (MFI) of wells achieving at least a 30-bead count per analyte were recorded. The *P. vivax* control curve was included on each plate to standardise data between plates.

Determination of seropositivity cut-offs

To determine seropositivity, antigen-specific cut-off values from log transformed MFI with background subtracted (MFI-bg) were calculated in R using the mixtools package (29). To ensure sufficient negatives for estimating population level exposure, we included individuals of all ages in cut-off determination for Bm33, Wb123, SAG2A, and VSP3/VSP5 antigens (30). Gaussian mixture models of data from individuals less than 3, 5, and 14 years were used to determine cut-offs for NIE, VSP3 + VSP5, Rp17 and TmpA, respectively. The mean of the lower component plus three standard deviations was then used to determine cut-off threshold. As multiple antigens were measured for specific diseases, we analyzed highly correlated antigens (Pearson's correlation co-efficient > 0.65) for the same pathogen together (Supplementary Figure 1). For filariasis (Bm14 and BmR1), trachoma (Pgp3 and Ct694) and giardiasis (VSP3 and VSP5), we used K-means clustering (three clusters) to classify seropositive and seronegative based on two antigens for each disease. We limited analysis of antigens for trachoma to children under 10 years old to excluded sexually acquired venereal chlamydia.

Statistical Analysis of Risk Factors

We assessed eight demographic, health and socioeconomic risk factors (Supplementary Table 1). Logistic regression was used to evaluate risk factors association to seroprevalence for each antigen, with household included as a random effect to control for sampling design. Associations with a $p < 0.05$ were considered statistically significant using adjusted odds ratios. Variables were assessed using variation inflation factor < 5 to assess for potential collinearity, and final models were selected using backwards elimination ($p<0.05$).

Spatial Patterns of Exposure Risks

To assess the spatial distribution of exposure risks, we additionally assembled potential spatial environmental covariates, including topographic measures, distance to land cover and forest types, population density, accessibility, and climatic variables (Table 2). Pearson correlation analysis was used to exclude highly correlated variables (correlation coefficient > 0.7) with the final dataset including 21 potential spatial and environmental predictors (Supplementary Tables 3 and 4). As

demographic data was not available for all locations within this region, we did not include additional questionnaire data. All covariates were resampled to 500m resolution for predictions.

Using the seropositivity thresholds defined above, we fit geostatistical models of household seroprevalence for each disease separately. Models were fit in a Bayesian framework with $p(x_i)$ denoting the seroprevalence at locations $x_i, i = 1 \ldots n$, with m_i individuals sampled per household location. The full model was specified as:

$$Y_i \sim \text{Binomial}(m_i, p(x_i))$$

With the linear predictor for the binomial model specified as:

$$\text{logit}(p(x_i)) = \beta_0 + \boldsymbol{d}(\boldsymbol{x_i})'\boldsymbol{\beta} + w_i$$

Where β_0 represents the intercept, $\boldsymbol{d}(\boldsymbol{x_i})'\boldsymbol{\beta}$ represents a vector of location specific covariate effects and w_i represents the spatial effect. Residual spatial autocorrelation was assessed using Moran's I, with spatial effects modelled as a Matern covariance function using the stochastic partial differential equation approach implemented in Integrated Nested Laplace Approximation (R-INLA) (31). Weakly informative priors of Normal (0, 100) were used for intercepts and fixed effect coefficients and penalized complexity priors were used for the spatial effect (32). Final models were assessed using the deviance information criteria (DIC) and root mean squared error. Posterior probabilities were estimated using 1,000 posterior samples. Additionally, to visualize the uncertainty around these predictions, we calculated exceedance probabilities using a 10% seroprevalence threshold (33). These exceedance probabilities represent the probability a location exceeds this threshold; locations with exceedance probabilities around 50% represent areas where there is high uncertainty around this threshold. All analysis was conducted in R statistical software (34), with maps visualized in ArcGIS (ESRI, Redlands, USA) and R statistical software.

Results

Seroprevalence

Cross-sectional serological survey data was available for 10100 individuals, with varying number of individuals available for analysis based on sample and antigen availability. Seroprevalence estimates of the whole study site in northern Sabah are shown in Table 1 and the spatial distribution of seroprevalence of antigens in Figure 2, Supplementary Figure 2. The seroprevalence of LF antigens in Bm33 were 10.9% and for Wb123 was 1.72%. Seroprevalence of strongyloidiasis NIE antigen was 16.8%, for toxoplasmosis SAG2A antigen was 29.9%, and giardiasis antigen GVSP3 + GVSP5 was

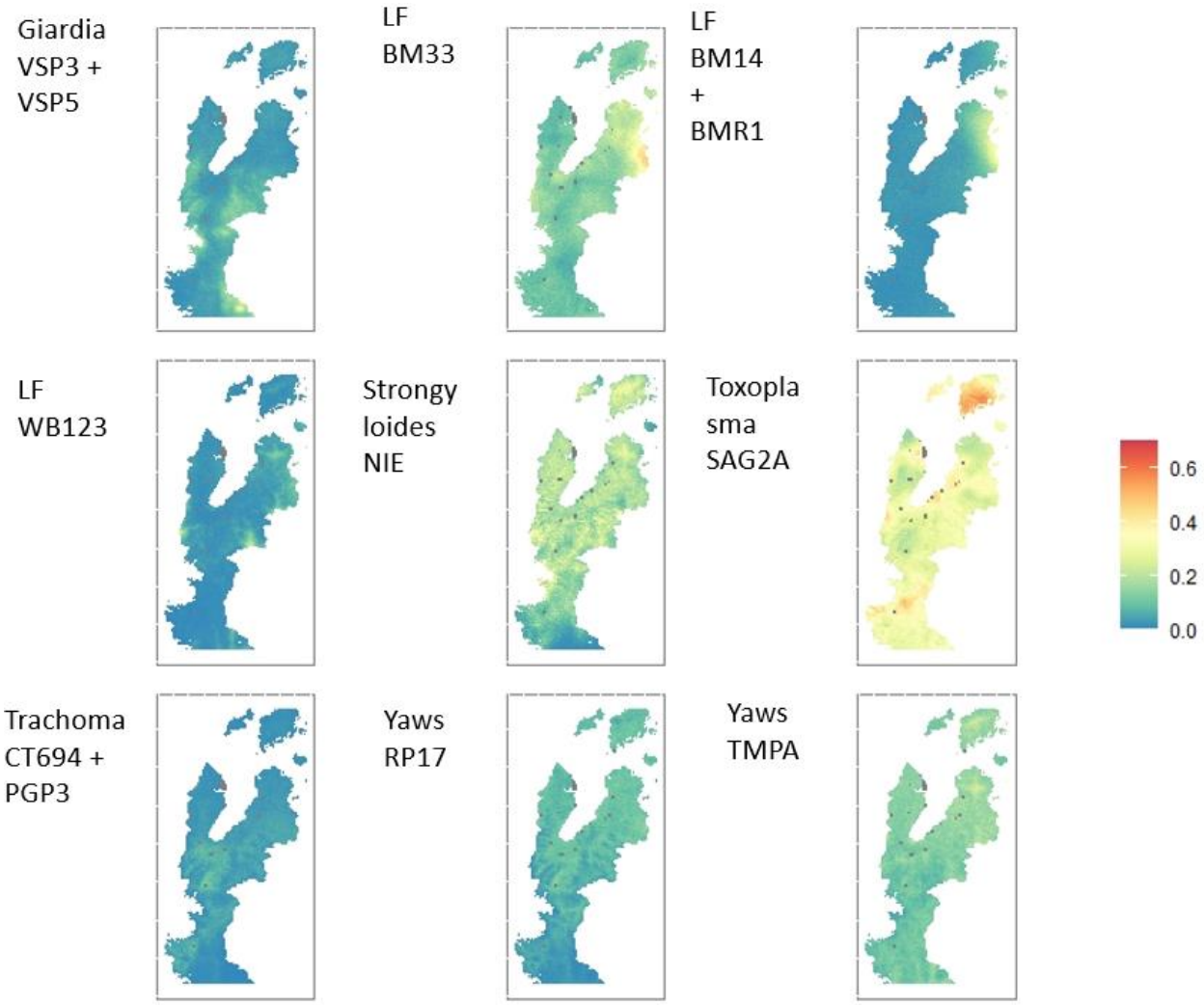
23.24%. For infections in school aged children less than 10, seroprevalence estimates for yaws antigens varied between the different antigens Rp17 (4.91%) and TmpA (4.81%). As Rp17 may indicate historical exposure and TmpA may indicate more recent exposure, double seropositivity to both antigens was 1.16%. Seroprevalence estimates for trachoma Pgp3 and Ct694 were 4.52%.

T4. 6 **Table 1. Cut-off method, seroprevalence and vaccine exposure in percentages, and number of individuals per antigen.**

Gaussian mixture model (2 distributions)			
Antigen, Pathogen	**Disease**	**Percent Seroprevalence with 95% CI**	**N**
Bm33, *Brugia malayi*	**Lymphatic Filariasis**	**10.9 (10.2, 11.6)**	**8129**
Wb123, *Wucheria Bancrofti*	**Lymphatic Filariasis**	**1.72 (1.46, 2.02)**	**8128**
NIE, *Strongyloides stercoralis*	**Strongyloidiasis**	**16.8 (16.7, 16.9)**	**8131**
SAG2A, *Toxoplasma gondii*	**Toxoplasmosis**	**29.9 (28.9, 30.1)**	**7430**
Rp17*, *Treponemal pallidum pertenue*	**Yaws**	**4.91 (3.93, 6.11)**	**1529***
TmpA*, *Treponemal pallidum pertenue*	**Yaws**	**4.81 (3.98, 5.79)**	**1660***
Rp17 TmpA double positive	**Yaws**	**1.16 (0.74, 1.80)**	**1638***
VSP3 + VSP5, *Giardia duodenalis*	**Giardiasis**	**23.24 (22.31-24.19)**	**7682**
K-means clustering (k=3)			
Bm14+BmR1, *Brugia malayi*	**Lymphatic Filariasis**	**3.53 (3.13, 3.99)**	**6855**
Pgp3+Ct694*, *Chlamydia trachomatis*	**Trachoma**	**4.52 (3.68, 5.53)**	**1970***

***Age group less than 10 years of age**

F4. 6 **Figure 2. Geostatistical maps showing mean posterior estimated seroprevalence per antigen**.



Seroprevalence of risk factor categories are listed in Supplementary Table 1. Seropositivity to all assessed antigens showed potential age effects, demonstrating differences in exposure by age category (Supplementary Figure 3-6). Study site characteristics included different demographic variables (gender, age, occupation, and ethnicity) and environmental variables (population density, elevation, normalized difference vegetation index (NDVI), temperature, seasonality, and spatial distance to natural environmental features (Table 2)). Additionally we assessed several lifestyle additional variables including socio-economic status based on a wealth index, owning animals, bathing location bed net use.

T4. 7 **Table 2. Study site characteristics.**

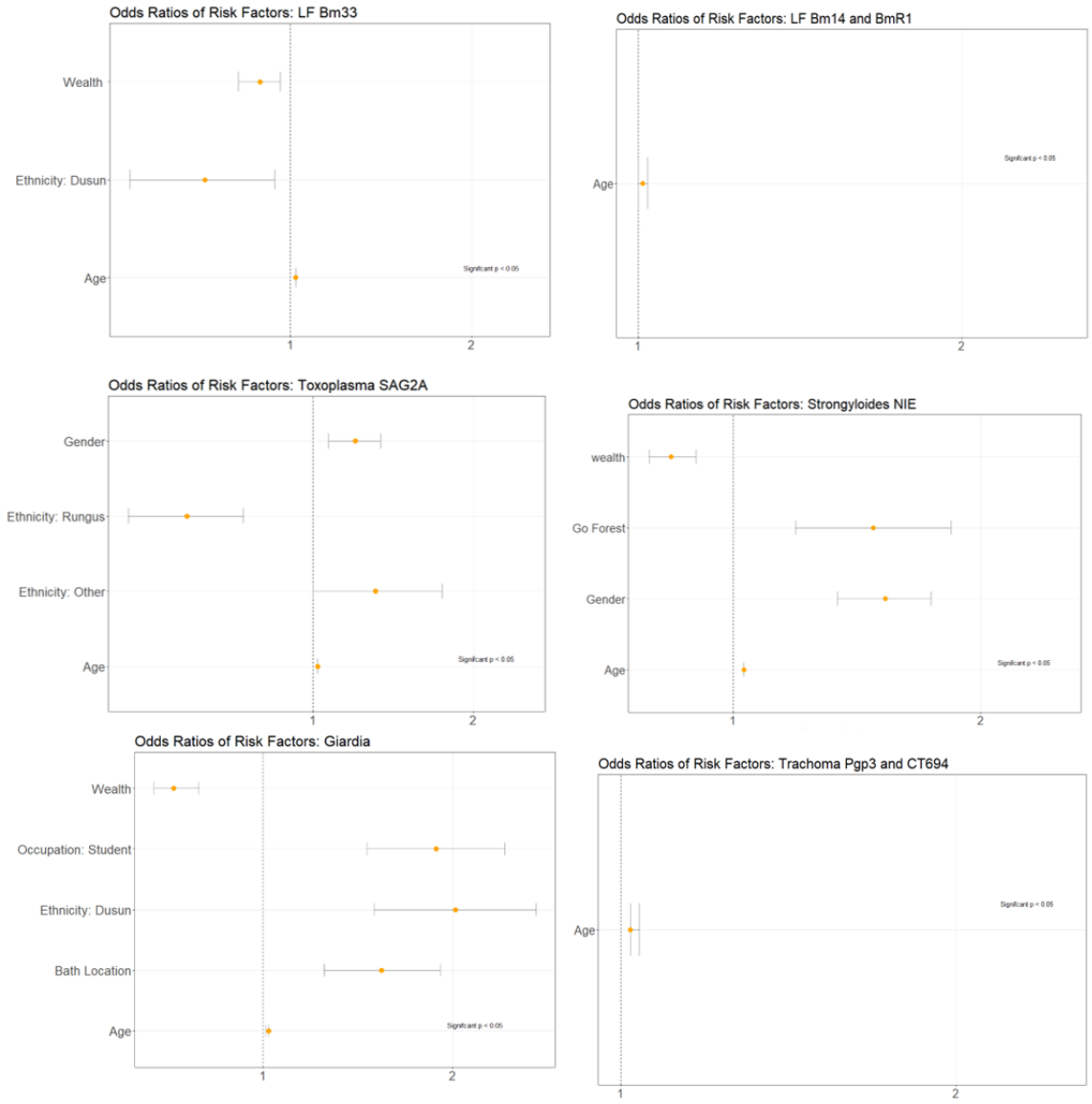
Demographic variable	N
Study Population size	8205
Males	3389
Females	4312
Age in mean years (range)	29 (0-105)
Occupation	N
Farmer	1153
Student	3745
Other Occupation	997
No Occupation	3745
Ethnicity	N
Bajau	752
Dusun	4137
Other	1135
Rungus	2091
Environmental Variable	Mean (range)
Population density (per km^2)	1.76 (0 – 183.42)
Elevation (meters above sea level)	166.4 (4.0 – 1258.0)
NDVI	0.47 (-0.24 – 0.86)
Average temperature, 1970 - 2000 (°C)	26.69 (21.46 – 27.53)
Mean diurnal range, 1970 - 2000 (°C)	8.22 (6.95 – 10.29)
Maximum temperature of warmest month, 1970 - 2000 (°C)	31.89 (28.09 – 32.78)
Minimum temperature of coldest month, 1970 - 2000 (°C)	21.46 (14.80 – 22.90)
Precipitation of the wettest month. 1970 - 2000 (mm)	2417 (2167 – 2754)
Precipitation seasonality, 1970 - 2000 (coefficient of variation)	44.14 (16.93 – 59.49)
Distance to intact forest (m)	3647 (0 – 19836)
Distance to irrigated farmland (m)	2794 (0 – 23716)
Distance to oil palm plantation (m)	1098 (0 – 20940)

Risk Factor Analysis

Multivariate analysis using logistic regression identified associations between seropositivity and risk factors that were considered significant at $p<0.05$ (Figure 5, Supplementary Table 2). For LF Bm33 antigen, significant associations were observed for age, wealth, and Dusun ethnicity. Higher socio-economic status and Dusun ethnicity demonstrated decreased odds of risk of exposure. For LF Wb123 antigens, no significant associations were observed, potentially due to the low overall seroprevalence in the population. For LF Bm14 + BmR1, significant associations were observed for age only. For T. gondii SAG2A antigen, significant associations were observed with age, gender, ethnicity, bath location. Increased odds of exposure were observed for males compared to females and ethnicity within the Other category. Decreased odds of exposure was observed for Rungus ethnic group. For S. stercoralis NIE antigen, significant associations were observed for age, wealth, going to the forest, and gender. Higher socio-economic status was observed with decreased odds of exposure, while going to the forest and being male demonstrated increased odds of exposure. For

giardiasis antigens, age, student occupation, Dusun ethnicity, and bath location (i.e. bathing outdoors or with water pipes) were shown to increase odds of exposure, while decreased odds of exposure was observed with higher socio-economic status. For Trachoma antigens, age was the only significant risk factor.

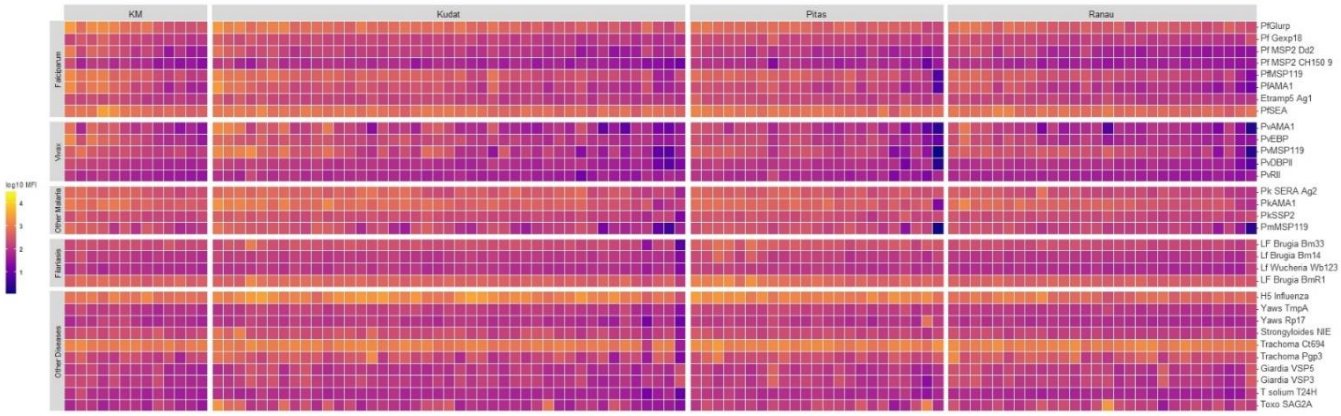
F4. 7 **Figure 5. Adjusted odd ratio plots for associated NTD risk factors.**



Environmental Risk Factors and spatial distribution of exposure

The study area represented a wide range of ecologies with varying land cover, topography, and population densities (Table 2, Supplementary Table 3). Marked heterogeneities in exposure were identified between villages and antigens (Figure 6). Using these data, we additionally identified predictive spatial and environmental factors for exposure to diseases (Supplementary Table 4).

F4. 8 **Figure 6. Mean MFI value per village cluster for all antigens assayed, including malaria antigens previously reported by (27)**



Discussion

Serological surveys provide a platform for integrated monitoring of numerous pathogens. In our study, we applied multiplex bead assays to assess seroprevalences and associated risk factors to six NTDs. The seroprevalence results provided evidence of exposure for all NTDs in Malaysia during 2015. Integrating this data within a geostatistical framework enables visualization of spatial distribution of exposure, identifying priority areas for follow up and surveillance.

Analysis of disease specific responses allowed identification of risk factors and spatial distribution of exposure for all diseases, showing broad agreement with other sources of epidemiological data. For example, persistent LF transmission and LF MDA was on-going during the year of the survey (World Health Organization [WHO] Global Health Observatory [GHO], accessed August 19, 2020). Preventative chemotherapy for strongyloidiasis (prevalence =16.8%) and other STHs were also administered to the country during the same year of this survey (according to WHO GHO, accessed August 19, 2020), and prevalence estimates were similar to previously reported estimates (31.5%, using ELISA) in Malaysia (Orang Asli) (2). It is possible that this estimate may also reflect some potential cross reactivity to other prevalent nematode infections (35). Seroprevalence estimates for toxoplasmosis in this study were 29.9%% (CI: 28.9%-30.1%) and consistent to sero-estimates of previous studies (10, 36). For giardiasis, seroprevalence estimates were 23.24% (CI: 22.31-24.19%), which is higher than prior estimates using molecular techniques varying from 0.2%-20% (37, 38). Compound antibody responses for trachoma were detected among 4.52% (CI: 3.68%- 5.53%) of the children 1 to 9 years of age. This is similar to what is seen in areas suspected not to be endemic for trachoma in Pacific Island nations of Solomon Islands, Fiji, and Vanuatu (39-41).

For LF, trachoma, yaws, and giardiasis, multiple antigens were included in determining seropositivity. For LF prevalence estimates varied using antigens of the same pathogen. This may be due to differing immunogenicity of antigens, antibody kinetics as markers of recent or historical exposure, or possible cross reactivity to other antibodies (42, 43). For yaws antigens, seroprevalence was 4.91% (CI: 3.93%-6.11%) for Rp17 and 4.81% (CI: 3.98%-5.79%) for TmpA. We observed a lack of correlation between the two yaws antigen, which may be due to individual antigen function (Supplementary Figure 7). For example, Cooley et al have found that Rp17 captures long-lived treponemal antibodies, while TmpA can be used to differentiate between active and low infections based on antibody titers (44). To describe potential current infection, we presented double seropositivity for both antigens (1.16%, CI: 0.74-1.80) For highly correlated antigens of LF and trachoma, we determined seropositivity by applying K-means clustering approach to classify seroprevalence. This new approach to classifying antibody responses may potentially enhance seroprevalence approximations by examining multiple highly correlated antigens within the population, maximizing the use of information from multiple antigens.

We examined several risk factors in this study. Given the age effect on antibody acquisition, we hypothesized that this association would be present among the antigens within our study population. We found age to be associated with seroprevalence for all antigens. For giardiasis, overall low transmission or low seroprevalence and consistent exposure among children and adults may dampen any age effects on seroprevalence.

Previous studies in Malaysia have found associations with low socioeconomic backgrounds and unbalanced burden of disease, which is attributable to impacts on living standards, working conditions and access to health care (45, 46). We hypothesized that high wealth index would be an acceptable indicator of adequate nutrition, better living conditions, and easier access to health care, thus reducing seroprevalence in higher socio-economic classes for all NTDs (14, 47, 48). We found associations of greater wealth status and decreased seroprevalence among antigens of LF, strongyloides and giardiasis but no associations were observed for toxoplasmosis, or trachoma.

Common socio-demographic risk factors such as gender, ethnicity, education, occupation, toilet usage, and contact with animals have also been previously studied for LF, toxoplasmosis, and giardiasis in Malaysia (5, 15, 36, 49, 50). We examined these potential risk factors for all NTDs in our panel of antigens. In this study, significant risk of exposure for occupation was not observed for the any NTDs. Previous studies have found limited data on animal seroprevalence for toxoplasmosis in

Malaysia, including domestic and livestock animals (51), although Ngui et al found significant associations with seropositivity for individuals coming in close contact with cats and other pets (10). In our study, we did not find any significant associations with owning animals and increased odds of exposure for any NTD. We also included bath location in this risk factor analysis, as clean water is important in the prevention of diseases such as STHs and giardiasis, and we found significant associations in decreased prevalence with the use of bathrooms compared to outside bathing for giardiasis in this study.

In addition to identifying risk factors, we demonstrate how serological data can be used to characterise the spatial distribution of exposure. Simple visualisations of cluster level mean antibody responses can be used to quickly identify clusters with high responses to multiple pathogens. By integrating serological data into geostatistical models, we identified areas with differential exposure of diseases such as filariasis; this data can be used to supplement available infection reports to support elimination campaigns. Conversely, we also identify diseases with widespread transmission, such as giardiasis. Characterising these differences in spatial distribution allows development of appropriate control and surveillance strategies for diseases with vastly different transmission levels.

Within this study there are several limitations. Serological standards to determine cut-offs have not been established for most pathogens on this panel, and choice of cut-off method may have impacted the accuracy of seroprevalence approximations. Another limitation within the survey is the lack of individual migratory data for coastal Sabah, thus it is unclear whether serological responses represent regional or imported cases. Lastly, we applied a non-conventional method to cluster seropositives using k-means algorithm for correlated antigens of the same pathogens. While Trachoma estimates were similar to what was found previously, for LF antigens, discrepancy in seroprevalence estimates among mixture models and k-means clustering implores further exploration of using this method paired with clinically confirmed data or gold standard approaches.

Despite these limitations, this study demonstrates the utility of MBAs for simultaneous disease monitoring of diverse pathogens in low transmission settings. As integrated disease management is being adopted in the WHO NTD Roadmap of 2021 (52). MBAs with serological surveys can provide rich information regarding population exposure and associated socio-demographic or environmental risk factors impacting transmission of numerous co-endemic pathogens.

