THE IMMUNO-EPIDEMIOLOGY OF HELMINTH AND *PLASMODIUM FALCIPARUM* CO-INFECTION IN INDIVIDUALS IN THE MIDDLE BELT OF GHANA

By

DENNIS ADU-GYASI (PG9922713)

A Thesis Submitted to the Department of Molecular Medicine, School of Medical Sciences, Kwame Nkrumah University of Science and Technology in Partial Fulfilment of the Requirement for the Degree of

DOCTOR OF PHILOSOPHY (IMMUNOLOGY)

Department of Molecular Medicine, School of Medical Sciences

November, 2018

DECLARATION

The work described in this thesis and submitted to the Department of Molecular Medicine, KNUST was carried out at the Kintampo Health Research Centre in Collaboration with Noguchi Memorial Institute for Medical Research, Ghana, and the West African Centre for Cell Biology of Infectious Pathogens (WACCBIP) of the University of Ghana, and Laboratory of Vaccines for the Developing World, Institute for Glycomics, Griffith, Australia. This work has not been submitted for any other degree.

CANDIDATE NAME	**SIGNATURE**	**DATE**
Mr. Dennis Adu-Gyasi	..…….……..	……..……..

SUPERVISORS

Professor (Mrs) Margaret Frempong	..…….……..	……..……..

(Department of Molecular Medicine, School of Medical Sciences, Kwame Nkrumah University of Science and Technology)

Professor Ben Gyan	..…….……..	……..……..

(Department of Immunology, Noguchi Memorial Institute of Medical Research, University of Ghana, Legon, Accra)

Professor Robert A. Ngala	..…….……..	……..……..

Head of Department

DEDICATION

I dedicate this work and write-up to God Almighty, greatly my wife Portia and children Gyidie, Adom and Nhyira. I also dedicate this work to Nana Abena Ankamu Pokuaa (formerly Francisca Abena Pokuaa Fredua) my mother. I again dedicate this work to Paul Adu-Gyasi and Samuel Adu-Gyasi for their support.

ACKNOWLEDGEMENT

First thanks go to the Almighty God for the life and grace throughout. It has been fruitful and a journey worth pursuing.

I also thank the director and staff of Kintampo Health Research Centre (KHRC) for funding this study for my thesis. Again, to the training committee, Scientific Review Committee (SRC) and Ethics Committee of KHRC, the Ethics Committee of Noguchi Memorial Institute for Medical Research, University of Ghana, I say thank you. I also want to acknowledge the contributions of the participants and community leaders of Kintampo North and South Districts to this study.

I wish to thank my supervisors Professor Margaret Frempong of Kwame Nkrumah University of Science and Technology and Professor Ben Gyan of Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana for their support to the work and this thesis. Again, my special acknowledgement goes to Dr. Mohammed Mutocheluh who played vital role in the initial stages of this programme.

My special acknowledgement to Ghana Education Trust fund (GETFund) for the scholarship to undertake a two-month training and sample analysis trip to Griffith University, Australia in 2016.

To Professor Seth Owusu-Agyei and Dr. Kwaku Poku Asante of KHRC, I am grateful for their immense motivation and support. To Mr David Dosoo and Mr. Dennis Konadu Gyasi of the Clinical Laboratory and all staff of KHRC, I am grateful.

I appreciate Professor Michael F. Good, AO and the team in the Laboratory of Vaccines for the Developing World, Institute for Glycomics, Griffith University, Australia for the training in immunodiagnostic techniques and funding support for sample analysis from February to June 2016.

I owe my experience with flow cytometry to Dr. John Tetteh and the team in the Department of Immunology, NMIMR. Again, in the same department, I express my appreciation to Dr. Godfred Futagbi, Dr. Bright Adu and Dr. Kusi Asamoah for their constant pieces of advise and constructive criticism in the processes and methods used for this study.

I thank Professor Gordon Awandare, Dr. Yaw Aniweh, Mr. Prince Berko Nyarko, Mr. Felix Ansah and the team at West African Centre for Cell Biology of Infectious Pathogens (WACCBIP), University of Ghana, Legon for the massive support to complete sample analysis for this work by providing reagents and availing for my use the BD LSRFortessa X20 flow cytometer.

I owe a big gratitude to Professor Michael Wilson, Dr. Irene Ayi, Mr. Joseph Otchere, Mrs Yvonne Ashong, Mr. Senyo Kojo Botchie, Miss Isabella Georgina Djameh and the entire team from the Parasitology Department of NMIMR for the training in the use of the Kato Katz stool test kit and support for molecular analysis aspects of the study.

Greatest appreciation goes to Mr. Augustine Nii O. Sowah of Parasitology Unit of Korle-Bu Teaching Hospital who gave me preserved stool samples with known parasites to train researchers for this study.

My heartfelt appreciation goes to my wife, Portia and the children Gyidie, Adom and Nhyira for bearing with the many times I had to be absent and dedicated to the completion of the work for this thesis. To my mother, Nana Pokuaa Ankamu Boatemaa (formerly, Francisca Pokuaa Fredua), I say thank you. Mummy Veronica Ivy Dzreke, thank you for your continuous encouragement which kept me focusing on this work. With the good advise of "not to fear man but God" and the attitude to get things done is owed to my dad Mr. Kwame Fredua. No amount of words can be used to explain the appreciation I have for the support you all gave me.

To those who helped in sample sorting and processing for analysis in the laboratory, I say thank you.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND ACRONYMS

ADCC	Antibody Dependent Cellular Cytotoxicity
AnCyPC1	Principal Component 1 from only plasma antibodies and cytokines variables
AnCyPC2	Principal Component 2 from only plasma antibodies and cytokines variables
AnPC1	Principal Component 1 from only plasma antibodies variables
AnPC2	Principal Component 2 from only plasma antibodies variables
APC	Antigen Presenting Cells
APC1	Principal Component 1 from all variables used in the Principal Component Analysis for plasma variables
APC2	Principal Component 2 from all variables used in the Principal Component Analysis for plasma variables
ApPC1	Principal Component 1 of the variables from parasitized red blood cells treatment in Principal Component Analysis
ApPC2	Principal Component 2 of the variables from parasitized red blood cells treatment in Principal Component Analysis
ACT	Artemisinin Combination Therapy
CCL	CC Chemokine ligand
CD	Cluster of differentiation
CDC	Centers for Diseases Control and Prevention
CePC1	Principal Component 1 from variables of cells and Cytokines from cultures
CePC2	Principal Component 2 from variables of cells and Cytokines from cultures

CyPC1	Principal Component 1 from only plasma Cytokines variables
CyPC2	Principal Component 2 from only plasma Cytokines variables
DALY	Disability-Adjusted Life Years
DC	Dendritic Cells
FcεR1	High Affinity Receptor for IgE
FcεRII	Low Affinity Receptor for IgE
FISH	Fluorescence In-Situ Hybridization
FoxP3	Forkhead transcription factor Protein 3
g/dl	Gram per deciliter
GATA3	GATA-binding protein 3. Zinc finger proteins that bind the consensus DNA sequence (T/A)GATA(A/G)
GITR	glucocorticoid-induced tumour necrosis factor receptor (TNFR)-related protein
GPIs	Glycosylphosphatidylinositols
Hb	Haemoglobin
Hct	Haematocrit
HEL	Helminth
HELMAL	Helminth-Malaria
Hgb	Haemoglobin
HKW	Hookworm
HKWMAL	Hookworm-Malaria
IFN-Ɣ	Interferon gamma
Ig	Immunoglobulin
IL	Interleukins
KAP	Knowledge, Attitude and Practices

KHDSS	Kintampo Health and Demographic Surveillance System
KHRC	Kintampo Health Research Centre
MAL	Malaria
MCH	Mean Cell Haemoglobin
MCHC	Mean Cell Haemoglobin Corpuscle
MCV	Mean Cell Volume
MDA	Mass Drug Administration
MHC I & II	Major Histocompatibility Complex I & II
NMIMR	Noguchi Memorial Institute for Medical Research
NoI	No Infection
PBMCs	Peripheral Blood Mononuclear Cells
PC1	Principal Component 1 of the variables in PCA
PC2	Principal Component 2 of the variables in PCA
PCR	Polymerase Chain Reaction
Pf	*Plasmodium falciparum*
pRBC	Parasitized red blood cell
QALY	Quality-Adjusted Life Years
RBC	Red Blood Cells
RDT	Rapid Diagnostic Test
RORɣt	retinoic acid receptor-related orphan receptor gamma-T
SNP	Single Nucleotide Polymorphisms
STAT6	signal transducer and activator of transcription 6
STH	Soil Transmitted Helminths
STHi	Soil Transmitted Helminths Infection
T-bet	T-box family transcription factor

TCR γδ	T Cell receptor gamma delta cells
TGFβ	Transforming Growth Factor beta
Th	T-helper cell
TNF-α	Tumour Necrosis Factor alpha
WBC	White Blood Cells
WHO	World Health Organization

ABSTRACT

Helminths infections affect over 2 billion people in the world. These infections are known to be associated with poverty, poor sanitation and lack of adequate clean drinking water. Malaria parasite infection on the other hand affect about 3 billion people worldwide with varying transmission patterns. In endemic areas, about 60–97% of the cases are attributable to *Plasmodium falciparum* (*Pf*) infection which is responsible for 13–28% of deaths in children under 5 years of age. Helminths are known Th2 inducers. Mainly IL-4 cytokine and characterized with high-level tissue eosinophilia, and production of IgE. Malaria parasite infection is traditionally associated with Th1 effector cells with the build-up of IFN-γ cytokines. Helminths have a complex life cycle which involve larvae penetrating the skin and further undergo a heart-lung migration before establishing to become an adult worm in a suitable place within the host. The host immune response to such infection involves both Th1 and Th2 arms of the immune system. A similar presentation is seen in the pre-erythrocytic and erythrocytic life cycles of the malaria parasite. Helminthic infections are also common in malaria endemic areas and their influence on the course of infection and the epidemiology of malaria has not been adequately investigated.

This study was set up to investigate the immunoepidemiology of helminths and malaria parasites co-infections in the middle-belt of Ghana in sub-Saharan Africa. Informed consents were sought from 1836 participants, randomly selected in an observational cross-sectional study from September 2015 to August 2016. Biological samples (stool, urine and blood) were collected to screen for parasites at baseline. Those found with infections were followed-up post-treatment. Peripheral Blood Mononuclear Cells (PBMCs) were cultured and flow cytometry was used to analyse for cell phenotypes with surface markers (CD3, CD4, CD8, CD11c TCR-γδ, HLA-DR) and intracellular markers (Foxp3, IL-4 cytokine and IFN-γ cytokine). Again, from culture supernatant and plasma, using BD Cytometric Bead Array

(CBA) Human Thl/Th2/Th17 kit and flow cytometry, concentrations of IL-2, IL-4, IL-6, IL-10, TNF, IFN-γ and IL-17A cytokines were estimated. Plasma from participants were also used to estimate concentrations of IgG, IgG1, IgM, IgE antibodies to helminth and malaria parasite crude antigens using ELISA. Data analysis was carried out using Stata statistical software version 13, GraphPad Prism 6 and R Statistical Software where necessary. Ethical approvals were obtained and required quality assurance processes were respected as approved.

Of the total 1569 participants with complete recruitment requirements to produce all needed samples at baseline, a prevalence of 19.3% crude helminth and 28.1% crude malaria parasite were reported over the study period. The prevalence of helminth and malaria parasite co-infection was 11.0%. The study documented 55.4% of hookworm infected participants with heavy infection, 83.3% of *A. lumbricoides* was light infection and 44.4% of *T. trichiura* with heavy infection. Helminth infection had significant association with climate (OR=1.72, p=0.030), inappropriate footwear use (OR=1.64, p=0.044), farming (OR=1.43, p=0.048), inadequate hand washing (OR=0.68, p=0.048) and traditional religion (OR=3.02, p=0.007). The presence of malaria parasite infection increased the risk of contracting helminth infection (OR=1.94, p<0.001). The prevalence of malaria infections was significantly different when one considers the rainy and dry seasons. Prevalence of 15.8% anaemia was recorded and malaria parasite (p=0.001) significantly reduced haemoglobin compared to helminth infection and with malaria co-infection. With helminth, leukocytosis and eosinophilia were found to be associated significantly. Generally, populations of CD8+ (p=0.028), CD8+/IFN-γ+ (p=0.047) and CD8+/HLA-DR+ (p=0.005), gamma delta T cells (CD3+/TCR-γδ+) (p=0.005) were different among the infection groups. Upon stimulation of Peripheral Blood Mononuclear Cells (PBMCs), among malaria infected individuals, CD4+ regulatory cells producing IFN-γ+ (CD4+/Foxp3+/IFN-γ+, p=0.016), IFN-γ+ and IL-4+ production by activated CD4+ cells (CD4+/HLA-DR+/IFN-γ+ (p=0.020), CD4+/HLA-DR+/IL-4+ (p=0.032)) were significantly

higher. Activated CD4+ (CD4+/HLA-DR+) cells, IL-6 (p=0.017) and IL-4 (p=0.021) were significantly higher among hookworm and malaria co-infected individuals. For antibodies, IgG1 to crude malaria parasite antigens were higher in hookworm infected than even malaria infected individuals.

Low prevalence of helminth and malaria as well as their co-infections were respectively recorded in the study. Children less than school-age had helminths infections. Hookworm infections were found to boost immune response to malaria parasite antigens whereas malaria tend to suppress the necessary response needed against hookworm. The study suggests that hookworm and malaria parasite co-infection maintain the Th2 effector cell activation *in-vitro* using malaria parasite antigens. In hookworm and malaria endemic regions, there is the need for careful evaluation of anthelminthic use particularly in Mass Drug Administration (MDA).

1 CHAPTER ONE

INTRODUCTION

1.1 General Introduction

Helminths (including *Necator americanus, Ancylostoma duodenale, Ascaris lumbricoides, Trichuris trichiura, Taenia spp., Schistosoma spp., Hymenolepis spp., Strongyloides stercoralis*) which are multicellular worms have adapted successfully to a parasitic lifestyle and approximately 2 billion people mainly children, get infected worldwide and mainly among children (Hotez et al., 2014, Efunshile et al., 2015, Salazar-Castañon et al., 2014). They can be classified into three taxonomic groups: cestodes (e.g., *Taenia solium*), nematodes (e.g., *Ascaris lumbricoides, Trichuris trichiura* and hookworm), and trematodes (e.g., *Schistosoma mansoni and Schistosoma haematobium*). Helminths usually cause asymptomatic and chronic infections (Salazar-Castañon et al., 2014).

An estimated 1.2 billion, 800 million and 740 million people worldwide are infected with *Ascaris lumbricoides, Trichuris trichiura,* and hookworm, respectively (Keiser and Utzinger, 2008, Knopp et al., 2012, Bethony et al., 2006). These infections are most prevalent in tropical and sub-tropical regions of the developing world where there is inadequate water and sanitation (Knopp et al., 2012).

In sub-Saharan Africa, hookworms (*Ancylostoma duodenale and Necator americanus*) prevalence is approximately 30% (De Silva et al., 2003), and in north-eastern Ghana, the prevalence has been reported to be as high as 50% (Yelifari et al., 2005). In the middle belt of Ghana, the prevalence of hookworms was equally reported to be 45% and that of other intestinal parasites' infection including *Hymenolepis spp, Taenia solium*, and *Trichuris trichiura* was 3% (Humphries et al., 2011).

Schistosomiasis can be found in 74 tropical countries in Africa, the Caribbean, South America, East Asia, and the Middle East, with 62% of the burden occurring in 10 countries in Africa. Worldwide, more than 700 million people are at risk of infection and more than 207 million people are infected (Hotez and Ehrenberg, 2010, WHO, 2008) with *Schistosoma* parasites. *Schistosoma* species are important cause of disease in many parts of the world, most commonly in places with poor sanitation just as most helminths. Commonly found and distributed species in areas affected with this condition across the globe are *Schistosoma mansoni, S. japonicum, S. mekongi, S. intercalatum* and *S. haematobium.*

Helminths are T-helper 2-cell (Th2) inducers in both humans and experimental models. It is usually marked with high-level tissue eosinophilia, mucosal mastocytosis and production of IgE (Maizels and Yazdanbakhsh, 2003, Ateba-Ngoa et al., 2015, Duarte et al., 2012). Helminths skew the production of Th2-cell which leads to immunoglobulin production and other immunological factors that ultimately lead to parasite elimination (McSorley and Maizels, 2012). There is an exception at the infective stage of the helminth which stimulate Th1 cell response just as in the case of some intracellular parasites like in malaria. The response switches to a Th2-cell production when the eggs of the helminths are released (Corrêa-Oliveira et al., 2002).

In endemic tropical areas, polyparasitism is common including infections with malaria parasites. Such coexistence of several parasite species may confound the development of acquired immunity against individual parasites (Doetze et al., 2000, Geiger et al., 2002). The influence of polyparasitism on the course of infection and the epidemiology of malaria needs to be adequately investigated (Adegnika and Kremsner, 2012).

Malaria contributes largely to deaths in children under 5 years of age (Bhattarai et al., 2007, WHO, 2014). Protection against *Plasmodium falciparum (Pf)* has been found to be associated with the preferential production of IgG, its subclasses and IgM antibodies as a result of their

ability to cooperate with blood monocytes in an Antibody-Dependant Cellular Cytotoxicity (ADCC)- like mechanism (Nebie et al., 2008, Abbas et al., 2014). Immunity to malaria parasites also lead to the production of Th1 cells and other immunological factors which are similar in helmenthiasis (Delves et al., 2011, Taylor-Robinson, 2010, Riley, 1999, Geiger et al., 2002).

Susceptibility to malaria attack may vary according to the intensity of parasite transmission, load and age of the infected individual (Sokhna et al., 2004, Brooker et al., 2007). This could be as a result of a more complex immunological mechanism than a simple linear link between the frequency of malaria infection and the degree of helminth infestation.

Studies on host immune response to helminth co-infection have come from experimental rodent models and in human. These have looked at the immune interactions in helminth and *Plasmodium* infections but with contradictory reports on which of the cell differentiations play active role in fighting to eliminate the parasites.

Whilst some of these studies demonstrated competition between the co-infections, with one infection usually leading to the rapid expulsion of the other (Behnke, 2008, Yoshida et al., 1999), others suggest it increases infection intensities by down modulating Th2 cytokine responses. This in turn reduces intestinal inflammation, leading to slower worm expulsion and increased worm burdens in co-infected animals (Behnke, 2008, Behnke et al., 2003, Grencis, 1997). In the former case, the mechanism is attributed to cross-reactive antibodies offering cross protection.

The biological interaction between helminth and malaria parasite co-infection could support a protective approach in some instances as well, additive or synergistic effect and even as one that pose a detrimental challenge depending on the organisms involved in the co-infection (Adegnika and Kremsner, 2012, Courtin et al., 2011, Roussilhon et al., 2010, Diallo et al., 2010).

The effects of helminth infection especially among children and in pregnancy include growth delay, anaemia and poor pregnancy outcomes (Hotez, 2008, Abanyie et al., 2013, Njunda et al., 2015). Helminth infection control efforts have traditionally focused on community-based treatment of high-risk populations (for instance, school-age children) with anthelminthic (Bentwich, 2000, Humphries et al., 2011). Though periodic deworming has been shown to increase growth in some population, it is important to also target the effects the environment contributes to the spread of helminth infection to make the available control strategies effective. Helminth infection is prevalent in poor rural communities (Brooker et al., 2007), because transmission occurs primarily through physical contact with soil contaminated by infective larvae. In these settings, co-infection with other endemic infectious diseases (e.g., malaria, human immunodeficiency virus (HIV), and tuberculosis) is also common, potentially compounding the negative effect of helminth infections (Mayer et al., 2007).

With this knowledge, it is important to identify factors and their influences on immune response to helminths and *Plasmodium* infections particularly in the middle-belt of Ghana where vaccine and antimalarial efficacy trials are often undertaken. This would help in the management of patients with helminths and *Plasmodium* co-infection and augment existing control measures.

1.2 Problem Statement

Commonly, helminth and malaria parasite infections contribute greatly to mortality and morbidity (Hotez et al., 2013). Their infections also affect the Quality/Disability Adjusted Life Years (QALYs/DALYs) (Hotez et al., 2013) of their hosts.

During an infection, T-cell receptors complexes with MHC I or II with peptide of processed antigens on an Antigen Presenting Cell (APC). These complexes, coupled with CD3 activation, cause the T cells to proliferate and differentiate targeting the antigen for a successful clearance (Ridley, 2012, Abbas et al., 2014, Actor, 2014, Luckheeram et al., 2012, Kenneth et al., 2016).

Interactions from the two infections have basically been described to involve two antagonistic responses (Th1 and Th2) (Diallo et al., 2004, Abbas et al., 2014) which could accelerate the process of parasite clearance.

Malaria parasite infections induce Th1 response by the body to get rid of the infection while with helminth, the Th2 arm is activated. In the early stages of helminth infection the Th1 arm of the immune response is activated and when that happens, malaria parasites are cleared faster with a better control of malaria parasite density which in a malaria endemic region could be said to be beneficial to the host (Diallo et al., 2004, Mulu et al., 2014, Naing et al., 2013, Nebie et al., 2008, Briand et al., 2005).