References

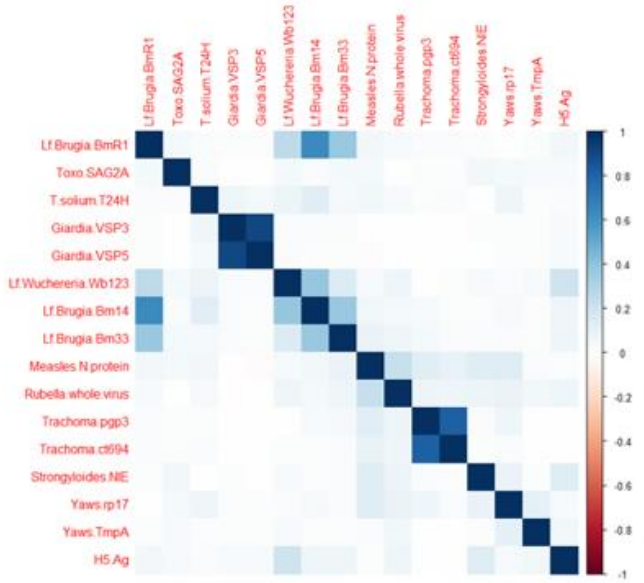
1. Hotez PJ. Aboriginal populations and their neglected tropical diseases. PLoS Negl Trop Dis. 2014;8(1):e2286.
2. Ahmad AF, Hadip F, Ngui R, Lim YAL, Mahmud R. Serological and molecular detection of Strongyloides stercoralis infection among an Orang Asli community in Malaysia. Parasitology Research. 2013;112(8):2811-6.
3. Lim YAL, Romano N, Colin N, Chow SC, Smith HV. Intestinal parasitic infections amongst Orang Asli (indigenous) in Malaysia: Has socioeconomic development alleviated the problem? Tropical Biomedicine. 2009;26(2):110-22.
4. Lim-Leroy A, Chua TH. Prevalence and risk factors of geohelminthiasis among the rural village children in Kota Marudu, Sabah, Malaysia. PLoS One. 2020;15(9):e0239680.
5. Al-Abd NM, Nor ZM, Ahmed A, Al-Adhroey AH, Mansor M, Kassim M. Lymphatic filariasis in Peninsular Malaysia: a cross-sectional survey of the knowledge, attitudes, and practices of residents. Parasites & Vectors. 2014;7.
6. Al-Mekhlafi HMS, Azlin M, Aini UN, Shaik A, Sa'iah A, Fatmah MS, et al. Protein-energy malnutrition and soil-transmitted helminthiases among Orang Asli children in Selangor, Malaysia. Asia Pacific Journal of Clinical Nutrition. 2005;14(2):188-94.
7. Dreyfuss ML, Stoltzfus RJ, Shrestha JB, Pradhan EK, LeClerq SC, Khatry SK, et al. Hookworms, malaria and vitamin A deficiency contribute to anemia and iron deficiency among pregnant women in the plains of Nepal. Journal of Nutrition. 2000;130(10):2527-36.
8. Simonsen PE, Derua YA, Magesa SM, Pedersen EM, Stensgaard AS, Malecela MN, et al. Lymphatic filariasis control in Tanga Region, Tanzania: status after eight rounds of mass drug administration. Parasites & Vectors. 2014;7.
9. Sinniah B, Hassan AKR, Sabaridah I, Soe MM, Ibrahim Z, Ali O. Prevalence of intestinal parasitic infections among communities living in different habitats and its comparison with one hundred and one studies conducted over the past 42 years (1970 to 2013) in Malaysia. Tropical Biomedicine. 2014;31(2):190-206.
10. Ngui R, Lim YAL, Amir NFH, Nissapatorn V, Mahmud R. Seroprevalence and Sources of Toxoplasmosis among Orang Asli (Indigenous) Communities in Peninsular Malaysia. American Journal of Tropical Medicine and Hygiene. 2011;85(4):660-6.
11. Yusof R, Lau YL, Mahmud R, Fong MY, Jelip J, Ngian HU, et al. High proportion of knowlesi malaria in recent malaria cases in Malaysia. Malaria Journal. 2014;13.
12. Ramdzan AR, Ismail A, Mohd Zanib ZS. Prevalence of malaria and its risk factors in Sabah, Malaysia. Int J Infect Dis. 2020;91:68-72.
13. Choy SH, Al-Mekhlafi HM, Mahdy MAK, Nasr NN, Sulaiman M, Lim YAL, et al. Prevalence and Associated Risk Factors of Giardia Infection among Indigenous Communities in Rural Malaysia. Scientific Reports. 2014;4.
14. Al-Mekhlafi HM, Al-Maktari MT, Jani R, Ahmed A, Anuar TS, Moktar N, et al. Burden of Giardia duodenalis infection and its adverse effects on growth of schoolchildren in rural Malaysia. PLoS Negl Trop Dis. 2013;7(10):e2516.
15. Sahimin N, Lim YAL, Ariffin F, Behnke JM, Basanez MG, Walker M, et al. Socio-demographic determinants of Toxoplasma gondii seroprevalence in migrant workers of Peninsular Malaysia. Parasites & Vectors. 2017;10.
16. Thayaparan S, Robertson ID, Fairuz A, Suut L, Abdullah MT. Leptospirosis, an emerging zoonotic disease in Malaysia. Malaysian Journal of Pathology. 2013;35(2):123-32.
17. Benacer D, Thong KL, Min NC, Bin Verasahib K, Galloway RL, Hartskeerl RA, et al. Epidemiology of human leptospirosis in Malaysia, 2004-2012. Acta Tropica. 2016;157:162-8.
18. Hotez PJ, Bottazzi ME, Strych U, Chang LY, Lim YAL, Goodenow MM, et al. Neglected Tropical Diseases among the Association of Southeast Asian Nations (ASEAN): Overview and Update. Plos Neglected Tropical Diseases. 2015;9(4).

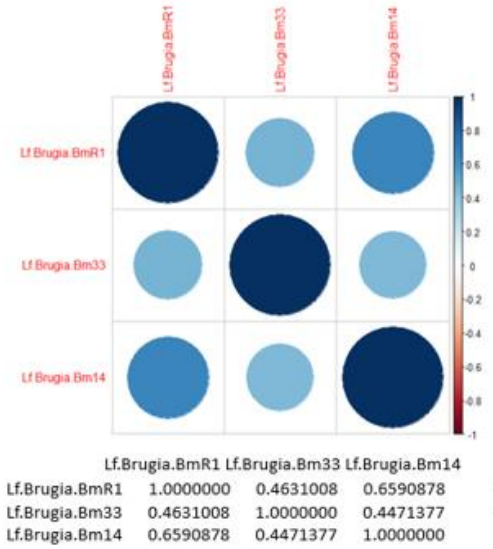
19. Lo EKC. Yaws in Malaysia. Reviews of Infectious Diseases. 1985;7:S251-S3.
20. Hotez PJ, Molyneux DH, Fenwick A, Kumaresan J, Sachs SE, Sachs JD, et al. Control of neglected tropical diseases. N Engl J Med. 2007;357(10):1018-27.
21. Lammie PJ, Moss DM, Brook Goodhew E, Hamlin K, Krolewiecki A, West SK, et al. Development of a new platform for neglected tropical disease surveillance. Int J Parasitol. 2012;42(9):797-800.
22. Arnold BF, van der Laan MJ, Hubbard AE, Steel C, Kubofcik J, Hamlin KL, et al. Measuring changes in transmission of neglected tropical diseases, malaria, and enteric pathogens from quantitative antibody levels. PLoS Negl Trop Dis. 2017;11(5):e0005616.
23. Njenga SM, Kanyi HM, Arnold BF, Matendechero SH, Onsongo JK, Won KY, et al. Integrated Cross-Sectional Multiplex Serosurveillance of IgG Antibody Responses to Parasitic Diseases and Vaccines in Coastal Kenya. Am J Trop Med Hyg. 2020;102(1):164-76.
24. Ondigo BN, Muok EMO, Oguso JK, Njenga SM, Kanyi HM, Ndombi EM, et al. Impact of Mothers' Schistosomiasis Status During Gestation on Children's IgG Antibody Responses to Routine Vaccines 2 Years Later and Anti-Schistosome and Anti-Malarial Responses by Neonates in Western Kenya. Front Immunol. 2018;9:1402.
25. Nissapatorn V, Suwanrath C, Sawangjaroen N, Ling LY, Chandeying V. Toxoplasmosis-serological evidence and associated risk factors among pregnant women in southern Thailand. Am J Trop Med Hyg. 2011;85(2):243-7.
26. Ngui R, Halim NA, Rajoo Y, Lim YA, Ambu S, Rajoo K, et al. Epidemiological Characteristics of Strongyloidiasis in Inhabitants of Indigenous Communities in Borneo Island, Malaysia. Korean J Parasitol. 2016;54(5):673-8.
27. Fornace KM, Brock PM, Abidin TR, Grignard L, Herman LS, Chua TH, et al. Environmental risk factors and exposure to the zoonotic malaria parasite Plasmodium knowlesi across northern Sabah, Malaysia: a population-based cross-sectional survey. Lancet Planet Health. 2019;3(4):e179-e86.
28. Wu L, Hall T, Ssewanyana I, Oulton T, Patterson C, Vasileva H, et al. Optimisation and standardisation of a multiplex immunoassay of diverse Plasmodium falciparum antigens to assess changes in malaria transmission using sero-epidemiology. Wellcome Open Res. 2019;4:26.
29. Benaglia T CD, Hunter DR, Young D. mixtools: An R Package for Analyzing Finite Mixture Models. Journal of Statistical Software. 2009;32:1-29.
30. Arnold BF, Martin DL, Juma J, Mkocha H, Ochieng JB, Cooley GM, et al. Enteropathogen antibody dynamics and force of infection among children in low-resource settings. Elife. 2019;8.
31. Lindgren F, Rue H. Bayesian Spatial Modelling with R-INLA. Journal of Statistical Software. 2015;63(19).
32. Simpson DP, Illian JB, Lindren F, Sorbye SH, Rue H. Penalising model component complexity: a principled, practical approach to constructing priors. Statistical Science. 2017.
33. Giorgi E, Diggle PJ, Snow RW, Noor AM. Geostatistical methods for disease mapping and visualisation using data from spatio-temporally referenced prevalence surveys. International Statistical Review. 2018;86(3):571-97.
34. R Core Team. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing; 2017.
35. Rascoe LN, Price C, Shin SH, McAuliffe I, Priest JW, Handali S. Development of Ss-NIE-1 recombinant antigen based assays for immunodiagnosis of strongyloidiasis. PLoS Negl Trop Dis. 2015;9(4):e0003694.
36. Yahaya N. Review of toxoplasmosis in Malaysia. Southeast Asian J Trop Med Public Health. 1991;22 Suppl:102-6.
37. Anuar TS, Azreen SN, Salleh FM, Moktar N. Molecular epidemiology of giardiasis among Orang Asli in Malaysia: application of the triosephosphate isomerase gene. Bmc Infectious Diseases. 2014;14.

38. Norhayati M, Penggabean M, Oothuman P, Fatmah MS. Prevalence and some risk factors of Giardia duodenalis infection in a rural community in Malaysia. Southeast Asian J Trop Med Public Health. 1998;29(4):735-8.
39. Butcher R, Sokana O, Jack K, Sui L, Russell C, Last A, et al. Clinical signs of trachoma are prevalent among Solomon Islanders who have no persistent markers of prior infection with Chlamydia trachomatis. Wellcome Open Res. 2018;3:14.
40. Cocks N, Rainima-Qaniuci M, Yalen C, Macleod C, Nakolinivalu A, Migchelsen S, et al. Community seroprevalence survey for yaws and trachoma in the Western Division of Fiji. Trans R Soc Trop Med Hyg. 2016;110(10):582-7.
41. Butcher R, Handley B, Garae M, Taoaba R, Pickering H, Bong A, et al. Ocular Chlamydia trachomatis infection, anti-Pgp3 antibodies and conjunctival scarring in Vanuatu and Tarawa, Kiribati before antibiotic treatment for trachoma. J Infect. 2020;80(4):454-61.
42. Kubofcik J, Fink DL, Nutman TB. Identification of Wb123 as an early and specific marker of Wuchereria bancrofti infection. PLoS Negl Trop Dis. 2012;6(12):e1930.
43. Hamlin KL, Moss DM, Priest JW, Roberts J, Kubofcik J, Gass K, et al. Longitudinal monitoring of the development of antifilarial antibodies and acquisition of Wuchereria bancrofti in a highly endemic area of Haiti. PLoS Negl Trop Dis. 2012;6(12):e1941.
44. Cooley GM, Mitja O, Goodhew B, Pillay A, Lammie PJ, Castro A, et al. Evaluation of Multiplex-Based Antibody Testing for Use in Large-Scale Surveillance for Yaws: a Comparative Study. J Clin Microbiol. 2016;54(5):1321-5.
45. Houweling TA, Karim-Kos HE, Kulik MC, Stolk WA, Haagsma JA, Lenk EJ, et al. Socioeconomic Inequalities in Neglected Tropical Diseases: A Systematic Review. PLoS Negl Trop Dis. 2016;10(5):e0004546.
46. Ngui R, Lim YA, Chong Kin L, Sek Chuen C, Jaffar S. Association between anaemia, iron deficiency anaemia, neglected parasitic infections and socioeconomic factors in rural children of West Malaysia. PLoS Negl Trop Dis. 2012;6(3):e1550.
47. Bangert M, Molyneux DH, Lindsay SW, Fitzpatrick C, Engels D. The cross-cutting contribution of the end of neglected tropical diseases to the sustainable development goals. Infect Dis Poverty. 2017;6(1):73.
48. Addiss DG. Soil-transmitted helminthiasis: back to the original point. Lancet Infect Dis. 2015;15(8):871-2.
49. Lim KC, Pillai R, Singh M. A study on the prevalence of antibodies to Toxoplasma gondii in Singapore. Southeast Asian J Trop Med Public Health. 1982;13(4):547-50.
50. Brandon-Mong GJ, Che Mat Seri NA, Sharma RS, Andiappan H, Tan TC, Lim YA, et al. Seroepidemiology of Toxoplasmosis among People Having Close Contact with Animals. Front Immunol. 2015;6:143.
51. Nasiru Wana M, Mohd Moklas MA, Watanabe M, Nordin N, Zasmy Unyah N, Alhassan Abdullahi S, et al. A Review on the Prevalence of Toxoplasma gondii in Humans and Animals Reported in Malaysia from 2008-2018. Int J Environ Res Public Health. 2020;17(13).
52. Organization. WH. Ending the neglect to attain the sustainable development goals: a framework for monitoring and evaluating progress of the road map for neglected tropical diseases 2021−2030. World Health Organization. 2021.

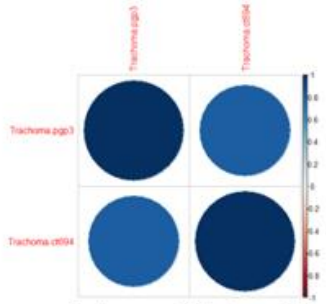
Supplementary Information.

F4. 9 **Supplementary Figure 1. Pearson's correlation among antigens within the study.**

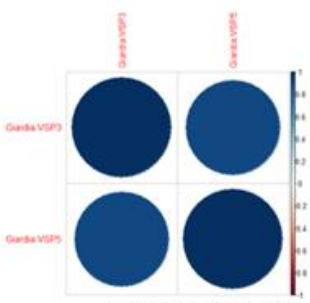




	Lf.Brugia.BmR1	Lf.Brugia.Bm33	Lf.Brugia.Bm14
Lf.Brugia.BmR1	1.0000000	0.4631008	0.6590878
Lf.Brugia.Bm33	0.4631008	1.0000000	0.4471377
Lf.Brugia.Bm14	0.6590878	0.4471377	1.0000000

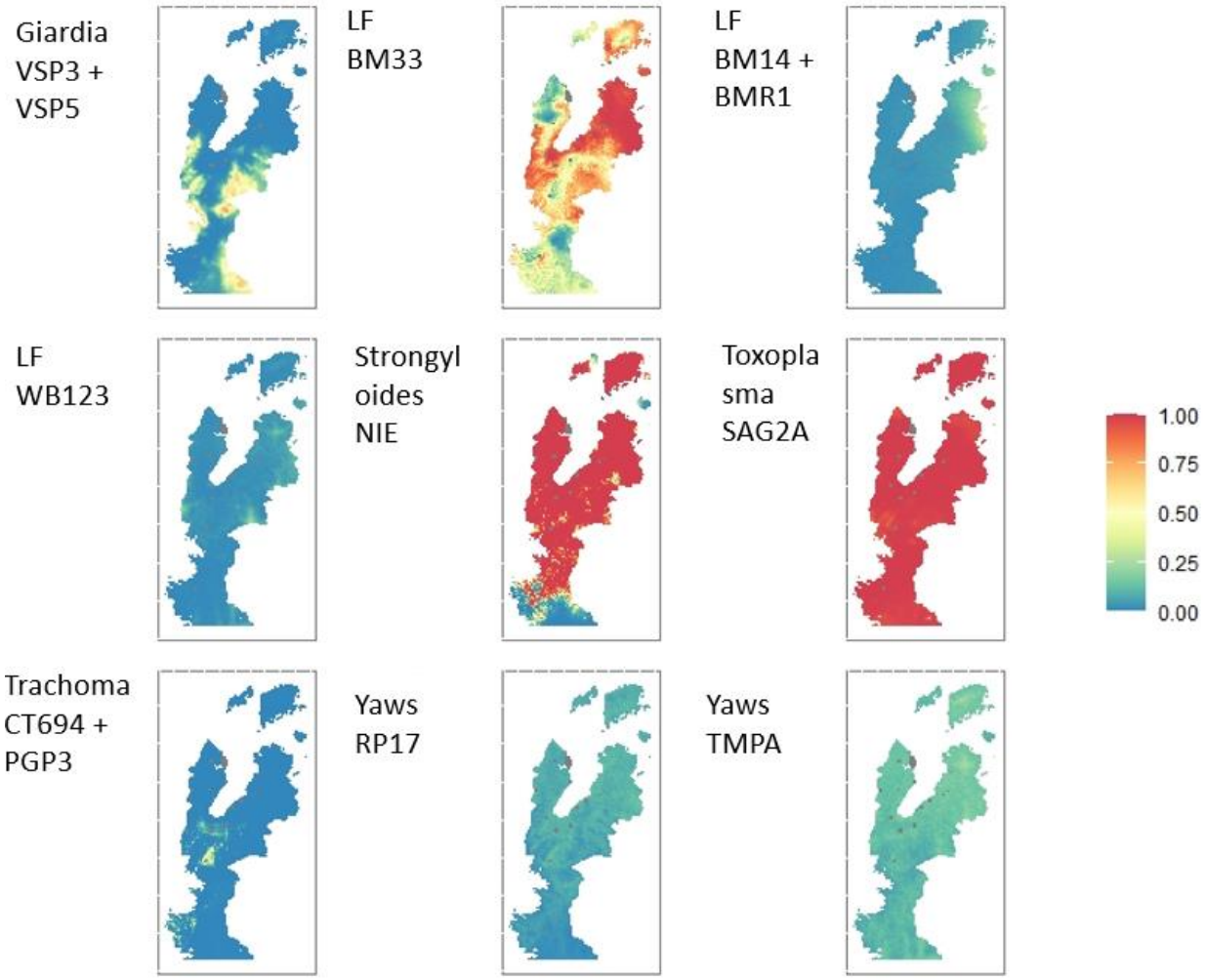


	Trachoma.pgp3	Trachoma.ct694
Trachoma.pgp3	1.0000000	0.8275665
Trachoma.ct694	0.8275665	1.0000000

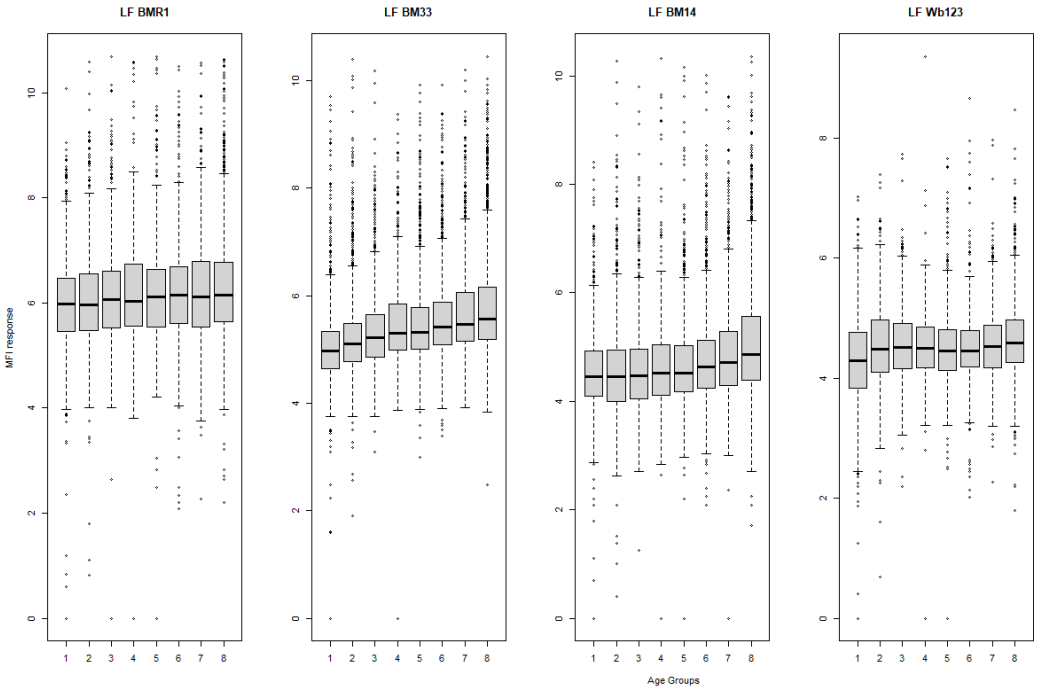


	Giardia.VSP3	Giardia.VSP5
Giardia.VSP3	1.0000000	0.9008358
Giardia.VSP5	0.9008358	1.0000000

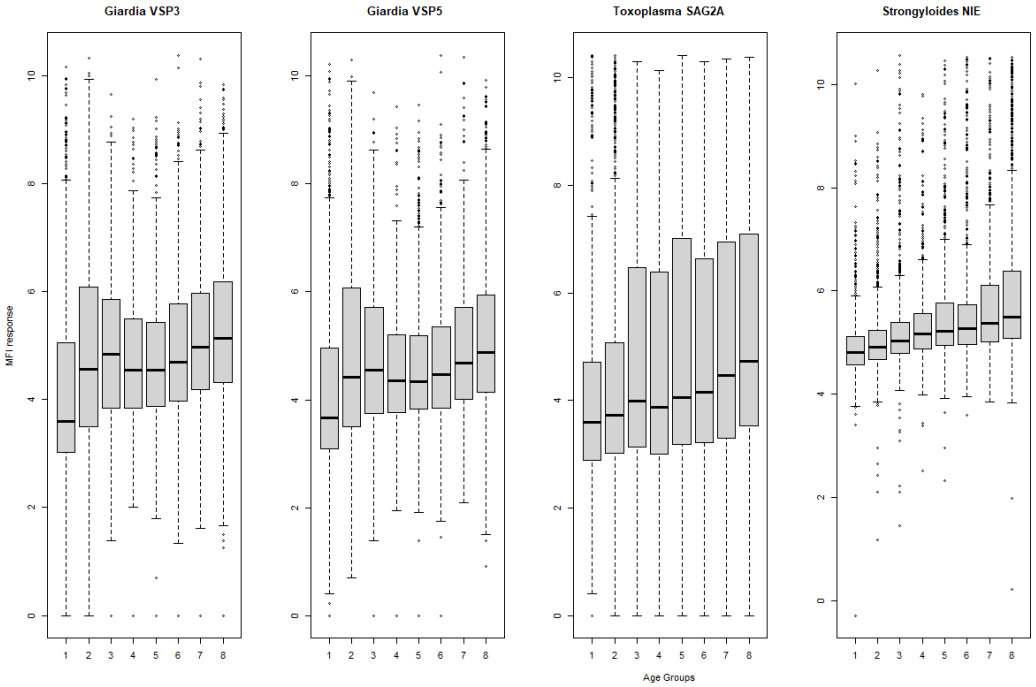
F4. 10 **Supplementary Figure 2. Maps show exceedance probability of seroprevalence estimates using an arbitrary 10% threshold per antigen.**



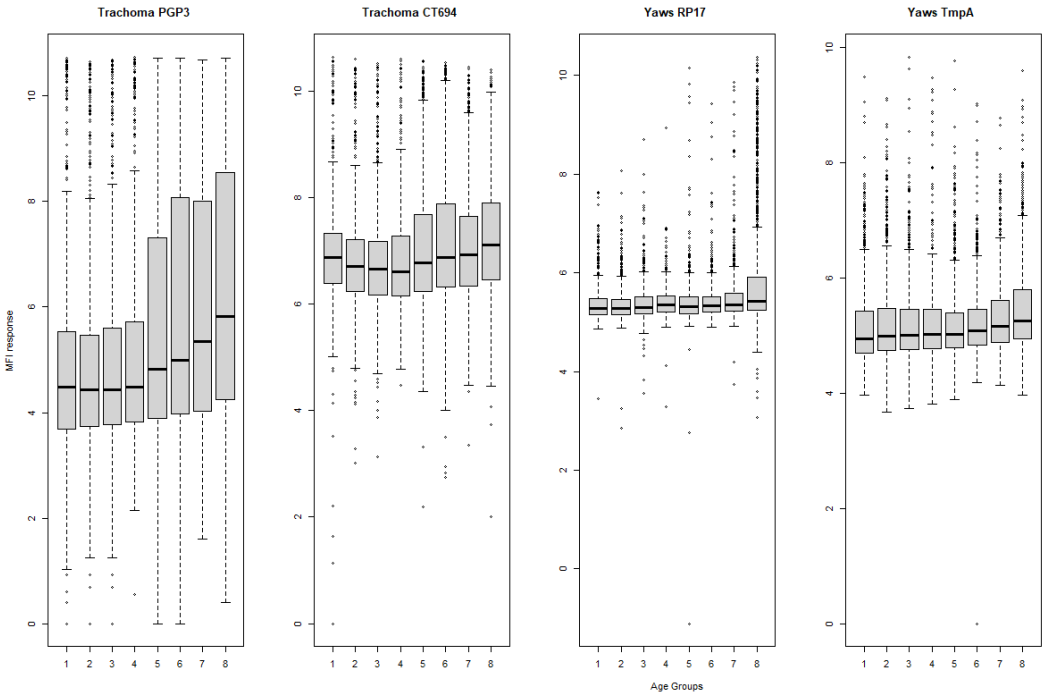
F4. 11 **Supplementary Figure 3. MFI response per age category for Lymphatic Filariasis antigens**



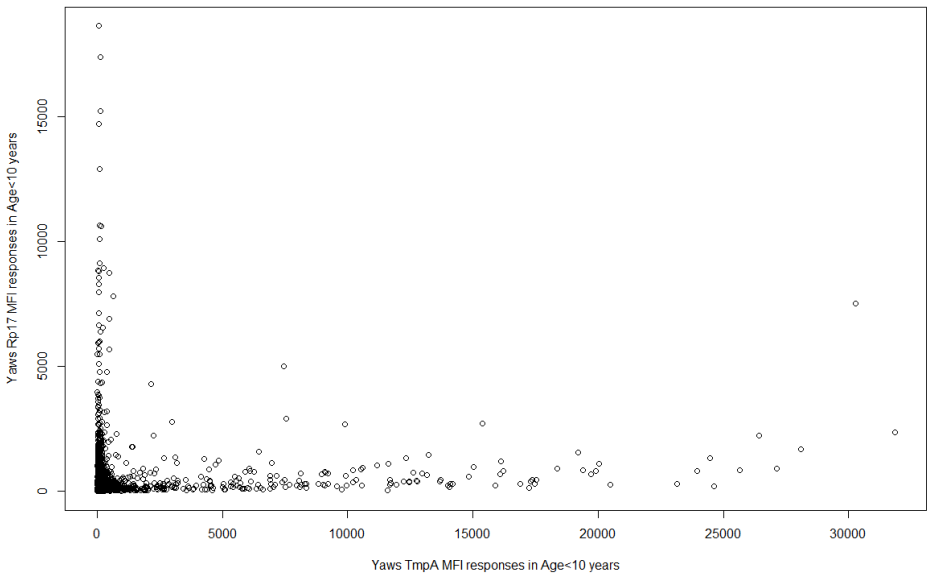
F4. 12 **Supplementary Figure 4. MFI response per age category for Giardia, Toxoplasma and Strongyloides antigens**



F4. 13 **Supplementary Figure 5. MFI response per age category for Yaws and Trachoma antigens**



F4. 14 **Supplementary Figure 6. Logged MFI antigen responses to Yaws Rp17 and TmpA is children less than 10 years of age**



T4. 8 **Supplementary Table 1. Seropositivity by antigens of risk factors**

Risk Factors (n)	Bm33	Wb123	BmR1+ 14	SAG2A	NIE	Rp 17	TmpA	Pgp+Ct	VSP3+5
N (8205)	8129	8128	6855	7430	8131	1529	1660	1970	7682
Gender									

M (3893)	11.62 (10.90-12.29)	1.87 (1.60-2.19)	3.96 (3.54-4.45)	31.26 (30.24-32.35)	19.69 (18.84-20.56)	14.31 (12.6-16.0)	27.20 (25.0-29.4)	3.32 (2.45-4.14)	23.67 (22.74-24.65)
F (4312)	10.20 (9.54-10.85)	1.52 (1.33-1.87)	3.15 (2.69-3.50)	28.62 (27.57-29.63)	14.22 (13.44-14.95)	13.14 (11.4-14.8)	23.98 (21.9-26.1)	4.55 (3.52-5.48)	22.85 (21.86-23.73)
Go Forest									
Y (615)	14.43 (13.63-15.17)	1.97 (1.41-1.98)	6.11 (5.54-6.65)	35.50 (36.59-34.41)	30.00 (29.00-30.99)	13.90 (12.3-15.7)	22.12 (20.1-24.1)	0.00 (0.00-0.00)	27.58 (26.59-28.60)
N (7538)	10.62 (9.93-11.27)	1.71 (1.69-2.31)	3.35 (2.98-3.82)	29.45 (30.44-28.36)	15.80 (16.59-15.00)	14.18 (11.9-15.3)	25.76 (23.7-27.9)	3.92 (2.97-4.82)	22.94 (21.95-23.84)
Wealth									
1 (1797)	13.00 (12.27 - 13.73)	1.92 (1.60-2.19)	4.49 (4.02-4.98)	31.61 (30.54-32.65)	20.72 (19.82-21.58)	17.11 (15.2-18.9)	30.99 (28.8-33.3)	3.40 (2.54-4.26)	30.82 (29.76-31.83)
2 (2037)	11.34 (10.61-20.12)	1.83 (1.51-2.09)	3.95 (3.44-4.35)	27.38 (26.38-28.41)	18.13 (17.26-18.94)	16.83 (14.9-18.7)	24.59 (22.5-26.7)	3.82 (2.89-4.71)	26.28 (25.31-27.28)
3 (2212)	10.23 (17.2-18.91)	1.92 (1.61-2.68)	3.56 (3.14-4.05)	29.23 (28.18-30.21)	16.57 (15.76-17.43)	12.95 (11.1-14.9)	26.01 (23.9-28.1)	4.69 (3.79-5.61)	20.40 (19.41-21.38)
4 (2159)	9.33 (14.4-16.23)	1.26 (1.01-1.58)	2.29 (1.85-2.75)	31.44 (30.39-32.41)	12.59 (11.76-13.43)	8.50 (6.63-10.4)	21.54 (19.4-23.6)	3.71 (2.79-4.61)	17.09 (16.11-18.08)
Occupation									
Farmer (1153)	15.91 (15.11-16.69)	2.20 (1.87-2.52)	5.67 (5.15-6.24)	37.12 (35.99-38.20)	28.21 (27.22-29.18)	-	-	-	26.46 (25.51-27.49)
None (3745)	10.91 (10.22-11.58)	1.77 (1.51-2.09)	3.20 (2.78-3.61)	29.67 (28.66-30.74)	16.07 (15.29-16.09)	-	-	-	21.46 (20.57-22.42)
Other (997)	12.74 (12.02-13.38)	1.31 (1.01-1.59)	4.60 (4.18-5.01)	35.78 (34.75-36.84)	23.84 (22.99-24.60)	-	-	-	21.99 (21.07-22.92)
Student (2245)	7.53 (6.82-8.17)	1.62 (1.31-1.89)	2.60 (2.18-3.01)	23.94 (22.86-24.94)	9.47 (8.69-10.30)	-	-	-	25.47 (24.57-26.42)
Ethnicity									
Bajau (752)	12.82 (12.08-13.53)	1.20 (0.96-1.44)	5.30 (4.77-5.83)	31.75 (30.74-32.86)	13.89 (13.41-14.65)	17.52 (15.6-19.4)	24.65 (22.5-26.7)	0.78 (0.37-1.22)	16.92 (16.05-17.74)
Dusun (4137)	9.59 (8.96-10.24)	1.20 (0.96-1.49)	2.28 (1.94-2.65)	30.81 (29.74-31.85)	17.14 (16.28-17.92)	11.16 (9.6-12.8)	22.20 (20.2-24.2)	4.82 (3.78-5.82)	26.49 (25.50-27.49)
Other (1135)	13.09 (12.45-13.74)	2.48 (2.26-2.74)	4.30 (3.95-4.65)	37.49 (36.44-38.56)	13.70 (12.88-14.52)	13.66 (12.1-15.3)	34.93 (32.9-36.9)	2.07 (1.07-3.12)	16.88 (15.90-17.89)
Rungus (2091)	11.69 (11.06-12.34)	2.57 (2.36-2.84)	4.79 (4.45-5.15)	23.67 (22.64-24.75)	19.20 (18.38-20.02)	16.37 (14.8-17.9)	26.46 (24.5-28.5)	3.51 (2.47-4.52)	22.84 (21.80-23.79)
Bednet									

Y (3175)	11.89 (11.19-12.06)	2.09 (1.78-2.41)	4.07 (3.63-4.56)	29.30 (28.26-31.6)	17.18 (16.38-18.02)	14.64 (12.4-15.9)	26.50 (24.6-28.9)	2.95 (2.19-3.18)	25.72 (24.71-26.68)
N (4964)	10.23 (9.53-10.86)	1.49 (1.23-2.41)	3.19 (2.78-3.61)	30.30 (29.25-31.34)	16.55 (15.78-17.41)	13.79 (11.5-14.9)	24.73 (22.3-26.5)	4.62 (3.60-5.59)	21.57 (20.67-22.52
Animals									
None (1900)	10.56 (9.93-11.27)	1.54 (1.23-1.76)	2.64 (2.23-2.97)	32.21 (31.13-33.26)	14.38 (13.63-15.16)	11.59 (1.91-21.3)	26.85 (13.45-40.25)	3.89 (2.98-4.81)	23.94 (22.94-24.85)
Domestic (1987)	10.41 (9.73-11.06)	1.79 (1.51-2.09)	3.40 (3.07-3.92)	27.93 (26.88-38.91)	17.49 (16.67-18.32)	28.57 (26.3-30.9)	38.1 (35.7-40.5)	6.28 (5.15-7.45)	23.30 (22.35-24.24)
Farm (4318)	11.23 (10.51-18.89)	1.77 (1.51-2.09)	3.95 (3.54-4.46)	29.67 (28.66-30.73)	17.57 (16.77-25.1)	13.3 (11.6-15.0)	25.2 (23.1-27.3)	2.83 (2.02-3.58)	22.90 (21.96-23.83)
Bath Location									
Bathroom (5152)	10.57 (9.93-11.27)	1.60 (1.32-1.87)	3.50 (3.07-3.92)	30.25 (29.15-31.24)	15.34 (14.51-16.09)	12.50 (6.85-18.1)	25.68 (18.3-33.1)	4.44 (3.42-5.38)	20.27 (19.39-21.20)
Outdoors (1638)	11.85 (11.19-12.61)	2.37 (2.07-2.73)	4.11 (3.63-4.56)	30.32 (29.25-31.34)	20.39 (19.52-21.28)	17.52 (11.0-23.9)	29.45 (21.8-37.2)	2.51 (1.75-3.24)	29.53 (28.47-30.52)
Water Pipe (1381)	11.15 (10.49-11.91)	1.49 (1.16-1.83)	3.03 (2.53-3.46)	28.11 (27.05-29.15)	18.60 (17.72-19.47)	15.84 (9.32-22.3)	23.97 (16.3-31.7)	3.57 (2.86-4.34)	27.37 (26.37-28.42)

T4. 9 **Supplementary Table 2. Odds ratios of risk factors for antigens.**

P-values of p<0.05 are bolded.

a. Bm33

Risk Factors	**Bm33**	**P Value**		
	Crude OR		**Adjusted OR**	**P-value**
Age				
	1.02(1.02-1.02)	<2e-16	1.02 (1.02-1.02)	2.2e-16
Gender (F)				
M	1.75 (1.01-1.36)	0.0318		
Go Forest (N)				
Y	1.48 (1.14-1.92)	0.00319		
Wealth (1)				
2	0.85 (0.68-1.08)			
3	0.73 (0.57-0.92)	0.0071		
4	0.66 (0.52-0.84)	0.0006	0.89 (0.82-0.96)	0.002076
Occupation (Farmer)				
None	0.15 (0.49-0.75)	3.56e-06		
Other	0.72 (0.55-0.94)	0.0169		
Student	0.40 (0.31-0.51)	8.54e-14		
Ethnicity (Bajau)				

Dusun	0.73 (0.55 -0.97)	0.0306	0.72 (0.54-0.94)	8.72e-05
Other	1.05 (0.75-1.45)		1.01 (0.74-1.39)	
Rungus	0.92 (0.68-1.24)		0.85 (0.63-1.14)	
Animals (N)				
Domestic	0.97 (0.76-1.24)			
Farm	1.07 (0.87-1.31)			
Bath Location (Bathroom)				
Outdoor	1.15 (0.94- 1.42)			
Water pip	1.11 (0.88 -1.39)			
Bednet (N)				
Y	1.21 (1.02- 1.42)	0.0253		

b. Wb123

Risk Factors	**Wb123**			
	Crude OR	**P Value**	**Adjusted OR**	**P-value**
Age				
	1.02 (1.01-1.02)	<2e-16		
Gender (F)				
M	1.05(1.04-1.06)	<2e-16		
Go Forest (N)				
Y	1.08 (0.44-2.65)			
Wealth (1)				
2	0.94 (0.42-2.09)			
3	0.98 (0.97-0.98)	<2e-16		
4	0.68 (0.68-0.68)	<2e-16		
Occupation (Farmer)				
None	0.70 (0.36-1.36)			
Other	0.39 (0.14-1.07)			
Student	0.45 (0.22-0.92)	0.0304		
Ethnicity (Bajau)				
Dusun	1.35 (0.34-5.96)			
Other	4.52 (1.01-20.17)	0.0484		
Rungus	2.73(0.62-11.95)			
Animals (N)				
Domestic	1.09 (1.08-1.01)	<2e-16		
Farm	1.09(0.62-1.92)			
Bath Location (Bathroom)				
Outdoor	1.48 (0.67-3.61)			
Water pip	1.71 (0.69-4.24)			
Bednet (N)				
Y	0.99 (0.56-1.77)			

c. BmR1 and Bm14

Risk Factors	**BmR1 and Bm14**			
	Crude OR		**Adjusted OR**	
Age				
	1.03 (1.02-1.03)	1.71e-08	1.01 (1.01-1.02)	5.65e-08
Gender (F)				

M	2.99 (1.64-5.48)			
Go Forest (N)				
Y	2.99 (1.64-5.47)	0.000353		
Wealth (1)				
2	0.87(0.39-1.95)			
3	0.68 (0.29- 1.56)			
4	0.465(0.18- 1.12)			
Occupation (Farmer)				
None	0.36 (0.21-0.60)	8.76e-05		
Other	0.65 (0.34-1.26)			
Student	0.28 (0.16-0.50)	1.60e-05		
Ethnicity (Bajau)				
Dusun	0.45 (0.17-1.18)		0.37 (0.19-0.71)	1.135e-06
Other	0.83 (0.30-2.37)		0.92 (0.48-1.78)	
Rungus	0.69 (0.26-1.81)		0.72 (0.38 -1.38)	
Animals (N)				
Domestic	1.39 (0.54-3.57)			
Farms	1.53 (0.68-3.46)			
Bath Location (Bathroom)				
Outdoor	1.15 (0.58-2.28)			
Water pip	0.78 (0.36—1.70)			
Bednet (N)				
Y	0.96 (0.56-1.66)			

d. SAG2A

Risk Factors	**SAG2A**			
	Crude OR		**Adjusted OR**	
Age				
	1.02 (1.02-1.02)	<2e-16	1.02 (1.02-1.02)	2.2e-16
Gender (F)				
M	1.15 (1.15-1.16)	<2e-16	1.20 (1.07-1.34)	0.00177
Go Forest (N)				
Y	1.39 (1.37-1.38)	<2e-16		
Wealth (1)				
2	0.79 (0.66-0.79)	0.0132		
3	0.88 (0.73-1.05)			
4	0.98 (0.82-1.18)			
Occupation (Farmer)				
None	0.67 (0.67-0.67)	<2e-16		
Other	0.93 (0.93-0.93)	<2e-16		
Student	0.50 (0.50-0.50)	<2e-16		
Ethnicity (Bajau)				
Dusun	0.92 (0.74-1.16)		0.89 (0.71-1.13)	4.71e-13
Other	1.32 (1.02-1.72)	0.036488	1.31 (1.00-1.75)	
Rungus	0.62 (0.48-0.79)	0.000112	0.58 (0.45-0.74)	
Animals (N)				
Domestic	0.79(0.79-0.79)	<2e-16		

Farm	0.88 (0.88-0.88)	<2e-16		
Water Bath Location				
Outdoor	1.02 (0.86-1.20)			
Water pip	0.90 (0.76-1.07)			
Bednet (N)				
Y	0.98 (0.97-0.98)			

e. NIE

Risk Factors	**NIE**			
	Crude OR		**Adjusted OR**	**P-value**
Age				
	1.03 (1.03-1.04)	<2e-16	1.03 (1.03-1.05)	< 2e-16
Gender (F)				
M	1.51 (1.33-1.71)	7.29e-11	1.53 (1.34-1.74)	3.14e-10
Go Forest (N)				
Y	2.44 (1.98-3.01)	<2e-16	1.48 (1.19-1.84)	0.000442
Wealth (1)				
2	0.22 (0.19-0.26)		0.84 (0.79-0.90)	2.53e-07
3	0.76 (0.63-0.92)	0.00425		
4	0.53 (0.43-0.65)	8.61e-10		
Occupation (Farmer)				
None	0.45 (0.38-0.54)	<2e-16		
Other	0.78 (0.63-0.97)	0.0269		
Student	0.24 (0.19-0.30)	<2e-16		
Ethnicity (Bajau)				
Dusun	1.27 (0.98-1.64)			
Other	0.96 (0.71-1.30)			
Rungus	1.49 (1.13-1.94)	0.00407		
Animals (N)				
Domestic	1.28 (1.05-1.58)	0.01698		
Farm	1.30 (1.09-1.56)	0.00365		
Bath Location (Bathroom)				
Outdoor	1.44 (1.22-1.72)	1.59e-05		
Water pip	1.26 (1.05 -1.52)	0.0112		
Bednet (N)				
Y	1.05 (0.91-1.21)			

f. VSP3 and VSP5

Risk Factors	**VSP3 and VSP5**			
	Crude OR		**Adjusted OR**	**P-value**
Age				
	1.02 (1.01-1.02)	< 2e-16	1.02 (1.01-1.02)	< 2e-16
Gender (F)				
M	1.08 (0.95-1.22)			
Go Forest (N)				

Y	1.31 (1.03-1.66)			
Wealth (1)				
2	0.78 (0.63-0.97)	0.0258	0.72 (0.67-0.79)	5.07e-13
3	0.51 (0.41-0.63)	2.94e-09		
4	0.39 (0.31-0.49)	9.53e-16		
Occupation (Farmer)				
None	0.72 (0.59-0.87)	0.000815	0.97 (0.78-1.18)	
Other	0.83 (0.64-1.06)		1.09 (0.85-1.43)	
Student	0.96 (0.78-1.18)		1.88 (1.46-2.42)	9.32e-07
Ethnicity (Bajau)				
Dusun	1.76 (1.33-2.34)	8.33e-05	2.02 (1.50-2.71)	3.94e-06
Other	0.97 (0.69-1.36)		0.97 (0.69- 1.37)	
Rungus	1.47 (1.09-1.99)	0.0119	1.39 (1.02- 1.91)	
Animals (N)				
Domestic	0.91 (0.72-1.15)	0.0400	0.76 (0.60-0.98)	0.7511
Farm	0.96 (0.79-1.18)	0.040	0.74 (0.61-0.92)	
Bath Location (Bathroom)				
Outdoor	1.87 (1.55-2.26)	7.33e-11	1.54 (1.25-1.91)	4.70e-05
Water pip	1.49 (1.21- 1.83)	0.000122	1.09 (0.87-1.36)	
Bednet (N)				
Y	1.32 (1.13-1.54)	0.000329		

g. Rp17

Risk Factors	**Rp17**		
	Crude OR	**Adjusted OR**	
Age			
	1.03 (1.02-1.04)		
Gender (F)			
M	0.65 (0.46-0.92)		
Go Forest (N)			
Y	0.00 (0.50-2.53)		
Wealth (1)			
2	1.02 (0.58-1.79)		
3	1.48 (0.84-2.59)		
4	1.13 (0.63-1.99)		
Occupation (Farmer)			
None	-	-	
Other	-	-	
Student	-	-	
Ethnicity (Bajau)			
Dusun	0.49 (0.24-0.96)	0.44 (0.22-0.89)	5.726e-06
Other	0.64 (0.29-1.41)	0.66 (0.31-1.42)	
Rungus	0.63 (0.31-1.27)	0.59 (0.28-1.25)	
Animals (N)			
Y	-	-	
Bath Location (Bathroom)			

Outdoor	0.86 (0.43-1.75)		
Water pip	0.89 (0.51-1.53)		
Bednet (N)			
Y	0.71 (0.47-1.05)		

h. Ct694 and Pgp3

Risk Factors	**Pgp3 and Ct694**			
	Crude OR		**Adjusted OR**	**P-value**
Age				
	1.07 (0.88-1.31)	0.494	1.02 (1.02-1.04)	1.26e-13
Gender (F)				
M	0.42 (0.15-0.12)			
Go Forest (N)				
Y	-			
Wealth (1)				
2	1.02 (0.08- 12.09)			
3	1.01 (0.09-13.50)			
4	0.96 (0.07-12.38)			
Occupation (Farmer)				
None	-			
Other	-			
Student	-			
Ethnicity (Bajau)				
Dusun	1.02 (0.08-12.09)			
Other	1.10 (0.09-13.31)			
Rungus	0.96 (0.08-12.37)			
Animals (N)				
Domestic	-			
Farm	-			
Bath Location (Bathroom)				
Other	0.17 (0.00-5.03)			
Water pip	0.14 (0.00-1.60)			
Bednet (N)				
Y	1.25 (0.27-5.78)			

T4. 10 **Supplementary Table 3. Spatial and environmental covariates**

Parameter	Description	Resolution	Source
Elevation	Elevation (metres above sea level)	30 m	ASTER Global Digital Elevation Map (51)
Slope and aspect	Slope and aspect (degrees)	30 m	Calculated from ASTER Global Digital Elevation Map
TWI	Topographic wetness index	30m	Calculated from ASTER Global Digital Elevation Map

NDVI	Normalised differential vegetation index	30 m	Calculated from NIR and Red Landsat8 bands (52)
Land cover	Euclidean distance to different land cover types	30 m	Calculated from land cover map of study site, described by (26)
Location of roads and houses	GPS coordinates	5 m	Mapped during GPS field surveys, described by (26)
Distance to roads and houses	Distance from nearest road and house	30 m	Calculated from GPS coordinates
Population density	UN-adjusted 2015 population density	100 m	World Pop (53)
Bioclimatic variables	Bioclimatic indicators of ecology, 1970 - 2000	1000 m	Calculated from (54)

T4. 11 **Supplementary Table 4. Mean posterior estimates of coefficients of fixed effects and spatial range for geostatistical models for household seroprevalence to:**

a. LF WB123

Covariate*	Mean	95% Bayesian Credible Interval (BCI)	
		2.5%	97.5%
NDVI	0.138	-0.021	0.300
Mean diurnal range	-2.713	-5.096	-0.143
Maximum temperature	1.626	-0.299	3.496
Minimum temperature	-2.514	-5.883	0.984
Precipitation	0.371	-0.359	0.876
Seasonality	-1.403	-2.807	-0.116
Distance to bush forest	0.382	0.055	0.689
Distance to old forest	0.128	-0.148	0.371
Spatial range (km)	86.17	75.31	96.64

* All covariates mean-centered and squared

b. LF BM14/ BMR1

Covariate*	Mean	95% BCI	
		2.5%	97.5%
Elevation	1.048	-0.517	2.612
Maximum temperature	0.734	-0.445	1.911
Distance to mangroves	-1.039	-2.576	0.496
Population density ^ 2	-0.199	-0.444	0.047
Spatial range (km)	183.9	40.63	592.73

* All covariates mean-centered and squared

c. LF BM33

Covariate*	Mean	95% BCI	
		2.5%	97.5%
Average temperature	-0.799	-3.103	1.281
Mean diurnal range	0.627	-0.905	2.291
Population density ^ 2	-0.218	-0.495	0.002
Spatial range (km)	197.31	42.435	630.12

* All covariates mean-centered and squared

d. Giardia

Covariate*	Mean	95% BCI	
		2.5%	97.5%
NDVI	-0.064	-0.179	0.050
Distance from sea	2.082	0.453	3.709
Average temperature	-3.854	-6.950	-0.761
Mean diurnal range	-1.905	-3.599	-0.213
Maximum temperature	2.368	0.153	4.582
Distance from agricultural land	-1.820	-3.017	-0.625
Spatial range (km)	21.76	4.810	64.255

* All covariates mean-centered and squared

e. Strongyloides

Covariate*	Mean	95% BCI	
		2.5%	97.5%
NDVI	0.060	0.007	0.112
Elevation	-0.879	-1.659	-0.099
Average temperature	-0.827	-1.626	-0.028
Mean diurnal range	-0.118	-0.299	0.063
Spatial range (km)	3.54	1.604	6.365

* All covariates mean-centered and squared

f. Trachoma (under 10 years old)

Covariate*	Mean	95% BCI	
		2.5%	97.5%
Aspect	-0.091	-0.200	0.014
Elevation	1.349	-0.511	3.221
Distance from sea	-2.380	-4.134	-0.652
Mean diurnal range	3.422	1.460	5.398
Minimum temperature	2.672	0.121	5.281

* All covariates mean-centered and squared

g. Yaws RP17 (under 10 years old)

Covariate*	Mean	95% BCI	
		2.5%	97.5%
Slope	-0.110	-0.257	0.038

Distance from the sea	-0.973	-1.615	-0.332
Maximum temperature	1.023	0.414	1.632
Minimum temperature	-1.242	-2.165	-0.319
Spatial range (km)	6.541	0.320	31.223

* All covariates mean-centered and squared

h. Yaws TMPA (under 10 years old)

Covariate*	Mean	95% BCI	
		2.5%	97.5%
Mean diurnal range	-1.181	-2.118	-0.256
Maximum temperature	0.749	-0.009	1.513
Minimum temperature	-1.142	-2.528	0.231
Precipitation	0.094	0.014	0.173
Distance from sparse forest	0.149	0.021	0.274
Distance from mangroves	0.451	-0.027	0.933
Distance from irrigated crops	-0.144	-0.324	0.028

* All covariates mean-centered and squared

i. Toxoplasmosis

Covariate*	Mean	95% BCI	
		2.5%	97.5%
NDVI	0.034	-0.014	0.082
Distance from sea	-0.612	-1.435	0.210
Maximum temperature	0.639	-0.002	1.280
Minimum temperature	-0.916	-2.003	0.169
Spatial range (km)	21.85	11.005	39.245

* All covariates mean-centered and squared

4.3.1 Research Conclusions

Similar to the study conducted in Haiti, serological MBAs were capable of assessing exposure to multiple pathogens in Malaysia. Compared to the previous section of this chapter, this work demonstrated the utility of serological MBAs to identify risk factors associated with exposure for many different pathogens simultaneously. Identifying specific risk factors and visualizing the distribution of transmission heterogeneity through predictive mapping can help to enable targeted interventions for integrated disease programs.

In Malaysia, serological MBAs were able to determine seroprevalence estimates for several pathogens where current burden of disease is unknown or thought to have been eliminated. While this may highlight the capacity of MBAs to capture unexpected burden of disease, it also brings to question the validity of some of these results. For example, seroprevalence of yaws was surprising to several co-authors, as this disease was previously eliminated. I also observed elevated antibody responses for trachoma in children, which is also unexpected in Malaysia. While some studies have seen similar antibody responses in other countries non-endemic for trachoma (as discussed in previous research paper), whether these estimates are reflective of transmission in Sabah should entail consulting clinical data and asking clinicians if they have seen actives cases in children. Additionally, I found that I frequently needed to seek individual expertise around specific pathogens to guide results and conclusions. Ideally, the analysis of MFI values may need to be supported by integrated disease programs with panels of experts for each of the co-endemic diseases assessed.

In trying to make sense of my results, I also examined existing literature. Some prior prevalence estimates for several diseases were not based on serological estimates and I found it difficult to make direct comparisons between my results of exposure prevalence and previous prevalence estimates. These limitations may underscore the need for validating antigens to clinical data to assess any potential biases in using serology for NTD surveillance, as discussed in the previous section.

4.4 Chapter Conclusions

The objective of Chapter 4 was to determine the utility of serological MBAs to support integrated disease monitoring and surveillance. To achieve **Objective 2**, two outcomes were evaluated in Haiti and Malaysia using serological MBAs (Chapter 4), which were to 1) assess multi-disease prevalence estimates and 2) assess associated risk factors of disease exposure.

Being able to produce multi-disease prevalence estimates is a considerable advantage of using serologic MBAs in surveillance, as these estimates can be assessed simultaneously to inform different disease programs and enable collaboration of targeted interventions. At the national level in Haiti, analysis of MFI data provided prevalence estimates to eleven different pathogens across ten Haitian administrative departments. The panel of sixteen antigens included biologically diverse pathogens from two arboviruses (chikungunya and dengue), three waterborne pathogens (*Escherichia coli*, *Vibrio cholerae,* and *Cryptosporidium parvum*), two vectorborne helminths (*Plasmodium falciparum and Wucheria bancrofti)*, three bacterial pathogens (Clostridium *tetani, Chlamydia trachomatis, Treponema pallidum), Strongyloides stercoralis, and Toxoplasma gondii*. At the state administrative level, prevalence was established for five different pathogens (*Toxoplasma gondii, Chlamydia trachomatis, Treponema pallidum, Strongyloides stercoralis, Wucheria bancrofit, Brugia malayi*) in Sabah, Malaysia.