In relation to the others, in the chronic state of helminth infection, the usual and expected Th2 activation when produced will be advantageous to *Pf* in thriving in their host increasing malaria parasite burden (Naing et al., 2013, Nebie et al., 2008, Briand et al., 2005) since the Th1 response needed to eliminate the malaria parasite including amplified antibody production will be suppressed by the Th2 produced (Mulu et al., 2014, Nebie et al., 2008).

The interaction gets complicated when the organisms involved in the infection have complex extracellular and intracellular stages of their life cycle such as the malaria parasite. With such organisms, the immune response produced by the host is very dependent on the stage of the parasite and the antigens produced.

The Th1/Th2 dichotomy has been found not to be the only immunological switches elicited in response to malaria and other infections but rather CD4+ T cells due to their plastic nature and depending on the cytokine *milieu* of the microenvironment differentiate into other forms. It further makes it difficult for immunologist to appreciate which particular arm of the immune response is involved in helminth infections, malaria parasite infections and their co-infection (Luckheeram et al., 2012, Perez-Mazliah and Langhorne, 2015).

It is known that helminth infection also occur in malaria endemic areas (Mayer et al., 2007). Vaccines for malaria which is a major public health disease are being developed. Some of the interpretations of the reduced efficacy from clinical trials of the vaccines (O'Brien, 2009, Hotez et al., 2013) could hinge on immunological responses that might be elicited in co-infections with other parasites. Immune response induced during chronic helminth infection affects not only the response to helminth antigens targeting parasite clearance but consequently downregulate the Th1 response which could create an environment to accommodate the co-infecting parasites (Diallo et al., 2004, Salazar-Castañon et al., 2014).

Control measures adopted for helminth infections include Mass Drug Administration (MDA) using Albendazole carried out periodically to targeted school going children in endemic and developing nations (Humphries et al., 2011, MoH, 2010 , Ortu et al., 2016). Aside programmes such as the MDA, and as part of public health, people are advised to take anthelminthic prophylaxis periodically (some suggest every three months).

It is not clear whether individuals who take these drugs for prophylaxis also induce similar immune responses as what happens in individuals who get the actual helminth infections. Having similar presentation from dosing and in individuals with malaria parasite infection might adversely affect the individuals. For this reason, it is important to understand the outcome of the immune interactions that present in helminth and malaria parasite co-infection (Lemaitre et al., 2014) factoring in the impact of environmental and risk factors such as personal hygiene and sanitation.

In the midst of the contradictory and controversial reports that have emanated from the various studies that considered effects of interactions from helminths and malaria co-infections (Diallo et al., 2004, Perez-Mazliah and Langhorne, 2015, Hotez et al., 2013), the impact of the complex immunological mechanisms that occur in their hosts and the varying immunological responses could adversely affect vaccine efficacy (Sokhna et al., 2004, Hartgers and Yazdanbakhsh,

2006, Hotez et al., 2013), and effective management and control of helminth and malaria infections (Taylor-Robinson et al., 2012, Humphries et al., 2013).

1.3 Research Questions

This proposed research in a malaria endemic site in the middle-belt of Ghana sought to elucidate the complexity surrounding the interactions between helminth and malaria parasite co-infection by finding answers to the following question:

- What is the prevalence of helminth and malaria parasite co-infection in the middle belt of Ghana?
- What are the environmental and behavioural factors that contribute to helminth and malaria parasite co-infection?
- Does helminth co-infection with *Plasmodium* parasite suppress or enhance malaria immunity and malarial parasitaemia?
- What immunity does helminth and malaria parasite co-infection offer to their hosts?

1.4 Justification

Helminth infection induce the production of Th2 response and cell phenotypes in human. This host immune response varies considerably depending on species of parasite causing the infection. In the acute phase, Th1 immune response predominates, and depending on both the time of infection and the developmental stage of the helminth a switch between Th1 and Th2 could persist or be sustained (Perez-Mazliah and Langhorne, 2015, Luckheeram et al., 2012). *Plasmodium* parasite infection largely induce a Th1 response as compared to helminthic infection (Perez-Mazliah and Langhorne, 2015).

It has been shown that Th1, Th2, and Th17 cells can migrate into the B-cell areas of secondary lymphoid organs and acquire the functional capacity and biomarkers of Follicular T- (Tfh) cells. Conversely, the Tfh subset can become Th1, Th2, and Th17 (Perez-Mazliah and Langhorne, 2015, Selzer and Caffrey, 2012, Damania and Dittmer, 2014).

In the acute phase of helminth infection, progress of malaria parasite infection is suppressed due to the Th1 production and later be promoted when there is a switch to Th2. This further downregulates Th1 (Selzer and Caffrey, 2012).

Reports from studies on the impact of all these interactions have been contradictory. It is important to assess the distribution and interaction of helminths infection in a malaria endemic area (Owusu-Agyei et al., 2009) in the middle-belt of Ghana where research into most malaria controls occur. This will help to add knowledge to the prevalence of helminth and malaria parasite infections, the immune response elicited to these infections and the possible outcome from interactions among co-infected individuals.

Most importantly, the added information from this study will contribute positively to the management of both diseases in Ghana. The outcome of this study might shed light on the possible impact of such interactions on vaccinations against infectious diseases in control programmes (Lemaitre et al., 2014, Boef et al., 2013, Hotez et al., 2013).

With the complexity of immune responses at play in helminth and malaria parasite infections, the cytokines and cell types of advantageous effect in preventing autoimmune conditions could be evaluated as well. It will also help to shed light on the possible effect the MDA programme can have on the immunity in helminth endemic regions.

1.5 Hypothesis

The study hypothesized that during *P. falciparum* and helminths co-infections, helminths alter Th2-type immune responses with regulatory cellular phenotypes and cytokine production increases susceptibility to malaria parasitaemia.

1.6 Objectives

1.6.1 Main Objective

To determine the prevalence of, and immunological response elicited to, helminths and *Plasmodium falciparum* co-infection in humans the middle belt of Ghana.

1.6.2 Specific Objectives

The study sought specifically to;

I. Determine the most prevalent helminth infection, *Plasmodium* species and their co-infections and environmental, socio-cultural and economic factors that contribute to their distribution.

II. Assess the impact of helminth, and malaria parasites co-infection on blood cell lines and the haematological indices in their hosts.

III. Characterize the cell phenotypes and the role they play in response to hookworm and hookworm with malaria parasites co-infection.

IV. Classify the humoral immune response to helminth and malaria infections by measuring immunoglobulin (IgE, IgM and IgG with sub-classes) and cell mediated immune responses (Th1/Th2/Th17) by measuring the cytokine levels to antigens of same.

2 CHAPTER TWO

LITERATURE REVIEW

2.1 General overview

Humans are hosts to nearly 300 species of parasitic worms and over 70 species of protozoa which have co-evolved with their mammalian hosts over hundred million years (Ridley, 2012, Voeks and Sercombe, 2000, Zaiss et al., 2015) (Figure 2-1). Some derived from primate ancestors and others acquired from the animals we have domesticated or come in contact with while on Earth (Ridley, 2012, Voeks and Sercombe, 2000, Zaiss et al., 2015).

Helminths have been around for long, and the impact in terms of mortality, morbidity and contributions to the Quality/Disability Adjusted Life Years (QALYs/DALYs) (Hotez et al., 2013) have been huge. It is surprising not much attention has been given to helminth infections neglected compared to other infectious conditions including *Plasmodium spp*, Ebola virus, Zika virus, HIV and some bacteria.

These infections which thrive in the tropical regions also occur where poverty and sanitation issues are grave. It has been reported that the distribution of worm burdens in human populations is a major determinant of both the dynamics of transmission and the level of community morbidity (Butterworth et al., 1988, Njua-Yafi et al., 2016, Campbell et al., 2016b). Though diagnosis, treatment, management and control of infections with helminths by all standards are not herculean, very little is achieved with regards to the listed criteria.

Till recently, funding and support towards minimizing the disease burden, control and eradication of helminth infection had been scarce and this at all levels of infectious disease control had been less developed (Feasey et al., 2010, Freeman et al., 2013).

This is in addition to the lack of commitment to obtain and distribute improved and affordable diagnostic methods for helminth infections. For instance, wet mount for routine stool

examination technique for detecting worm ova needs to be performed on at least two samples from each person to increase the sensitivity of picking an infection to about 70% (Nikolay et al., 2014). This routine wet mount process with its limitations is not practiced in most clinical laboratories in developing countries (Cheesbrough, 2006).

This means the worse affected population with helminth infection might report to the health facilities and return undiagnosed because of insensitive diagnostic methods. This also contribute to the harbouring of the parasites in affected and endemic population. The reservoir of the parasites created in the population become a link for continuous transmission and even outbreaks (Beldomenico and Begon, 2010).

The public is educated to take periodic anthelminthic therapy to get rid of helminths in our homes. Ideally, each home is expected to carry-out self-deworming every three months (Taylor-Robinson et al., 2012, Kirwan et al., 2010) since the drug used in the Mass Drug Administration programme with anthelminthic is also available over the counter and one does not require prescription to buy. These drugs are used devoid of clinical laboratory testing for parasites in patients, a practice which could lead to poor compliance and huge abuse (Kouyos et al., 2014, Sangster, 1999). These influence the development of resistant strains of the parasites which might pose a bigger biological threat in infectious disease management. It is proper to admit that, control of helminthiasis should not be only of therapeutic approach but with preventive measures taking cognizance of the beliefs (Fosu, 1981, Taylor-Robinson et al., 2012) and socioeconomic status (Gyapong et al., 2016) during education.

In endemic places, the immunological response in helminth infection and infections with other pathogens is much desired considering the parasite burden in poor environments (Lertanekawattana et al., 2005). Some of the helminth infections have been found to be associated with poor prognosis of certain diseases. For instance, urinary schistosomiasis has been implicated in cancer of the bladder in affected people (Gonçalves et al., 2003, Katie et al.,

2014a). Hookworm has been associated with great blood loss and neurological deraignment due to autoimmunity triggered by some parasite antigens (Hotez and Ehrenberg, 2010, Hotez and Kamath, 2009).

Interestingly, the presence of hookworm in individuals with Crohn's (Inflammatory Bowel) disease has been found to be beneficial in managing such condition. In fact, some specific proteins of the hookworm, *Ancylostoma* Secreted Protein 2 *(ASP-2*), found in 'soup' are targets to minimize attacks in asthmatic patients and equally work on a possible vaccine for the control of hookworm infections (Hawdon, 2014, Loukas et al., 2006, Hotez et al., 2013). Helminths, at initial phase of infection induce Th1 response and later in the chronic stage mainly switch over to Th2 response. Conversely, a Th1 response is mounted by the host in malaria parasite infection to get rid of the parasite or keep it under control. It makes sense to understand why malaria parasitaemia is controlled when a host is co-infected with hookworm and *Plasmodium falciparum* parasites (Humphries et al., 2013, Mulu et al., 2014, Naing et al., 2013).

On the contrary, immunological interactions from co-infections of hookworm and malaria parasite have been found to be detrimental to their host (Adegnika and Kremsner, 2012, Boel et al., 2010, Naing et al., 2013, Nacher, 2011). Some studies have observed and reported the beneficial effect of malaria parasitaemia kept under control in a host that had a *Schistosoma* co-infection and *A. lumbricoides* infections (Katie et al., 2014b, Lemaitre et al., 2014, Nacher, 2011).

Communication in the immunological labyrinth in a host involves cells, cytokines and antibodies together with host natural defense mechanism. It is important to understand the mechanisms that might be involved in the immunological response to helminths and even those that play direct role under co-infected conditions (Allen and Maizels, 2011, Boel et al., 2010, Nacher, 2011).

How much of influence does the environment have on the level of immune response to an infection? This would need more knowledge contributed through a structured study. This project sought to combine environmental factors and some host dynamics to study the immune response elicited by host to helminth infections and in malaria parasites co-infection status in endemic area in the middle-belt of Ghana, West Africa.

2.2 Helminth epidemiology and infection

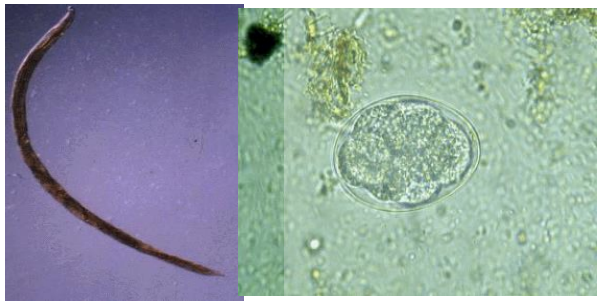
In the tropics, all the conditions favouring transmission of parasitic (helminths and protozoa) infections, including a humid climate, unsanitary environments and poor socio-economic conditions abound (Hotez and Kamath, 2009, Humphries et al., 2011, Loukas et al., 2006).

These parasites are a major cause of morbidity with incidence estimated at approximately 50 % in developed countries and reaches up to 95 % in developing countries, with sub-Saharan Africa having the highest burden (Hotez et al., 2009, Njunda et al., 2015). Approximately 2.7 billion people who live in low-income countries in Africa, South America, and Asia are thought to have some type of helminth infection (Brooker et al., 2007, Damania and Dittmer, 2014). This remains the most common infection in over 230 million preschool-aged children (Hotez et al., 2013, Hotez and Kamath, 2009).

2.2.1 Helminths

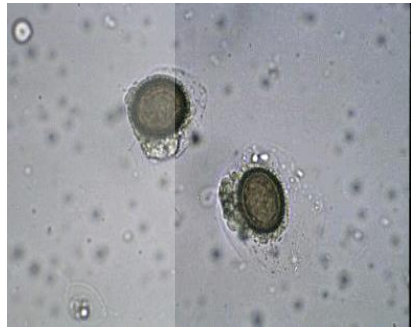
The mammalian intestine is home to many pathogens, including commensal bacteria, helminths, and viruses. Among these, helminths represent some of the earliest recorded human infections in history and remain a significant source of infection today (Damania and Dittmer, 2014, Adegnika and Kremsner, 2012, Toma et al., 1999). The four major genera of helminths (*Ascaris (eg. Ascaris lumbricoides), Trichuris (eg. Trichuris trichiura), Schistosomes (eg. Schistosoma haematobium)* and the hookworms (egs. *Ancylostoma duedenale* and *Necator americanus*)) which differ in their mode of infection share common characteristics (Anderson and May, 1985, Bethony et al., 2006). Unlike microparasites, these organisms are not known

to multiply within the host and as a result, each worm represents a unit of transmission. It is therefore important to know that the worm burden directly translate into the transmission intensity of infection (Anderson and May, 1982, Bethony et al., 2006).



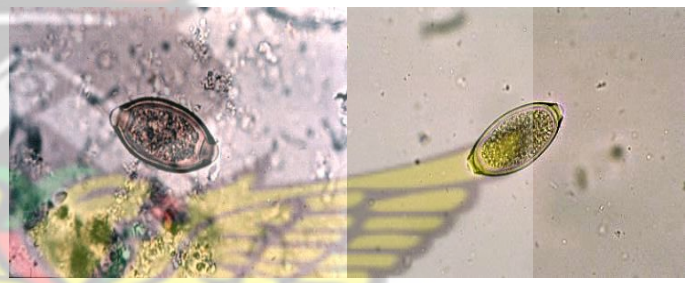
Hookworm adult worm and ovum

Strongyloides stercoralis rhabditiform larva

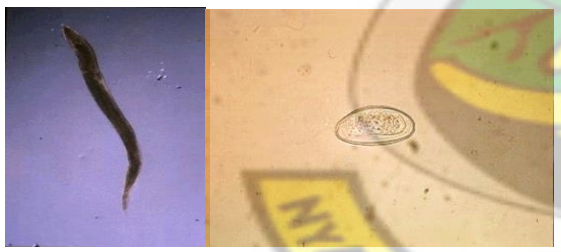


Taenia spp ova

A. Iumbricoides male and female adult worms. *A. Iumbricoides* egg (Fertilized)



Trichuris trichiura ovum (in human) and *Trichuris vulpis* ovum (in dogs and are larger



Enterobious vermicularis adult worm and ovum

Hymenolepis species ova

Figure 2-1: Micrographs of some selected helminths screened from stool samples (Atlas of Medical Parasitology, 1996, First Edition)

2.2.2 Nematodes

Most of the medically important intestinal nematodes are geohelminths, i.e. soil-transmitted (spread by faecal contamination of the soil). Person becomes infected by swallowing infective eggs in the case of; *A. lumbricoides, T. trichiura,* and *Enterobius vermicularis,* or by infective larvae penetrating the skin as in hookworms and *Strongyloides stercoralis* infection (Anderson and Medley, 1985, Cheesbrough, 2009, Beldomenico and Begon, 2010). Before becoming adults in their human host, the larvae of hookworms, *A. lumbricoides*, and *S. stercoralis* undergo the heart-lung migration for about 10 days. The worm is coughed out of the lungs and into the intestines to find a suitable place, grow and develop into adult.

Nematodes are cylindrical worms. They have a body cavity and a cuticle (skin) which may be smooth, spined, or ridged. The adults of some species of the nematodes considered are very long, e.g. *Dracunculus medinensis,* can measure 1 metre or more.

The mouth of these parasites is surrounded by lips, or papillae. In some species, e.g. hookworms, the lips open into a buccal cavity which has cutting or tooth-like plates (Cheesbrough, 2009). The digestive system is a simple tube which ends in an anus. Sexes are separate with the male worms being smaller than the females. Females of the nematodes could be either viviparous (produce larvae) or oviparous (lay eggs) within their host or outside. The discharged eggs may hatch directly into infective larvae or they may require special conditions in which to hatch and up to three developmental stages (1st, 2nd and 3rd stage larvae) by shedding off old cuticle before becoming infective larvae (Cheesbrough, 2009, Schmidt and Roberts, 2009, Actor, 2012, Agrawal, 2012, Ridley, 2012).

Helminthiasis, have been described as infection commonly associated with poverty, poor sanitation and affects people mainly in the tropics. Considering organisms that are involved in causing intestinal helminth infections in humans, Ascaris, *Trichuris trichiura,* and hookworm are the three most common type of helminth infection, each is estimated to affect between

600-800 million people worldwide each year (De Silva et al., 2003, Bethony et al., 2006, Brooker et al., 2006, Hotez and Kamath, 2009, Taylor-Robinson et al., 2012). More than a quarter of the world's population is reported to be infected with one or more of the soil-transmitted intestinal worms (Chan, 1997).

There is evidence that some protection are associated with high worm burden in some studies with small sample size. Similar studies have also given contradictory evidence of harm associated with high worm burden infections. All these studies have not been corroborated by larger trials (Taylor-Robinson et al., 2012). Rather, studies involving larger sample sizes demonstrated a decline in infection intensity in the contemporary population (Anderson and Medley, 1985, Nacher et al., 2001, Taylor-Robinson et al., 2012).

2.2.2.1 Hookworm

Hookworm infection had in the early 1800s inflicted pain on miners in the United States and virtually people who worked in close contact with the soil, particularly where people defecated. As it has been elaborated in the life cycle of hookworm in Figure 2-2, the link is clear that the larvae of the worms penetrated the skin of affected people who initially reported of itching.

This is not too different to the frequent lack of proper footwear in developing countries in this age of farming or embarking on activities that involve close contact with hookworm infested formites. Even with better understanding of the route of transmission of helminths infection, open-field defecation is still a major problem in places largely affected and with high prevalence of the worm infestation (Freeman et al., 2013, Campbell et al., 2016a).

Getting potable water as prescribed by public health is still a major challenge since majority of people in affected communities and environments resort to rivers, streams and at best hand-dug wells as their sources of drinking water. Transmission of the infection described hundreds of years ago still plaque humanity in the tropical and poor regions

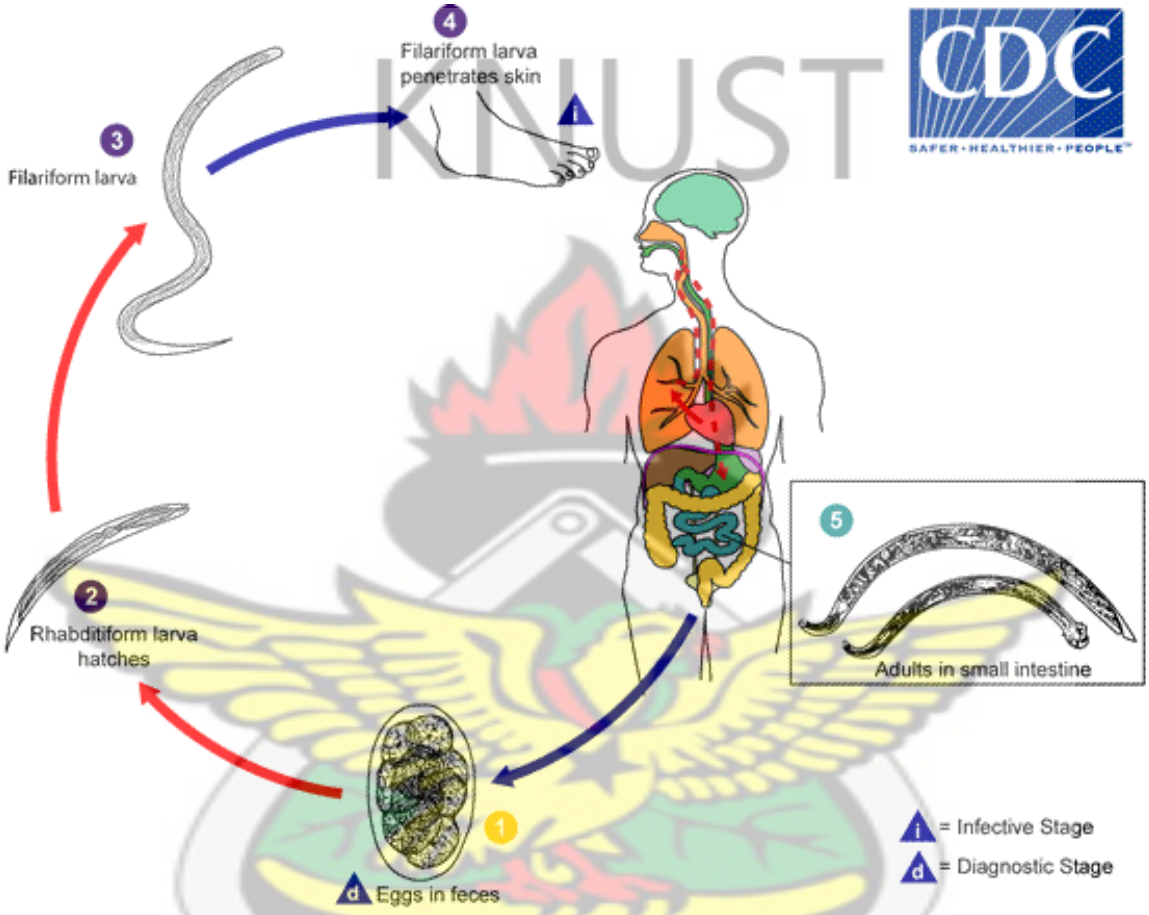


Figure 2-2: Life cycle of hookworm.