Additionally, serological MBAs can monitor pathogens without current epidemiological estimates, as demonstrated in these studies with yaws, trachoma, and strongyloidiasis. This feature is especially important as the global health community pushes forward in the elimination of NTDs. Identifying potential disease recrudescence or inception will engender attention for interventions or additional surveillance.

In Haiti, epidemiological analysis of MFI data with age as a risk factor (and proxy for time) showed significant changes in population antibody levels over time for most antigens in the panel. In Malaysia, age, gender, socio-economic status, occupation, spatial, and ethnicity risk factors were found to be associated with seroprevalence of most antigens assessed. This information can be useful in advising programs where to divert resources to maximize resources for control activties.

Within this chapter, I focused primarily on determining the potential utility of MBAs without formal analysis of co-endemicity (results typically were presented for individual diseases, despite being simultaneously assayed). A more vigorous evaluation of co-endemicity may provide additional insight into concomitant exposure patterns of tropical diseases and may be a relevant topic for future research using serological MBA data. For example, pinpointing geographic clusters of complete seronegatives to all antigens may implore investigating past interventions that lead to

exposure reduction in those clusters, or perhaps is attributable to a dearth of disease vector due to climatological changes or programmatic interventions. In contrast, finding geographic clusters of complete seropositives to all antigens may indicate the need integrated disease interventions.

Assessing individual co-exposure can also be done using antigen seropositivity as individual risk factors for other diseases. This may help to understand any associated exposure between pathogens (taking into consideration any cross-reactivity), in addition to known demographic, socio-economic, and transmission risk factors. The presence of co-exposure may also justify the need for integrated control measures, such as MDA and IVM within targeted populations.

Chapter 4 provided evidence of the capability of serological MBAs to understand risk factors associated with exposure to multiple tropical diseases simultaneously and is a significant chapter in the overarching aim of the thesis to determine the appropriateness of these platforms. There are several important public health implications of these findings, including the use of serological MBAs to support integrated disease initiatives, routine surveillance, and epidemiological research of NTDs, that will be further deliberated and discussed in Chapter 6. In the next Chapter, I will further explore the potential for serological MBAs to support integrated disease surveillance using easy access groups as part of convenience sampling.

4.5 References

1. Ending the neglect to attain the Sustainable Development Goals – A road map for neglected tropical diseases 2021–2030. Geneva: World Health Organization; 2020. Licence: CC BY-NC-SA 3.0 IGO.
2. Hotez PJ, Molyneux DH, Fenwick A, Kumaresan J, Sachs SE, Sachs JD, et al. Control of neglected tropical diseases. N Engl J Med. 2007;357(10):1018-27.
3. Tambo E, Ai L, Zhou X, Chen JH, Hu W, Bergquist R, et al. Surveillance-response systems: the key to elimination of tropical diseases. Infect Dis Poverty. 2014;3:17.
4. Hotez PJ, Fenwick A, Savioli L, Molyneux DH. Rescuing the bottom billion through control of neglected tropical diseases. Lancet. 2009;373(9674):1570-5.

CHAPTER 5: APPLICATION OF SEROLOGICAL MBA TO EASY ACCESS GROUPS SAMPLING

5.1 Background and Rationale

Research Rationale

Surveillance plays a critical role in the control of infectious diseases (1). Current approaches to surveillance for many NTDs depend on passive surveillance. However, passive surveillance has several important limitations, as outlined in the introduction of this thesis, that can lead to under reporting of cases due to asymptomatic infections. Implementing active surveillance to complement potential shortcomings of passive surveillance as part of an integrated surveillance framework should help to determine more complete estimates of disease burden.

In active disease surveillance, community wide household surveys can generate very robust prevalence estimates, but they are often expensive, labour intensive, and may not be feasible to be implemented frequently (2, 3). One sampling approach, known as convenience sampling, offers a less resource intensive sampling alternative to community wide household surveys, and may generate more complete estimates of prevalence than relying on care-seeking behaviour in passive surveillance. However, an expected challenge to convenience sampling is the lack of generalizability to the target population, due to over or under representation of subpopulations within the sample population, leading to biased estimates (4). For example, sub populations using children sample may have different exposure responses compared to adults, while sub populations able to attend health facilities may have different ability to access care and economic potential compared to those who cannot attend (5).

There is evidence showing that convenience sampling of easy access groups (EAG) are able to reflect trends of community exposure to malaria (3). EAG sampling occurs at strategic locations such as community centres, schools, and health facilities that are then used to represent associated geographic catchments in the community (6, 7). Compared to community household sampling (HH), EAG sampling is less resource demanding and more convenient to employ (3). In theory, EAG sampling could also be applied to multi-disease serosurveillance of NTDs but has not yet been assessed in comparison to community household sampling.

Overview

Previous chapters investigated several important gaps of knowledge pertaining to using serological MBAs toward understanding multi-disease exposure and associated risk factors. The research in this chapter instead applies serological MBAs to evaluate whether EAG surveys can generate seroprevalence estimates that reflect exposure in the corresponding community to support surveillance of NTDs and VPDs. If shown to provide consistent estimates, EAGs may provide an operational sampling approach for routine monitoring of NTDs using serological MBAs.

Chapter 5 address **Objective 3.** The objectives of Paper 5 are to use serological MBAs to assess whether EAG seroprevalence estimates are concordant with community surveys from the same target population employing MBAs in Artibonite, Haiti.

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT
T: +44 (0)20 7299 4646
F: +44 (0)20 7299 4656
www.lshtm.ac.uk

RESEARCH PAPER COVER SHEET

Please note that a cover sheet must be completed for each research paper included within a thesis.

SECTION A – Student Details

Student ID Number	1603698	**Title**	Mr
First Name(s)	YuYen		
Surname/Family Name	Chan		
Thesis Title	Comparability of Easy Access Groups and Household Serological Surveys to Monitor Neglected Tropical Diseases and Vaccine Preventable Diseases in Artibonite District, Haiti		
Primary Supervisor	Chris Drakeley		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?			
When was the work published?			
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion			
Have you retained the copyright for the work?*	Choose an item.	Was the work subject to academic peer review?	Choose an item.

*If yes, please attach evidence of retention. If no, or if the work is being included in its published format, please attach evidence of permission from the copyright holder (publisher or other author) to include this work.

SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	Undecided
Please list the paper's authors in the intended authorship order:	YuYen Chan, Lotus Van denHoogen, Thomas Druetz, Karen E. S. Hamre, Eric Rogier, Vena Joseph, Jean Frantz Lemoine, Michelle Chang, Thomas P. Eisele, Chris Drakeley, Gillian Stresman

Stage of publication	**Not yet submitted**

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	Writing and drafting manuscript, analysis included determing seroprevalence and assessing seroposivity to each antigen, assessing agreement and concordance

SECTION E

Student Signature	YuYen Chan
Date	20/09/2021

Supervisor Signature	Chris Drakeley
Date	16/09/2021

5.2 Application of multiplex bead assays to support monitoring using easy access groups

Comparability of Easy Access Groups and Household Serological Surveys to Monitor Neglected Tropical Diseases and Vaccine Preventable Diseases in Artibonite District, Haiti

Authors and Affiliations

YuYen Chan[1], Lotus Van denHoogen[1,2], Thomas Druetz[2,3], Karen E. S. Hamre[4,5], Eric Rogier[4], Vena Joseph[2,6], Jean Frantz Lemoine[7], Michelle Chang[4], Thomas P. Eisele[2], Chris Drakeley[1], Gillian Stresman[1]

1. Department of Infection Biology, London School of Hygiene and Tropical Medicine, London, United Kingdom
2. Center of Applied Malaria Research and Evaluation, Department of Tropical Medicine, Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, USA
3. Department of Social and Preventive Medicine, School of Public Health, University of Montreal, QC, Canada
4. Centers for Disease Control and Prevention, Center for Global Health, Division of Parasitic Diseases and Malaria, Malaria Branch, Atlanta, Georgia, USA
5. CDC Foundation, Atlanta, Georgia, USA
6. Malaria Zero Alliance, CDC Foundation, Port-au-Prince, Haiti
7. Programme National de Contrôle de la Malaria, Ministère de la Santé Publique et de la Population (MSPP), Port-au-Prince, Haiti

Abstract

Background: Despite scaled up control efforts, many endemic countries continue to struggle to achieve elimination targets of neglected tropical diseases (NTDs). One of the challenges is limited surveillance associated with many of these diseases. 'Easy access' subpopulations combined with multiplex serology technology provide a novel, operationally attractive approach to enhance surveillance for multiple infectious diseases, but limited evidence exists for how these compare with other surveillance, more standard estimates.

Methods: Easy access group (EAG, here, comprising primary schools and health facilities) and a household cross-sectional survey were conducted in Artibonite, Haiti in 2017. Antibodies to two neglected tropical diseases (lymphatic filariasis and strongyloidiasis) and two vaccine preventable diseases (measles and tetanus) were assessed using multiplex bead assay (MBA). Concordance of log-mean antibody concentrations and seroprevalence were assessed between school and health facility catchments of the the two surveyed populations using Lin's Concordance Correlation.

Results: For school catchments, the mean seroprotection and range for measles was 99.9% (99.1%-100.0%) in schools and 99.4% (95.3%-100.0%) in the community; the mean seroprotection and range for tetanus was 99.9% (98.0%-100.0%) in schools and 99.5% (97.6%-100.0%) in the community; the mean seroprevalence for strongyloidiasis was 11.9% (0.0%-24.4%) in schools and 0.0% (0.0%-2.2%) in the community; the mean seroprevalence and range for LF Wb was 7.0% (0.0%-24.6%) in schools and 1.9% (0.0%-4.7%) in the community; the mean seroprevalence and range for LF Bm was 18.3% (2.0%-26.8%) in schools and 18.8% (2.0%-26.1%) in the community. For health facility catchments, the mean seroprotection and range for measles was 100.0% (100.0%-100.0%) in health facilities and 99.5% (98.3%-100%) in the community; the mean seroprotection and range for tetanus was 100.0% (100.0%-100.0%) in schools and 99.5% (98.3%-100%) in the community; the mean seroprevalence for strongyloidiasis was 8.1% (0.0%-14.8%) in schools and 0.7% (0.0%-2.1%) in the community; the mean seroprevalence and range for LF Wb was 7.4% (0.0%-13.0%) in schools and 1.5% (0.0%-11.4%) in the community; the mean seroprevalence and range for LF Bm was 18.5% (10.0%-34.7%) in schools and 17.1% (12.8%-20.4%) in the community. No concordance was observed for logged mean median fluorescent intensities (MFI) or seoprevalance between the household and easy access group surveys for any antigen. Consistency of seroprevalence rankings per catchment area was identified for only lymphatic filariasis BM33 antigen between surveys (Rank sum, $p = 0.05$). Hypothetical, programmatic seroprevalence thresholds highlighted potential comparability between surveys that were antigen specific.

Conclusion: Lack of concordance of mean logged MFI and seroprevalence of all studied pathogens highlights inherent differences in population characteristics between EAG venues and the community. EAG surveys may not be reliable in assessing community seroprevalence estimates for these diseases within the corresponding catchment area. However, agreement in mean differences in seroprevalence across all catchments suggests that EAG surveys could be informative at larger spatial aggregations. Additionally, EAGs may be useful for certain antigens with predefined programmatic thresholds and EAGs include age groups of interest.

Introduction

Monitoring neglected tropical diseases (NTD) remains a challenge in resource-constrained settings, despite significant improvements in surveillance and diagnostics of NTDs within the past two decades (1). Asymptomatic and sub-clinical cases, which may not be captured within health facility-based surveillance efforts for NTD, may hinder control and elimination initiatives due to incomplete knowledge of disease epidemiology. Active surveillance is therefore necessary to provide critical information to NTD control programs regarding areas of transmission interruption, ongoing transmission, and to prioritize intervention (1, 2). In recent years, attention has increasingly focused towards monitoring these pathogens using serology, which examines antibodies in human sera (3, 4). An advantage of using serology as a surveillance tool is its ability to detect historical exposure to pathogens, thus greatly enhancing the capacity to understand transmission heterogeneity and exposure even in low endemic settings (5, 6).

Population estimates are ideally ascertained through community-based cross-sectional surveys involving household visits. These surveys can estimate population prevalence due to representative sampling designs, including an assessment of geographic distribution and demographic distribution (7). However, these surveys are often resource-intensive, time-consuming, and difficult to employ routinely (8, 9). Additionally, in very low or focal transmission environments, these surveys require large sample sizes to provide informative estimates (10, 11). NTD and vaccine preventable disease (VPD) monitoring therefore may benefit from integrating suitable and cost-effective methods for active surveillance using surveys in easy access groups (EAGs) and enrolling people where they gather (e.g., schools, health facilities, and churches). Ideally, the convenience sample of the population, may provide meaningful inferences of the target population, as has been found for certain settings and diseases (9, 12-15). For NTDs, school-aged children and health facilities are potential targets of control and surveillance programs (4, 16-18). Although EAGs have typically been

used in the context of a single disease, they could provide an opportunity for multi-disease surveillance.

In serological surveillance, seropositivity provides a measure of disease exposure whereas for VPD, seropositivity can be used to assess the degree of seroprotection within a community (8, 19-21). Multiplex bead assays (MBA) can simultaneously quantify antibody responses to manifold antigens from a single sample, thus facilitating integrated surveillance of multiple diseases (4). MBA have been used in various settings to monitor NTD and VPD, such as assessing immunity gaps (22) and the evaluation of public health program success towards NTD control (23). Since NTD epidemiology and implementation of vaccine campaigns targets similar population (24), integrated surveillance could be particularly cost-effective for control programs to assess concurrent exposure to multiple pathogens (25).

To our knowledge, there is limited evidence of comparability of NTD disease serological estimates between EAGs and community surveys. The objective of this study was to determine if primary school and health facility surveys can provide estimates comparable with more conventional community surveys in terms of prevalence to antigens of two NTDs (lymphatic filariasis and strongyloidiasis) and to two antigenic targets employed as part of vaccination campaigns (measles and tetanus). We therefore compared both continuous antibody data and seroprevalence estimates between populations sampled during an EAG survey and a household survey in Artibonite, Haiti during 2017.

Methods

Sampling

Sampling took place as part of a malaria study conducted in Artibonite, Haiti in 2017. This region is also endemic for multiple tropical diseases (26). The EAG and household surveys were sampled from April to May, and from July to October, respectively. For the EAG venues, 21 schools with a minimum of 100 enrolled pupils were purposefully selected using a stratified random sampling procedure to ensure equal distribution across sub-communes. A maximum of 25 pupils per class between the grades of two and six were selected, with a maximum of 150 pupils sampled per school (27). The head-teachers consented on behalf of their school and parents were informed of the full study procedure, including the option for their children to opt out, during community consultations prior

to visiting the school. All children provided their assent and had the option to refuse participation. All nine functioning health facilities in Artibonite, were included with all outpatients and accompanying individuals eligible for sampling. Those who had previously visited the health facility during the study period, required urgent medical attention, or were under 6 months of age were excluded. A maximum of 150 people per each age category (6 months-5 years, 6-15 years, >15 years) at each facility were enrolled (28). For the household survey, a geographically weighted random sample of all households in the study area was performed using georeferenced census data with all consenting individuals residing in sampled households greater than 6 months of age eligible for inclusion. Blood spots were obtained through finger prick from all participants sampled in the two surveys and key demographic information including participants' age, gender, temperature, date and location of collection were recorded.

Catchment Areas

There was spatial overlap between the household and EAG survey areas for twenty-two primary schools and nine health facilities. The catchment areas per venue were defined according to a geostatistical model ascribing the probability of attendance according to a friction surface accounting for distance, barriers to travel, and facility-specific characteristics and informed by the location of a subset of participants where spatial coordinates were available (29). To control for spatial bias, data in both EAG and household surveys were restricted to the subset of the population that resided within the venues' catchment area (Figure 1). Data were available for 2,127 and 2,116 individuals sampled from school and health facilities, respectively. Household data were available for 18,559 individuals in both school and health facilities of corresponding catchments to serve as the gold standard (Table 1).

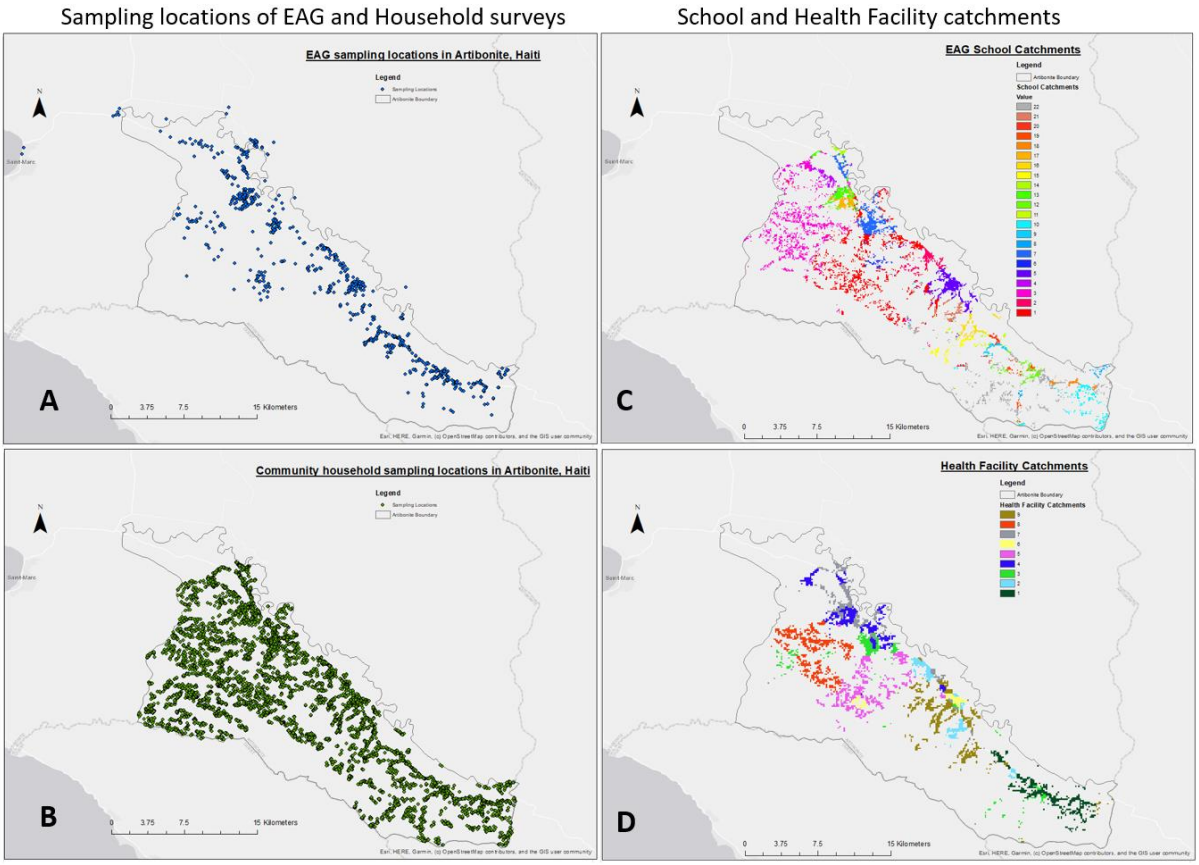


Figure 1. Household location for EAG (A) and Household (B) survey participants and catchment areas for included schools (C) and health facilities (D) in the EAG survey.

Map of study area showing the location of children sampled in the primary school survey (only a subset of EAG was geolocated and shown in A); B) the household locations of the individuals sampled as part of the household survey; C) school catchments and D) health facility catchments based on geostatistical models including EAG sampling locations in A) within defined Artibonite boundaries.

T5. 1 **Table 1. Summary of key demographic information within school and health facility catchments per survey.**

The total number of individuals within catchments used for analysis from the EAG and community Household surveys with median age, gender percentage, and range of the number individuals within school and health facility (Health F.) catchments sampled.

Survey	Total N	Median Age in Years (range)	Gender (Male)	Range of individuals in catchments
EAG	6004			
School	2086	10 (2-24)	50.19%	(20-146)
Health F.	2126	24 (0-88)	33.37%	(148-291)
Household	21222			
School	18859	26 (0-108)	45.9%	(104-4373)
Health F.	18859	26 (0-108)	45.9%	(290-6331)

Laboratory

Briefly, median fluorescence intensity (MFI) was quantified using Luminex MBA through a one-step protocol for all antigens in both EAG and household surveys and processed concurrently (30). This included antigens measuring exposure to strongyloidiasis (ss-NIE) and lymphatic filariasis (Wb123 and Bm14), and exposure to the vaccine derived antigens for tetanus (TT74) and measles (MEA73). The one-step protocol is a cursive assay method that was used to ensure programmatic feasibility for processing large volumes of the samples quickly, where reagents were added simultaneously rather than in a stepwise approach that requires removal and washing of each reagent at multiple steps. Levey Jennings plots were used to screen for abnormalities or significant MFI outliers in plate readings for each antigen.

Data Analysis

Descriptive statistics were used to characterize the population sampled in EAG and household surveys. As immunological competence, antibody avidity, and antibody quantity is known to vary by age (31, 32), we explored age-adjusted log transformed MFI using linear regression and restricted the community dataset to school aged children (ages 6-17) to match the age distribution when comparing with the school survey (ages 6-17) in school and health facility catchments between surveys to minimize potential age-related bias. Mean MFI values from school aged children residing within each EAG catchment was then compared to the average MFI value from all households residing within the corresponding catchment.

Bland-Altman plots were used to evaluate whether there is a consistent bias in survey approach according to the pattern of agreement and number of outliers between the two measurements of EAG and household surveys as well as the range of agreement (1.96 standard deviations from the mean difference) (33). To further assess the strength of comparability, we used Lin's concordance coefficient to evaluate agreement, with 1.0 being perfect concordance between two measurements (34). As both continuous MFI and seroprevalence can be informative (35, 36), evidence of comparability was assessed for both outcome measures. Thresholds for seropositivity per survey were determined using two component Gaussian mixture models according to the mean of the lowest component plus three standard deviations. Seroprotection was calculated for VPD using pre-determined thresholds based on international units (MFI>100 IU) (37). Rank preservation of

seropositivity was assessed to evaluate whether prevalence estimates of catchment areas ranked similarly between EAG and household surveys.

Additionally, to investigate whether EAGs could produce meaningful information compared to household surveys when using a predefined programmatic threshold, we set a hypothetical threshold of above 99% seroprevalence for measles to indicate 'seroprotection' and below 5% seroprevalence for lymphatic filariasis to indicate 'low transmission'. We assessed rank preservation and compared catchment responses using Wilcoxon rank sum and Fisher's exact tests.

Human Subjects Protection/Ethics

The procedures for both study regions were approved by the National Bioethics Committee in Haiti (1516-30), the London School of Hygiene & Tropical Medicine Ethics Committee (103939) and the Tulane Institutional Review Board (795709). All participants provided informed written consent and/or assent, with parental consent for the school surveys using an opt-out process approved by all ethics committees (38). Participation in the study was not remunerated.

Results

Schools

The mean differences (d) in logged MFI scale were higher in schools compared to the community for all pathogens, although the magnitude of this difference varied per antigen (Table 2, Supplementary Table 1). For strongyloidiasis, the mean difference of logged MFI was 0.275 higher in schools compared to the community. For the lymphatic filariasis Wb and Bm, the logged mean difference was higher in schools compared to the community by 0.435 logged MFI and 0.468 logged MFI, respectively. For VPD, MFI values at schools were higher than the community for tetanus by 0.251 logged MFI and for measles by 0.266 logged MFI. Concordance was not observed for any antigen ($p>0.05$, Table 2, Supplementary Figure 2). Most observations fell within limits of agreement according to Bland Altman plots (Supplementary Figure 1.)

The seroprevalence estimates of exposure to NTDs in schools were higher than the community (strongyloidiasis (d= 11.2%) and LFwb (d=5.1%)) but not for LFbm ((d=-0.5%), Table 2, Supplementary Table 2). Additionally, for strongyloidiasis, Bland Altman plots revealed proportional bias, showing that both surveys do not agree across average MFI values (Supplementary Table 3). For VPDs, the estimated seroprotection was higher in schools compared to the community, although this difference was less than 1% (measles (d=0.6%) and tetanus (d=0.4%), Table 2). Concordance of

seroprevalence and seroprotection was not observed in schools for any antigen (Table 2, Supplementary Figure 4). Proportional bias was also observed for VPDs in schools (Supplementary Figure 3).

T5. 2 **Table 2. Summary of comparability methods in school venues.**
A summary of comparability methods and their results, with mean differences between survey, concordance correlation coefficients assessing agreement and biases, pearson's R measuring correlation, and Wilcoxon rank sum tests for consistency in catchment rankings.

Comparability Assessment	**Measles School**	**Tetanus School**	**Strongy -loides School**	**LFwb School**	**LFbm School**
Comparing Logged Mean MFI					
Mean Difference (95% confidence intervals)	**0.266** (-0.359, 0.891)	**0.251** (-0.553, 1.056)	**0.275** (-0.415, 0.966)	**0.435** (-0.105, 0.975)	**0.468** (-0.125, 1.060)
Lin's Concordance Coefficient (95% confidence interval)	**-0.083** (-0.315-0.150)	**-0.016** (-0.307, -0.298)	**0.086** (-0.156, 0.329)	**0.045** (-0.075, 0.166)	**0.068** (-0.079, 0.214)
Lin's Concordance Coefficient p-value	(0.485)	(0.913)	(0.486)	(0.460)	(0.364)
Pearson's R (p- value)	-0.157 (0.487)	-0.025 (0.914)	0.155 (0.490)	0.166 (0.459)	0.205 (0.360)
Comparing Seroprotection (VPD) and Seroprevalence (NTD)					
Mean Difference with 95% confidence intervals	**0.006** (-0.015, 0.027)	**0.004** (-0.013, 0.021)	**0.112** (-0.041, 0.266)	**0.051** (-0.052, 0.154)	**-0.005** (-0.172, 0.162)
Lin's Concordance Coefficient (95% confidence intervals)	**-0.037** (-0.156, 0.081)	**0.019** (-0.307, 0.345)	**-0.008** (-0.034, 0.018)	**-0.034** (-0.161, 0.094)	**-0.068** (-0.475, 0.339)
Lin's Concordance Coefficient (p-value)	(0.061)	(0.909)	(0.541)	(0.603)	(0.743)
Pearson's R (p-value)	-0.138 (0.540)	0.025 (0.910)	-0.138 (0.541)	-0.117 (0.605)	-0.073 (0.747)
Wilcoxon Rank Sum P-value	0.002	0.031	0.000	0.000	0.644

Health Facilities

Similarly, those sampled in health facilities had observed mean logged MFI values higher than the community (strongyloidiasis (d = 0.371), LFwb (d = 0.743), and LFbm (d = 0.715), Table 3, Supplementary Table 3). For VPD, differences between surveys for tetanus (d=2.307) and measles (d =1.578) were also higher in health facilities compared to the community. No concordance in mean MFI estimates was observed for any antigen (Table 3, Supplementary Figure 2) in health facility venues. Similar to school catchments, most observations fell within limits of agreement according to Bland Altman plots with some bias observed with LFbm (Supplementary Figure 1.)

Health facilities had consistently higher seroprevalence estimates compared to the community (strongyloidiasis (d=7.4%) and LFwb (d=5.9%) and LFbm (d=-1.4%), Table 3, Supplementary Table 4). For VPDs, the magnitude of the mean difference was less than 1% (measles (d=-0.7%) and tetanus (d=0.5%), Table 3). A proportional bias was observed for all antigens in health facilities (Supplementary Figure 3). Concordance of seropositive and seroprotection in health facilities was also not observed for any antigens (Table 3, Supplementary Figure 4).

Moreover, the consistency in ranking of catchment-level prevalence of disease/vaccine exposure between the household survey and EAGs assessed here were significantly different in school catchments, with the only exception being LFbm (Supplementary Table 5). In health facility catchments, ranking of catchment-level prevalence were consistent among all antigens assessed between surveys (Supplementary Table 5). These findings suggest that although there was a lack of concordance, surveys may agree in the classification of areas of high and low prevalence for LFbm antigen in school surveys and all antigens in health facility surveys.

T5. 3 **Table 3. Summary of comparability methods in health facility venues.**
A summary of comparability methods and their results. Mean differences, concordance correlation and significant p values for concordance correlation coefficient are also in bold.

Comparability Assessment	**Measles Health Facility**	**Tetanus Health Facility**	**Strongy -loides Health Facility**	**LFwb Health Facility**	**LFbm Health Facility**
Comparing Logged Mean MFI					
Mean Difference (95% confidence intervals)	**0.042** (-0.429, 0.513)	**0.111** (-0.984, 1.206)	**0.371** (-0.223, 0.965)	**0.742** (0.209, 1.274)	**0.715** (-0.022, 1.453)
Lin's Concordance Coefficient (p-value)	**0.146** (0.684)	**-0.050** (0.837)	**-0.047** (0.714)	**-0.034** (0.291)	**-0.021** (0.617)

Lin's Concordance 95% confidence interval	(-0.556, 0.847)	(-0.526, -0.483)	(-0.301, 0.207)	(-0.096, 0.291)	(-0.105, 0.063)
Pearson's R (p-value)	0.151 (0.699)	-0.077 (0.843)	-0.140 (0.720)	0.393 (0.296)	-0.193 (0.619)
Comparing Seroprotection (VPDs) and Seroprevalence (NTDS)					
Mean Difference (95% confidence intervals)	**0.007** (-0.003, 0.017)	**0.005** (-0.005, 0.015)	**0.074** (-0.010 0.158)	**0.059** (-0.027, 0.145)	**-0.014** (-0.172, 0.200)
Lin's Concordance Coefficient with p-value	**0.000** (0.520)	**0.000** (0.520)	**0.005** (0.871)	**0.048** (0.227)	**0.184** (0.372)
Lin's Concordance 95% confidence interval	(.0.000-0.000)	(0.000-0.000).	(-0.052, 0.061)	(-0.030, 0.126)	(-0.220, 0.588)
Pearson's R with p-value	0.249 0.518	0.249 0.518	0.061 0.875	0.488 0.183	0.316 0.408
Wilcoxon Rank Sum P-value	0.008	0.016	0.008	0.020	0.7344

In examining hypothetical programmatic thresholds applied to EAGs, results were different among the test antigens (Supplementary Table 6). 17 out of 21 catchments for measles antigen resulted in were classified the same. Based on our hypothetical program threshold, EAGs overestimated prevalence of those assumed seroprotected for four catchments in household surveys, however, this difference would not be observed if the threshold was set at 95%. 8 out of 21 catchments for LF Wb antigens resulted in the same categorical outcome. In contrast, 22 out of 22 catchments for LF Bm antigen resulted in the same categorical outcome between surveys.

Discussion

Whilst there are numerous factors that influence the persisting burden of NTD, the absence of effective, routine surveillance has been long recognized as a persistent problem (11, 39). EAGs have the potential to support routine surveillance by supporting passive with an approach for operationally attractive active surveillance. In this study we examined the comparability of serosurveillance data from EAGs with a gold standard community survey to support multi-disease surveillance using MBAs and estimated exposure to two pathogens (lymphatic filariasis, strongyloidiasis) and two vaccine preventable disease (measles, tetanus), in Artibonite, Haiti.

Our results generally showed a systematic bias in EAGs sampling for antibody responses to most antigens assessed. Concordance of seropositivity was not observed for any antigen, suggesting that health facilities and schools may not be reliable in assessing seroprevalence in the community, despite being age balanced. For NTDs, health facility-based surveys overestimated seroprevalence compared to the community, while seroprevalence from schools either overestimated or underestimated community seroprevalence. For measles and tetanus, schools over estimated community seroprotection while health facilities over and underestimated seroprotection compared to community catchments. Proportional bias was also observed for several antigens in both venues, indicating a lack of agreement based on the magnitude of responses between surveys.

The systematic biases observed draws attention to the characteristic differences, of the populations surveyed in primary school and health facilities compared to the target population in the community (14, 38). Elevated MFI levels of NTDs in school venues may be attributable to the high burden of NTDs in children, as children more biologically vulnerable to infection due to developing immune and other biological systems, and can be at higher risk for exposure due to close proximity in schools (40). Conversely, schools are often targets for mass chemotherapy programs, which may lead to lower expected seroprevalence, as observed in the differing trends (41, 42). In health facility venues, discordance in MFI levels of NTD is potentially attributable to the presence of individuals with active or severe infections. For VPDs, increased MFI values compared to the community may be expected due to more abundant vaccine coverage and immunization programs in people attending schools and health facilities, or those who are able to access these venues (7, 43). Additionally, proportional differences in mean logged MFI between surveys may also indicate areas of historical exposure in the community compared to schools and health facilities.

Despite these biases, the utility of EAG venues may be better understood with acceptable thresholds, as defined by programs or clinics. When we examined seropositivity rank preservation among the different catchments, results showed consistency between surveys for lymphatic filariasis Bm and all antigens of health facilities, indicating that both surveys may arrive to similar conclusions regarding areas of high and low seroprevalence for this specific antigen, depending on a comparable range of estimates. EAG and household surveys may also equally determine seropositivity above or below a prevalence threshold in the community designated *a priori,* irrespective of the difference in magnitude above or below the threshold. For example, our results from Bland Altman plots showed a mean difference of seroprotection less than one percent between EAGs and the community for VPD estimates, yet Lin's concordance correlation coefficient found discordance in VPD between

surveys based on this difference. In this situation, the question then becomes whether this difference has a meaningful impact on programmatic decision, or if estimates below or above that prevalence threshold, regardless of the difference, would be informative. EAGs could potentially be used to detect transmission below a certain prevalence threshold in which disease propagation is no longer sustainable, using minimum thresholds required for certain surveillance programs (17).

To investigate this question of whether EAG could produce meaningful information compared to household surveys when using a predefined programmatic threshold, we set hypothetical thresholds for EAGs. How EAGs performed with a notional program threshold suggests that EAG surveillance may be suitable for certain antigens when specific program targets have been defined. The hypothetical results demonstrated that EAGs may be suitable for measles and LF Bm surveillance but not for LF Wb. It is unclear why LF Bm and LF Wb performed differently in these settings, which could an artefact of the assay or pertains to specific antigen kinetics reflecting different biological process. EAGs may be useful in identifying areas to prioritize control measures, including targeted active surveillance in certain high burden EAG catchments or interventions to reduce transmission, depending on the antigenic target. Inclusion of vaccine targets also provided targeted insight into population vaccination coverage, as part of integrated, multi-disease surveillance. Additionally, since EAG overestimates community-level prevalence, finding transmission below the set threshold in EAGs could theoretically be a strong indicator of low disease transmission or program intervention success in the community (12).

Limitations

There are several limitations to highlight within this study. Firstly, sampling also was not executed the same time within the year, so there could be some temporal or season impact on prevalence estimates. Next, catchment areas were difficult to define. Individuals in Haiti also travel to EAG sites based on preference, instead of solely proximity, resulting in potentially overlapping catchment areas. Therefore, modeled catchments based on the probability an individual would attend a given catchment area were used to define our populations for comparison. Additionally, data were aggregated into a small number of catchments for comparison, and conclusions were drawn from paired cluster averages, which may have led to information loss. For example, the notable large limits of agreement reflect a large variability in seropositivity comparisons and low precision of these estimates in health facility venues may be a result of comparing the small number of catchments. Furthermore, number of individuals within certain catchments of this analysis may not have

sufficient power to ensure accurate seroprevalence of individual diseases. Future studies that aim to monitor multiple pathogens may need to consider the minimum number of samples needed to have sufficient power to estimate exposure in all pathogens assessed.

Another important limitation of this study is the use of the “one step” approach during laboratory technique of bead washing at that time, which has been shown with some non-specific binding, leading to artificially elevated MFI concentrations (44). However, the assay was consistently applied to the samples collected in both surveys, so the bias is expected to be non-differential and interpretations still valid (44). While this laboratory technique was applied uniformly between both surveys which allowed for some degree of comparisons at the very least, it draws attention to the importance of developing standard protocols for serologic MBAs that will enable confidence in procured MFI values. Lastly, we applied non-standard cut-off approaches for our VPD data with borrowed international standards for the assay used, therefore programmatic interpretation of VPD data is not possible, although comparisons between survey estimates were still reasonable.

Conclusion

As more countries are nearing elimination of tropical diseases, reliable and efficient monitoring will be crucial. Serological surveys with MBA provide an opportunity to monitor multiple diseases simultaneously. EAG are less resource intensive surveys as compared to community surveys, yet our results question the ability of surveys in primary schools and health facilities, including all-attendees, to monitor NTD at the catchment-level. While the results demonstrated that estimates were not perfectly concordant between EAGs and household surveys, relatively small differences of seropositivity and mean logged MFI across all catchments may indicate that EAG could be used as a monitoring tool for tropical diseases at a less spatially granular level than individual catchments. Additionally, EAGs may be suitable for surveillance of certain antigens with programmatic thresholds to guide decision making.

References

1. Tambo E, Ai L, Zhou X, Chen JH, Hu W, Bergquist R, et al. Surveillance-response systems: the key to elimination of tropical diseases. Infect Dis Poverty. 2014;3.
2. Hollingsworth TD. Counting Down the 2020 Goals for 9 Neglected Tropical Diseases: What Have We Learned From Quantitative Analysis and Transmission Modeling? Clin Infect Dis. 2018;66:S237-S44.
3. Lammie PJ, Moss DM, Goodhew EB, Hamlin K, Krolewiecki A, West SK, et al. Development of a new platform for neglected tropical disease surveillance. Int J Parasitol. 2012;42(9):797-800.
4. Arnold BF, Scobie HM, Priest JW, Lammie PJ. Integrated Serologic Surveillance of Population Immunity and Disease Transmission. Emerg Infect Dis. 2018;24(7):1188-94.
5. Bolzoni L, Real L, De Leo G. Transmission Heterogeneity and Control Strategies for Infectious Disease Emergence. Plos One. 2007;2(8).
6. Hotez PJ, Molyneux DH, Fenwick A, Kumaresan J, Sachs SE, Sachs JD, et al. Current concepts - Control of neglected tropical diseases. New Engl J Med. 2007;357(10):1018-27.
7. Cutts FT, Izurieta HS, Rhoda DA. Measuring coverage in MNCH: design, implementation, and interpretation challenges associated with tracking vaccination coverage using household surveys. PLoS Med. 2013;10(5):e1001404.
8. Metcalf CJE, Farrar J, Cutts FT, Basta NE, Graham AL, Lessler J, et al. Use of serological surveys to generate key insights into the changing global landscape of infectious disease. Lancet. 2016;388(10045):728-30.
9. Sesay SSS, Giorgi E, Diggle PJ, Schellenberg D, Lalloo DG, Terlouw DJ. Surveillance in easy to access population subgroups as a tool for evaluating malaria control progress: A systematic review. Plos One. 2017;12(8).
10. Harris I, Sharrock WW, Bain LM, Gray KA, Bobogare A, Boaz L, et al. A large proportion of asymptomatic Plasmodium infections with low and sub-microscopic parasite densities in the low transmission setting of Temotu Province, Solomon Islands: challenges for malaria diagnostics in an elimination setting. Malar J. 2010;9:254.
11. Mackey TK, Liang BA, Cuomo R, Hafen R, Brouwer KC, Lee DE. Emerging and Reemerging Neglected Tropical Diseases: a Review of Key Characteristics, Risk Factors, and the Policy and Innovation Environment. Clin Microbiol Rev. 2014;27(4):949-79.
12. Stevenson JC, Stresman GH, Gitonga CW, Gillig J, Owaga C, Marube E, et al. Reliability of School Surveys in Estimating Geographic Variation in Malaria Transmission in the Western Kenyan Highlands. Plos One. 2013;8(10).
13. Sserwanga A, Harris JC, Kigozi R, Menon M, Bukirwa H, Gasasira A, et al. Improved Malaria Case Management through the Implementation of a Health Facility-Based Sentinel Site Surveillance System in Uganda. Plos One. 2011;6(1).
14. Chacky F, Runge M, Rumisha SF, Machafuko P, Chaki P, Massaga JJ, et al. Nationwide school malaria parasitaemia survey in public primary schools, the United Republic of Tanzania. Malaria J. 2018;17.
15. Walldorf JA, Cohee LM, Coalson JE, Bauleni A, Nkanaunena K, Kapito-Tembo A, et al. School-Age Children Are a Reservoir of Malaria Infection in Malawi. Plos One. 2015;10(7).
16. Chu BK, Deming M, Biritwum NK, Bougma WR, Dorkenoo AM, El-Setouhy M, et al. Transmission assessment surveys (TAS) to define endpoints for lymphatic filariasis mass drug administration: a multicenter evaluation. PLoS Negl Trop Dis. 2013;7(12):e2584.
17. Zhou XN, Bergquist R, Tanner M. Elimination of tropical disease through surveillance and response. Infect Dis Poverty. 2013;2.
18. Ortu G, Williams O. Neglected tropical diseases: exploring long term practical approaches to achieve sustainable disease elimination and beyond. Infect Dis Poverty. 2017;6.

19. Winter AK, Martinez ME, Cutts FT, Moss WJ, Ferrari MJ, McKee A, et al. Benefits and Challenges in Using Seroprevalence Data to Inform Models for Measles and Rubella Elimination. J Infect Dis. 2018;218(3):355-64.
20. Rogier E, Wiegand R, Moss D, Priest J, Angov E, Dutta S, et al. Multiple comparisons analysis of serological data from an area of low Plasmodium falciparum transmission. Malar J. 2015;14:436.
21. Raafat N, Blacksell SD, Maude RJ. A review of dengue diagnostics and implications for surveillance and control. Trans R Soc Trop Med Hyg. 2019.
22. Scobie HM, Patel M, Martin D, Mkocha H, Njenga SM, Odiere MR, et al. Tetanus Immunity Gaps in Children 5-14 Years and Men >= 15 Years of Age Revealed by Integrated Disease Serosurveillance in Kenya, Tanzania, and Mozambique. Am J Trop Med Hyg. 2017;96(2):415-20.
23. Priest JW, Jenks MH, Moss DM, Mao B, Buth S, Wannemuehler K, et al. Integration of Multiplex Bead Assays for Parasitic Diseases into a National, Population-Based Serosurvey of Women 1539 Years of Age in Cambodia. Plos Neglect Trop D. 2016;10(5).
24. Hotez PJ. Mass Drug Administration and Integrated Control for the World's High-Prevalence Neglected Tropical Diseases. Clin Pharmacol Ther. 2009;85(6):659-64.
25. Standley C, Boyce MR, Klineberg A, Essix G, Katz R. Organization of oversight for integrated control of neglected tropical diseases within Ministries of Health. Plos Neglect Trop D. 2018;12(11).
26. Lemoine JF, Desormeaux AM, Monestime F, Fayette CR, Desir L, Direny AN, et al. Controlling Neglected Tropical Diseases (NTDs) in Haiti: Implementation Strategies and Evidence of Their Success. PLoS Negl Trop Dis. 2016;10(10):e0004954.
27. Gitonga CW, Karanja PN, Kihara J, Mwanje M, Juma E, Snow RW, et al. Implementing school malaria surveys in Kenya: towards a national surveillance system. Malar J. 2010;9:306.
28. van den Hoogen LL, Stresman G, Presume J, Romilus I, Mondelus G, Elisme T, et al. Selection of Antibody Responses Associated With Plasmodium falciparum Infections in the Context of Malaria Elimination. Front Immunol. 2020;11:928.
29. Van den Hoogen L. EAG Catchment Calculations in Artibonite, Haiti. In Prep.
30. Rogier EW, Moss DM, Mace KE, Chang M, Jean SE, Bullard SM, et al. Use of Bead-Based Serologic Assay to Evaluate Chikungunya Virus Epidemic, Haiti. Emerg Infect Dis. 2018;24(6):995-1001.
31. Doria G, Dagostaro G, Poretti A. Age-Dependent Variations of Antibody Avidity. Immunology. 1978;35(4):601-11.
32. Stiasny K, Aberle JH, Keller M, Grubeck-Loebenstein B, Heinz FX. Age Affects Quantity but Not Quality of Antibody Responses after Vaccination with an Inactivated Flavivirus Vaccine against Tick-Borne Encephalitis. Plos One. 2012;7(3).
33. Giavarina D. Understanding Bland Altman analysis. Biochem Medica. 2015;25(2):141-51.
34. Watson PF, Petrie A. Method agreement analysis: A review of correct methodology. Theriogenology. 2010;73(9):1167-79.
35. Won KY, Sambou S, Barry A, Robinson K, Jaye M, Sanneh B, et al. Use of Antibody Tools to Provide Serologic Evidence of Elimination of Lymphatic Filariasis in The Gambia. Am J Trop Med Hyg. 2018;98(1):15-20.
36. Goodhew EB, Priest JW, Moss DM, Zhong G, Munoz B, Mkocha H, et al. CT694 and pgp3 as serological tools for monitoring trachoma programs. PLoS Negl Trop Dis. 2012;6(11):e1873.
37. Ondigo BN, Muok EMO, Oguso JK, Njenga SM, Kanyi HM, Ndombi EM, et al. Impact of Mothers' Schistosomiasis Status During Gestation on Children's IgG Antibody Responses to Routine Vaccines 2 Years Later and Anti-Schistosome and Anti-Malarial Responses by Neonates in Western Kenya. Front Immunol. 2018;9:1402.
38. Druetz T, Stresman G, Ashton RA, van den Hoogen LL, Joseph V, Fayette C, et al. Programmatic options for monitoring malaria in elimination settings: easy access group surveys to investigate Plasmodium falciparum epidemiology in two regions with differing endemicity in Haiti. BMC Med. 2020;18(1):141.

39. Parker M, Allen T. 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. Health Res Policy Sy. 2011;9.
40. Rees CA, Hotez PJ, Monuteaux MC, Niescierenko M, Bourgeois FT. Neglected tropical diseases in children: An assessment of gaps in research prioritization. Plos Neglect Trop D. 2019;13(1).
41. Linehan M, Hanson C, Weaver A, Baker M, Kabore A, Zoerhoff KL, et al. Integrated Implementation of Programs Targeting Neglected Tropical Diseases through Preventive Chemotherapy Proving the Feasibility at National Scale. Am J Trop Med Hyg. 2011;84(1):5-14.
42. Amor A, Rodriguez E, Saugar JM, Arroyo A, Lopez-Quintana B, Abera B, et al. High prevalence of Strongyloides stercoralis in school-aged children in a rural highland of north-western Ethiopia: the role of intensive diagnostic work-up. Parasite Vector. 2016;9.
43. Jaber SM. A serological survey of measles, mumps and rubella immunity among school aged children in western Saudi Arabia. Saudi Med J. 2006;27(1):63-9.
44. Rogier E, van den Hoogen L, Herman C, Gurrala K, Joseph V, Stresman G, et al. High-throughput malaria serosurveillance using a one-step multiplex bead assay. Malar J. 2019;18(1):402.

Supplementary Materials

T5. 4 **Supplementary Table 1. Mean logged MFI of school catchments per survey**.
A comparison of average MFI values per each individual school catchment (n=22) between EAG and Household (HH) Surveys for antigens of measles, tetanus, strongyloidiasis, and lymphatic filariasis.

School	EAG Measles	HH Measles	EAG Tetanus	HH Tetanus	EAG Strongyloides	HH Strongyloides	EAG LFWB	HH LFWB	EAG LFBM	HH LFBM
1	8.582	8.012	9.723	9.500	4.438	4.125	4.912	4.394	3.418	3.016
2	8.050	8.193	9.591	10.132	4.514	4.437	4.885	4.66	3.919	3.276
3	8.717	7.995	10.477	9.354	4.760	3.927	5.051	4.328	3.809	3.118
4	8.383	8.407	9.908	9.901	4.611	4.449	4.941	4.242	3.465	2.582
5	8.599	8.205	10.057	9.616	4.550	4.284	4.972	4.486	3.493	3.380
6	8.362	8.410	9.778	9.583	4.500	3.878	4.971	4.107	3.497	3.013
7	8.673	8.219	10.219	9.727	4.762	4.539	5.130	4.461	3.876	3.021
8	8.442	8.693	10.052	10.536	4.375	4.908	4.708	5.112	3.649	3.247
9	8.308	8.201	10.088	9.819	4.667	4.555	4.946	4.684	3.478	3.349
10	8.757	8.039	9.999	9.884	4.963	4.714	5.175	4.844	3.827	3.124
11	8.620	8.357	10.270	9.641	4.624	4.061	4.844	4.431	3.286	3.223
12	8.393	8.124	10.121	9.757	4.731	4.259	4.862	4.583	3.571	3.444
13	8.355	8.342	9.936	9.347	4.378	3.717	4.794	4.002	3.134	3.114
14	8.722	8.287	10.151	9.520	4.674	4.958	4.946	4.778	3.628	3.249
15	8.507	8.264	9.944	9.881	4.603	4.634	5.095	4.855	3.844	3.282
16	8.641	8.179	10.332	10.439	5.120	4.090	5.362	4.598	3.960	3.286
17	8.694	8.386	10.251	9.828	4.761	4.744	5.119	4.661	3.729	3.199
18	7.869	8.182	9.965	9.965	4.728	4.459	5.196	4.668	4.219	3.462
19	8.550	8.564	10.250	10.041	4.754	4.635	4.958	4.674	3.809	2.914
20	8.722	8.419	10.125	10.536	4.642	4.476	4.956	4.511	3.504	3.642
21	8.787	7.978	10.307	9.729	4.998	4.433	5.211	4.799	3.893	3.431
22	8.682	8.110	10.469	9.748	4.754	4.575	5.036	4.598	3.902	3.248

T5. 5 **Supplementary Table 2. Mean seroprevalence (seroprotection for VPDs) of school catchments per survey**.

A comparison of average seroprevalence per each individual school catchment (n=22) between EAG and Household (HH) Surveys for antigens of measles, tetanus, strongyloidiasis, and lymphatic filariasis.

School	EAG Measles	HH Measles	EAG Tetanus	HH Tetanus	EAG Strongyloides	HH Strongyloides	EAG LFWB	HH LFWB	EAG LFBM	HH LFBM
1	1.000	0.993	0.980	0.992	0.000	0.008	0.078	0.017	0.118	0.173
2	1.000	0.983	1.000	0.983	0.049	0.017	0.062	0.017	0.198	0.190
3	1.000	0.996	1.000	0.996	0.148	0.009	0.056	0.015	0.204	0.202
4	1.000	1.000	1.000	1.000	0.082	0.000	0.000	0.013	0.098	0.066
5	1.000	0.991	1.000	0.997	0.088	0.003	0.059	0.018	0.167	0.197
6	1.000	1.000	0.991	1.000	0.336	0.000	0.246	0.012	0.373	0.163
7	1.000	0.995	1.000	1.000	0.083	0.005	0.097	0.018	0.250	0.142
8	1.000	1.000	1.000	1.000	0.047	0.013	0.023	0.063	0.233	0.253
9	1.000	1.000	1.000	1.000	0.167	0.000	0.091	0.015	0.152	0.182
10	1.000	0.993	1.000	0.997	0.179	0.007	0.077	0.017	0.256	0.160
11	1.000	1.000	1.000	1.000	0.107	0.019	0.027	0.000	0.107	0.200
12	1.000	0.990	1.000	1.000	0.161	0.020	0.036	0.020	0.161	0.224
13	0.991	1.000	1.000	0.993	0.082	0.000	0.036	0.007	0.073	0.201
14	1.000	0.953	1.000	0.977	0.088	0.000	0.029	0.023	0.157	0.233
15	1.000	0.989	1.000	0.995	0.083	0.005	0.095	0.027	0.214	0.170
16	1.000	0.992	1.000	1.000	0.244	0.000	0.098	0.016	0.268	0.180
17	1.000	1.000	1.000	0.989	0.099	0.022	0.076	0.022	0.137	0.187
18	1.000	1.000	1.000	1.000	0.000	0.000	0.100	0.000	0.200	0.200
19	1.000	1.000	1.000	1.000	0.137	0.000	0.042	0.000	0.200	0.091
20	1.000	1.000	1.000	1.000	0.097	0.000	0.069	0.050	0.139	0.250
21	1.000	0.988	1.000	0.976	0.189	0.012	0.074	0.048	0.189	0.262
22	1.000	0.997	1.000	0.995	0.148	0.005	0.074	0.009	0.130	0.200

T5. 6 **Supplementary Table 3. Mean logged MFI of health facility catchments per survey**.

A comparison of average logged MFI per each individual health facility catchment (n=9) between EAG and Household (HH) Surveys for antigens of measles, tetanus, strongyloidiasis, and lymphatic filariasis.