Eggs are passed in the stool (1). Larvae hatch in 1 to 2 days. The released rhabditiform larvae grow (2), and after 5 to 10 days become infective filariform larvae (3). These infective larvae can survive 3 to 4 weeks in favourable environmental conditions. On contact with the human host, the larvae penetrate the skin and undergo a heart-lungs migration. They penetrate into the pulmonary alveoli, ascend to the pharynx, and are swallowed (4). The larvae reach the small intestine, where mature into adults in the host (5). Most adult worms are eliminated in 1 to 2 years, but the longevity may reach several years (https://www.cdc.gov/parasites/hookworm/biology.html).

The infection is directly related to causing anaemia and its associated challenges among its sufferers (Sant-Rayn et al., 2008, Ridley, 2012) and specifically iron-deficiency and hypoalbuminemia (Hotez et al., 2013).

Although one cannot link hookworm infections to severe mortality, its towing effect on morbidity cannot be ruled out. The marked effect of hookworm (*Ancylostoma duodenale* and *Necator americanus*) infection causing chronic gastrointestinal blood loss leading to iron deficiency infection (Hotez and Ehrenberg, 2010, Humphries et al., 2013, Cheesbrough, 2006, Sant-Rayn et al., 2008) is seen in children and pregnant women. This also affect the cognitive and reasoning power of children especially in association with iron-deficiency. It can also cause pregnancy lose in addition to the dire consequences of foetus malformation in infected mothers (Yatich et al., 2009, Adegnika and Kremsner, 2012).

Many have attributed the increased rate of absenteeism among school-age children to hookworm infection in the developing countries (Humphries et al., 2011, Humphries et al., 2013).

Though effective therapy is available for all helminth and in this case hookworm infection, the routine methods for diagnosis are not highly sensitive. The routine processes of collecting stool samples to screen for ova and parasites barely reveal more than 70% of the positive cases (Nikolay et al., 2014).

A more sensitive and direct method of identification of the worm would have been appropriate. In the absence of such and seeing the therapy (albendazole) for hookworm as efficacious, public health officials and managers have resorted to Mass Drug Administration (MDA) among school children and also advise prophylactic treatment at three-month intervals in each home (Ortu et al., 2016, Taylor-Robinson et al., 2012). Due to insufficient funding, the MDA of albendazole happens once every year and even only in years when funding is available. Looking at the reinfection rate of most helminths, a prophylactic therapy should have been

taken at least four times each year (Humphries et al., 2011, KMHD, 2015, Kepha et al., 2017, Salam et al., 2015).

The fight against hookworm and other worms mainly from the therapy point of view has contributed to the development of resistant strains of the parasites and it is not surprising there are reports of minimized efficacy of the drug among some infected individuals (Beech et al., 2011, Humphries et al., 2013). It is refreshing that the control measures have also targeted simultaneously hygiene and poor sanitation issues. But of course, a lot needs to be done to increase the number of farmers or individuals whose occupation bring them into constant contact with the soil to have appropriate attire and footwear for their jobs or trade (Esrey et al., 1991, Freeman et al., 2013, Campbell et al., 2016a).

Potable treated drinking water which should have been a right to all, is a privilege of a few just as appropriate place of defecation and refuse management in our communities (Campbell et al., 2016a). Schools that have toilet facilities and provisions for children to wash their hands after attending to "natures call" are countable in the developing countries of which Ghana and for that matter Kintampo in the middle-belt cannot be left out (Freeman et al., 2013, Campbell et al., 2016a). These keep making hookworm and other helminths burden a menace to society and the human race.

2.2.2.2 Ascaris lumbricoides

Ascaris lumbricoides (A. lumbricoides) (Figure 2-1) commonly known as "large roundworms" described as far back in the medieval period in coprolites (Ridley, 2012, Schmidt and Roberts, 2009) accounts for the majority of helminth infections in an estimated one billion individuals worldwide (Bethony et al., 2006, Hotez et al., 2009).

The worm which became one of the parasites described by Linnaeus is found globally due to the presence of infective eggs in soil that is commonly used to fertilize farm land. This is one of the few worms that reinfection is highly impossible since eggs could only become fertile

and could release infective larvae upon contact with soil. For that matter, *Ascaris* ova need to be passed out in faeces and be re-ingested in contaminated soil (Abanyie et al., 2013, Bethony et al., 2006) as presented in Figure 2-3.

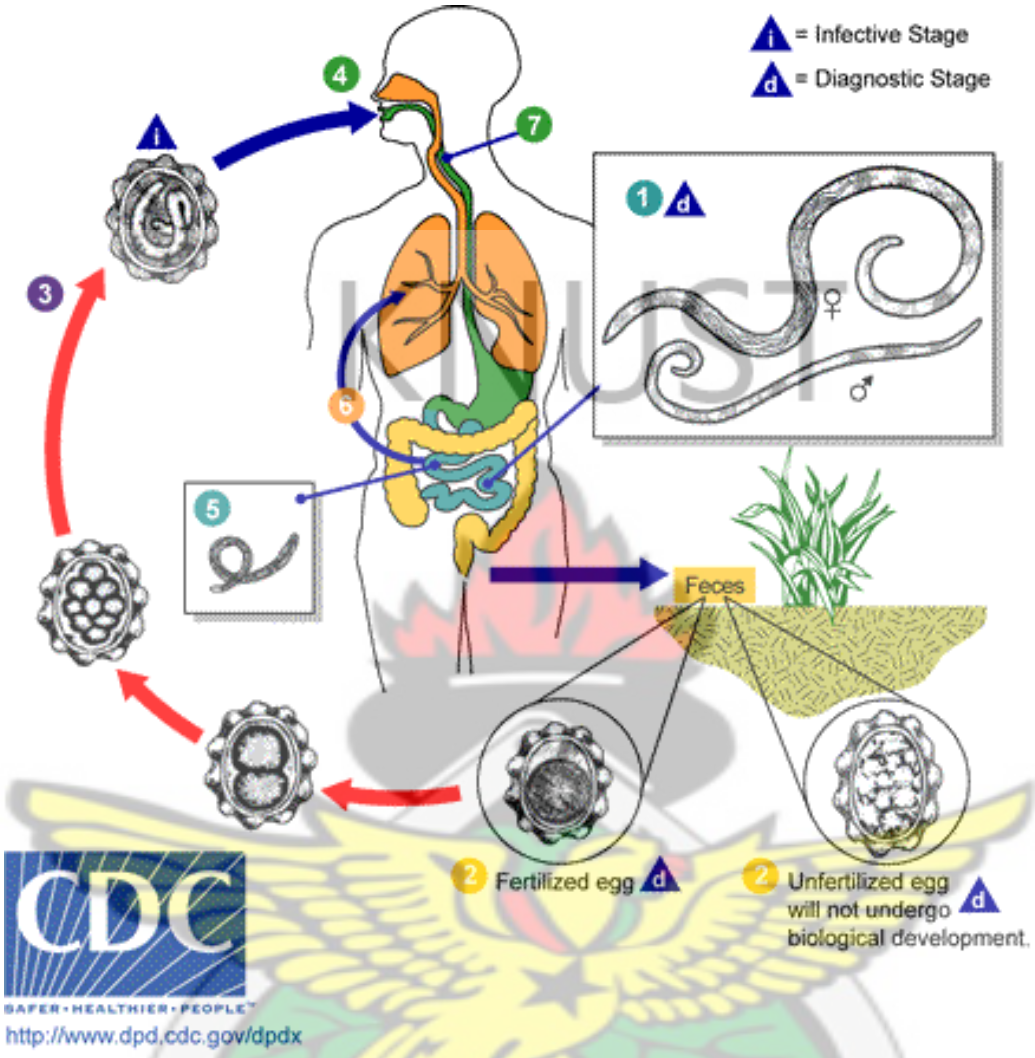


Figure 2-3: Life cycle of *Ascaris lumbricoides*. *Ascaris suum* (roundworm found in pigs).

Adult worms (1) produce approximately 200,000 eggs per day in the small intestine and are passed with the faeces (2). Fertile eggs embryonate and become infective after 18 days to several weeks (3), depending on the environmental conditions. After infective eggs are swallowed (4), the larvae hatch (5), and undergo the heart-lungs migration (6). The larvae mature and are coughed up to the throat, and are swallowed (7). They develop into adult worms in the intestines (1). Between 2 and 3 months are required from ingestion of the infective eggs to oviposition by the adult female. Adult worms can live 1 to 2 years. (https://www.cdc.gov/parasites/ascariasis/biology.html).

2.2.2.3 Hymenolepis species

Hymenolepis nana, (Figure 2-1) commonly referred to as "dwarf" tapeworm, has ova that are infective immediately they are passed out in stool but assuredly cannot survive for more than 10 days without its host (CDC, 2012). The parasitic infection which is commonly transmitted from infected person to another via the faeco-oral route has also another zoonotic form, *Hymenolepis dimunita* (in rats) which can also infect human accidentally (CDC, 2012, Shiba et al., 1996).

When eggs are ingested (in contaminated food or water or from hands contaminated with faeces), the oncospheres contained in the eggs are released and the cycle continues as described in Figure 2-4.

The infection which is most common in children is estimated to affect about 50-70 million people worldwide and it is one of the helminthic infections that has not been associated with death though morbidity is common (Harbir, 2015, Shiba et al., 1996).

In the middle-belt of Ghana, as part of other intestinal parasites present in fewer than 3% of the population, *Hymenolepis nana* (3%), *Taenia solium* (1.1%), and *Trichuris trichiura* (1.2%) prevalence was reported (Humphries et al., 2011).

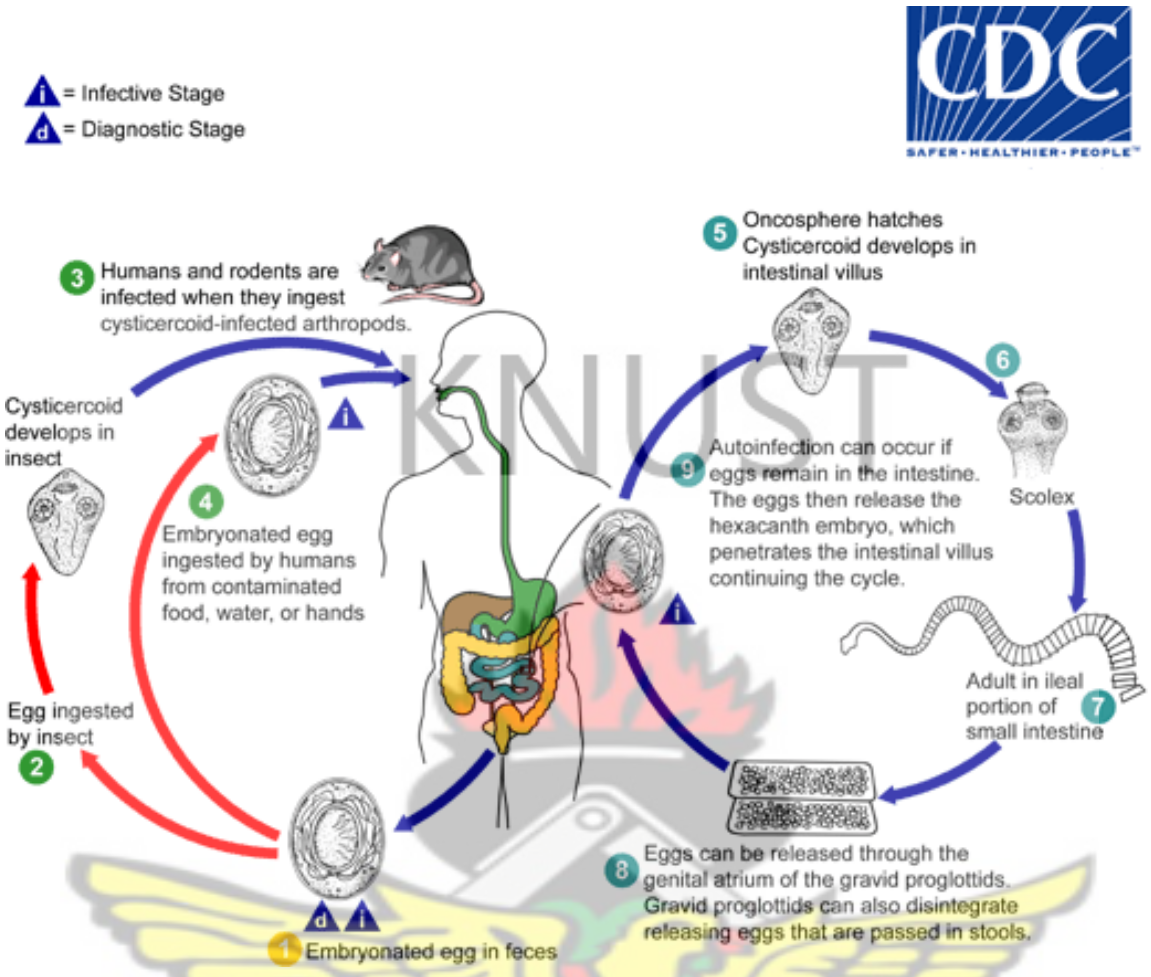


Figure 2-4: Life cycle of *Hymenolepis* spp.

Eggs of *Hymenolepis nana* are immediately infective when passed with the stool [1]. When ingested by an arthropod intermediate host [2] they develop into cysticercoids, which can infect humans or rodents upon ingestion [3] and develop into adults in the small intestine. When eggs are ingested, [4] the oncospheres contained in the eggs are released which develop into cysticercoid larvae [5]. The cysticercoids rupture, return to the intestinal lumen, evaginate their scoleces [6], attach to the intestinal mucosa and develop into adults. They produce gravid proglottids [7]. Eggs are passed in the stool in the small intestine [8]. An alternate mode of internal autoinfection can occur without passage through the external environment [9]. **(https://www.cdc.gov/parasites/hymenolepis/biology.html).**

2.2.2.4 Strongyloides stercoralis

Strongyloides stercoralis, (Figure 2-1) is a nematode common in the tropical and subtropical areas. *Strongyloides* life cycle is more complex than that of most nematodes with its alternation between free-living and parasitic cycles, and its potential for autoinfection and multiplication within the host (CDC, 2012, David and William, 2006). It has two complex life cycle as presented in Figure 2-5. To date, occurrence of autoinfection in humans with helminthic infections is recognized only in *Strongyloides stercoralis* and *Capillaria philippinensis* infections.

In the case of *Strongyloides*, autoinfection may explain the possibility of persistent infections for many years in persons who have not been in an endemic area and of hyperinfections in immunosuppressed individuals (CDC, 2012, David and William, 2006).

It infects about 30-300 million people worldwide and with a prevalence of 23.5% in an endemic area in North-Eastern Thailand, is a serious threat to public health (Jongsuksuntigul et al., 2003, Ridley, 2012, Shiba et al., 1996). The clinical spectrum of strongyloidiasis could be asymptomatic and symptomatic (including mild symptomatic abdominal diseases, skin diseases as well as fatal presentations in immunosuppressed patients) with leading to disseminated strongyloidiasis particularly among AIDS patients (Ridley, 2012, Shiba et al., 1996).

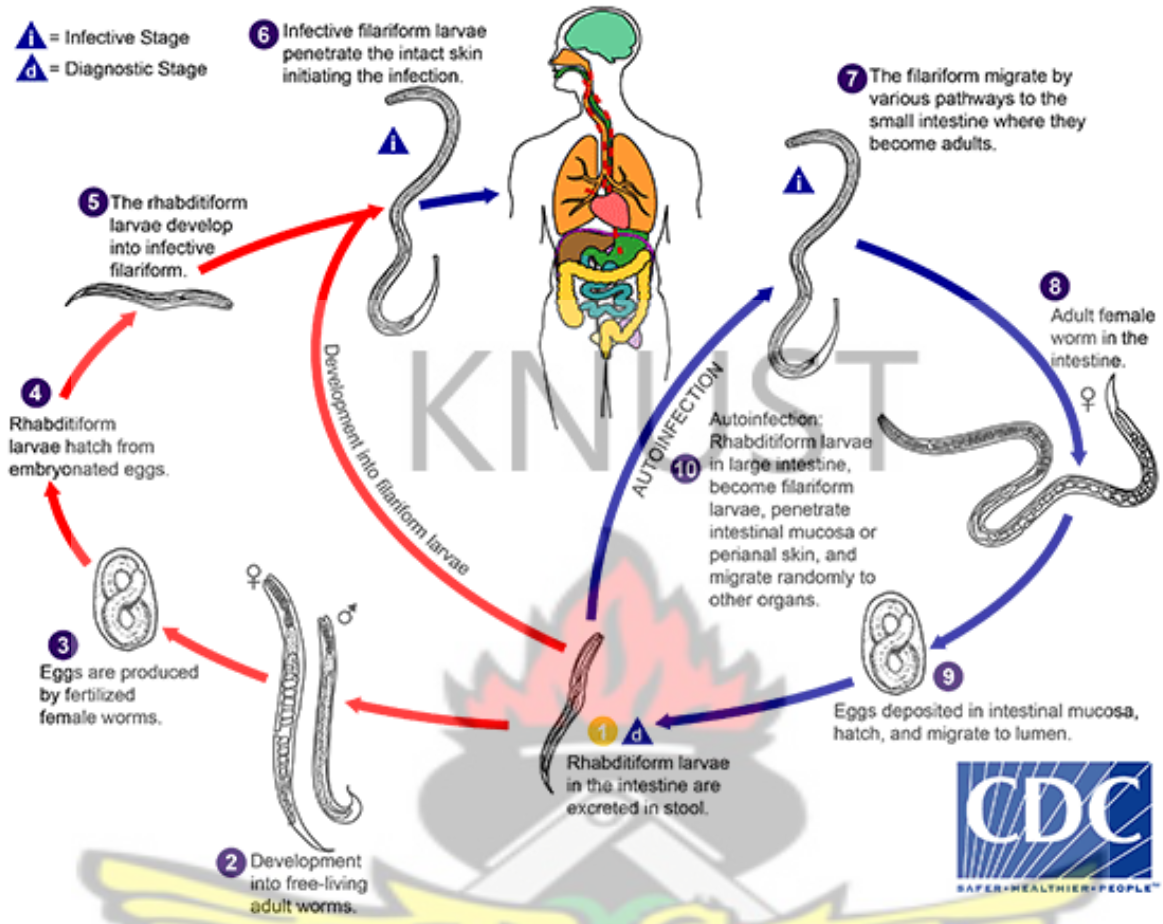


Figure 2-5: Life cycle of *Strongyloides*.

<u>Free-living cycle:</u> The rhabditiform larvae passed in the stool (1) can either become infective filariform larvae (6) or free living adult males and females (2) that mate and produce eggs (3) from which rhabditiform larvae hatch (4). and eventually become infective filariform larvae (5). The filariform larvae penetrate the human host skin to initiate the parasitic cycle (6). <u>Parasitic cycle:</u> Filariform larvae in contaminated soil penetrate the human skin (6), and migrate into the small intestine (7). In the small intestine they develop into adult female worms (8). The worms produce eggs (9) and the rhabditiform larvae is passed in the stool (1), or can cause autoinfection (10). (https://www.cdc.gov/parasites/strongyloides/biology.html).

2.2.2.5 Trichuris trichiura

Trichuris trichiura, (Figure 2-1), commonly known as whipworm affects approximately 10% of the world's population (Shiba et al., 1996). The adult worms (approximately 4 cm in length) live in the cecum and ascending colon. The adult worms are fixed in that location, with the anterior portions threaded into the mucosa. The females begin to oviposit 60 to 70 days after infection. Female worms in the cecum shed between 3,000 and 20,000 eggs per day. The life cycle is presented in Figure 2-6.