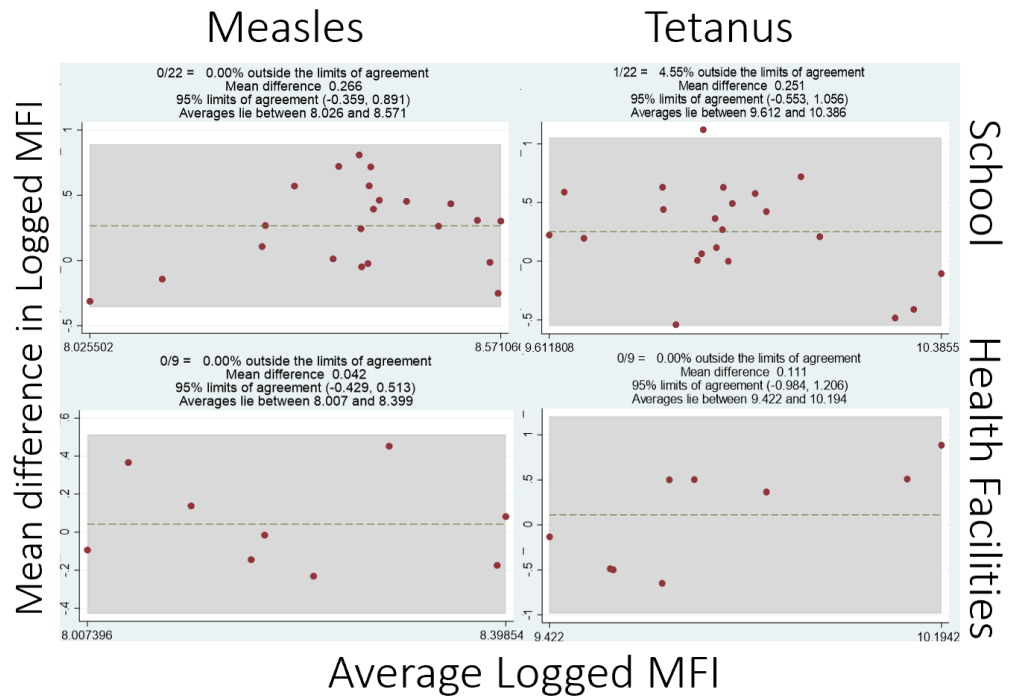
Health Facility	EAG Measles	HH Measles	EAG Tetanus	HH Tetanus	EAG Strongyloides	HH Strongyloides	EAG LFWB	HH LFWB	EAG LFBM	HH LFBM
1	8.165	8.181	10.382	9.872	4.595	4.526	5.333	4.699	3.750	3.280
2	8.174	8.035	9.320	9.968	4.648	4.415	4.935	4.538	3.675	3.120
3	8.088	8.233	10.033	9.666	4.633	4.352	5.327	4.212	4.324	3.149
4	8.103	8.334	9.299	9.797	4.408	4.208	5.152	4.367	3.486	3.174
5	7.961	8.054	9.356	9.488	4.621	4.229	5.284	4.372	3.987	2.875
6	8.303	8.478	9.298	9.785	4.728	4.151	5.107	4.584	3.395	3.235
7	8.440	8.357	10.637	9.751	4.617	4.570	4.972	4.564	3.745	3.092
8	8.516	8.063	9.959	9.455	4.521	4.002	5.175	4.370	3.986	3.124
9	8.229	7.863	9.909	9.407	5.175	4.158	5.389	4.292	4.350	3.211

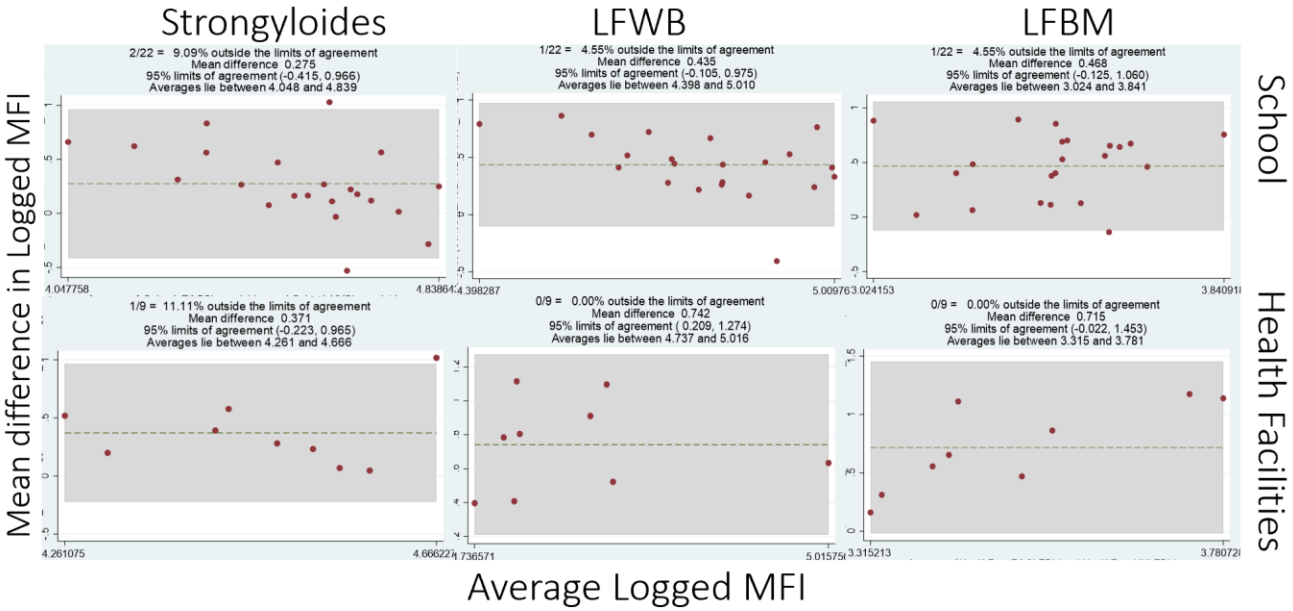
T5. 7 **Supplementary Table 4. Mean seroprevalence (seroprotection of VPDs) of health facility catchments per survey**.

A comparison of average seroprevalence per each individual health facility catchment (n=9) between EAG and Household (HH) Surveys for antigens of measles, tetanus, strongyloidiasis, and lymphatic filariasis.

Health Facility	EAG Measles	HH Measles	EAG Tetanus	HH Tetanus	EAG Strongyloides	HH Strongyloides	EAG LFWB	HH LFWB	EAG LFBM	HH LFBM
1	1.000	0.996	1.000	0.996	0.114	0.006	0.114	0.019	0.143	0.197
2	1.000	0.983	1.000	0.983	0.083	0.017	0.000	0.009	0.167	0.130
3	1.000	0.990	1.000	1.000	0.087	0.000	0.130	0.010	0.348	0.182
4	1.000	0.996	1.000	0.996	0.080	0.004	0.080	0.021	0.080	0.204
5	1.000	0.990	1.000	0.993	0.000	0.006	0.114	0.015	0.200	0.136
6	1.000	1.000	1.000	1.000	0.083	0.000	0.083	0.026	0.083	0.128
7	1.000	0.995	1.000	0.995	0.100	0.021	0.000	0.005	0.100	0.161

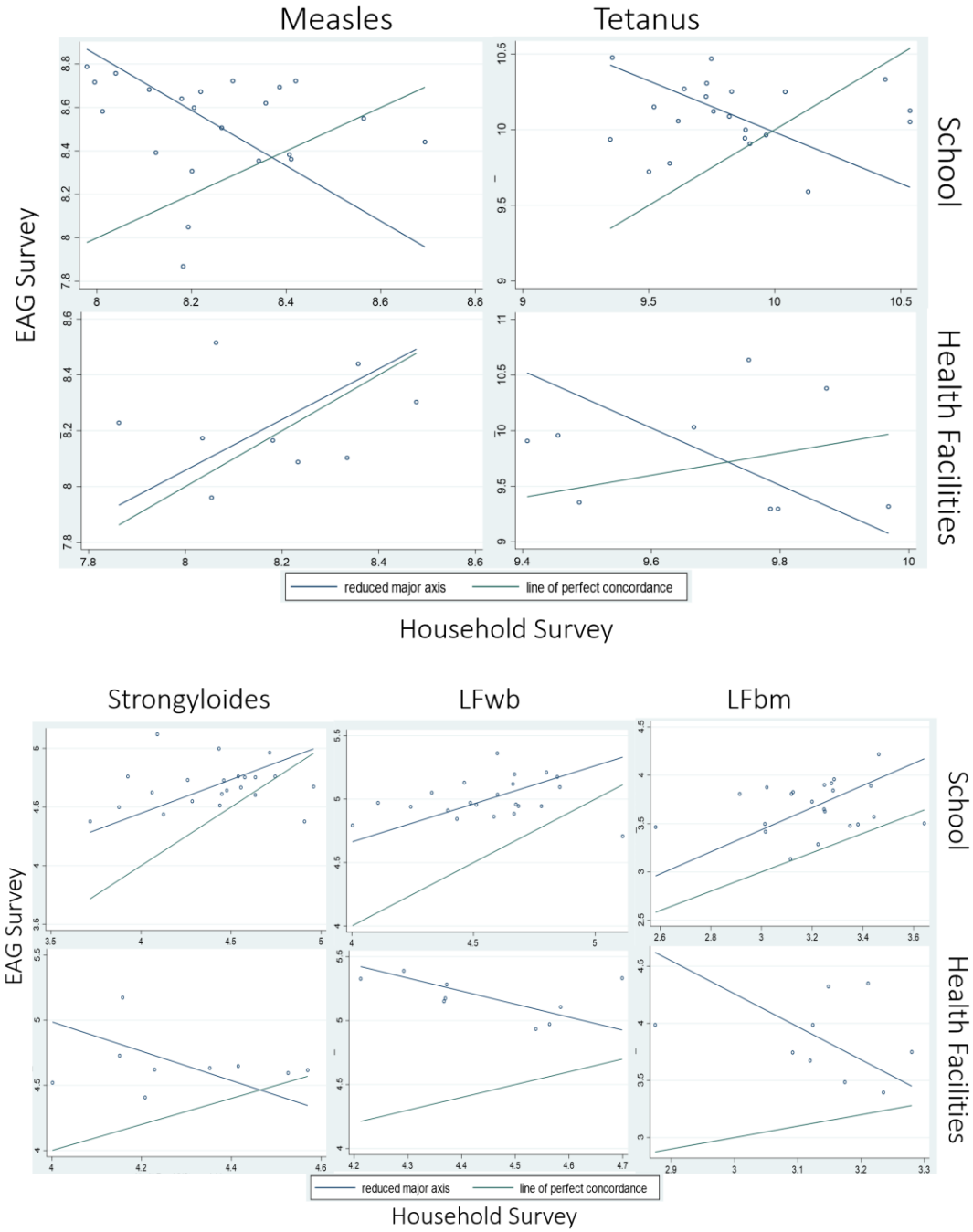
8	1.000	0.996	1.000	0.995	0.036	0.007	0.071	0.017	0.214	0.200
9	1.000	0.989	1.000	0.995	0.148	0.005	0.074	0.016	0.333	0.204





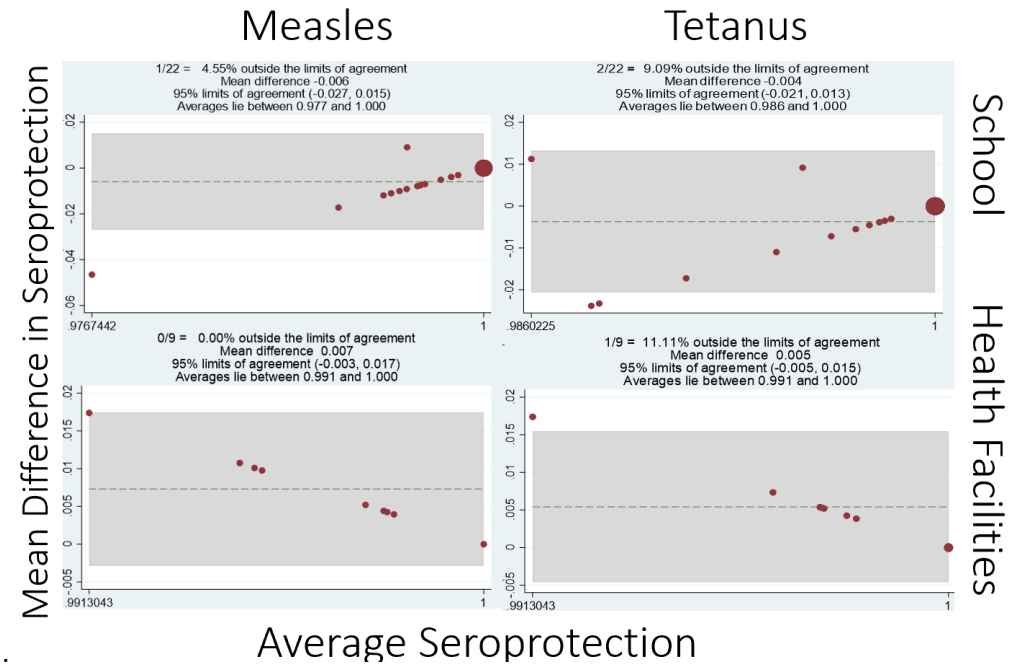
F5. 2 **Supplementary Figure 1. Assessing differences in mean logged MFI using Bland-Altman plots between EAG and Household Surveys.**

Bland Altman plots of mean logged MFI show most catchments fall within level of agreement (gray area) and trends in proportional differences among schools and health facilities.

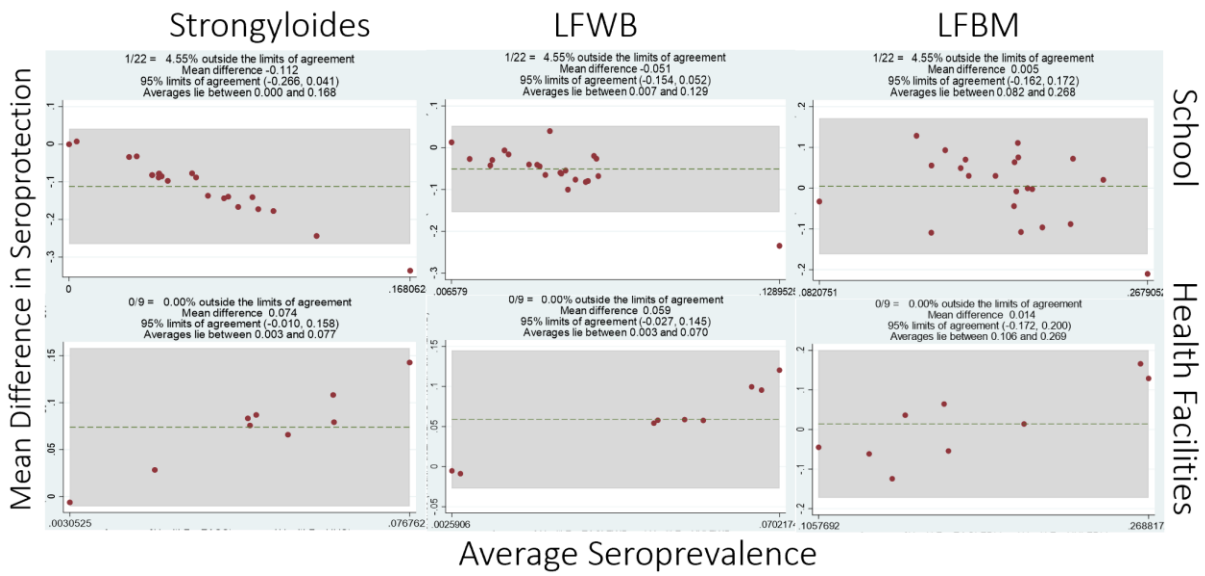


F5. 3 **Supplementary Figure 2. Assessing agreement of mean MFI values using Lin's Concordance Correlation Coefficient.**

Scatterplot of school and health facility venues comparing EAG and household surveys. Line of perfect concordance (green) and reduced major axis (accounting for variability between in both surveys, blue) was used to assess agreement and bias between surveys.

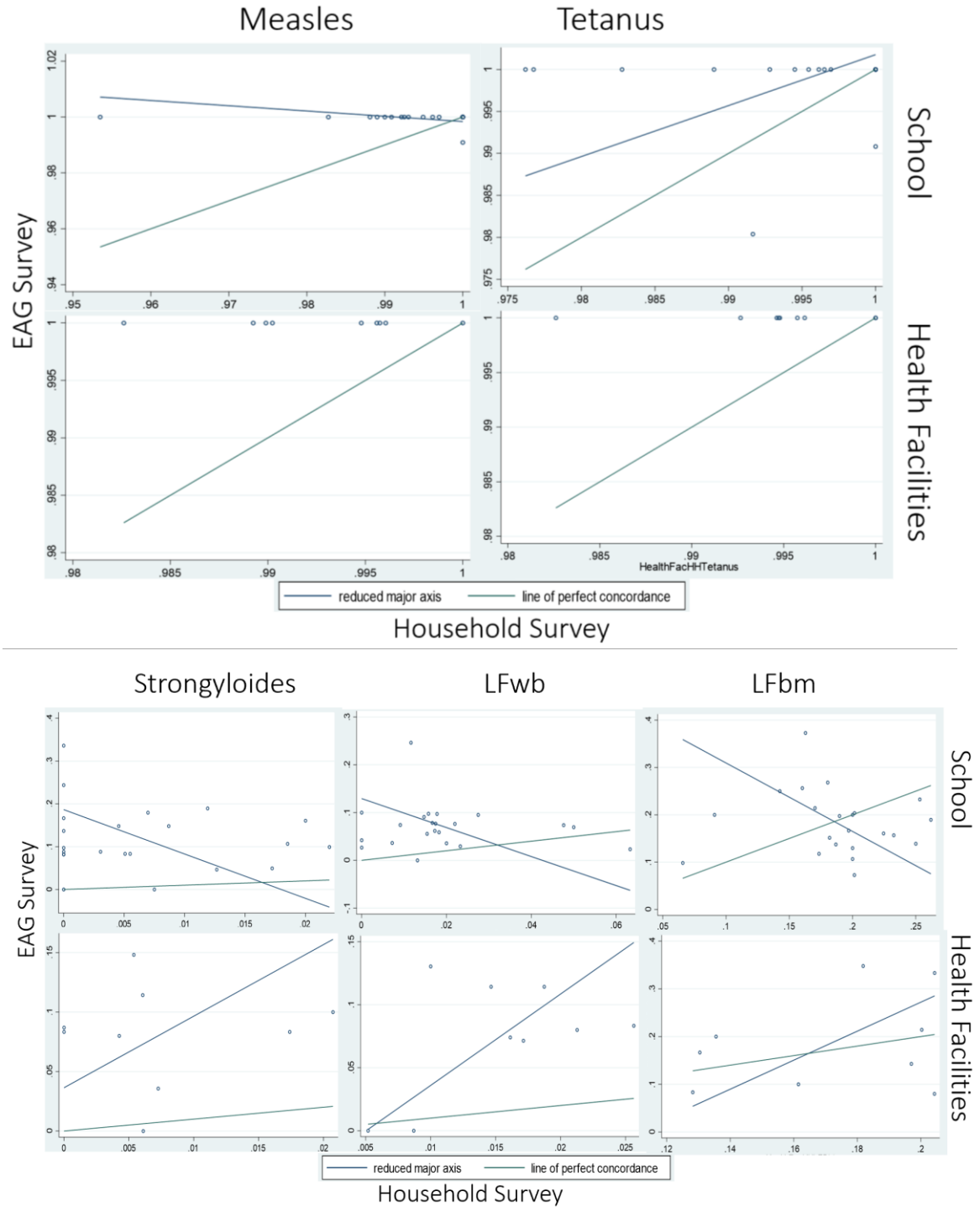


.



F5. 4 **Supplementary Figure 3. Assessing differences in seropositivity using Bland-Altman plots.**

Bland Altman plots showing seropositivity falling within limits of agreement (gray area) when comparing both surveys. Trends highlights any proportional bias by the magnitude of seroprevalence.



F5. 5 **Supplementary Figure 4. Assessing agreement of seropositivity using Lin's Concordance Correlation Coefficient.**

Discordance in seropositivity measurements among EAG and household surveys using line of perfect concordance (green) and reduced major axis (blue).

T5. 8 **Supplementary Table 5. Wilcoxon rank sum test comparing school and health facility catchment rankings between EAG and Household surveys.**
P<0.05 indicates that rankings are significantly dissimilar.

School Catchments	EAG and Household Surveys
Diseases (Antigen)	Rank Sum P-value (α <0.05)
Measles (MeaV)	0.000
Tetanus (TT)	0.006
Strongyloidiasis (NIE)	0.000
Lymphatic Filariasis (Wb123)	0.000
Lymphatic Filariasis (Bm14)	0.511
Health Facility Catchments	
Measles (MeaV)	0.317
Tetanus (TT)	0.146
Strongyloidiasis (NIE)	0.479
Lymphatic Filariasis (Wb123)	0.965
Lymphatic Filariasis (Bm14)	0.691

T5. 9 **Supplementary Table 6. Comparison of School Catchments for measles and lymphatic filariasis antigens with hypothetical prevalence threshold.**

Hypothetical, arbitrary thresholds for measles (seroprotection greater than 99%) and LF (seroprevalence less than 5%) used to investigate the comparability of EAG to household surveys with predefined, notional program targets. Y indicates that the catchment met the programmatic threshold, whereas N indicates that it did not. The number of catchments that were identified the same is indicated on the bottom row, with Fisher's exact 1-sided p-value (looking at the overall comparison within Artibonite, with the assumption that EAGs may overestimate HH) and Wilcoxon rank sum tests (assessing the rankings of catchments) of binary variables.

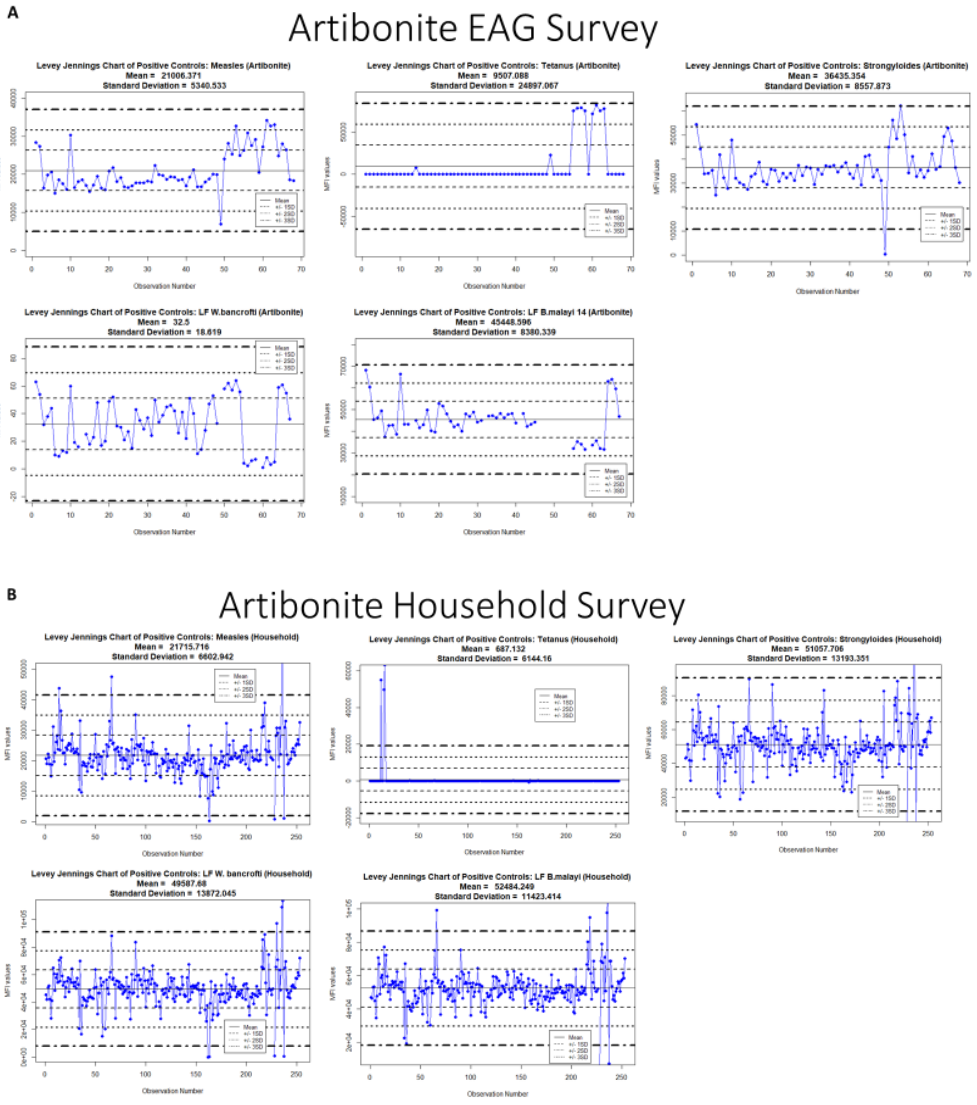
School Catchment Above 99% (VPD)	EAG Measles 21/21	HH Measles 17/21	School Catchment Below 5% (NTD)	EAG LFWB 8/21	HH LFWB 19/21	EAG LFBM 0/21	HH LFBM 0/21
1	Y	Y		N	Y	N	N
2	Y	N		N	Y	N	N
3	Y	Y		N	Y	N	N
4	Y	Y		Y	Y	N	N
5	Y	Y		N	Y	N	N
6	Y	Y		Y	Y	N	N
7	Y	Y		N	Y	N	N
8	Y	Y		Y	N	N	N
9	Y	Y		N	Y	N	N
10	Y	Y		N	Y	N	N
11	Y	Y		Y	Y	N	N
12	Y	Y		Y	Y	N	N
13	Y	Y		Y	Y	N	N
14	Y	N		Y	Y	N	N
15	Y	N		N	Y	N	N
16	Y	Y		N	Y	N	N
17	Y	Y		N	Y	N	N
18	Y	Y		N	Y	N	N
19	Y	Y		Y	Y	N	N
20	Y	Y		N	N	N	N
21	Y	N		N	Y	N	N
22	Y	Y		N	Y	N	N
Same Category	**17/21**, Fisher's exact 1-sided p value: 0.503 Rank sum: 0.038			**8/21**, Fisher's exact 1-sided p value: 0.000 Rank sum: 0.000		**21/21**, Fisher's exact 1-sided p value: 1.000 Rank sum: -	

5.2.1 Research Conclusions

This research addressed the outcomes of Objective 3, which is to compare serological prevalence estimates for tropical diseases in Artibonite, Haiti for two different epidemiological sampling approaches. In this chapter I assessed agreement between serological outcomes from samples collected from participants in EAG and gold standard community surveys. In the analysis, I initially measured agreement of mean MFI values to compare the magnitude of antibody responses between surveys. I then applied consistent cut-off approaches between surveys and measured agreement of seroprevalence between both surveys. As part of ensuring data quality initially, I assessed any inter-plate variability using Levey Jennings (Figure 1).

The results from research paper 5 showed a general absence of agreement between the serological measurements from EAG venues assessed and household surveys for all antigens included in the panel. The differences observed may be impacted by different characteristics of sampling populations between surveys such as age effects on antibody acquisition or care-seeking behaviour, and potential seasonal effects given the slight time shift for when the surveys were conducted. However, EAGs may be broadly suitable using predefined program thresholds or catchment rankings of seroprevalence intensity to identify catchments that may require any public health interventions.