It is also generally a disease of poverty and inadequate water supplies and sanitation and its impact is deeply felt in developing countries in the tropical region (Shiba et al., 1996, Bethony et al., 2006, Ridley, 2012).

Infections with *T. trichiura* are considered tropical disease, most cases are found in Asia and to a lesser extent in Africa and South America. Mostly children are with high risk of exposure to the parasite (Bethony et al., 2006, Hotez et al., 2014, Ridley, 2012).

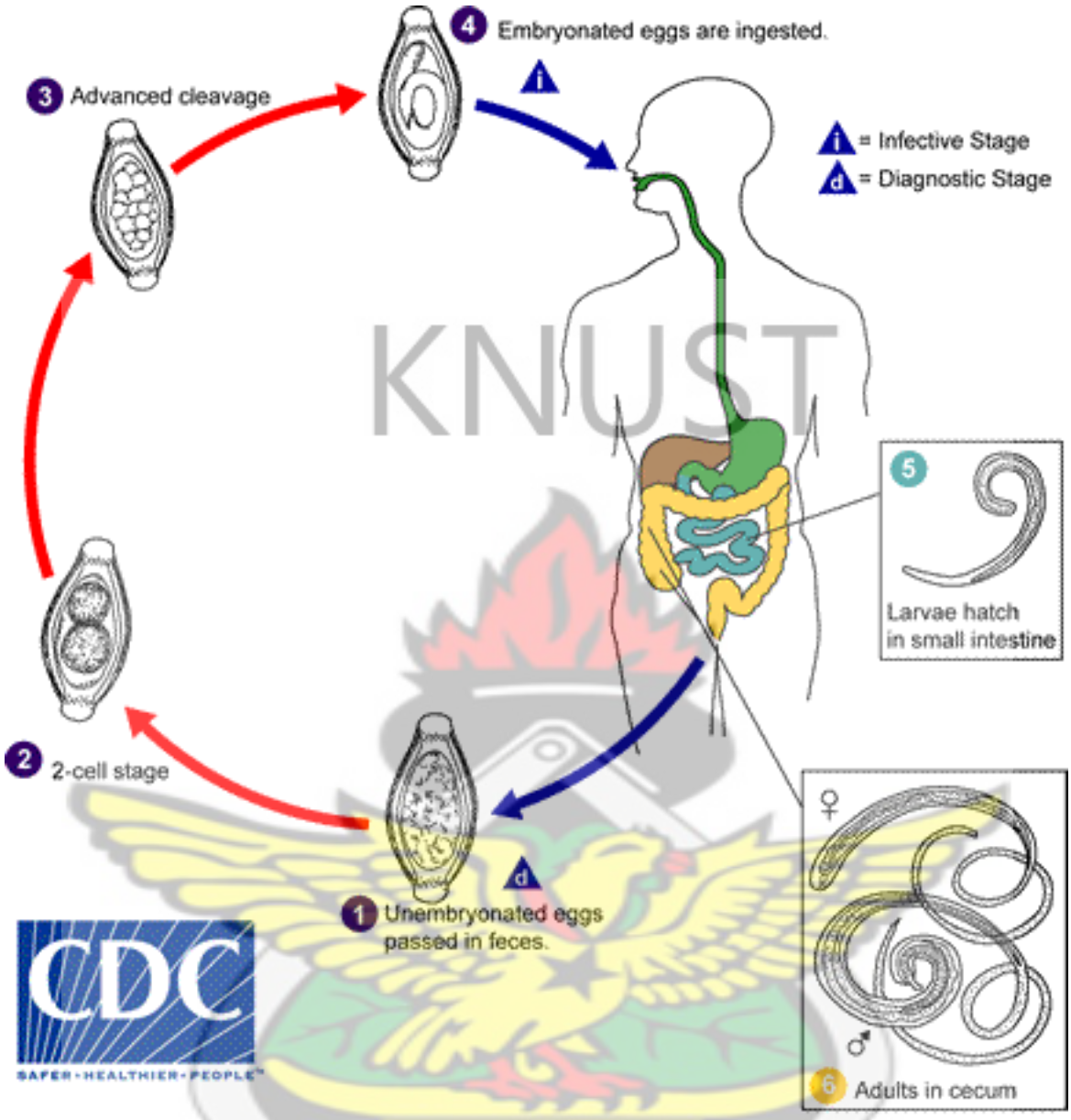


Figure 2-6: Life cycle of *Trichuris trichiura*.

The unembryonated eggs are passed with the stool 1 which develop into a 2-cell stage 2 , an advanced cleavage stage 3 , and then they embryonate 4 . The eggs become infective and after ingestion they hatch in the small intestine, and release larvae 5 that mature into adults in the colon 6 . The life span of the adults is about 1 year. (https://www.cdc.gov/parasites/whipworm/biology.html)

2.2.2.6 Enterobius vermicularis

The organism, *Enterobius vermicularis*, (Figure 2-1) infects a significant number of children annually around the world, including the United States. Symptoms are itching and irritation of the anus or vagina, digestive disorders, insomnia, irritability, or nervousness (Ridley, 2012).

On a global basis it is estimated that up to 500 million people may be infected at any given time with pinworms (Ridley, 2012). The infection is spread via the fecal-oral route by ingestion or inhalation of embryonated ova or from contaminated fomites, soiled fingers, dirty bed linens, toilet seats, clothing (Bethony et al., 2006, Gonçalves et al., 2003), curtains and carpeting.

It is common to get this parasite spread easily through families and children in groups due to the possibility of person-to-person transmission. It is necessary to treat all members in a family when one is identified to have the parasite (Haider et al., 2009, Salam et al., 2015).

The life cycle of the pinworm is presented in Figure 2-7. The time interval from ingestion of infective eggs to oviposition by the adult females is about one month. The life span of the adults is about two months.

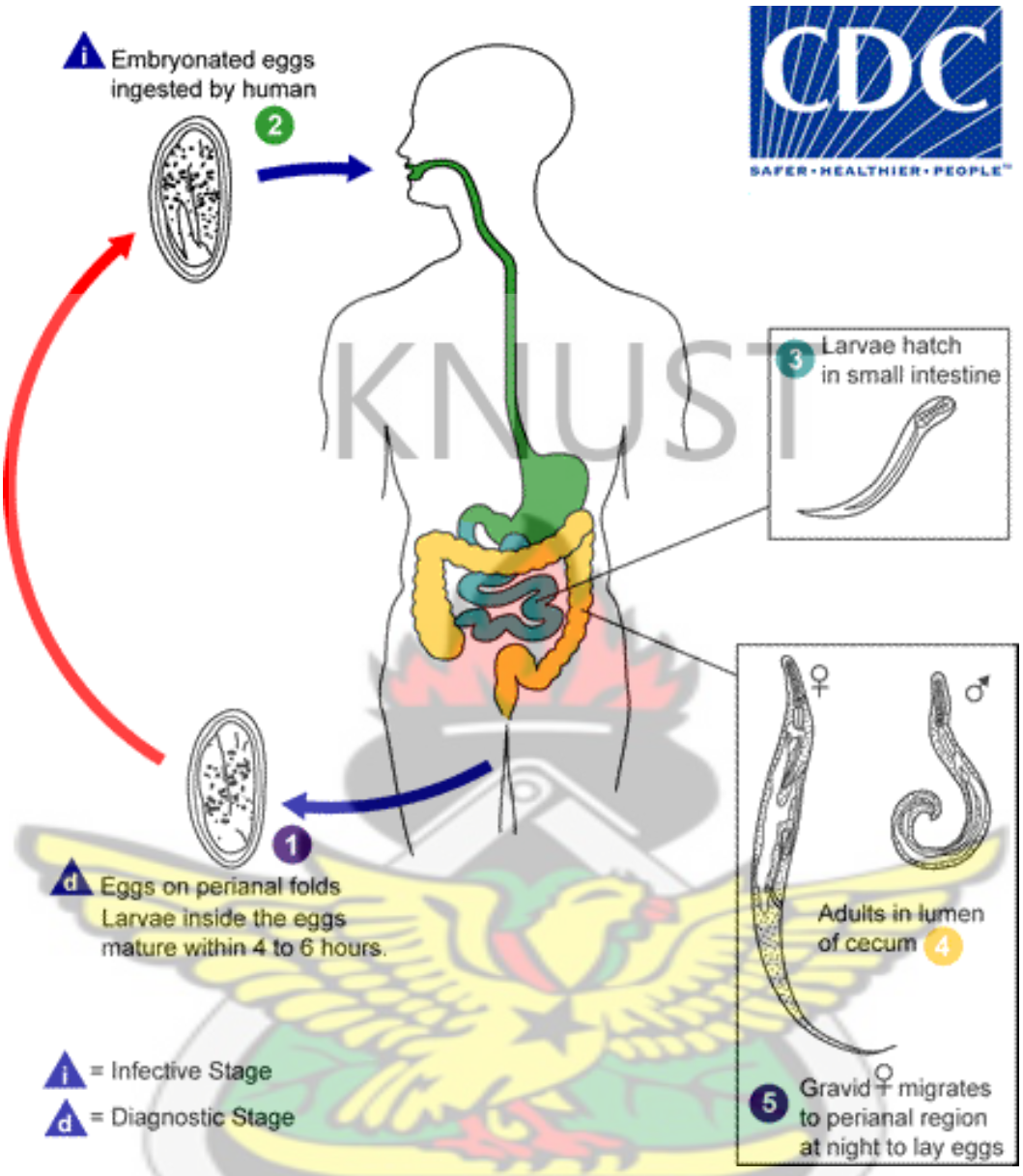


Figure 2-7: Life cycle of *Enterobius vermicularis*.

Eggs are deposited on perianal folds 1. Self-infection can occur when one scratches the perianal area 2. Eggs on contaminated sources would be swallowed and follow the same development as ingested eggs. Infective eggs hatch into the larvae in the small intestine 3 and the adults establish themselves in the colon 4. Gravid females migrate nocturnally outside the anus to lay eggs 5.

(https://www.cdc.gov/parasites/pinworm/biology.html)

2.2.2.7 Schistosomes

It is reported that almost 90% of all schistosomiasis cases worldwide are found in sub-Saharan Africa (Hotez et al., 2009). And specifically, *S. japonicum* is common and endemic causative agent in Asia while *S. mansoni* and *S. haematobium* are more located in Africa (Xu et al., 2012, Shiba et al., 1996). The life cycle of *Schistosoma* spp is presented in Figure 2-8.

The species commonly found to also cause infection in Ghana, and sometimes as mixed infection in the same person, are *Schistosoma haematobium* and *S. mansoni* (Adu-Gyasi et al., 2010, Danso-Appiah et al., 2010).

The prevalence of the two parasites commonly identified in Ghana ranges from 3.7% in adult pregnant women in the Southern part of Ghana (Tay et al., 2017) to 95% of *Schistosoma haematobium* among school-age children in the middle part of Ghana (Ayeh-Kumi et al., 2015). The Ghanaian population at risk of the heaviest burden of schistosomiasis are school-aged children. Just like in any other population, the disabling morbidity associated with schistosomiasis include anaemia, malnutrition and impaired development largely in children (Mazigo et al., 2018, Danso-Appiah et al., 2010).

Pathology of *S. mansoni* and *S. japonicum* infections can include: Katayama fever, hepatic perisinusoidal egg granulomas, Symmers' pipe stem periportal fibrosis, portal hypertension, and occasional embolic egg granulomas in brain or spinal cord (CDC, 2012, David and William, 2006, Helmby, 2015). Pathology of *S. haematobium* infections can include: hematuria, scarring, calcification, bladder cancer, and occasional ectopic egg granulomas in brain or spinal cord (CDC, 2012, David and William, 2006, Helmby, 2015). Human contact with water is thus necessary for infection by schistosomes.

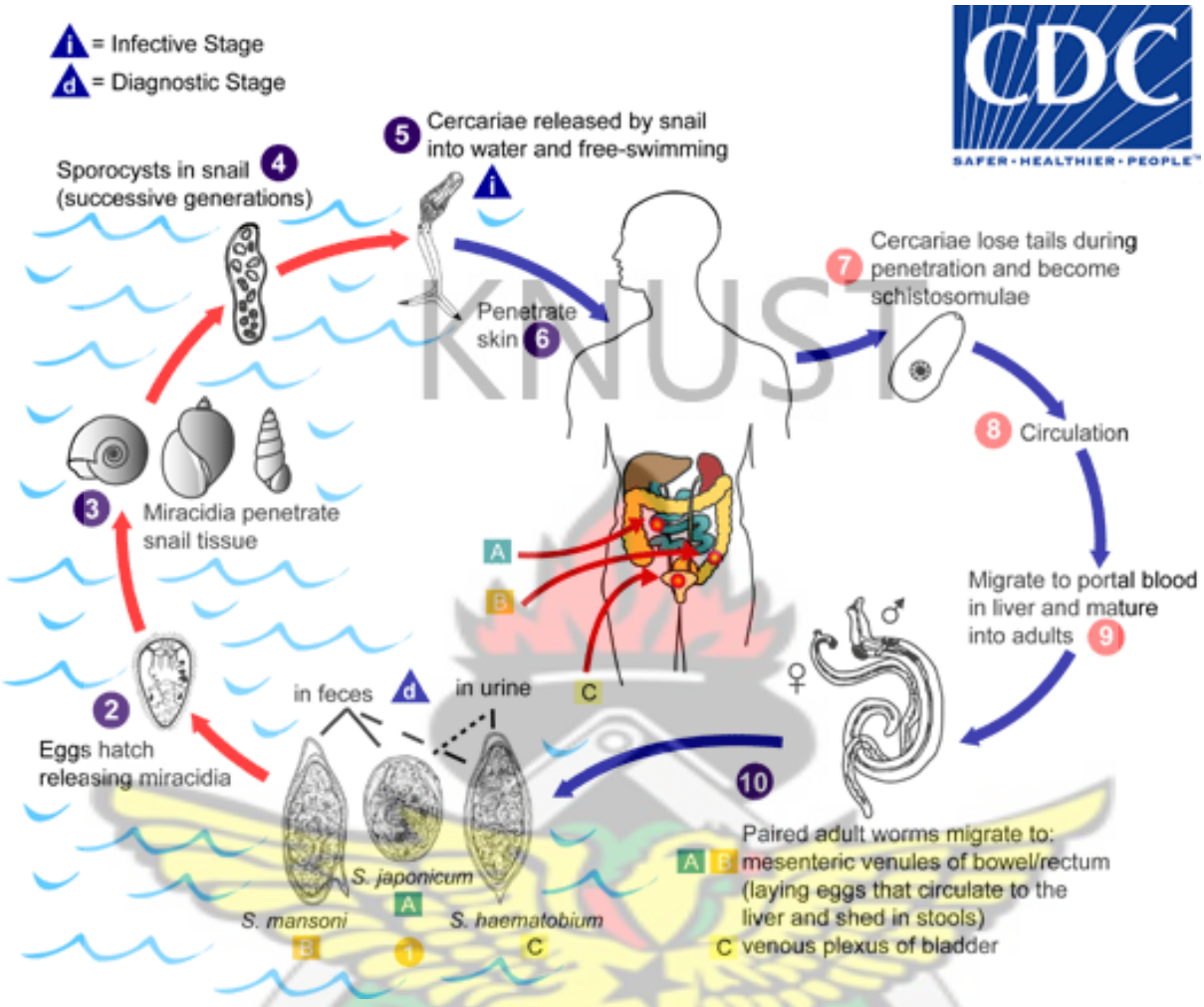


Figure 2-8: Life cycle of *Schistosoma* spp.

Eggs are eliminated with faeces or urine 1 and hatch and release miracidia 2 under optimal conditions. , They penetrate specific snail intermediate hosts 3 . In the snail, sporocysts 4 and cercariae 5 are produced. Infective cercariae from the snail penetrate to infect human host 6 and become schistosomulae 7 . The schistosomulae develop and resides in the veins (8 , 9). Adult worms in humans reside in the mesenteric venules in various locations, depending on the species 10 . The females deposit eggs which progressively move into the intestine (*S. mansoni* and *S. japonicum*) and of the bladder and ureters (*S. haematobium*) to be eliminated with faeces or urine, respectively 1 . (https://www.cdc.gov/parasites/schistosomiasis/biology.html)

2.2.3 Life cycles of helminths and protozoan parasites

Life cycle of most helminths involve adult worms passing eggs which develop into infective forms when in contact with the environment. The infective form can penetrate a host to initiate infection.

These processes of penetration cause ground itch and the larvae further undergo the heart-lung migration before establishing in the intestinal tract or any other suitable place within the host (Shiba et al., 1996, Cheesbrough, 2009, Agrawal, 2012, Ridley, 2012). Reinfection is usually impossible with some of these worms (e.g. *A. lumbricoides*) since their eggs can only be fertile when they get into contact with the soil (Geiger et al., 2002, Hagel et al., 2011). The fertile eggs after the soil contact can hatch to release its content when swallowed by its suitable host in contaminated formites.

With others like *E. vermicularis* and *S. stercoralis*, the eggs can hatch and release the larvae within the host making autoinfection common with infections by these parasites (Schmidt and Roberts, 2009, Ridley, 2012). Likewise, it is difficult to observe the eggs of these parasites in stools of infected people and for that matter sensitivity for their diagnosis in stool samples are increased with increased frequency of samples collected.

Some of the intestinal parasites (including *Entamoeba histolytica* (Figure 2-9), *S. stercoralis* (Figure 2-5), *Giardia lamblia* (Figure 2-9), *T. trichiura* (Figure 2-6), *A. lumbricoides* (Figure 2-3), *E. vermicularis* (Figure 2-7), *A. duodenale,* and *N. americanus* (Figure 2-2)) require only one species of host in which to complete their development. They are said to have a direct life cycle (Shiba et al., 1996, Agrawal, 2012, Ridley, 2012).

Others classified as having indirect life cycle require two or more hosts to complete their development. Some common helminths with indirect life cycle include; *Schistosoma species*, *Wucherera bancrofti*, *Taenia* species and *Diphyllobothrium latum* (Shiba et al., 1996, Agrawal, 2012, Ridley, 2012).

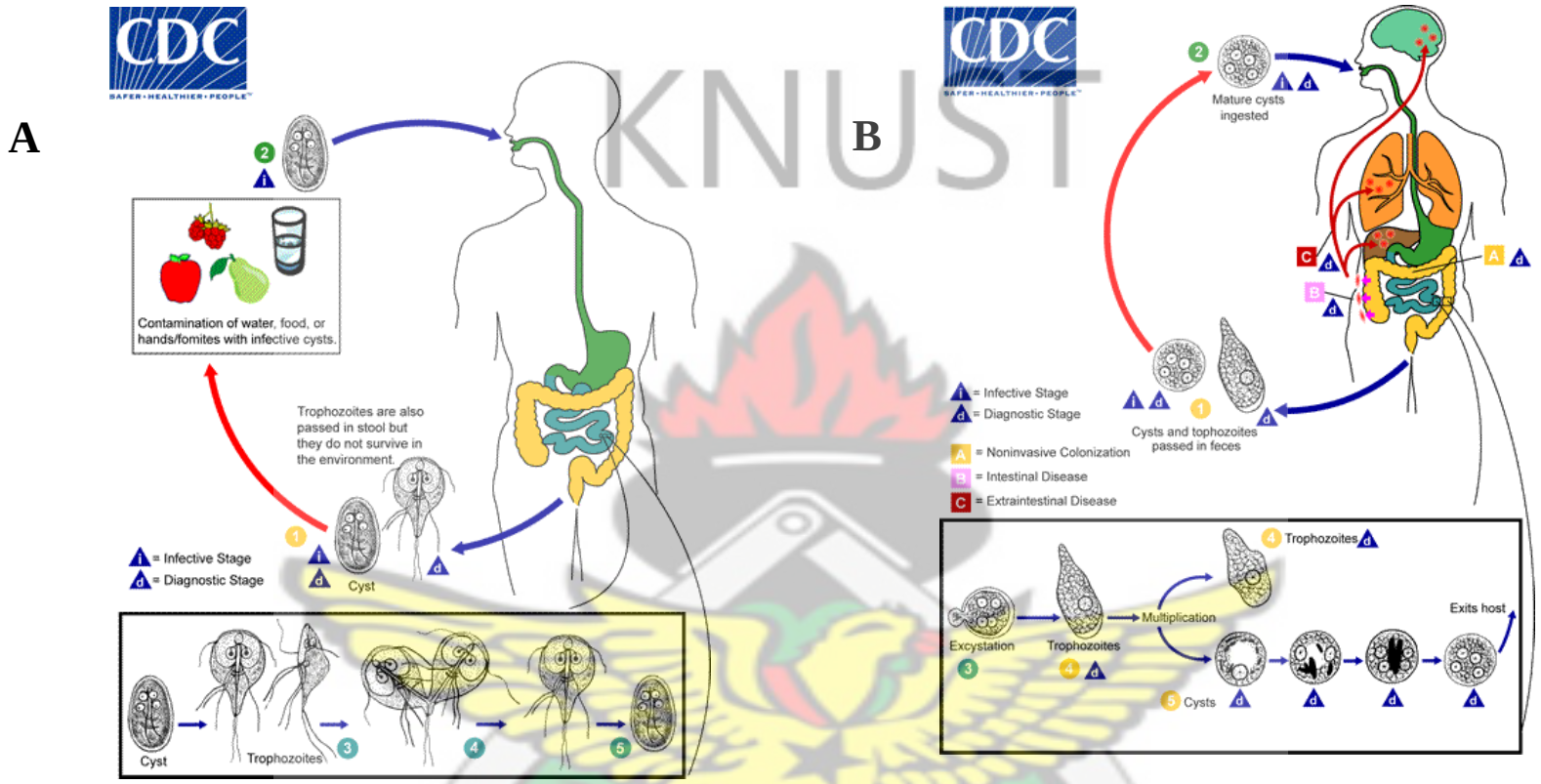


Figure 2-9: Life cycle of some protozoans

***Giardia* (A): Both cysts and trophozoites can be found in the faeces (diagnostic stages) (1). One becomes infected by ingesting cysts in contaminated sources (2). Cysts release trophozoites in the small intestine (3). Trophozoites can be free or attached to the mucosa (4). Encystation occurs in the colon and passed in faeces (5). Life cycle of *Entamoeba histolytica* (B): Cysts and trophozoites are passed in faeces (1) and infection occurs by ingesting mature cysts (2). Excystation (3) occurs in the small intestine and trophozoites (4) are released to mature in the large intestine. The trophozoites produce cysts (5), and both stages are passed in the faeces (1).**

2.2.4 Life cycle stages and related immune responses

Life cycle of an organism in a host contributes largely to the complex nature with which the host might have to elicit immune response to the various proteins at all stages of the parasites' development (Abbas et al., 2014, David and William, 2006, Ridley, 2012). This really contributes to the ability of helminth to induce both Th1 and Th2 response and the other forms of T cell differentiation depending on the cytokine *milieu* and even the dose of the antigen the host is exposed to (Luckheeram et al., 2012).

Having said this, it is also important to acknowledge that, even helminths that might have akin life cycle and stages might induce the immune system differently because of their location even within the host (Abbas et al., 2014, Agrawal, 2012, Ridley, 2012).

These contribute greatly to which cells get activated, proliferated, differentiated and even to what forms of T-cells and by what specific proteins in an infected person to fight the infection (Luckheeram et al., 2012, Nacher et al., 2000, Nakayamada et al., 2012).

Knowing which proteins are involved in inducing immune response, some potent therapies (example, use of *ASP2* protein from hookworm) and even vaccines have been prospected (Hotez et al., 2008, Loukas et al., 2006, Hotez et al., 2013).

2.2.5 Factors that contribute to spread and incidence of helminths infections

The knowledge of helminths has elucidated some factors that might influence the spread of helminth infections. Inadequate sanitation (Campbell et al., 2016a, Freeman et al., 2013) and unhygienic living conditions leading to faecal contamination of the environment, insufficient water and contaminated water supplies together with opportunities that improve parasite breeding are some of the factors that contribute to the epidemiology of helminths in Africa (Campbell et al., 2016a, Freeman et al., 2013, Hotez, 2008).

Lack of health education, poverty and malnutrition are also known to increase our susceptibility to getting helminth infection (Adkinson et al., 2014, Efunshile et al., 2015, Humphries et al., 2013, Loukas et al., 2006).

One cannot conclude on the subject matter without mentioning lack of effective control measure and failure of drugs to target and eliminate the parasite (Taylor-Robinson et al., 2012, Campbell et al., 2016a).

Climatic factors such as rains, temperature, humidity, soil moisture and water content are the factors that largely determine if a particular helminth could survive in a region whether tropical or temperate.

Population dynamics could also not be left out. Issues of war that could create common over-crowding conditions and habitats to enable the parasites to thrive also contribute to parasite development (Campbell et al., 2016a, Freeman et al., 2013, Adkinson et al., 2014, Brooker et al., 2006, Brooker et al., 2015).

2.2.6 Humoral and cell mediated immunity in infection

T cell precursors from lymphoid haematopoietic stem cells in the bone marrow move to the thymus for maturation (Delves et al., 2011, Actor, 2012, Luckheeram et al., 2012).

T-cell precursors (thymocytes) migrate through the medullary microenvironments and contact with Major Histocompatibility Complexes (MHC) on antigen-presenting cells (APCs) to differentiate. This shapes the T cell repertoire for antigen recognition (Delves et al., 2011, Actor, 2012, Luckheeram et al., 2012, Abbas et al., 2014).

T-cell Receptors (TCR) on T-cells complexes with MHC I or II with peptide on an APC (Ridley, 2012, Kenneth et al., 2016). This coupled with CD3 activation, a network of molecules including cytokines are induced. The concentration of the cytokines induced relate to the amount of antigens. TCR could consist of $\alpha\beta$ or $\gamma\delta$ chains bonded with five CD3 subunits (γ, δ, μ, π, and Σ). These lead to proliferations and differentiations of the naïve T-cells to produce

effector cells which will target the antigen whose peptide was processed and presented by the APCs (Ridley, 2012, Abbas et al., 2014, Actor, 2014, Luckheeram et al., 2012, Kenneth et al., 2016).

2.2.7 T-Helper 1 (Th1) cells differentiation and function

When APCs (DCs) are activated through their pattern recognition receptors by whatever pathogenic antigen (Abbas et al., 2014, Kenneth et al., 2016), the large amount of IL-12 which are produced stimulate NK cells. The cells produce IFN-γ (Luckheeram et al., 2012, Nacher et al., 2000, Perez-Mazliah and Langhorne, 2015) which dictates Th1 differentiation (Figure 2-10 & Figure 2-11).

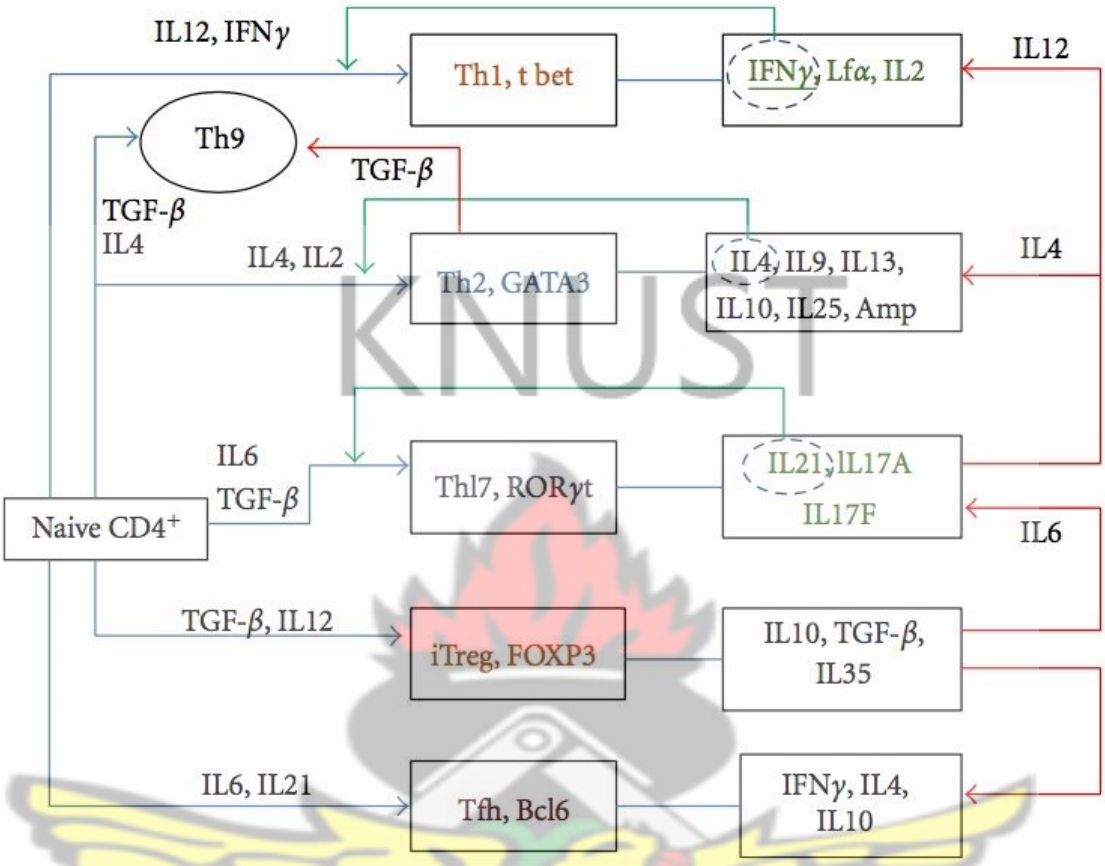


Figure 2-10: Influence of distinct cytokine *milieu* in the differentiation of $CD4^{+}$T cells. Blue arrows showing the differentiation of naive cells, the green arrows represent the self-amplification phase by the encircled cytokines, and the switch to a T cell subset under the influence of cytokines as the red arrows together with their master regulators (Source: Luckheeram et al, 2012).

At this stage, the master regulator of Th1 differentiation, T-box transcription factor (T-bet) comes into play to activate genes to promote the Th1 cell phenotypes and molecules while suppressing other opposing cell lineages (Nakayamada et al., 2012, Abbas et al., 2014, Luckheeram et al., 2012). This could be done by inhibiting the crucial IL4 gene for the development of Th2 cells and inhibiting the encoding of RORγt which promotes the Th17 lineage (Luckheeram et al., 2012).

It must also be understood that IL-12 together with IL-18 can induce the production of IFN-γ independent of T cell activation to enhance the Th1 differentiation. T-bet again acts as a repressor gene to prevent over-production of IFN-γ and its subsequent immunopathology (Delves et al., 2011, Luckheeram et al., 2012, Nakayamada et al., 2012).

The IFN-γ produced activates mononuclear phagocytes including macrophages to enhance phagocytic activity. IFN-γ (Th1) is expected to block some of the functions of IL-4 (Th2) on B-cells for IgE production as well as upregulation of *FcεRII* (Conrad, 1990, Luckheeram et al., 2012, Mulu et al., 2014, Nakayamada et al., 2012).

IFN-γ is found to increase levels of *FCcRII* on monocytes, eosinophils, platelets, and Langerhan's cells, a function similar to what is largely a preserve of IL-4 (Conrad, 1990, Mulu et al., 2014). This suggest similar role plays by the two cytokines traditionally assigned to Th1 for IFN-γ and Th2 for IL-4.

2.2.8 T-Helper 2 (Th2) cells differentiation and function

Helminth infection polarizes the mammalian immune system locally and beyond the gut-associated lymphoid environment (Damania and Dittmer, 2014). Th2 response is the arm of the immune system that minimize the pathogenesis associated with helminth infection.

It plays key roles in immune disorders such as asthma and allergies (Figure 2-10 & Figure 2-11). IL-4 and, IL-12, IL-2, IL-6 and IL-21 are crucial for Th2 cells differentiation (Nakayamada et al., 2012, Luckheeram et al., 2012, Urban et al., 1998, Voehringer et al., 2006).

There is evidence of IL-4 independent pathway for Th2 differentiation (Nakayamada et al., 2012, Luckheeram et al., 2012, Urban et al., 1998, Voehringer et al., 2006) with key chemokines such as the CC-chemokine receptor 3 (CCR3) ligand, CC-chemokine ligand 11 (CCL11 also known as eotaxin 1) (Allen and Maizels, 2011).

IL-6 is produced by APCs upon antigen presentation with MHC II. This further promote the production of Th2 cell phenotypes by enhancing the production of IL-4 by naïve CD4+ cells and inhibit the Th1 lineage (Abbas et al., 2014, Luckheeram et al., 2012, Nakayamada et al., 2012).

The effector function of Th2 mounts immune response to extracellular parasites including helminths and even some stages of the life cycle of malaria parasite infections as well as in asthma and allergic diseases using key cytokines such as IL-4, IL-5, IL-9, IL-13, IL-10 and IL-25 (Adkinson et al., 2014, Abbas et al., 2014, Luckheeram et al., 2012, Nakayamada et al., 2012).

IL-4 plays a major role in the IgE class switching and secretion by B-cells. The cytokine also upregulates low-affinity IgE receptor (*FcεRII*) on B-cells and mononuclear phagocytes (Adkinson et al., 2014, Conrad, 1990, Luckheeram et al., 2012).

High-affinity IgE receptor (*FcεRI*) on mast cells and basophils (Adkinson et al., 2014, Conrad, 1990, Luckheeram et al., 2012) in addition to some proinflammatory mediators (example IL-6) are also upregulated. It is necessary to note that in Th1 T-cell clones, some *FcεRII* upregulation occurs without the addition of IL-4 cytokine but then up to 50-fold upregulation (termed *FcεRII* superinduction) is seen when exogenous IL-4 is added to Th1/B-cell cultures (Conrad, 1990, Keegan et al., 1989, Adkinson et al., 2014). This function is further complimented by the activation of eosinophils by IL-5 since these cells have the most receptors (IL-5R) for the chemical molecule.

One other important function worthy to note is the enhancement of Th2 response by IL-25 through mucus production to help expel foreign protein and parasites. This includes, eosinophilia, immunoglobulin class switching, inhibition and suppression of Th17 response (Actor, 2014, Allen and Maizels, 2011, Luckheeram et al., 2012, Nakayamada et al., 2012).

As usual and expected, the beauty to calm down the barrage of cytokines produced in exposure to antigens from the Th2 cells is the presence of IL-10 and its homeostatic role as an anti-inflammatory cytokine by suppressing Th1 cells and even IgE production (Adkinson et al., 2014). In addition, Th2 responses promote the "walling off" of eggs or larvae in tissues via granuloma formation as well as promoting tissue repair mechanisms (Helmby, 2015).

It is evident that mice lacking the IL-4 receptor a-chain (IL-4Ra); a component of both IL-4 and IL-13 receptors, signal transducer and activator of transcription 6 (STAT6) or the transcription factor GATA-binding protein 3 (GATA3) show highly compromised anti-helminth immunity since the type 2 effector mechanism cannot be driven by IL-4 and/or IL-13 (Urban et al., 1998, Zhu et al., 2004, Voehringer et al., 2006).

2.2.9 T-Helper 17 (Th17) cells differentiation and function

IL-6 and IL-21 which are major players of the Th1 cell differentiation are together with IL-23, TGF-β and the master gene regulator, retinoic acid receptor-related orphan receptor gamma-T (RORγt), are involved in Th17 cells differentiation (Figure 2-10 & Figure 2-11).

The differentiation process can be split into 3 stages, including the differentiation stage mediated by TGF-β and IL-6, the self- amplification stage by IL-21, and the stabilization stage by IL-23 (Actor, 2012, Luckheeram et al., 2012, Nakayamada et al., 2012).

In cell differentiation, particularly for Th1 and Th2, production of IFN-γ and IL-4 respectively amplifies the differentiation processes. But this is not the case in Th17 since IL-21 instead of IL-17A contributes to the Th17 differentiation. This cell phenotype is expected to mount immune response to bacteria and fungi through its major effector cytokine.

The vast distribution and the presence of the receptor (IL-17R) in multiple tissues (haematopoietic tissue, skin, lung, intestine, and joints) suggest the possible influence the Th17 differentiation might bring on board when activated.

The cytokine, IL-17A, is also known for its induction of IL-6, IL-1 and TNF which all together enhance chemotaxis (Kenneth et al., 2016, Urban et al., 1998, Voehringer et al., 2006, Luckheeram et al., 2012).

2.2.10 Regulatory T cells (Treg) differentiation

Natural T regulated (nTreg) cells are released from the thymus Forkhead transcription factor (FOXP3) already expressed (Luckheeram et al., 2012, Chen et al., 2003, Abbas et al., 2014) (Figure 2-10 & Figure 2-11) .

When CD4+ T cells are primed with antigens in the peripheral lymphoid organs, an induced regulatory T cells (iTreg) are developed which are marked as CD4+/CD25+/FOXP3+/CD127- cells (McKee and Pearce, 2004, Sanou et al., 2012, Christine, 2010).

These regulatory cells produce specific cytokine environment (for example IL-10) to induce, to promote, or suppress the necessary cell phenotypes that are required to regulate an immune response with the major aim of avoiding immunopathology (Adkinson et al., 2014, Bustinduy et al., 2015).

Both Th17 and Tregs have demonstrated their plasticity nature just as the switch of naïve T cells into Th1 and Th2. It is documented that Th17 in the presence of IL-12 switched to Th1 and Th2 in the presence of IL-4 (Kenneth et al., 2016, Luckheeram et al., 2012, Mohrs et al., 2001, Nakayamada et al., 2012, Wilson, 1993).

2.2.11 TCR gamma delta (TCR γδ) cells

T cells expressing the $\gamma\delta$ receptor are naturally negative for both CD4 and CD8 but make up in their matured form, the dominant population of T cells in the skin, intestinal epithelium and the pulmonary epithelium (Sanou et al., 2012, Christine, 2010).

TCR-γδ because of their ability to bind to a broad range of cell surface molecules in their unprocessed forms have been described to act as NK cells and especially when they could recognize antigen independent of MHC proteins (Luckheeram et al., 2012, Nakayamada et al., 2012, Kenneth et al., 2016).

In the immune response to parasitic infections, TCR-γδ cells have been identified as important bridge between the natural and adaptive immunity (Abbas et al., 2014, Kenneth et al., 2016, Christine, 2010). TCR-γδ cells have been implicated in malaria pathogenesis particularly in gastrointestinal pathology (Perera et al., 1994, Abbas et al., 2014, Kenneth et al., 2016) and its role is worth exploring especially in helminth and malaria co-infections.

2.2.12 Antibody production

Effective immunity against *Pf* malaria in humans develops slowly after repeated exposure (Stanisic et al., 2009). The fact that several leading malaria vaccines are made from merozoites suggest that protective immunity are obtained mainly from antibodies against merozoite antigens (Lamb, 2012, Stanisic et al., 2009).

IgG1 and IgG3 are the predominant subclasses produced in response to merozoite antigens and IgG1 has been identified to be associated with protection against symptomatic malaria parasite infection (Stanisic et al., 2009).

When the body is exposed to hookworm/helminth antigens, the Th2 effector cells with IL-4 produced activate B-cells to secrete IgM, IgA and IgG and further switch to secrete anti-IgE and other subclasses; IgG1and IgG4, which are necessary for protective immunity (Loukas and Prociv, 2001, Quinnell et al., 2004, Wilson, 1993).

The events leading to the production of the antibodies rely largely on repeated infections and of importance to note is the fact that some isotypes of antibodies produced could cross-react with other helminths (Quinnell et al., 2004).

It has been reported previously that only 6% to 17% of the polyclonal IgE produced is parasite specific, allowing ample opportunity for non–parasite-specific IgE to interfere with histamine release towards allergens (Pritchard et al., 2007, Abbas et al., 2014).

2.2.13 Immune responses to Helminth Infections

The parasite's size and location of infection, as well as the actual immune response the organism stimulates, may either result in physical damage to the host or destruction of the parasite (Ridley, 2012).

Hookworms survive relatively well in an immunologically hostile environment, although the Th2 phenotype is associated with partial protection. The parasite may promote its survival by secreting a molecular screen of immune- suppressive agents and, possibly, by stimulating the appearance of regulatory T-cell populations (Conrad, 1990, Hewitson et al., 2009, Maizels and Yazdanbakhsh, 2003).

Hookworm has the capacity to downregulate the FcεR1 on basophils and mast cells which in turn explains the protective effect in hookworm infection against the development of respiratory wheeze against environmental allergens and even the promotion of the immune response that lessens the detrimental effects in autoimmunity and inflammatory bowel disease (Adkinson et al., 2014, Conrad, 1990, Smallwood et al., 2017).

Fortunately, a study showed that from a parasitological standpoint, potentially protective FcεRI-dependent immune responses are not blocked in human necatoriasis but rather mediated by secreted parasite immune suppressants and the induction of regulatory leukocyte populations (Pritchard et al., 2007).

In a Schistosome-endemic area, egg-positive people had significantly higher levels of specific antibodies, IL-2, IFN-γ and IL-23 in contrast to egg negative individuals that had significantly higher circulating IL-10, IL-4, IL-13 and IL-21 (Milner et al., 2010).

Previous studies suggesting that anti-helminth immune responses fall into a Th1 (pro-inflammatory) and Th2 (anti-inflammatory) dichotomy with resistance to infection being associated with Th2 responses (Wilson, 1993, Dunne and Mountford, 2001) failed to fully explain resistance, susceptibility to infection and re-infection in people resident in helminth endemic areas. For example, both Th1 and Th2 responsiveness appear compromised in schistosomiasis patients, and within the Th2 compartment, IL-5 responses are suppressed while IL-4 production is relatively intact (Grogan et al., 1998).