Serologic MBAs were able to generate multi-disease estimates in both EAG and household surveys, demonstrating their potential to support integrated surveillance frameworks. The results in this research also underlined the utility of MBAs to support public health research using antigens of different pathogens to draw conclusions regarding the feasibility of EAGs for multi-disease surveillance. Due to the limitations of the one-step approach (which is no longer applied) for serological MBAs, this study also indirectly highlighted the need for standard lab protocols to consistent and constant data reporting.



F5. 6 **Figure 1. Assessing inter-plate variability using Levey Jennings Plots.**

Panel A shows antigens from EAG survey and Panel B show antigens in Household survey. Controls on individual plates were assessed for variability outside normal MFI range (more than three standard deviations from the mean, denoted by dotted lines). Plates consistently outside this range for all antigens were removed from analysis.

5.3 Chapter Conclusions

While multi-disease surveillance is possible using serological MBAs, it is important to investigate how they can be implemented operationally in endemic countries. Objective 3 evaluated the potential of using convenience sampling to support integrated disease monitoring with serological MBAs. In comparing a panel of two diseases and two vaccine targets between catchments of EAGs venues assessed and community household surveys, I found that EAGs typically overestimated disease prevalence, which may highlight the characteristic differences of the sample populations between surveys, as well as potential differences in transmission based on season in which samples were collected. In applying hypothetical thresholds and comparing seroprevalence intensity-rankings per catchments, prevalence estimates between surveys resulted in similar conclusions for several antigens tested and provided some insight into a possible application of EAGs to support routine surveillance. For example, powered critical cut-offs are currently used in Transmission assessment surveys (TAS), which are school based surveys to determine whether mass drug administration should continue (8). A similar approach could be applied within the context of EAGs for the pathogens of interest if appropriate and programmatically meaningful cut-offs can be identified and validated, either in terms of the number of seropositive individuals, seroprevalence or based on the continuous MFI value (8).

Before implementing EAGs into routine, multi-disease surveillance, there are several research areas that should be further examined considering the results of my work. As this research highlighted potential biases in using schools and health facilities, identifying, and integrating other venues may provide added diversity for convenient sampling populations and improve representation of the target population. This may include sampling other easy to access locations such as churches, community centres, or town centres to help reduce selection bias. Additionally, the results of seroprevalence rankings with program thresholds indicated that EAGs could be suitable for certain antigens due to specific antibody dynamics within the population. For example, understanding antibody dynamics between LF Wb and LF Bm antigens may help to explain the discrepancy between antigen rankings of prevalence thresholds of the same disease in this study. As Wb has been shown to be an infection marker (9), potential differences in recent exposure among school-aged populations compared to the community may explain why seroprevalence of this antigen is higher in school surveys. A future EAG assessment using serological MBAs should include more NTDs antigens to identify antigens that would be appropriate (taking into consideration antibody dynamics and transmission biology in the sample population compared to the

target population) for EAG sampling. Additional consideration should also be placed toward appropriate sample sizes and sample strategies needed to estimate disease burden for a particular disease on multi-disease panels, and whether EAGs would be able to generate accurate prevalence estimates, especially as localities progress toward elimination. Severely convalescent individuals who are captured by EAG surveillance (e.g., at health facilities) with GPS tracking may provide clues on areas with potential ongoing transmission that may require additional attention, however, it is likely that active surveillance will be needed to uncover asymptomatic exposure in the community.

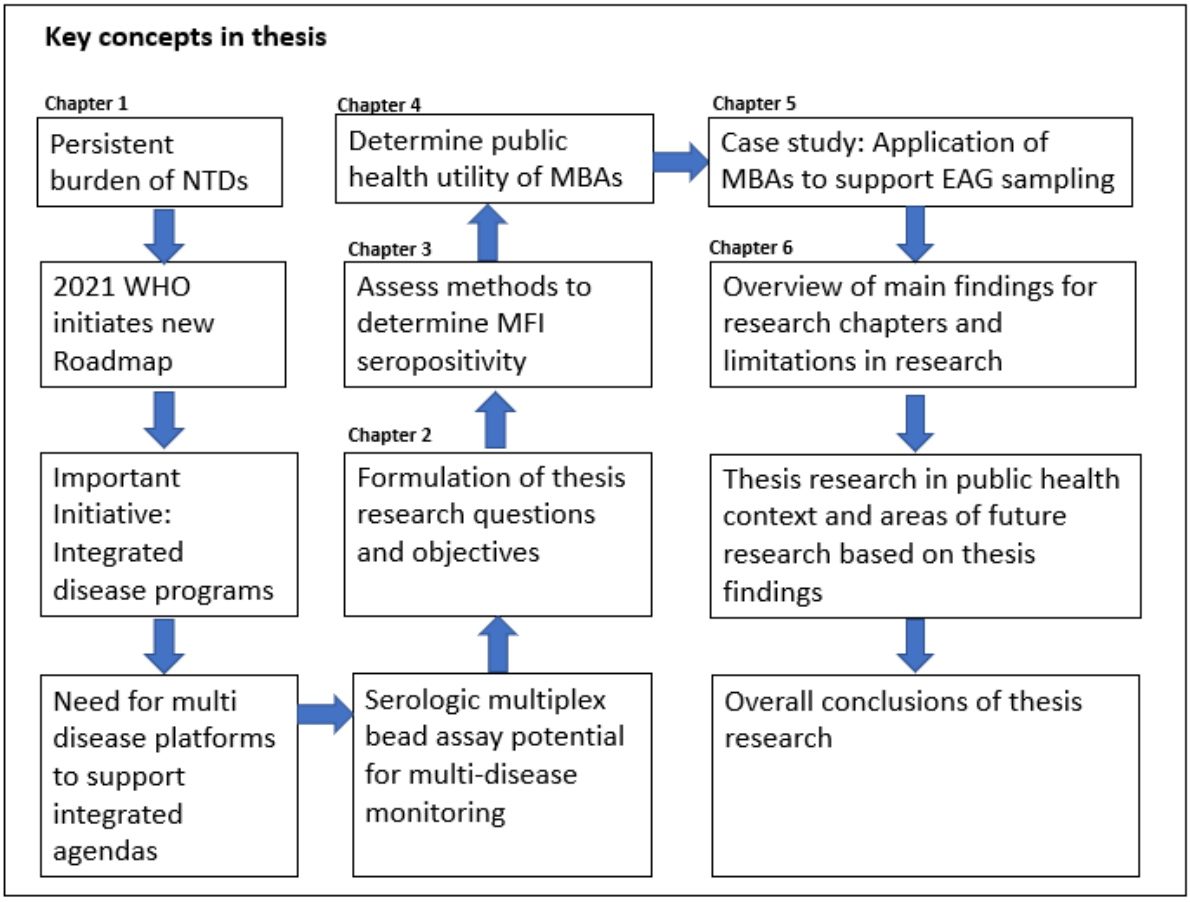
In the context of this thesis, serologic MBAs demonstrated the potential for multiple disease surveillance in surveys targeting different populations. MBAs could likely be integrated into an enhanced active surveillance approach for monitoring exposure to NTDs. The conclusions from the research chapters of this thesis (Chapter 3-5) provides evidence to support the application of serologic MBAs in integrated disease agendas moving forward.

5.4 References

1. Tambo E, Ai L, Zhou X, Chen JH, Hu W, Bergquist R, et al. Surveillance-response systems: the key to elimination of tropical diseases. Infect Dis Poverty. 2014;3:17.
2. Kolaczinski JH, Hanson K, Robinson E, Picon D, Sabasio A, Mpakateni M, et al. Integrated surveys of neglected tropical diseases in southern Sudan: how much do they cost and can they be refined? PLoS Negl Trop Dis. 2010;4(7):e745.
3. Sesay SSS, Giorgi E, Diggle PJ, Schellenberg D, Lalloo DG, Terlouw DJ. Surveillance in easy to access population subgroups as a tool for evaluating malaria control progress: A systematic review. PLoS One. 2017;12(8):e0183330.
4. Jager J, Putnick DL, Bornstein MH. Ii. More Than Just Convenient: The Scientific Merits of Homogeneous Convenience Samples. Monogr Soc Res Child Dev. 2017;82(2):13-30.
5. Banerjee A, Chaudhury S. Statistics without tears: Populations and samples. Ind Psychiatry J. 2010;19(1):60-5.
6. Sserwanga A, Harris JC, Kigozi R, Menon M, Bukirwa H, Gasasira A, et al. Improved malaria case management through the implementation of a health facility-based sentinel site surveillance system in Uganda. PLoS One. 2011;6(1):e16316.
7. Stevenson JC, Stresman GH, Gitonga CW, Gillig J, Owaga C, Marube E, et al. Reliability of school surveys in estimating geographic variation in malaria transmission in the western Kenyan highlands. PLoS One. 2013;8(10):e77641.
8. Chu BK, Deming M, Biritwum NK, Bougma WR, Dorkenoo AM, El-Setouhy M, et al. Transmission assessment surveys (TAS) to define endpoints for lymphatic filariasis mass drug administration: a multicenter evaluation. PLoS Negl Trop Dis. 2013;7(12):e2584.
9. Kubofcik J, Fink DL, Nutman TB. Identification of Wb123 as an early and specific marker of Wuchereria bancrofti infection. PLoS Negl Trop Dis. 2012;6(12):e1930.

CHAPTER 6: DISCUSSION

The aim and objectives of this thesis was to enhance knowledge of the public health utility of serologic MBAs in monitoring tropical diseases to support integrated diseases agendas. This was done by assessing methods to characterise MFI responses into seroprevalence with context to programmatic application; determining MBA capacity to support integrated disease frameworks through multi-disease surveillance and identifying associated risk factors of exposure; and applying MBA to support public health research through a case study of the suitability of EAG sampling in for integrated disease monitoring (Figure 1). This chapter provides an overview of the main research findings, discusses potential public health implications of research results, addresses limitations pertaining to serological MBAs in the scope of this thesis, and suggests areas of potential future research to improve the understanding of the utility of serological MBAs.



F6. 1 **Figure 1. Overview of key concepts, aims, and objectives within thesis.**

Flowchart of thesis chapters and thesis topics examined in sequential order.

6.1 Summary of main research findings

6.1.1 Research Objective 1

The first objective of this thesis addressed the knowledge gap pertaining to appropriate methods of determining MFI seropositivity and potential programmatic implications based on method choice. To assess current methods used to determine MFI seropositivity, I researched the literature and identified seven applied cut-off approaches, with individual strengths and limitations of methods to establish seroprevalence (Chapter 3, Objective 1: Outcome 1). ROC curves, presumed negative populations, and mixture models were the most frequently applied methods identified in the review, but methods were observed to be applied inconsistently among antigens and settings, which may be due to the lack of standard approaches to determining seroprevalence.

To investigate potential programmatic implications accorded to different statistical or mathematical methods to determine seroprevalence, I compared prevalence estimates from three cut-off approaches in Haiti and Malaysia case studies. The results demonstrated some inconsistencies in prevalence estimates (Chapter 3, Objective 1: Outcome 2). While all methods evaluated were able to identify similar areas of high exposure, this was not the case for areas of zero to low prevalence. These incongruities may cause concern if there were to result in conflicting programmatic inferences.

The conclusion from this chapter highlighted the importance of judicious consideration of cut-off approaches and the need for internationally standard cut-offs or consistent approaches to ensure confident program inferences and avoid misclassification of seronegatives and seropositives in the context of program interpretation.

6.1.2 Research Objective 2

The second objective of the thesis targeted the knowledge gap pertaining to the public health utility of serological MBAs to support integrated disease frameworks. To address this gap in knowledge, I assessed simultaneous prevalence estimates to numerous tropical diseases and evaluated potential risk factors of disease exposure. In a national survey in Haiti, data from MBAs were used generate prevalence estimates for eleven pathogens and assessed potential impacts of time (using age as a proxy) on exposure (Chapter 4, Objective 2: Outcome 1). In a state survey in Sabah, Malaysia, multiple risk factors were evaluated for five pathogens concurrently using spatial and demographic data collected within serosurveys. In doing so, I identified several associated risk factors of diseases that were supported by the existing epidemiology estimates found in literature and also identified exposure for

diseases assumed to have been previously eliminated or non-endemic (Chapter 4, Objective 2: Outcome 2).

The conclusions from this chapter highlighted the capability of serological MBAs to support multi-disease monitoring in two distinct settings and the assessment of overlapping risk factors of different diseases that may be beneficial in helping integrated control programs to ascertain exposure and transmission patterns.

6.1.3 Research Objective 3

The third objective of this thesis was to evaluate serologic MBAs in a case study to assess the feasibility of EAGs for multi-disease surveillance (Chapter 5, Objective 1: Outcome 1 and 2). To do this, I measured the agreement of catchment-level seroprevalence and mean logged MFI values between easy access groups (EAGs) and community household surveys. The results showed no agreement in exposure estimates between catchments for both continuous MFI values and seroprevalence, with EAGs generally overestimating community burden of diseases. This may be due to differences in sample population between surveys and also limitations within assays procedures of the one-step approach. However, programmatic thresholds highlighted potential utility in using EAGs for measles and LF antigens to identify stratified catchments that exceed a predefined prevalence.

The conclusion of this chapter with context to the aim of the thesis highlights the ability of MBAs to support multi-disease surveillance using two different active sampling strategies and can be applied to assist public health research. The single setting of this study may not be applicable to all settings, thus further research implementing EAGs in other settings are needed to establish their utility in NTD monitoring.

6.2 Research in context

With the recent launch of the WHO NTD Roadmap of 2021, substantial emphasis has been placed on the importance of cross-sectorial, integrated disease approaches to managing NTDs (as discussed in Chapter 1: Section 2). This is due to the geographic overlap of transmission, shared risk factors, and cost-efficiency of coordinated control activities. New surveillance tools are needed to support these integrated disease initiatives. Serological MBAs have demonstrated promising potential in the capacity to simultaneously assay manifold antigens of numerous, biologically diverse pathogens (1). Initially discussed in the conclusions of Chapter 4, there are several important, public health implications when serological MBAs are applied to integrated disease control (Figure 2).

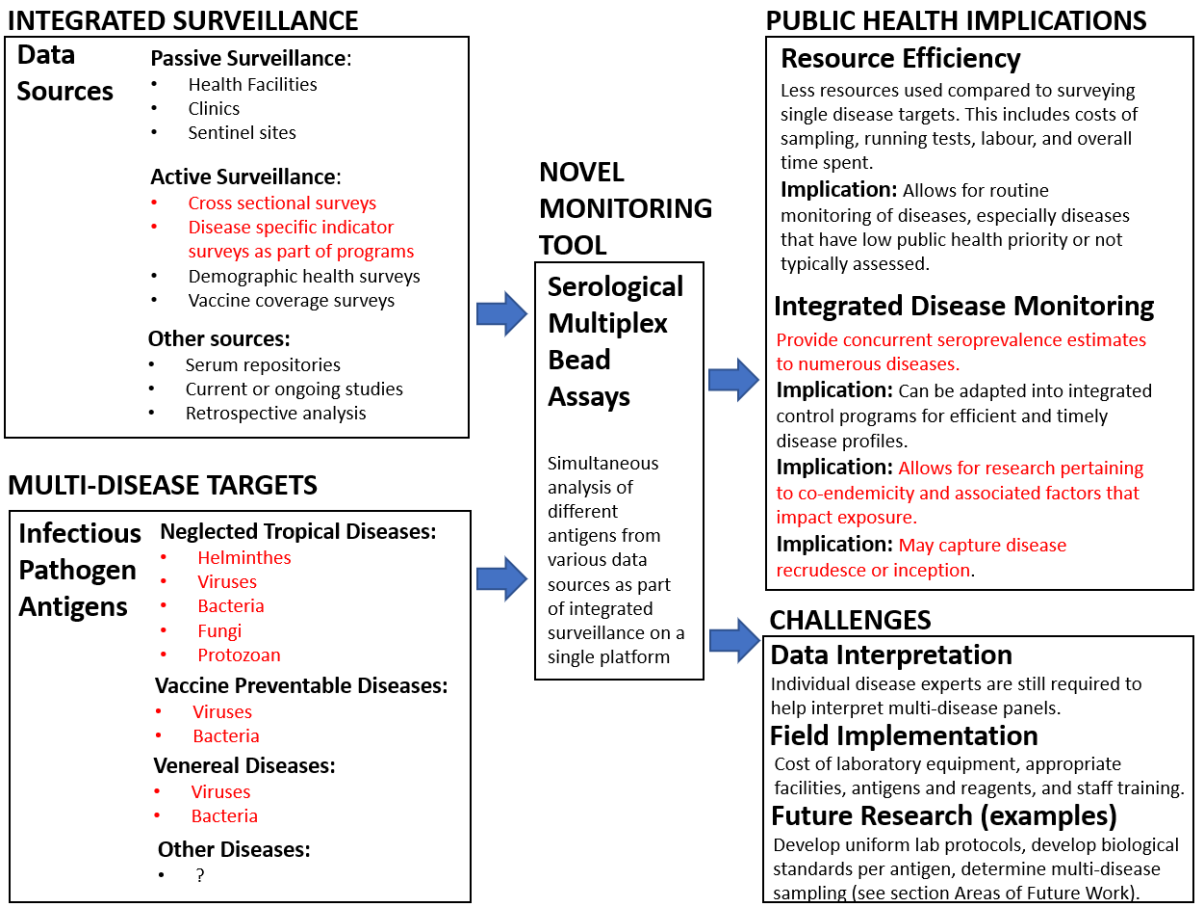


Figure 2. Application of serologic MBA in public health settings.

Samples collected using different surveillance strategies and multi-disease antigen panels can be assayed using serological MBAs, resulting in several beneficial public health implications. Red highlights topics examined in this thesis.

6.2.1 Enabling multi-diseases monitoring and coordinated interventions

Informing integrated programmatic action

In the context of control and elimination, multi-disease monitoring using serological MBAs may be used to inform multisectoral stakeholders and public health entities regarding current disease burdens and the impact of different control interventions (2, 3). Furthermore, serological MBAs with serosurveys that collected spatial information can help to identify areas with higher intensity of exposure to numerous diseases (as demonstrated in Chapter 4, Paper). Consequently, this information may leverage integrated disease control due to common epidemiological features among diseases surveyed. To illustrate, I will consider the provisional results from the Haiti TRaC survey in Chapter 4, Research Paper 3. In this study, prevalence estimates to eleven NTDs were determined using serological MBAs. Some hypothetical examples of how these estimates could support integrated disease initiatives are listed in Table 1.

T6. 1 **Table 1. Example integrated initiatives based on estimates generated in the Haiti 2015 TRaC National Survey.**

Example Integrated Diseases Initiatives	Targeted diseases monitored within survey	Common disease features (in a co-endemic setting)
Integrated Vector Management	Malaria Lymphatic Filariasis Dengue Chikungunya	Vector borne transmission (mosquitoes)
Integrated Water Sanitation and Hygiene	Toxoplasmosis Giardiasis Strongyloidiasis	Transmitted via contaminated water, food, and soil
Integrated Mass Drug Administration Example 1	Trachoma Yaws	Can be treated with same drug
Integrated Mass Drug Administration Example 2	Lymphatic Filariasis Strongyloidiasis Toxoplasmosis Trachoma Yaws	"Rapid-effect packages" (see Chapter 1) include treatments for the listed diseases
IVM + WASH + MDA (+ Vaccine campaigns?)	All diseases within panel	Geographic overlap

In the first example, simultaneous prevalence estimates were generated for Malaria, LF, Dengue, and Chikungunya. As these are vector-borne diseases, IVM programs may prioritise interventions around sampling locations where seroprevalence was high (as defined by program guidelines) for multiple diseases. Serological MBAs can further support IVM programs with post-intervention monitoring of these diseases. The second example includes concurrent prevalence estimates of pathogens that are spread by inadequate sanitation or contamination (toxoplasmosis, giardiasis, strongyloidiasis, and other waterborne pathogens). The information regarding these collective diseases may help to advise WASH programs concerning the need for interventions. Habitual surveillance of water and foodborne pathogens could also provide support in assessing progress of WASH initiatives. The next two examples consist of strategizing disease control based on shared preventative chemotherapy, with serological MBAs supporting multi-disease monitoring (e.g., the need for rapid-effect packages if multiple NTDs require attention). These examples offer some speculation of how MBAs can be applied to inform integrated disease activities (with limitations in interpreting serological data in this thesis discussed in Section 6.3).

Assessing co-endemicity and associated risk factors

In the context of research, multi-disease monitoring can help to facilitate studying co-exposure of diseases and enable the evaluation of risk factors associated with co-endemicity. While the geographic overlap of tropical diseases and their association with poverty and sanitation is well documented, demographic, socio-economic, and spatial risk factors of co-endemic transmission may vary from region to region. For example, cultural practices, residences and habitations, or certain occupations are likely unique to different endemic regions across the world. Identifying shared epidemiological risk factors that are site-specific may aid integrated disease initiatives in implementing informed, shared interventions to reduce co-transmission in particular locations or populations (4, 5). Additionally, identifying new risk factors across antigens among different settings may further advise new integrated approaches to managing co-endemicity.

6.2.2 Incorporating serological MBAs into integrated surveillance

Integrated surveillance is an important initiative of the WHO NTD Roadmap of 2021, which focuses on combining different surveillance strategies to obtain better estimates of disease prevalence. While previous monitoring of NTDs relied primarily on passive surveillance, the current WHO NTD Roadmap initiatives aims to integrate both active and passive surveillance strategies (6). In this thesis, data from several different cross-sectional surveys were used: a national administrative survey in Haiti; a departmental administrative survey in Artibonite, Haiti; easy access groups survey within Artibonite, Haiti; and state administrative survey in Sabah, Malaysia. Serologic MBAs were able to support sampling at different spatial scales to estimate burden of different diseases and demonstrated potential to be incorporated in integrated surveillance.

While the research in this thesis was drawn entirely from active surveillance sampling, the capacity of serological MBAs for multi-disease monitoring should theoretically support any surveillance approach. For example, in Chapter 5, I investigated the application of serological MBAs in health facility venues as part of EAG sampling to support multi-disease surveillance. Equally, samples collected in health clinics or sentinel sites as part of passive surveillance can also be analysed to assess exposure to numerous diseases and stimulate control action if needed, based on standardized approaches for characterising MFI responses (further discussed in the following section 6.3). Serologic MBAs may also be integrated into longitudinal studies to assess antibody kinetics and changes within the population of different diseases (7). Assessing changes in antibody responses that reflect transmission (with and without treatment) may help to identify optimal approaches for future surveillance based on program goals (9).

The analysis in this thesis used retrospective data, which underlines the potential of applying serological MBAs to existing serum or fluid samples previously stored. This may enable programs that adopt serological MBAs to assess burden of numerous NTDs, especially diseases that are not routinely surveyed, with already collected samples. Seroprevalence estimates from the existing samples may then be used to promote future surveillance activities or provide past estimates to compare to current or future estimates to assess changes in seroprevalence based on interventions or lack thereof.

The resource efficiency of multi-disease surveillance in terms of cost, labour, and time compared to individual disease surveillance (see Chapter 1, Section 2.2) may also enable more habitual surveillance (depending on sampling approaches and program goals) of numerous diseases simultaneously. This will allow for more frequent reports of multi-disease estimates, which may help countries and governments to stay on track toward their public health elimination goals (8). Additionally, routine and habitual surveillance may help to better understand antigen exposure and infection trends. Future studies may be interested in developing specific surveillance strategies, such as explored in Chapter 5, Paper 5, that would allow for cost-effect routine monitoring with MBAs, while addressing program targets.

Serological MBAs may also integrate antigens panels with non-endemic diseases that could be used to screen for imported exposure or disease recrudesce from previous elimination (or elimination as a public health problem) status, thus helping to ensure public health actions are rendered when needed. This includes diseases that were not habitually collected in previous surveillance due to limited funding or low public health priority. For example, in Chapter 4, I was able to determine prevalence estimates for strongyloidiasis, yaws, and trachoma in Sabah Malaysia in 2015 that were thought to not be a public health problem. According to the WHO global health observatory, these diseases did not have current estimates (9), and this information may help to alert the public health sector for potential action in Sabah, Malaysia.

6.2.3 Supporting NTD efforts in the current pandemic

The current global pandemic has detrimentally impacted the control of NTDs as resources among endemic countries were diverted towards control of SARS COVID-19 (10). Additionally, numerous interventions were interrupted or delayed due to the need for quarantining to prevent transmission. For example, WHO has recommended that community-based surveys, active case findings, and MDAs be postponed until transmission of COVID-19 is under control (10, 11). When surveillance of NTDs resume, serological MBAs can help to support the prompt evaluation of the current state of numerous NTDs through multi-disease surveillance. Simultaneously capturing disease burdens of different diseases may

aid countries in resuming their elimination goals and assess potential changes in transmission due to the interruption of treatment, interventions, and care seeking behaviour. With the necessary SARS-COV2 specific antigens, tropical disease panels can also incorporate monitoring of COVID-19, thus helping to harmonise limited resources to both NTDs and COVID-19 surveillance. Conversely, mucosal samples used to test for COVID-19 have the potential to be integrated with serological MBAs for multi-disease surveillance, although additional research in various aspects will be needed to validate COVID-19 surveillance in multi-disease panels, such as the potential for cross-reactivity with other antigens, the quantity needed for the use of non-serum samples in multi-disease sampling, and interpretation of antibodies (kinetics) found with in mucus.

6.3 Limitations in Theis Research and Implications for Areas of Future Research

While this thesis has addressed several important knowledge gaps pertaining to the application of serological MBAs to integrated disease surveillance, there are limitations and challenges that have been identified during my research. This section provides a general overview of limitations within my thesis, broader limitations in the application of serological MBAs in the field, and suggestions for further research based on these limitations. For example, according to the WHO, diagnostic tools should perform according to ASSURED criteria (accuracy, accessibility, and affordability) (12). While ASSURED criteria is typically applied to point of care testing (which serological MB platforms may not be used for at present), several criteria of ASSURED should be considered in future research of serological MBAs and respective antigens (with subsequent interpretation as appropriate to historical exposure or recent infection) included in multi-disease panels. To date, several studies have investigated potential cost-utility, sensitivity/ specificity, and user-friendliness of serological MBAs (see: Chapter 1, Section 1.3) and have found potential benefits of using a panel of antigens to monitor many diseases simultaneously. Yet, a possible area for future research may entail a more formal review of the cost utility of serological MBAs compared to standard diagnostic tools in the context of multi-disease surveillance, and if cost-utility may not be beneficial when fewer diseases need to be regularly monitored. The summary of future research areas, their significance, and suggestions are included in Table 4.