There is no clear pattern between either Th1 or Th2 cytokine responses and infection intensity. It is suggested that the balance between Th1, Th2 and Treg responses determine the outcome of helminth infections (Milner et al., 2010, Belkaid, 2007, Perez-Mazliah and Langhorne, 2015, Nakayamada et al., 2012)

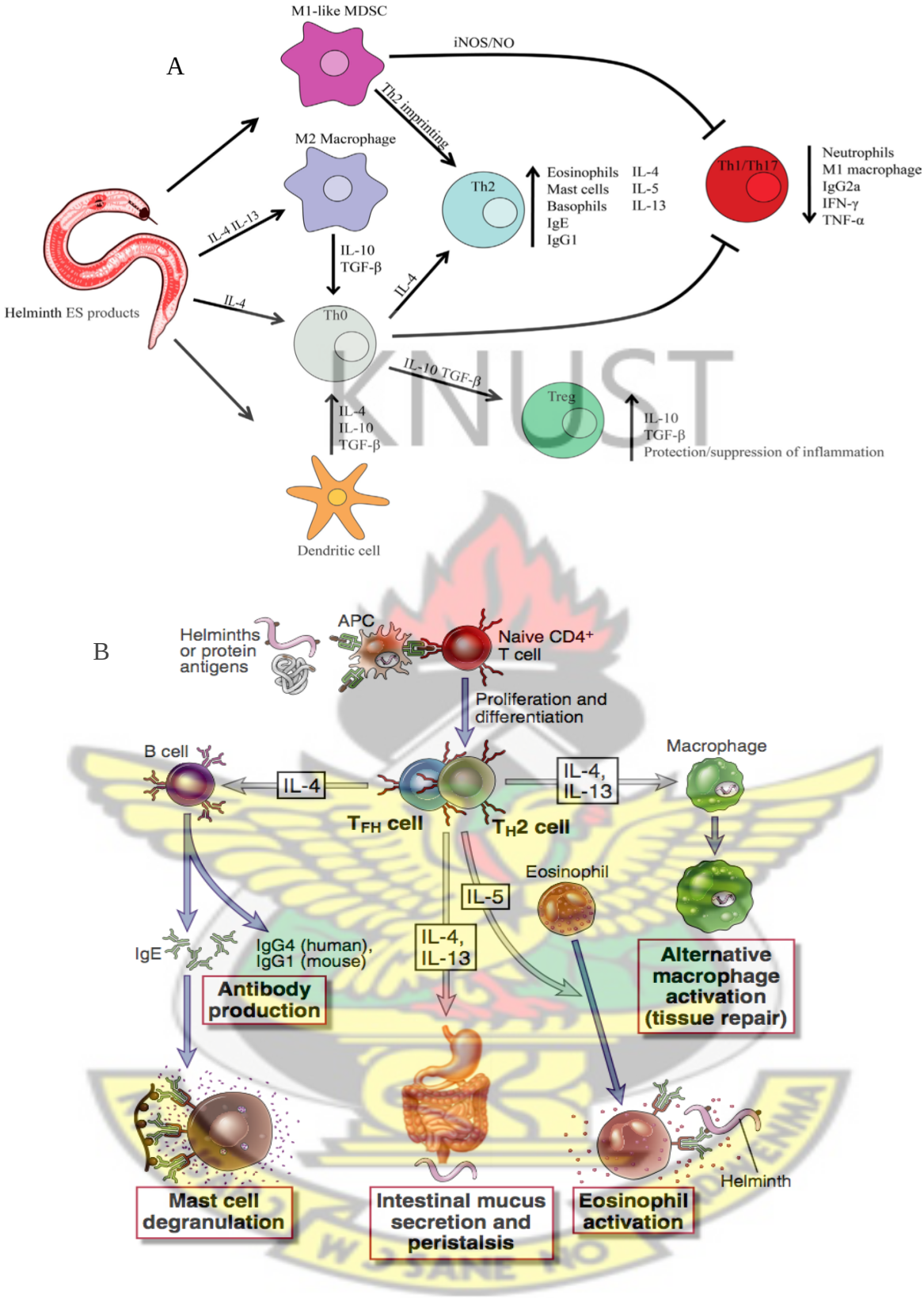


Figure 2-11: Diagrammatic presentation (A and B) showing the pathways involved in helminths eliciting immune response in their host with the necessary effector cells and cytokine build up (Abbas et al., 2015, Smallwood et al., 2017).

2.3 Diagnosis of helminth infections

Along with the morphology and characteristics of the parasitic organism, the eggs of some parasites are used to identify them while others are by their larvae (Ridley, 2012). Stool examination is routinely employed to screen for the presence of ova (eggs) or the possibility of seeing parasites (larvae, segments, etc) in the stool.

Because of its low level of sensitivity especially on one-time sample test, there is a tendency of getting a lot of false negative results (Hirata et al., 2007). As earlier stated, using parasite eggs to differentiate some species (for example, hookworm) is problematic.

Diagnosis of helminths infections could adopt use of direct saline smear method (Cheesbrough, 2009, Koga et al., 1991) which is suitable for observing all forms of ova and parasites. The use of saline also help to maintain the cellular architecture of the parasites or ova observed. Also, agar plate culture technique for development of larval forms of parasites and, quantitative formalin ethyl acetate concentration technique (Agrawal, 2012, David and William, 2006, Elkins et al., 1986) are effective techniques.

Formalin–ethyl acetate sedimentation-digestion (FEA–SD) technique (Xu et al., 2012) which though is sensitive one cannot observe the organism in its live state but preserved. Mostly for quantification and estimation of infection intensity, Kato Katz (WHO, 2012), Mini-Flotac (Levecke et al., 2011) and McMaster (Barda et al., 2013) are effective methods used for such purposes.

2.4 Treatment and control of Helminth Infections

Currently, a large scale promotion of preventive chemotherapy by WHO in the form of prophylaxis using multiple anthelminthic drugs to treat conditions including STHs, schistosomiasis, and filariasis through the Mass Drug Administration (MDA) programme is being undertaken (Efunshile et al., 2015, Taylor-Robinson et al., 2012, Humphries et al., 2013, Ortu et al., 2016).

In the case of treating and managing strongyloidiasis, albendazole and ivermectin have been identified as the drugs of choice for infections in human with cure rates between 67% to 100% (Kotze et al., 2004, Verweij et al., 2009).

Unlike in filariasis where there is no reliable documentation of resistance to ivermectin despite the prolonged use and distribution in the onchocerciasis control program (OCP) (Sen et al., 2002), some studies have reported pyrantel and benzimidazole resistance in human hookworm infection in Africa and Australia (Kotze et al., 2004, Sen et al., 2002).

2.5 *Plasmodium* species epidemiology and infection

Malaria which is caused in human by five strains of the genus *Plasmodium* (*Plasmodium vivax*, *P. ovale*, *P. malaria*, *P. knowlesi* and *P. falciparum* (Figure 2-12)), is the most prevalent disease on a world-wide scale (Njunda et al., 2015, Kenneth et al., 2016). The life cycle of the malaria parasite is presented in Figure 2-13.

With the ubiquitous nature of this parasite in various geographical areas, *P. falciparum* among the five remains the most malignant accounting for more than 80% of the malaria infections particularly in Africa (Njunda et al., 2015, WHO, 2014). In endemic areas, individuals live with the malaria parasites asymptomatically.

The support received from the varying funding bodies has contributed significantly to the decline in the global prevalence of malaria. In 2013, 198 million cases of malaria and 584,000 deaths were recorded. The huge burden of the dangerous infection is felt among children mostly below 10 years of age in sub-Saharan Africa (WHO, 2014). Thus malaria happens to be one of the leading causes of morbidity and mortality in the developing world.

In endemic areas, about 60–70% of the cases are attributable to *Plasmodium falciparum* (*Pf*) infection while 30–40% are attributable to other malaria parasite infections (Alonso et al.,

2011, Dev et al., 2001). *Pf* is responsible for 13–28% of deaths in children under 5 years of age (Bhattarai et al., 2007). The episode of clinical malaria and severe malaria occurring annually among children less than 5 years of age is high in the study area, in the middle-belt of Ghana (Owusu-Agyei et al., 2009, Asante et al., 2010).

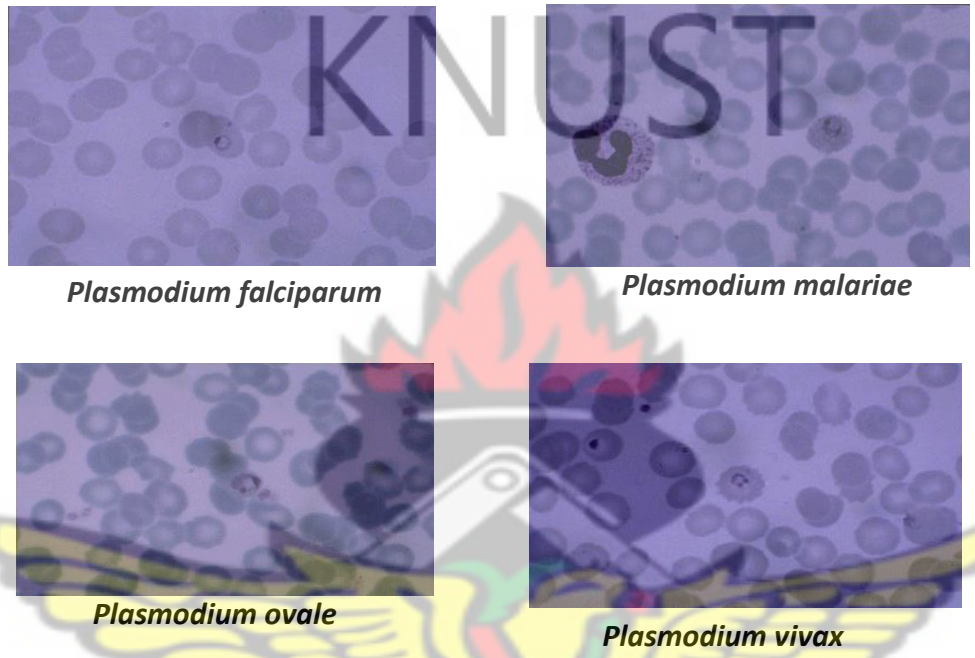


Figure 2-12: Micrographs of *Plasmodium species* screened from blood samples (Atlas of Medical Parasitology, 1996, First Edition)

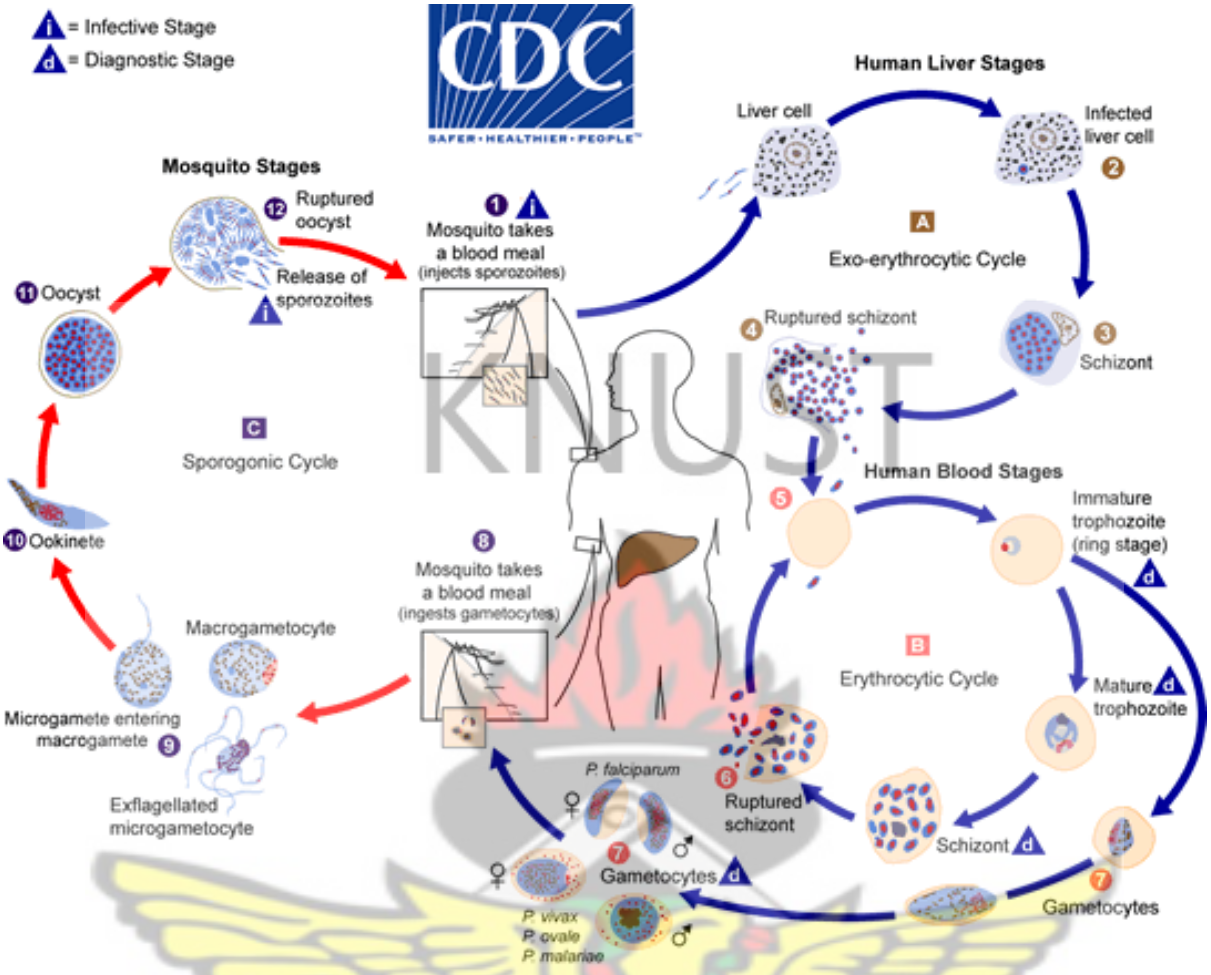


Figure 2-13: The malaria parasite life cycle

Infected female Anopheles mosquito inoculates sporozoites into the human host ❶. Sporozoites infect liver cells ❷ and mature into schizonts ❸. They release merozoites ❹. Merozoites infect red blood cells ❺ and some differentiate into gametocytes ❼. The gametocytes, male (microgametocytes) and female (macrogametocytes), are ingested by an Anopheles mosquito during a blood meal ❽. While in the mosquito's stomach, the microgametes penetrate the macrogametes generating zygotes ❾. The zygotes in turn become motile and elongated (ookinetes) ❿ which invade the midgut wall of the mosquito where they develop into oocysts ⓫. The oocysts grow, rupture, and release sporozoites ⓬, which make their way to the mosquito's salivary glands. Inoculation of the sporozoites ❶ into a new human host perpetuates the malaria life cycle. (https://www.cdc.gov/malaria/about/biology/index.html)

2.6 Immune responses to *Plasmodium* species infection

Malaria pathogenesis is complex and this mostly involves immunologic and non-immunologic mechanisms. The life cycle of the malaria parasite involves two stages. The extracellular stage (an early clinically silent liver stage) elicit humoral immune response and cell mediated immune (CMI) response; involving largely T-cells, to the intracellular phase of the parasites' development (Abbas et al., 2015, Boef et al., 2013, Luckheeram et al., 2012, Righetti et al., 2012).

Some peripheral blood cells such as lymphocytes, neutrophils, basophils, natural killers and lymphocyte subsets are involved in malaria parasite control (Kenneth et al., 2016). It has been alluded that CD4+ T-cell responses are associated with controlling the intracellular phase of malaria parasites' infection (Abbas et al., 2015, Luckheeram et al., 2012, Walther et al., 2009).

However, since the parasite biology and kinetics at the various stages of the life cycle differ significantly, one would expect the immune response elicited to the parasite stages to be dissimilar. Even if CD4+ plays a significant role, it might be in a different subset bearing in mind the concept of CD4+ plasticity (Luckheeram et al., 2012, Perez-Mazliah and Langhorne, 2015, Walther et al., 2009, Nakayamada et al., 2012).

Little has been documented of the very role CD4+ T cells play in the immune response to the pre-erythrocytic stage of malaria parasite infection. However, records of antibodies, such as in vaccination (Abbas et al., 2015, Imoukhuede et al., 2010, Stanisic et al., 2009, Walther et al., 2009), which indicate response to antigens of the extracellular stage, including circumsporozoite protein (CSP), liver-stage antigen 1 (LSA1), and sporozoite threonine–asparagine-rich protein (STARP) suggest the involvement of CD4+ cells in receiving antigens from APCs and activating B-cells (Nebie et al., 2008, Sanou et al., 2012, Stanisic et al., 2009, Abbas et al., 2014).

Another form of T cells which make up to 1-10% of circulating lymphocytes in human and reported to exhibit marked increase in human *Plasmodium* spp parasite infections are the *TCR-γδ* cells (Kenneth et al., 2016, Sanou et al., 2012, Dieli et al., 2001).

These cells could remain elevated for more than a month even after treatment (Kenneth et al., 2016, Sanou et al., 2012, Dieli et al., 2001). It is also suggested that an important role of *TCR-γδ* in malaria parasite infection is their cytotoxic role as well as how these cells inhibit growth of the asexual blood stages seen *in vitro* (Dieli et al., 2001, Farouk et al., 2005, Sanou et al., 2012). These cells operate through cytolytic and proinflammatory molecules and their increase correspond to an increase in IFN-γ produced by T-bet+ Th1 cells, Natural Killer (NK) cells, natural regulatory cells (Tregs; to suppress immune reaction), natural killer T (NKT) cells, CD8+ T-cells and *TCR-γδ*-cells in addition to TNF (Abbas et al., 2014, Belkaid, 2007, Dieli et al., 2001, Sanou et al., 2012, Villegas-Mendez et al., 2012).

When dendritic cells (APC) encounter malaria parasite antigens from destroyed infected hepatocytes, they pick them and process them to present to CD4+ T cells which in turn prime CD8+ cytotoxic T cells and B-cells for antibody production. This mechanism is known to involve STAT6 and IL-4 suggesting CD4+ cells of Th2 phenotype (Luckheeram et al., 2012, Perez-Mazliah and Langhorne, 2015, Nakayamada et al., 2012).

Basically, Th1/Th2 paradigm has been proposed to fight against malaria infection in human and this has not been enough to explain the immune response put forward in infected people. Follicular CD4+ T (Tfh) cells producing IL-21 cytokines have been reported in immune adults living in endemic areas of *Pf*. Though activation of Th17 subset of CD4+ cells producing IL-17A cytokine occurs, there has not been any identified and defined role during *Plasmodium* infections (Luckheeram et al., 2012, Perez-Mazliah and Langhorne, 2015, Nakayamada et al., 2012).

Plasmodium can modulate the response of antigen presenting cells, such as macrophages and dendritic cells (DC), which leads to suppression of the immune response. DC function is affected by the presence of TNF-α (Abbas et al., 2014, Ateba-Ngoa et al., 2015). The regulatory T cells are extremely important to control the inflammatory process in malaria and the number of $CD4^{+/}CD25^{+/}Foxp3^{+}$ regulatory T cells (Treg) increase in *Plasmodium* parasite infection as demonstrated in studies involving mice (Abbas et al., 2014, Luckheeram et al., 2012, Perez-Mazliah and Langhorne, 2015, McKee and Pearce, 2004, Ateba-Ngoa et al., 2015).

IgE, which is usually known to be under the influence of IL-4 from Th2 cells causing antibody class switching and its production from B-cells, has been seen as the preserve of helminth infection (Nacher et al., 2000). However, total and parasite-specific IgE levels have been reported in cases of malaria which supports the fact that IFN-γ also cause upregulation of low affinity IgE receptor because of IgE elevation (Conrad, 1990) with high levels of IgE correlating with protection against severe malaria (Duarte et al., 2012, Bereczky et al., 2004).

2.7 Diagnosis of *Plasmodium species* infections

Malaria is routinely diagnosed from blood samples by staining blood films on slide with giemsa to observe using a microscope (Adu-Gyasi et al., 2015a). This procedure is recognized as the gold standard in malaria diagnosis. One is afforded the capability to estimate the density of malaria parasite causing infection in an individual using either an absolute or relative white blood cell count of the person (Adu-Gyasi et al., 2012, Adu-Gyasi et al., 2015a).

Malaria microscopy with all its positive attributes requires time, electricity and special skills and competence to be apt in the diagnosis and to even pick less dense infections which are mostly submicroscopic (Adu-Gyasi et al., 2015b).

For this reason, other equally sensitive rapid diagnostic test (RDT) methods and molecular techniques (for example PCR and FISH) have been developed that are comparably effective in diagnosing the parasitic infection (Kwarteng et al., 2015, Adu-Gyasi et al., 2018). All these

methods have been useful even in the trials of newly developed malaria therapy and control measures.

2.8 Treatment of malaria disease

The severity of malaria disease and the species of the malaria parasite contribute to the treatment to be selected out of the available treatment options (MoH, 2010 , WHO, 2015). Due to the possibility of the parasites developing resistance to therapy, combination of more than one malaria drugs have been accepted for the treatment of malaria and parasite clearance (WHO, 2014, WHO, 2015).

Combination Therapies, including Artemether Lumefantrine (AL), Artesunate Amodiaquine (AQ) and Sulphadoxine Pyrimethamine (SP) have been used largely to treat the uncomplicated form of the parasitic infection (Kwarteng et al., 2015, MoH, 2010).

Quinine and Primaquine have been used successfully for the management of severe and complicated malaria (MoH, 2010 , WHO, 2015).

For prophylactic purposes, some monotherapies including Artesunate and Malarone, can be used. With the safety profile of SP, it has been used as a prophylactic drug to prevent malaria among pregnant women (MoH, 2010 , WHO, 2015).

2.9 Epidemiology of helminth and *Plasmodium species* co-infections

In the developing and less endowed countries, parasitic infections are common because dwellers by virtue of their environment and occupation live in close proximity to where these parasites are haboured.

Concomitant parasite infections in humans can, therefore, not be ruled out and some of these infections have heavy economic and social burdens (Hotez, 2008, Hotez and Kamath, 2009, Njunda et al., 2015). Examples of such are *Plasmodium* spp and co-infections with *Schistosoma*

spp., Ascaris, hookworm, *Hymenolepis* spp, and *Trichuris trichuira* (Katie et al., 2014b, Lemaitre et al., 2014).

Co-infections of helminth and malaria parasites in children could affect their development and learning capabilities and this is demonstrated in the classical apathy to study and its consequent increased absenteeism in developing countries (Humphries et al., 2013, Nkuo-Akenji et al., 2006, Taylor-Robinson et al., 2012). The interactions that the human body will have in mono-infections with parasites will definitely be modified in the presence of another parasite (Lemaitre et al., 2014).

There is evidence supporting the fact that individuals with multiple parasitic infections have increased incidence of morbidity and also stand the risk of developing severe diseases (Nacher et al., 2002, Kinung'hi et al., 2014, Tshikuka et al., 1996). This is even seen in infections with organisms that have complex extracellular and intracellular life cycles as happens with malaria parasites.

Depending on the organisms that are involved in the co-infections, an outcome of the response could be negative (detrimental), positive (beneficial) or neutral where the association of the two parasites infections enables the body to accommodate parasites as though nothing is happening. With malaria and schistosomiasis, it was established in a study that the association was negative (Lemaitre et al., 2014, Briand et al., 2005)

2.10 Immune responses in co-infections of helminth and *Plasmodium species*

The Th2 phenotype in helminth infection further suppresses regulator genes of the Th1 arm of the immune response (Abbas et al., 2014, Adkinson et al., 2014) and that generates the immunopathology in *Plasmodium spp.* infected individuals.

Typically, in malaria, the major cytokine expected to be at play is Th1 (IFN-γ) upon activation by the blood stage infection.

The pre-erythrocytic phase of the infection has been described to activate the Th2 arm just as in helminth infections (Conrad, 1990, Nacher et al., 2000). It is therefore apparent that the stage of the life cycle of the infecting pathogens together with the cytokine environment determines the response one mounts to helminth, malaria parasites or their co-infections (Mulu et al., 2014).

The questions of how the coexistence of helminths and *Plasmodium* parasites within the same host might influence the immunological responses to each species and whether interactions affect resistance, susceptibility, and the clinical outcome of malaria has not gotten a straight forward answer (Mulu et al., 2014, Salazar-Castañon et al., 2014).

Inducing IgE has been found so important in the severity of malaria infection. Treatment of human schistosomiasis suppresses IgE immune complex formation (Bousquet and Michel, 1993, Nacher et al., 2000, Adkinson et al., 2014). This suggest the need to take a second look at the prescribed MDA programme targeting helminths and possible impact of the MDA on malaria control and its severity in the developing countries (Bousquet and Michel, 1993, Nacher et al., 2000, Adkinson et al., 2014). This should be done bearing in mind the immune benefits (withstanding inflammatory and autoimmune conditions) helminths offer to the people they infect at moderate intensity (Hewitson et al., 2009, Hotez et al., 2008, Loukas et al., 2006). It is also necessary to note that, elevated levels of IgE is found among individuals from malaria endemic areas (Bousquet and Michel, 1993, Adkinson et al., 2014). The concomitant helminth and malaria parasite infection cannot be a coincidence in endemic areas, probably a natural selection process to confer immunity to malaria parasites infected individuals.

If that observation could be true, then managers of helminthiasis and malaria control programmes need to assess the impact of eliminating these organisms together in order not to create an imbalance with unbearable consequential effects (Bousquet and Michel, 1993, Nacher et al., 2000, Adkinson et al., 2014).

Studies in Ghana and Mali has shown that, higher production of IL-10 was found among helminth infected compared to malaria co-infected subjects when their cells were exposed to *Plasmodium* antigens. Among Senegalese, IFN-γ was increased among the co-infected individuals and IL-10 was rather increased in *Schistosoma* and *Plasmodium* co-infection (Ateba-Ngoa et al., 2015, Ateba-Ngoa et al., 2014).

What adds to the confusion is that even among infection with organisms of the same genus, the pattern is not clear. In the studies in Mali and Senegal, *S. mansoni* had an increased incidence of clinical malaria in co-infection while a protective effect of *S. haematobium* infection against malaria was reported in Mali (Ateba-Ngoa et al., 2015, Sokhna et al., 2004, Lyke et al., 2005). In all those studies and where cytokines were measured, the presence of *Schistosoma* spp in co-infection did not restrict the malaria parasite in inducing the production of Th1 cytokines (IFN-γ).

2.11 Immunological presentation with other infections

Helminthic presence has been known to affect the response to certain antigens including vaccines like *Bacillus Calmette-Guerin* (BCG) and *Rubella* just as *Ascaris lumbricoides* has been found to reduce response to oral cholera vaccine which can be restored with albendazole treatment (Smith and Brooker, 2010, Taylor-Robinson et al., 2012, Adkinson et al., 2014).

On the other hand, exploration of the beneficial effect of helminth in the management of inflammatory and autoimmune conditions (Crohn's disease, ulcerative colitis and allergic diseases) have been enormous (Adkinson et al., 2014, Helmby, 2015, Hotez et al., 2013, Smallwood et al., 2017).

The anti-inflammatory or regulatory response by host to helminths could be potentially detrimental to the host if it interferes with the development of protection against other infections that require an inflammatory response, such as *Leishmania major* or *Trypanosoma cruzi* (Xu et al., 2007, Ricci et al., 2011, Belkaid, 2007). The hyporesponsive immune response

induced during chronic helminth infection affects not only the response to helminth antigens but also to other antigens.

Table 2-1: A: Global estimation of number of helminth infection, B: Some reported prevalence of helminth infection in Ghana and, C: Some selected prevalence outcome of helminth and malaria co-infections

Table 2-1 **A:**

Disease/Parasitic burden	Estimated Number of infected people	References
Ascariasis	819 million	(Hotez et al., 2014, Pullan et al., 2014)
Trichuriasis	465 million	(Hotez et al., 2014, Pullan et al., 2014)
Hookworm	439 million	(Hotez et al., 2014, Pullan et al., 2014)
Lymphatic filariasis	36 million	(Hotez et al., 2014, Pullan et al., 2014)
***Oesophagostomum bifurcum* (Nodule worm)**	0.25 million (largely Togo and Ghana)	(Ziem et al., 2006)
Strongyloidiasis	100 million	(Hotez et al., 2014, Pullan et al., 2014)
Onchocerciasis	30.4 million	(Hotez et al., 2014, Pullan et al., 2014)
Loa loa	13 million	(Feasey et al., 2010)
Schistosomiasis	252 million	(Hotez et al., 2014, Pullan et al., 2014)
Echinococcosis	1.1 million	(Hotez et al., 2014, Pullan et al., 2014)

Table 2-1 **B:**

Organism	Prevalence (%)	Study Area	Infection category	Reference
Hookworm	3.2%	Ghana	Mono-infection	(Kinung'hi et al., 2014)
Hookworm	50%	North-Eastern (Bawku), Ghana	Mono-infection	(Yelifari et al., 2005)
Hookworm	45%	Kintampo, Ghana	Mono-infection	(Humphries et al., 2011)
S. haematobium	14.4%	Ghana	Mono-infection	(Kinung'hi et al., 2014)
S. haematobium and Hookworm	0.7%	Ghana	Co-infection	(Kinung'hi et al., 2014)
H. nana, T. solium and T. trichiura	3%	Kintampo	Co-infection	(Humphries et al., 2011)
Hookworm	5.6%, 10.4%	Ethiopia	Mono-infection	(Habtamu et al., 2015, Deribe et al., 2012)

Table 2-1 **C:**

Country	Study design	Sample size	Prevalence (%)	Mean age/range in yrs	Reference
South Ethiopia (2012)	Cross sectional	1065	19.4	18.6	(Degarege et al., 2012, Naing et al., 2013)
Uganda (2010)	Cross sectional	1770	15.5	0-80	(Naing et al., 2013, Shapiro et al., 2005)
Ghana (2009)	Cross sectional	785	16.6, 45 (pregnant)	26.8	(Naing et al., 2013, Yatich et al., 2009)
Ghana (2011)	Cross sectional	258	10 (adult), 51 (children)	1-80	(Humphries et al., 2011, Naing et al., 2013)
Nigeria (2013)	RCT (nested Case control)	690	42.9	35.5	(Abanyie et al., 2013, Naing et al., 2013)
Kenya (2013)	Cross sectional	2013	4.7	5-18	(Bustinduy et al., 2015, Naing et al., 2013)

Generally, considering the immunology of parasitic infections and the role of the lymphocytes, one cannot sum it all up as more in agreement as captured by Perez-Mazliah and Langhorne, (2015) as follows:

"With the discovery of the wide array of possible $CD4^+$ T-cell subsets and their different activation requirements and functional capacities, it is becoming clear that $CD4^+$ T-cells may not be simply defined as individual subsets of Th cells producing a single cytokine, but rather they represent components of a dynamic and interactive response, in which these cells can be multifunctional, flexible, and plastic depending on the disease/infection and activation environment".

The interplay of the various cells after receiving antigens from APCs mainly the CD4+, CD8+, their activated forms and the *TCR-γδ* (has also an APC independent pathway of activation) with their regulatory role through the cytokines and molecules they produce is worth studying in helminth, malaria parasite or their co-infections in an endemic place in the middle-belt of Ghana, an African tropical region.

3 CHAPTER THREE

METHODS

3.1 Study design

An observational cross-sectional design was used to survey households within the study area of Kintampo Health Research Centre (KHRC) from September 2015 to August 2016 to describe the distribution and immunological response in helminth and *Plasmodium* species co-infections.

Also, a case control study design was adopted among a cohort of participants that had helminth and/or *Plasmodium* spp infection at baseline and followed them at least three-month post-treatment (initial visit, visit on day 14 and three-months post-treatment) (Figure 3-1).

Field workers contacted prospective participants in selected households and consented them for the study processes. Information sheet and informed consent forms were administered by the field workers to all selected participants in a household.

Participants who consented to participate in the study were offered the guide to collect appropriate stool and urine samples needed for the study in an appropriate container. They were given containers to collect their stool and urine samples separately in the following morning.

Participants were invited to a central point within the community for questionnaires (Appendix 6) to be administered and blood samples collected into appropriate test tubes. The questionnaires were administered by the field workers.

All participants with helminth (multiple- and mono-) infection except pregnant women in the first trimester were treated with single dose albendazole (400 mg/dose) and praziquantel (40 mg/kg body weight) for *Schistosoma* infection after the initial samples were collected. Malaria parasite infected participants were also treated with an Artemisinin Combination Therapy

(ACT). Participants were observed to take the treatment for helminths and the first dose of the antimalaria.

Stool, urine and blood samples were collected at each visit. These biological samples that were shipped to the clinical laboratory for processing within 6 hours.

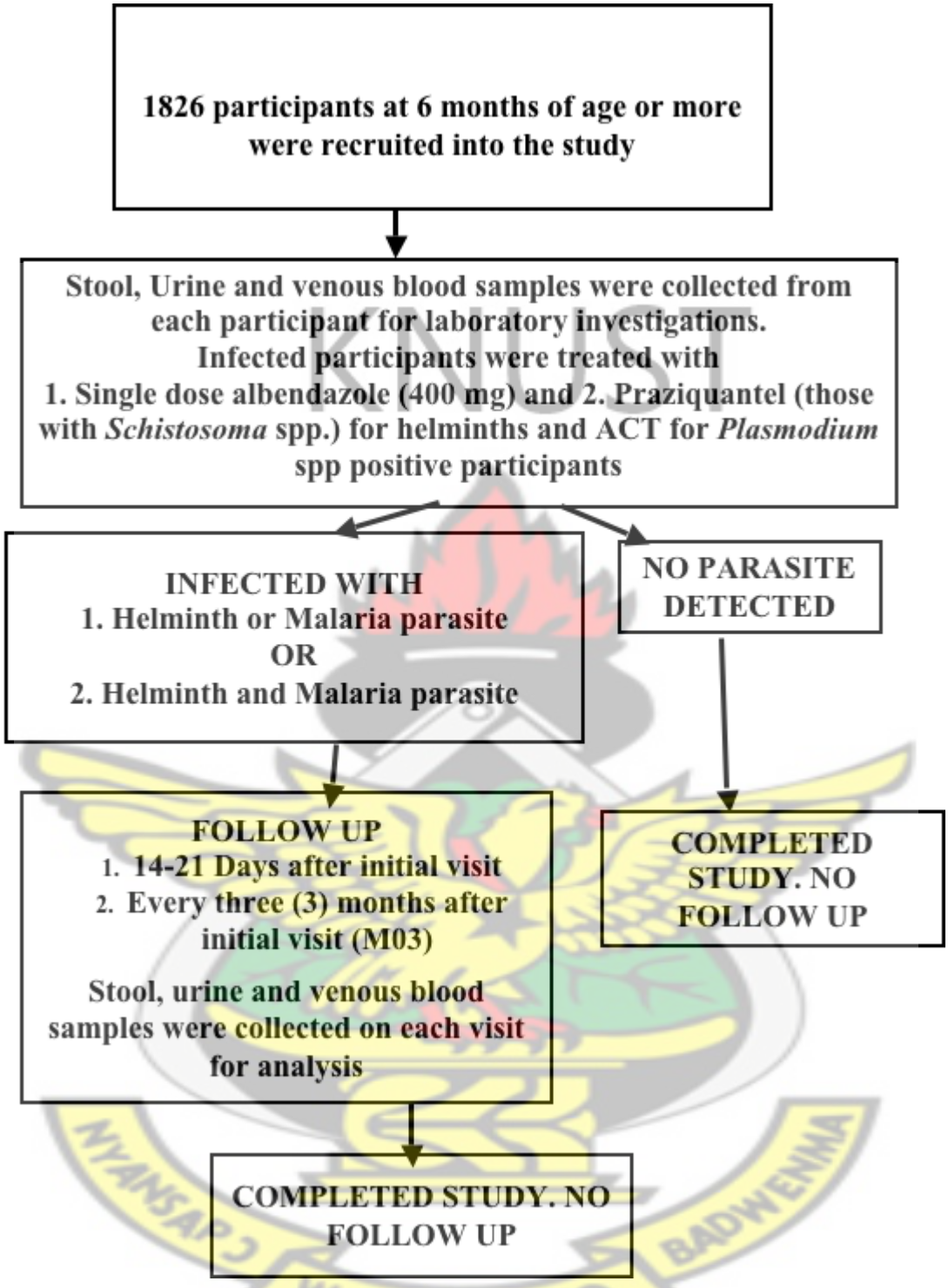


Figure 3-1: Flow chart of study implementation framework

3.1.1 Ethical considerations

The study involved collecting identifiable data and biological specimen of participants from selected households within the study area. Ethical and Scientific approvals were sought from the KHRC-Institutional Ethics Committee (IEC) (ID: 2014-20), Noguchi Memorial Institute for Medical Research Institutional Review Board (NMIMR-IRB) (#: 033/14-15) and the KHRC-Scientific Review Committee (SRC).

Permission was sought from the community opinion leaders before entry for study processes. Data collection commenced after ethical and scientific approvals were obtained from the respective institutions. Written informed consent was sought from each study participant.

3.2 Study area

The study was conducted in the Kintampo North Municipality and Kintampo South District. It is located within the forest-savannah transitional ecological zone in the middle part of Ghana. The area covers a total of 7162 km^2 with a resident population of approximately 140,000 in 32,329 households (KHRC, 2010, Owusu-Agyei et al., 2012) which are mapped geospatially and also with unique permanent identification numbers.

Residents are individuals who have lived in the study area for three months or more. These are followed-up at least once in a year for update on health demographic information.

Community members are predominantly subsistent farmers of crops and animal rearing. Farming in such ecological zone in the tropics coupled with rearing of some animals is found to be suitable and contribute to the risk of acquiring helminths infections (Agrawal, 2012, David and William, 2006) and malaria (Owusu-Agyei et al., 2009).

Pit latrine remains the most available toilet facility (57.5%) in the study area (KHRC, 2010). About 40% of the population use open-fields to defecate while 2.4% have access to water closet (WC).

The study area has twelve (12) clinics and three (3) hospitals (Adu-Gyasi et al., 2015b, Kwarteng et al., 2015). The health facilities routinely treat both suspected and confirmed STH infections with albendazole which is also used in Mass Drug Administration (MDA) and available over the counter in various pharmacy and chemical shops (MoH, 2010).

The routine laboratory examination of stool and urine samples in the area is based on wet film preparation with normal saline. The site is involved in the periodic MDA programme among basic school children (up to Junior High School). The deworming programme has to a large extent been beneficial to the population (Humphries et al., 2011).

Two of such deworming exercise offered to school aged children (SAC) from seven (7) years to sixteen (16) years occurred in the study area in November 2014 and November 2015. The exercises administered albendazole (400 mg) and praziquantel (600 mg). In each round of dosing, there was 74.1% and 77.1% coverage in the respective years as reported by the district assemblies. Recruitment of participants into the study occurred at least six months after the MDA programme.

KHRC has a HOBOware weather station installed onsite to monitor climatic indicators including temperature, rainfall, relative humidity and wind speed for the study area. The geographical position of houses and other facilities within the communities were recorded with global positioning system (GPS) to aid in mapping infections in the study area.

3.3 Study population

Kintampo Health and Demographic Surveillance (KHDSS) database was used to recruit participants who gave their informed consent. Listings of potential participants were generated at random from the database indexing on the household heads.

The lists when generated had children (6 months and beyond) and adults included at various age categories existing in the study area (Owusu-Agyei et al., 2012). All members of a selected

household were recruited to participate in the study by providing blood, stool and urine samples.

3.4 Inclusion and exclusion criteria

Inclusion: Individual residents within the HDSS site, for three months or more, within the major sub-districts including Kintampo, Jema, Amoma, Apesika, Mansie, Busuama, Asantekwa, Ampoma and Anyima., capable of giving informed consent and available for the study duration (one year) were included in the study.

Each participant had to provide; one stool, one urine samples and blood sample collected for the baseline. Those that were positive with helminth and *Plasmodium* spp infection also had to give similar samples per each scheduled period of follow up post-treatment.

Exclusion: Individuals who were less than 6 months were excluded from participating. Those who were not residents (stayed in the area for less than 3 months) were excluded. Potential participants who refused consenting were excluded from the study. I did not screen for other infections.

3.5 Sample size

At 95% confidence level with 90% power, using a population size of 140,000 with expected frequency of 41% of hookworm infection (Humphries et al., 2011) and Assuming a worst acceptable frequency of 33.2% of helminth infection among the population in this study, a minimum sample size of 1,660 (Stata software version 13) was calculated.

Considering a 10% refusal rate to obtain samples and complete data gave a total sample size of 1826 individual participant. Using an average of 6 members in a household in the study area, a total of 304 households from the HDS database was selected at random to recruit participants for this survey.

3.5.1 Cases of interest for immunological study and subgrouping

At 95% confidence level with 80% power, a minimum of 15 cases (Table 3-1) in each of the four groups was adequate to assess the impact of helminth and malaria mono- and co-infections on their respective immune responses in the study area (Katie et al., 2014b).

Table 3-1: Categorization of the group of participants selected for the immunological study

Category	Infection type	Sample size
Group 1	No helminth or *Plasmodium* infection	15
Group 2	Helminth infection, No *Plasmodium* infection	15
Group 3	Helminth and *Plasmodium* infection	15
Group 4	*Plasmodium* infection and no helminth	15

3.6 Sample collection and analysis

3.6.1.1 Blood samples

Blood samples were collected into 6 ml EDTA vacutainer test-tubes from each participant and used for peripheral blood mononuclear cell (PBMC) isolation, full blood count analysis and blood smear for malaria parasite identification. Plasma was obtained from the same blood sample and were stored at -80^{o}C freezer till analysed.

3.6.1.1.1 Full Blood Count Analysis

The Full Blood Counts (FBC) for each participant's sample was carried out using the ABX Micros Pentra 60 C+ (Horiba ABX, Montpellier, France) which is a 5-part differential autoanalyser.

The haematological parameters considered in this study were white blood cells (WBC), lymphocytes (%), lymphocytes (absolute), monocytes (%), monocytes (absolute), neutrophils (%), neutrophils (absolute), eosinophils (%), eosinophils (absolute), basophils (%), basophils (absolute), red blood cells (RBCs), haemoglobin (Hgb) level, haematocrit (Hct), mean cell

volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin corpuscle (MCHC) and platelets (Plt).

3.6.1.1.2 Blood Slide Preparation and Reading (BSR) for Blood parasites

Blood smear for blood borne parasites were prepared on microscope glass slides as described in a previous study for malaria parasites (Adu-Gyasi et al., 2012).

For the microfilaria, smears were first screened at a low magnification (4X and 10X objective lens) to detect suitable fields and presence of microfilaria. On positive slides, speciation was done using the 40X objective lens.

3.6.1.1.3 Peripheral Blood Mononuclear Cells (PBMC) Separation

PBMCs were separated from 5 ml of the collected blood samples using Standard Operating Procedure (SOP) (Appendix 1).

Briefly, whole blood was layered in a medium and subjected to density gradient centrifugation. Layers expected to contain the cells of interest were taken and washed in media enriched with nutrients to sustain the viable cells. The separated cells were then stored in a freezing medium (DMSO) with nutrients containing agents. These were stored in liquid nitrogen and -150 degrees freezer in KHRC.