6.3.1 Data Collection and Data Management for Multi-disease Panels

Survey Designs incorporating serological MBAs

All surveys used in this thesis were designed and powered to assess malaria prevalence. While this does have some impact on how representative these estimates are regarding community exposure of the NTDs/VPDs in my research and interpretation of my findings, including these antigens in malaria study

panels allowed for estimation of certain disease burdens that may not have otherwise been collected at that time.

Determining appropriate approaches for assessing sample size for multi-disease panels that will be sufficiently representative (depending on the aim of the survey) should be explored in future studies. This may involve powering sampling surveys to the rarest expected diseases within the panel, however, if diseases are extremely rare (or impacted by deliberate control activities), it may require a large quantity of resources in active surveillance. For example, in elimination settings, precision of prevalence estimates decreases as prevalence decreases without sufficient samples sizes. Finding the necessary sample sizes, without oversampling that would lead to wasted resources or undersampling, which would impact accuracy of prevalence estimates, for each disease remains a concern when using multiplex bead assays. Determining appropriate sample sizes may also need to take into consideration the costs that would ideally allow for routine surveillance and account for program goals or targets. For example, extensive household surveys as discussed in Chapter 5, may provide strong estimates of disease in the community but is also extremely resource intensive. EAG sampling, while less expensive to implement, may not be able to capture accurate disease estimates from asymptomatic or rural communities. Ideally, finding balance between the resource requirements and representative sampling may allow for more habitual surveillance.

As there are different protocols for active sampling of serological surveys, standard procedures may help to ensure consistent data collection that is representative of multi-disease targets. Standard procedures should be based on program goals and targets, and stages of public health control, such as control and elimination guidelines that may also support the decision for standard approaches to analyzing the data.

An example sampling strategy for multi-disease panel based on diseases included within this thesis would take into consideration the following:

- Appropriate antigens: Include appropriate antigens that can capture endemic strains (e.g. specific LF antigens for *Wucheria* and *Brugia* strains).
- Seasonality: Surveillance may account for the time of year to accurately capture certain burden of diseases (e.g. rainy seasons impacting vector populations, sanitary conditions impacting WASH conditions, or occupation impacting exposure and migration)

- Restricted populations: Use of certain age groups for sampling according to program guidelines (e.g. in TAS for lymphatic filariasis) or to avoid cross reactivity (e.g., trachoma vs. chlamydia and yaws vs. syphilis)
- Geographic distribution: Certain diseases may be more focal in certain environments based on host or vector population density (e.g., chikungunya in urban vs rural populations), and multi-disease panels would needed to be mindful of disease epidemiology when designing surveys.

Data Management

A universal data repository may be beneficial to the global NTD community, as way to track and monitor NTD spread internationally, as well as provide potential data to support NTD research. At local levels, an organized and consistent method may need to be developed to keep track of the progress of different diseases. Further considerations include entities responsible for managing this repository, accessibility, standard ethics protocols for data stored, and potential requirements for regular surveillance in sentinel locations across the world intended to monitor transmission.

6.3.2 Antigen Kinetics and Considerations

Cross Reactivity and Antigen Validation

Certain antigens may risk potential cross reactivity (CR) with other antibodies of other diseases that can lead to elevated or unusual responses and may confound results. This is due to some antibodies with non-specific affinity towards different antigens. CR has been observed in certain cases for filarial (e.g. specific LF antigens), bacterial (VPD antigens), and treponemal antigens (13-15). As more antigens may be introduced into multi-disease panels for NTD surveillance for existing or new diseases, determining potential CR between antigens is needed for accurate interpretation of MFI values.

Using well defined, validated targets, as in antibodies that bind selectively to specific antigens and have been tested to be suitable for monitoring particular organisms (16), may help to ensure confidence in the MFI values procured and reduce CR. This may involve reviewing antigen structures and protein sequences for potential similarities to other antigens used within the multi-disease panel, particularly with pathogens of similar biology. The use of monoclonal antibodies with specific binding to particular antigen epitopes may also help to avoid some cross reactivity, although this may vary by antigen (16).

Additionally, new antigens may likely undergo minimum performance requirements (as guided by the WHO) based on specific sensitivities and specificities, supported by clinical data. For example, the WHO

guidelines for antigen tests for SARS-CoV-2 have performance requirements of 80% sensitivity and 97% specificity (17). Ideally, antigenic targets would have very high specificity and sensitivities, based program and research based on program or research goals

In my analysis, I was provided data based on antigenic targets that had been previously designed and validated. I aimed to account for potential CR throughout this thesis by looking at antigen correlation plots (Chapter 4, Paper 3-Supplemental Table 4, Paper 4, Supplementary Figure 1) or reviewing the literature for known issues.

Antibody Kinetics

Although the study of antibody/antigen kinetics is outside the scope of this thesis, understanding these characteristics is necessary for the full interpretation of specific antigen responses (introduced in Chapter 4 conclusions). When exposed to diseases, the immune system will typically mount immune responses that leads to the production of unique antibodies to fight specific infection. The time from exposure until detectable antibodies are present will vary from antigen to antigen. Some antibodies have demonstrated decay within weeks after initial exposure, while other long-lived antibodies can circulate within the body for years (18). The rate of antibody decay can serve as a proxy for time since exposure, and when applied to catalytic models, certain antibodies can be used to estimate approximate time of exposure (19). The rate of this decay is also an important factor in determining whether these markers pertain to historical or recent exposure, as surveillance may capture elevated responses that may represent historical exposure without baseline estimates that identifies current or recent infection (18). Within this thesis I reviewed the literature to assess known and assumed kinetics of the antibodies included in this research (see Table 2) and discussed with collaborators regarding the interpretation of my results and any impact from antibody kinetics.

Additionally, assessing multiple antigens from the same pathogen given their known kinetics may also provide a better understanding of exposure history compared to using a single antigen and is a topic for future research. For example, in Chapter 4, Paper 4, double positivity was examined for giardiasis, yaws, trachoma and LF, as collaborators have suggested that multiple antigens may provide a more complete picture of disease prevalence.

Of interest is also investigating any immune modulation due to factors such as exposure to other pathogens or public health interventions. For example, diseases such as helminth parasitism, acquired immunodeficiency virus, or malaria may impact suppression or boosting in antibody production that

should be considered when interpreting detectable antibody levels and concentrations. Additionally, future studies may study the behaviour of antibodies in the presence of interventions (i.e., preventative chemotherapy), that may enhance antibody decay compared to the antibodies without interventions. This could potentially impact interpretation of serological biomarkers and surveillance schedules in post elimination settings, where routine surveillance is needed to ensure elimination status.

T6. 2 **Table 2. Summary of provenance and important kinetics of antigens from Introduction.**

Disease/Antigen	Provenance and Kinetics
Chikungunya **CHIKV**	• RNA protein for the Chikungunya RNA virus that is used for detection in other methods such as PCR and RT-PCR (20) • Included in rapid tests have been looking at IgM(21)
Dengue **DENV-2** **DENV-3**	• DENV-2 has epitopes for antibodies to dengue virus serotype 2 and 3; DENV-3 has epitopes for antibodies to serotypes 3 and 1(22) • DENV-2 and DENV-3 are unaffected by cross-reactivity(22)
Toxoplasmosis **SAG2A**	• SAG2A is easily expressed in bacterial cultures as GST fusion protein(23) • IgG ELISAs using recombinant SAG2A were shown to be sensitive (96% and specific 100%) compared to crude Toxoplasma antigens(23) • No evidence of cross reactivity between rSAG2 and GST(23)
Lymphatic Filariasis **Wb123** **BM33** **BM14**	• Bm14 and Bm33 are good estimations of transmission(24) • Differences in isotype responses show that Bm14 and Bm33 antibody responses are specific(24) • No cross reactivity between Bm33 and Bm14(24) • Bm33 was the first antibody response to be detected in children and Bm33 also had the highest seroconversions(25) • Evidence suggests that Bm14 and Bm33 antibody are infection markers(25) • Bm14 and Bm33 should be used only in areas without other filarial antigens known to illicit cross-reactivity(25) • Luciferase immunoprecipitation system has shown Wb123 to precede the appearance of antigenemia in two separate sample populations(25)
Strongyloidiasis **NIE**	• Developed for Immunodiagnostic(26) • The sensitivity and specificity for Luminex was 93% and 95%, respectively(26) • Can be used for routine screening and clinical diagnosis (in the US)(26)
Trachoma/ Chlamydia bacteria **Pgp3** **Ct694**	• Antigens selected based on recognition by serology from trachoma positive patients in published studies. Ct694 found to be involved in pathogenesis(27) • Pgp3 is the only plasmid encoded ORF and secreted into host cell cytoplasm during infection(27). • Pgp3 function is unknown but appears to play a role in pathogenesis(27). • It also could be a potential diagnostic marker for genital chlamydial and is related to both disease and infection status(27)
Yaws / Syphilis **Rp17** **TmpA**	• Rp17 is marker of historical infection and TmpA is a marker of recent or active infection(28). • Rp17 and TmpA detected with high sensitivity and specificity compared to responses with standard reference tests.(28)

	• Positivity correlation with RPR (previous standard of measure) with increasing TmpA levels on MBA(28)
Giardiasis **VSP (1-5)**	• VSP is a surface protein that covers entire parasite-host- immune system is exposed to many different VSP sequences/heterogeneity(29) • IgG antibodies correlate with infection status(29).
Cryptosporidiosis **Cry17** **Cry27**	• Used in ELISA, which detected IgG antibodies to Cry 17 and Cry 27with good sensitivity and specificity relative to the gold standard Western blot in both outbreak and non-outbreak pathogens(29). • Elevated Cry17 is the most recognizable feature of an outbreak.(29)
Amoebiasis **LecA**	• Recombinant LecA is shown to capture specific responses of the E. histolytica-specific IgG antigens(30).
Tetanus **Tetanus Toxin**	• International standard for monitoring vaccine coverage • Showed high sensitivity 99% and specificity 92%(31)
Cholera **Ct b4**	• CT toxin is documented as the major toxin during cholera manifestations(32)

Antibody Isotypes

This thesis focused on the interpretation of general serum IgG responses, without looking at IgM, IgA, or other specific immunoglobulin isotypes. In part, this was due to the retrospective, secondary analysis of this thesis that used bloodspots collected previously for other studies. Antibodies can be collected from most body fluid secretions, and within these different secretions, different antibodies can be present (33). Future research pertaining to MFI may benefit from investing the possibility of using MFI panels that include different sampling techniques (e.g., serum with saliva) and assessing MFI capabilities using multi-isotype approaches with respective antibody kinetics that may provide additional assessments to understanding seroprevalence. Moreover, these different samples may also be able to be incorporated into integrated surveillance approaches. For example, a passive surveillance strategy may collect saliva at a certain timepoint, while an active sampling approach collects plasma a different time point for a specific study within the same year. Collectively, assaying both samples using serological MBAs can be used to understand a broader range of different antigen responses and different isotype responses over a time period and monitor prevalence.

6.3.3 Seroprevalence

Binary transformation of continuous data

The research for this thesis focused primarily on interpretation of MFI data in context of seroprevalence, however, converting continuous data into binary metrics has been shown to lead to some loss of information (34). For example, MFI concentrations may indicate variations in exposure history, such as

recent or historical exposure based on antigen behaviour that may not be captured using a single cut-off. For VPDs, there is some evidence to show that certain seropositivity thresholds are unable to detect changes in MFI values above the defined threshold (35). These changes may provide insight into immunity/antibody decay, boosting, and seroconversion or seroreversion, which may help to accurately interpret data and understand antibody behaviour and dynamics in the population (discussed in the following sub-section). Part of the reason for examining antibody distributions with current approaches to determining cut-offs (i.e., mixture models), is to account for these potential kinetics that impact the interpretation of antibody responses . Additionally, continuous MFI titres may be used to decipher chronological historical exposure and active infections; or differentiate between natural and vaccine-induced responses (36, 37). While seropositivity may currently be a more intuitive approach to guide decision making, future NTD research may benefit from studying MFI concentrations of numerous NTDs assessed through serological MBAs to better inform seropositivity cut-offs or develop approaches aside from seroprevalence that can be translated into programmatic action.

Cut-off methodology

An important, overall challenge of using seroprevalence throughout this thesis was the determination of appropriate cut-off methods for a diverse panel of antigens. As I did not have ROCs or international standards are yet to be developed for many of these antigens, to address of this challenge I examined antigen distributions, discussed with experts, and reviewed literature to determine appropriate methods. Future research may need to focus on developing international serological standards or standard approaches that will allow for uniformity and confident data reporting across settings, as discussed in Chapter 3. Additionally, new cut-off methods may be applied that could be better suited for certain antigens. For example, in Chapter 4, Paper 4, I applied K-means clustering given the statistical and expected biological correlation in several antigens within the disease panel. Depending on how new cut-off approaches are developed, they would likely need to be validated against existing cut-off methods (such as ROC curves) or clinical data to ensure accurate reflection of population exposure or specific programs/research goals.

6.3.4 Summary of future work

This subsection contains a summary of future research as discussed in the previous sub-sections, with potential significance of specific tasks and preliminary suggestions.

T6. 3 **Table 3. Identified areas of future work to support serological MBAs**

Research Area	Areas for further MBA research	Potential Significance	Preliminary Suggestions
Laboratory Procedures	Account for antigen cross-reactivity	Reduces inflated MFI responses	Develop well-defined antigens
	Standard laboratory protocols and reagents cross validated across different MBA platforms	Enables comparison of MFI values across settings	Develop standard protocols based on antigen performance across different MBA platforms using various laboratory techniques
	Identify other diseases to include in panel aside from NTD, VPD, or malaria.	Further enables multi-disease monitoring	Incorporate diseases that already use antigens in monitor and develop antigens of diseases that do not use serology
Data Collection and Management	Sample size determination to for multi disease panels in active surveillance	Improves precision and accuracy of disease estimates for all diseases	Assess minimum representative samples needed per pathogen on disease panel
	Standard protocols for serosurveys involving multi-disease NTD targets	Provides conventional procedures specific to collecting multi-disease NTD targets	Different Standards should be based on program goals, and integrated disease initiatives,
	Multi-disease data repository	Improves data management and organization	Develop a universal organization system to complement serological MBAs.
Data Analysis	International standards or cut-offs to determine seropositivity thresholds (dynamic ranges)	Simplifies challenges in determining cut-off approach; enables comparison of seroprevalence across settings; may eliminate the need for cut-off approaches	Determine IS using serum from most endemic countries, using clinical data or population data for different cut-off points, or artificially procured, dependent on program goals
	Interpretation of specific antibody kinetics	Needed for the interpretation of antigen responses	Examine boosting effects after re-exposure and analyse antibody decay using longitudinal cohorts.

	Shared antigens of the same pathogen	May provide more complete estimates of seroprevalence	Assess seroprevalence using multiple antigen targets
	Co-endemicity and co-exposure	Knowledge of co-endemicity or associated patterns may enhance integrated efforts	

6.3.5 Summary of current WHO initiatives of NTDs studied and future with serological MBAs

This subsection contains a summary of the current WHO initiatives for each of the neglected tropical antigens assessed (38), with some considerations with how serological MBAs can be used to support these agendas based on the research done in this thesis.

Lymphatic Filariasis

The current WHO response to lymphatic filariasis is based on large-scale preventative chemotherapy and provision of essential packages to reduce suffering among chronic infections. Vector control will also supplement treatment where needed. Examples of how serological multiplex bead assays can be used to support these public health measures include:

- Provide evidence of transmission interruption with appropriate antigens
- Supplement ongoing TAS surveys with periodic serosurveillance and in post elimination
- Identify geographical areas to target interventions

Chikungunya

The current WHO response to chikungunya includes several key initiatives such as preparation of outbreak management plans, improved reporting systems, provide training on clinical management of disease, and diagnosis and vector control at the regional. Examples of how serological multiplex bead assays can be used to support these public health measures include:

- Determine baseline prevalence estimates
- Monitor changes of transmission during active control
- Provide routine surveillance to capture outbreaks
- Identify geographical areas to target interventions

Dengue

The current WHO response to dengue involves several key initiatives such as immunization, prevention and control (vector control including insecticides, environmental modification to prevent breeding sites, household and personal protection, and active monitoring and surveillance), improved reporting among countries to manage outbreaks, and provide training on clinical management of diseases. Examples of how serological multiplex bead assays can be used to support these public health measures include:

- Determine baseline prevalence estimates
- Provide routine surveillance to capture outbreaks
- Identify geographical areas to target interventions
- Assess immunity coverage and gaps

Yaws

The current WHO response to yaws eradication includes activities to guide planning and eradication and yaw efforts, develop training material to support health and community workers in managing disease, improved data collection and reporting, and administration of preventative chemotherapy. Examples of how serological multiplex bead assays can be used to support public these health measures include:

- Determine baseline prevalence estimates of subclinical exposure
- Identify geographical areas to target interventions
- Provide routine surveillance to capture recrudescence
- Aid in confirmation of elimination

Trachoma

The current WHO response to trachoma consists of following SAFE (surgery, antibiotics, facial cleanliness, and environmental improvement) strategy for elimination of trachoma. Examples of how serological multiplex bead assays can be used to support these public health measures include:

- Determine baseline prevalence estimates of subclinical exposure
- Identify geographical areas to target interventions
- Provide routine surveillance to capture recrudescence

Soil-transmitted helminths infections

The current WHO response to STH is implement control through habitual treatment of at-risk populations in endemic areas, which includes deworming (preventative chemotherapy), community education and awareness, and sanitation. Examples of how serological multiplex bead assays can be used to support these public health measures:

- Determine baseline prevalence estimates of subclinical exposure
- Identify geographical areas to target interventions
- Provide routine surveillance to monitor transmission

6.4 Conclusions

This thesis aimed to address several important knowledge gaps pertaining to the interpretation of MFI values and the capability of serological MBAs to support integrated disease surveillance in Haiti and Malaysia. Although several areas of future work are needed to address additional identified knowledge gaps, this thesis has contributed to the understanding of the utility of serological MBAs in public health settings.

The findings within this thesis can help guide programs that implement serological MBAs and the analysis of MFI data as part of integrated disease initiatives. In addition, the research of thesis provides a foundation for implementing serological MBAs in context to integrated NTD surveillance to support elimination of these diseases.

6.5 References

1. Lammie PJ, Moss DM, Brook Goodhew E, Hamlin K, Krolewiecki A, West SK, et al. Development of a new platform for neglected tropical disease surveillance. Int J Parasitol. 2012;42(9):797-800.
2. Arnold BF, Scobie HM, Priest JW, Lammie PJ. Integrated Serologic Surveillance of Population Immunity and Disease Transmission. Emerg Infect Dis. 2018;24(7):1188-94.
3. Taylor EM. NTD Diagnostics for Disease Elimination: A Review. Diagnostics (Basel). 2020;10(6).
4. Ng'etich AKS, Voyi K, Mutero CM. Evaluation of health surveillance system attributes: the case of neglected tropical diseases in Kenya. BMC Public Health. 2021;21(1):396.
5. Mackey TK, Liang BA, Cuomo R, Hafen R, Brouwer KC, Lee DE. Emerging and reemerging neglected tropical diseases: a review of key characteristics, risk factors, and the policy and innovation environment. Clin Microbiol Rev. 2014;27(4):949-79.
6. Hatherell HA, Simpson H, Baggaley RF, Hollingsworth TD, Pullan RL. Sustainable Surveillance of Neglected Tropical Diseases for the Post-Elimination Era. Clin Infect Dis. 2021;72(Suppl 3):S210-S6.
7. Pinsent A, Solomon AW, Bailey RL, Bid R, Cama A, Dean D, et al. The utility of serology for elimination surveillance of trachoma. Nat Commun. 2018;9(1):5444.
8. Ending the neglect to attain the Sustainable Development Goals – A road map for neglected tropical diseases 2021–2030. Geneva: World Health Organization; 2020. Licence: CC BY-NC-SA 3.0 IGO.
9. Organization TWH. The Global Health Observatory [Available from: https://www.who.int/data/gho.
10. de Souza DK, Picado A, Bieler S, Nogaro S, Ndung'u JM. Diagnosis of neglected tropical diseases during and after the COVID-19 pandemic. PLoS Negl Trop Dis. 2020;14(8):e0008587.
11. Organization TWH. COVID19 WHO interim guidance implementation NTD programmes [Available from: https://www.who.int/neglected_diseases/news/COVID19-WHO-interim-guidance-implementation-NTD-programmes/en/.
12. Land KJ, Boeras DI, Chen XS, Ramsay AR, Peeling RW. REASSURED diagnostics to inform disease control strategies, strengthen health systems and improve patient outcomes. Nat Microbiol. 2019;4(1):46-54.
13. Njenga SM, Kanyi HM, Arnold BF, Matendechero SH, Onsongo JK, Won KY, et al. Integrated Cross-Sectional Multiplex Serosurveillance of IgG Antibody Responses to Parasitic Diseases and Vaccines in Coastal Kenya. Am J Trop Med Hyg. 2020;102(1):164-76.
14. de Caprariis PJ, Della-Latta P. Serologic cross-reactivity of syphilis, yaws, and pinta. Am Fam Physician. 2013;87(2):80.
15. Frikha-Gargouri O, Gdoura R, Znazen A, Gargouri J, Rebai A, Hammami A. Diagnostic value of an enzyme-linked immunosorbent assay using the recombinant CT694 species-specific protein of Chlamydia trachomatis. J Appl Microbiol. 2009;107(6):1875-82.
16. Weller MG. Ten Basic Rules of Antibody Validation. Anal Chem Insights. 2018;13:1177390118757462.
17. Homza M, Zelena H, Janosek J, Tomaskova H, Jezo E, Kloudova A, et al. Covid-19 antigen testing: better than we know? A test accuracy study. Infect Dis (Lond). 2021;53(9):661-8.
18. Achtman AH, Bull PC, Stephens R, Langhorne J. Longevity of the immune response and memory to blood-stage malaria infection. Curr Top Microbiol Immunol. 2005;297:71-102.
19. Sepulveda N, Stresman G, White MT, Drakeley CJ. Current Mathematical Models for Analyzing Anti-Malarial Antibody Data with an Eye to Malaria Elimination and Eradication. J Immunol Res. 2015;2015:738030.

20. Waggoner JJ, Gresh L, Mohamed-Hadley A, Ballesteros G, Davila MJ, Tellez Y, et al. Single-Reaction Multiplex Reverse Transcription PCR for Detection of Zika, Chikungunya, and Dengue Viruses. Emerg Infect Dis. 2016;22(7):1295-7.
21. Johnson BW, Russell BJ, Goodman CH. Laboratory Diagnosis of Chikungunya Virus Infections and Commercial Sources for Diagnostic Assays. J Infect Dis. 2016;214(suppl 5):S471-S4.
22. Poirier MJP, Moss DM, Feeser KR, Streit TG, Chang GJJ, Whitney M, et al. Measuring Haitian children's exposure to chikungunya, dengue and malaria. B World Health Organ. 2016;94(11):817-25.
23. Priest JW, Moss DM, Arnold BF, Hamlin K, Jones CC, Lammie PJ. Seroepidemiology of Toxoplasma in a coastal region of Haiti: multiplex bead assay detection of immunoglobulin G antibodies that recognize the SAG2A antigen. Epidemiol Infect. 2015;143(3):618-30.
24. Moss DM, Priest JW, Boyd A, Weinkopff T, Kucerova Z, Beach MJ, et al. Multiplex bead assay for serum samples from children in Haiti enrolled in a drug study for the treatment of lymphatic filariasis. Am J Trop Med Hyg. 2011;85(2):229-37.
25. Hamlin KL, Moss DM, Priest JW, Roberts J, Kubofcik J, Gass K, et al. Longitudinal monitoring of the development of antifilarial antibodies and acquisition of Wuchereria bancrofti in a highly endemic area of Haiti. PLoS Negl Trop Dis. 2012;6(12):e1941.
26. Rascoe LN, Price C, Shin SH, McAuliffe I, Priest JW, Handali S. Development of Ss-NIE-1 recombinant antigen based assays for immunodiagnosis of strongyloidiasis. PLoS Negl Trop Dis. 2015;9(4):e0003694.
27. Goodhew EB, Priest JW, Moss DM, Zhong G, Munoz B, Mkocha H, et al. CT694 and pgp3 as serological tools for monitoring trachoma programs. PLoS Negl Trop Dis. 2012;6(11):e1873.
28. Cooley GM, Mitja O, Goodhew B, Pillay A, Lammie PJ, Castro A, et al. Evaluation of Multiplex-Based Antibody Testing for Use in Large-Scale Surveillance for Yaws: a Comparative Study. J Clin Microbiol. 2016;54(5):1321-5.
29. Priest JW, Moss DM, Visvesvara GS, Jones CC, Li A, Isaac-Renton JL. Multiplex assay detection of immunoglobulin G antibodies that recognize Giardia intestinalis and Cryptosporidium parvum antigens. Clin Vaccine Immunol. 2010;17(11):1695-707.
30. Leo M, Haque R, Kabir M, Roy S, Lahlou RM, Mondal D, et al. Evaluation of Entamoeba histolytica antigen and antibody point-of-care tests for the rapid diagnosis of amebiasis. J Clin Microbiol. 2006;44(12):4569-71.
31. Scobie HM, Patel M, Martin D, Mkocha H, Njenga SM, Odiere MR, et al. Tetanus Immunity Gaps in Children 5-14 Years and Men >= 15 Years of Age Revealed by Integrated Disease Serosurveillance in Kenya, Tanzania, and Mozambique. Am J Trop Med Hyg. 2017;96(2):415-20.
32. Kumar P, Jain M, Goel AK, Bhadauria S, Sharma SK, Kamboj DV, et al. A large cholera outbreak due to a new cholera toxin variant of the Vibrio cholerae O1 El Tor biotype in Orissa, Eastern India. J Med Microbiol. 2009;58(Pt 2):234-8.
33. Isho B, Abe KT, Zuo M, Jamal AJ, Rathod B, Wang JH, et al. Persistence of serum and saliva antibody responses to SARS-CoV-2 spike antigens in COVID-19 patients. Sci Immunol. 2020;5(52).
34. Altman DG, Royston P. The cost of dichotomising continuous variables. BMJ. 2006;332(7549):1080.
35. Arnold BF, van der Laan MJ, Hubbard AE, Steel C, Kubofcik J, Hamlin KL, et al. Measuring changes in transmission of neglected tropical diseases, malaria, and enteric pathogens from quantitative antibody levels. PLoS Negl Trop Dis. 2017;11(5):e0005616.
36. Krammer F. The human antibody response to influenza A virus infection and vaccination. Nat Rev Immunol. 2019;19(6):383-97.
37. Hay JA, Laurie K, White M, Riley S. Characterising antibody kinetics from multiple influenza infection and vaccination events in ferrets. PLoS Comput Biol. 2019;15(8):e1007294.

38. Organization TWH. Fact sheets [cited 2022. Available from: https://www.who.int/news-room/fact-sheets.