3.6.1.2 Stool

Stool samples (not exceeding 50 g) were collected from each participant, prepared, and examined for the presence of helminth eggs, larvae and other intestinal parasites' larvae on day of collection according to SOP (Appendix 2).

Different diagnostic methods were concurrently used to enhance the sensitivity and specificity for identifying and diagnosing common soil-transmitted helminth (*Ascaris lumbricoides*, hookworm and *Trichuris trichiura*) infections (Jeandron et al., 2010) and other intestinal parasites.

Stool samples were examined using the routine wet mount, the Kato-Katz (Magalhães et al., 2011) and the ether-based concentration methods to assess the true extent of mono- and poly-parasitism. Portions of stool samples that were found to have parasites or ova were stored in -80^{o}C freezer for molecular and other exploratory analysis.

3.6.1.2.1 Routine wet mount method

The wet mount method was used to identify ova and larvae of helminths, and also any protozoan, trophozoites and cysts which are usually missed by some methods for stool analysis.

3.6.1.2.2 Kato-Katz method

This technique was of value for the semi-concentration and semi-quantitative estimation of eggs of intestinal parasites and Schistosome eggs.

Small amount of the faecal sample was placed on a paper and a nylon screen was pressed on top of the faeces. Using a spatula, the sieved faecal material was scraped through the screen to prevent collecting debris. The scraped faecal material is used to fill the hole in the Kato Katz template. The template delivers consistently 41.7 mg of stool on the microscope slide. The template was carefully lifted and placed it in a bucket of water mixed with concentrated detergent so that it can be reused. One piece of the cellophane, soaked overnight in methylene blue glycerol solution, was placed over the faecal sample. This was pressed downwards to evenly spread the faeces on the slide for observation using the microscope (Agrawal, 2012, Jeandron et al., 2010). Egg count and intensity were calculated and expressed as Egg Per Gram (EPG) of faeces.

3.6.1.2.3 Formol-ethyl acetate concentration method

This was used to screen samples for parasites ova. This technique is reproducible, inexpensive, simple and safe. Its level of parasite detection and quantification is comparable to results obtained by Real time PCR especially when products are digested (Xu et al., 2012).

To about 1g (to the size of a match stick head) of faeces in 15ml centrifuge tube, 5ml of 10% formalin was added. The sample was mixed until a suspension was obtained. The suspension was topped up to 10 ml 10% formalin to have slightly cloudy suspension. This was filtered through a gauze filter (40–60 mesh) into a clean centrifuge tube (falcon tube). About 2ml of ethyl acetate was added and mixed well for at least one minute. The suspension was Centrifuged for ten (10) minute at 500g. All the fatty plug at the top were broken with a stick applicator, and the supernatant poured away by inverting the tube to remove the debris.

The sediments at the bottom of the tube were transferred to a microscope slide for examination covering with a cover glass. Slides were examined with X10 and X40 objectives and counted ova, cysts or larvae if present.

3.6.1.3 Urine sample collection and analysis

A minimum of 10 ml of urine samples were collected from each participant into a clean container with boric acid which stabilized the urine chemistry prior to preparation and examination. Concentrations of urine chemistry parameters were measured using urinalysis dip stick (Mission Urine Reagent Strips, USA).

Urine samples were then centrifuged and sediments examined on microscope slides for each participant. *Schistosoma haematobium*, *Candida* spp, and protozoan parasites (example *Trichomas vaginalis*) were largely looked out for in examination of samples. Parasites and cellular compositions of the sediments were observed and counted under high power field magnification (Cheesbrough, 2009).

Results were basically put into dichotomy without estimating intensity of infection due to the inability to use the quantification method.

3.6.1.4 Measurement of Immunologlobulins with Enzyme Linked Immunosorbent Assay (ELISA)

ELISA techniques were employed to estimate the concentrations of immunoglobulins (IgG, IgG1, IgM, IgE) in the plasma that were separated from the blood collected from each of the participants. These were carried out in the Laboratory of Vaccines for the Developing World, Institute for Glycomics, Griffith University, Australia with SOPs adopted from this laboratory and another in James Cook's University, Australia (Appendix 3).

In brief, Enzyme-Linked Immunosorbent Assay (ELISA) using a biotin-conjugated secondary antibody with streptavidin-HRP and an OPD chromogen system in serum or plasma from humans was used to estimate antibody concentrations. IgA and IgE against malaria parasite antigens and also IgA against worm extracts were not measured.

Crude antigens were titrated to assess the working dilutions to use with the secondary antibodies and the streptavidin concentrations needed for the assays. Samples from known positive and non-exposed individuals were added on each plate during the assays as controls.

3.6.1.4.1 Hookworm antigen

Hookworm somatic extract from *Ancylostoma caninum,* which can cross-react with antibodies of hookworm of human origin were used to coat plates for the estimation of concentrations of IgG, IgG1 and IgE in the selected samples of study participants.

Optical Densities (OD) from the assays in duplicates for each sample at four different dilutions were compared among participants and their infection status by microscopy.

3.6.1.4.2 Trichuris trichiura antigen

Whipworm somatic antigen extract from *Trichuris muris* also cross react with human strains of *Trichuris trichiura* antibodies. The extract was used to estimate the concentration of IgG, IgG1 and IgE measuring the ODs from the duplicate assays of each sample.

The difference in ODs measured were compared among participants and their infection status by microscopy.

3.6.1.4.3 Plasmodium falciparum antigen (NF54 and 7G8 strain)

Crude *Plasmodium falciparum* extract from NF54 and 7G8 strains were prepared respectively from parasites in culture. These were used as antigens to coat ELISA plates to estimate the concentrations of IgG, IgG1, IgM respectively using their ODs.

3.7 Flow cytometry

3.7.1 Cell culture and stimulation

Stored PBMCs were thawed using a working SOP. Cell viability were assessed using 0.4% Trypan blue stain (fresh and filtered). Cell suspension in complete media (10% Heat-Inactivated Human Sera) were plated into wells, each with 2 x10^5 cells in 100µl of cell suspension.

To each sample, a minimum of two wells were set for each treatment (complete media (no stimulant), 1% phytohaemagglutinin (PHA-positive stimulant) and *Plasmodium falciparum* parasitized RBC (pRBC, 2% parasitaemia in 6 x10^5 cell suspension). The plates were incubated in 5% CO_2 for 8 hours together with 0.4µl of BD GolgiStop Protein Transport Inhibitor (10 µg/ml containing Monensin) in wells that were dedicated for intracellular staining. This was intended to increase the secretion of the intracellular cytokines from the cells.

Supernatant was separated from the corresponding wells and stored for cytokine analysis. Cells were pooled together for surface and intracellular staining as described in the SOP presented in Appendix 5.

3.7.1.1 Sample Treatment with Media, 1%PHA and pRBC

Briefly, for each sample, all cells with similar treatments were pooled giving a total of three-tubes (Media, PHA and pRBC) per sample.

After washing the cells with PBS 1X, 1µl of the prepared viability dye (Fixable Viability Stain 620) was added to each tube and incubated. The samples were stained with prepared antibodies (volume used was obtained from the respective titrations at validation) for the target surface markers (CD3-APC H7, CD4-PE, CD8-Alexa fluor 700, CD11c PerCP-Cy5.5, TCR-γδ -APC, HLA-DR BB515).

With commercially prepared fixing and permeabilization buffers (Transcription Factor Buffer Set, BD), antibodies to intracellular markers (IL-4 BUV737, IFN-γ BUV395 and FoxP3 PECy7) were used as described in the SOP. With the compensation settings obtained using unstained and stained cells, samples stained and prepared were acquired on BD LSRFORTESSA X-20 flow cytometer and BD FACDIVA acquisition software. Fluorescence Minus One (FMO) controls were used for positive and unspecific fluorescence threshold setting.

The geometric mean percentage (%) population of the various cell phenotypes and expressions of the specific markers on their surfaces and intracellular cytokine production were carried out for T-Helper (Th1) cells (CD4+), Cytotoxic T Cells (CD8+), Activated CD4+ cells (CD4+/HLA-DR+), Activated CD8+ cells (CD8+/HLA-DR+), CD4+/IFN-γ+, CD4+/IL-4+, CD8+/IFN-γ+, CD8+/IL-4+, CD4+/Foxp3+, CD8+/Foxp3+, CD3+/TCR-γδ+ and CD11c+/HLA-DR+.

Cytokine concentrations were also estimated for Thl/Th2/Th17 differentiation (IL-2, IL-4, IL-6, IL-10, TNF, IFN-γ and IL-17A). Concentrations were estimated from supernatant of PBMCs of study participants cultured with parasitized red blood cells and controlled with Complete Media (No Stimulant) and 1%_PHA as a positive stimulant.

3.7.2 Gating strategy

At acquisition, a threshold of 50,000 was selected to cut-off as much debris and dead cells after setting the SSC and FSC voltages with fresh unstained as well as frozen PBMCs. Primary gate

was set to cover as much of the acquired cells with focus on the lymphocyte and monocyte/macrophage cell population (Figure 3-2).

Live cells were selected using the fixable viability dye, from which CD3+/CD4+ as well as CD3+/CD8+ were used for further analysis (Figure 3-2). Likewise, from the live cells, CD11c/HLA-DR were used for the analysis of Antigen Presenting Cell (largely macrophages/monocytes/dendritic cells) (If resources were available, the best approach would had been to add CD123 marker. Nonetheless, the proportion of cells that will be missed would be similar across the samples considered).

TCR-γδ cells were gated on the CD3+ population (CD3+/TCR-γδ+) (this was done being mindful of the fact that 1-5% of the T-cells are CD3+) (Kenneth et al., 2016) (Figure 3-2).

Statistics were performed on percentage mean cell population.

3.7.3 Measurement of Cytokine concentrations from plasma

BD Cytometric Bead Array (CBA) non-human primate Thl/Th2 Cytokine kit was used according to manufacturer's instructions. Volumes advised in the kit for samples and standards were scaled down since comparable results were achieved as with validated volumes.

The pre-composed array of beads of Interleukin-2 (IL-2), Interleukin-4 (IL-4), Interleukin-6 (IL-6), Interferon-y (IFN-γ), Tumor Necrosis Factor (TNF) and Interleukin-5 (IL-5) made it possible to measure the protein levels of these cytokines in a single sample as presented in an SOP in Appendix 4.

Briefly, assay diluent was added to the cytokine standards. The standard was serially diluted to cover a range of low and high concentrations of the cytokines to measure. Samples were added to predetermined volumes of the mixture of detecting beads in tubes, incubated and acquired using the flow cytometer (BC Cyan ADP 9). The FCS files obtained data were analysed with FCAP Array (vl.01 for windows) software (BD).

3.7.4 Measurement of Cytokine concentrations from culture supernatant

BD Cytometric Bead Array (CBA) Human Thl/Th2/Th17 Cytokine kit (Catalog No. 560484) was used according to manufacturer's instructions. Volumes advised in the kit for samples and standards were scaled down since comparable results were achieved as with validated volumes. This kit was used to measure Interleukin-2 (IL-2), Interleukin-4 (IL-4), Interleukin-6 (IL-6), Interferon-y (IFN-γ), Tumor Necrosis Factor (TNF), Interleukin-17A (IL-17A), and Interleukin-10 (IL-10) protein levels in the supernatant produced from the cell cultures and stimulation, and also as described previously in Appendix 5.

The voltages (PMT-V) for the fluorophores APC and PE were set up using the setup beads in the kit provided by BD. Different bead types were identified based on their level of APC fluorescence and PE was used as the reporter detector to measure how much of the respective cytokines were bound to the beads.

Prepared samples were acquired using the BD LSRFORTESSA X-20 flow cytometer and BD FACDIVA acquisition software. The FCS files obtained data were analysed with FCAP Array (v3 .01 for windows) software (BD) to obtain the final concentrations of the seven cytokines in each sample.

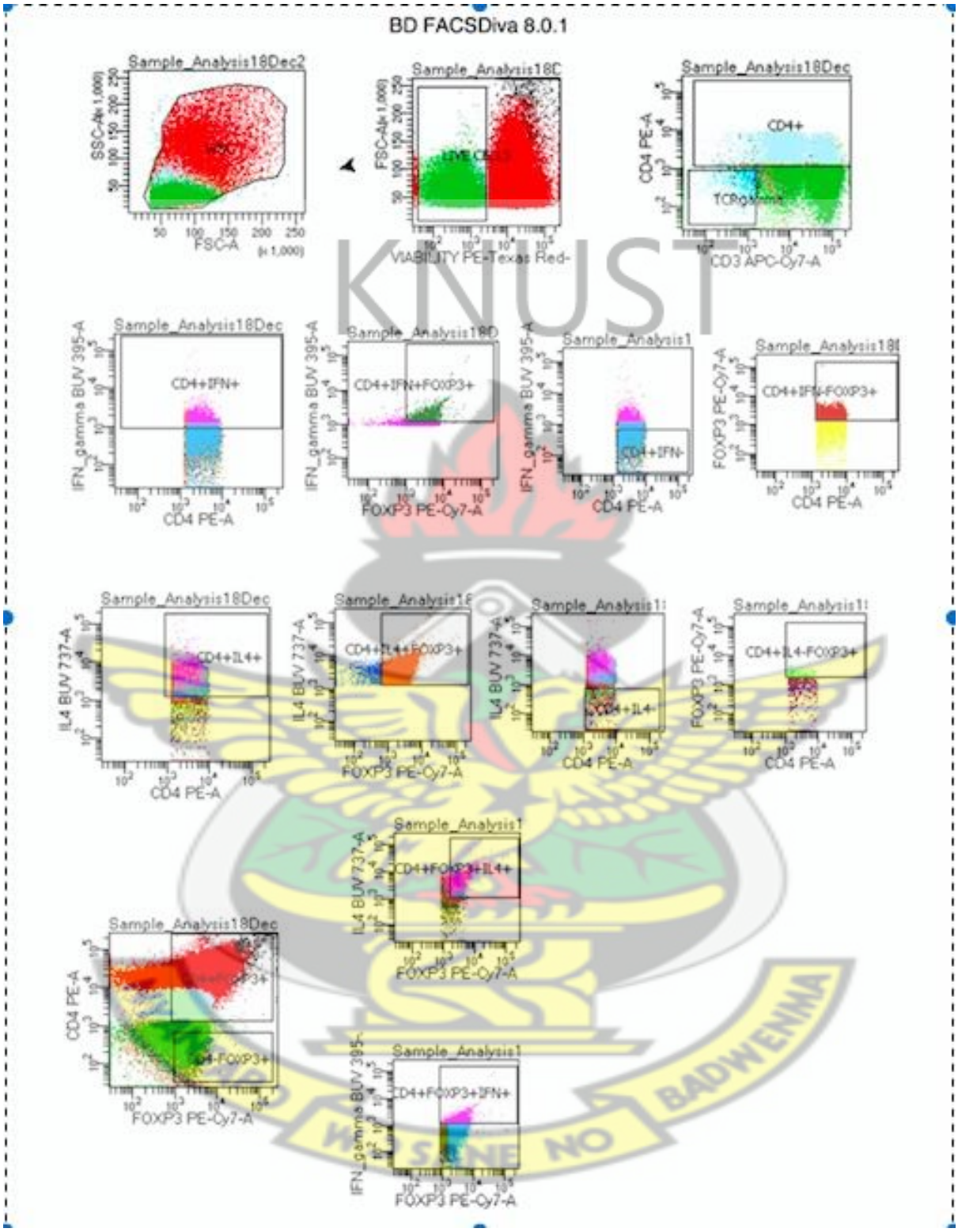


Figure 3-2: Gating and population selection for analysis

3.8 Quality Assurance and Control employed in the study and samples analysis

Before the survey, the team was trained with the tools for data collection. Laboratory staff were trained to evaluate the consistency of parasite detection and egg counting. Picture guide from bench aids for identification of parasites of medical importance were adopted and made available to all technical staff that were involved in the study (Agrawal, 2012, David and William, 2006, Ridley, 2012, Shiba et al., 1996, WHO, 1991).

At least 10 slides of stool samples with known ova present and concentrated were prepared and the reading of each slide by each laboratory staff in the study team was compared with the expected results and count for the evaluation. A discrepancy of up to 5-10% for egg counts was accepted as normal. Each personnel with larger discrepancy was identified and corrected with refresher training.

All quality assurance measures in handling samples, measuring blood counts and blood slide reading for malaria parasites, stool preparations and reading, routine urine analysis needed for acceptable analysis of study data, results and immunological assays were strictly adhered to.

Routinely, three levels of daily quality controls are performed to be certain of the analyser's performance in estimating Low, Normal and High parameters of blood counts. Each printed result was checked and signed by the operator and the unit supervisor according to standard operating procedures (SOPs) before being sent for data entry.

Single stain controls for the fluorochromes used in the flow cytometry analysis were prepared and used for the compensation. The set voltages were used for the cell acquisition after samples processing. Fixable viability dye was added to each tube to work with Live cells.

3.9 Data management and analysis

Data collected were batched for double entry and verified using Microsoft Access software 2010. Template of the forms were created to facilitate data entry. Data was checked for inconsistencies and verified before being analysed. Stata statistical software version 13, R

Software (version 3.2.4), MedCalc Statistical Software version 12.7 and GraphPad Prism 5.0 Statistical software were used for data analysis.

Data in Flow Cytometry Standard (FCS) format were analysed using BD FACSDiva and FlowJo v10.2. Participant was declared positive for helminth infection if any of the three methods used identified a parasite ovum or larva.

Basic descriptive analyses were performed on the demographic characteristics and some immunological parameters (blood cell indices). Proportions of infections and means of concentrations of immunological factors were compared among participants and their infection status using the students' t-test. Chi square ($\chi 2$) analysis was performed for continuous variables where necessary.

Where needful especially with data that were not normally distributed, the concentrations of the immunological factors were transformed and used for further analysis. Linear regression, ANOVA and/or Kruskal Wallis tests were performed among the various infection groups (Bowers, 2014, Sokal and Rohlf, 1995) with post-hoc analysis (Fisher-Hayter (FH) for ANOVA and Dunn's test for Kruskal wallis test) of variables that were found significant *a priori* and at exploration. All reported p-values were 2-sided and significance was taken as p-<0.05 (Campbell et al., 2016a).

Before selecting the variables to consider the influence of parasitic infection and age on their production, series of univariate regression were run to select variables that were at significance level of ≤0.25. This value was selected rather than using a smaller p-value which might conceal the true effect of a particular variable in the model (Bowers, 2014, Sokal and Rohlf, 1995). All those variables were then put together in a multiple regression model to identify the influence of parasitic infection and adjusted with age among samples that were treated in the cell culture this time at p-value of <0.05. Means of cell phenotypes and their population percentage means were considered among participants and their infection status.

The cell populations from the flow cytometry, cytokines measured from plasma and also the supernatant of the cultures of the PBMCs using the CBA technique, together with the antibody (IgG, IgG1 and IgM) concentrations were estimated. ELISA data were explored using heatmaps from their correlation coefficients and also with Principal Composite Analysis (PCA) (Sanchez-Arcila et al., 2014).

Cell types and the intracellular and extracellular cytokines they produce were used in exploratory PCA to observe which of the variables could explain the Th1/Th2/Th17 switch and their contributions in infection with helminth (Hookworm), malaria or their co-infections if any. The parameters; demographic (Age, Weight, Height), Blood Cell Counts (WBC, lymphocytes, monocytes, eosinophils, basophils, neutrophils, RBCs, Hgb and Hct), antibody to Cell phenotypes (CD3/CD4/IFN-γ/IL-4/Foxp3/CD11c/HLA-DR/CD8/TCR-γδ) and cytokines (IL-2, IL-4, IL-17A, IL-6, IL-10, TNF, IFN-γ) were used in an initial PCA to determine which of them correlates to the possible variations that might be observed among the infection groups (No-Infection (None), Hookworm, Malaria and Hookworm-Malaria).

The variables were used over the Infection groups that were present in the study to detect possible explanatory variables that might influence the variations in the immune response pose by infection with any of the helminths, malaria or their co-infections. The extent of dissimilarity of the various variables among the groups and their contribution to whatever differences existing were determined using the eigenvalues and subsequently the principal composite scores.

For the purposes of exploration to identify how the infections influence the immune responses studied, all components that explained more than 50% of the variation were used to select the number of components to use for further exploration (Salazar-Castañon et al., 2014, Zuur et al., 2007, Mori et al., 2016). Prior to the analysis, rather than omitting some observations with missing values, each missing record was replaced by the average of the observations that had

measured values. Zero values in each of the variables used in the PCA were eliminated by adding 1 unit of measurement to all records for those variables. The value was subtracted after final analysis and back transformation.

Relationship between identified infections with the factors; age groupings, season and climate, footwear use, malaria parasite co-infection and others were explored

Presentation of spatial distribution and analyses of data were carried out using the ArcMap Desktop 10.2 software. Purely spatial analysis using the Discrete Poisson model was done with Software for the Spatial and Space-Time scan statistics (SaTScan version 9.4.4, Harvard, USA) to identify clusters of the infections in the study area.

The infection groups were defined as individuals with No Infection (NoI), Helminth (HEL), Hookworm (HKW), Malaria (MAL), Helminth-Malaria co-infection (HELMAL) and Hookworm-Malaria (HKWMAL).

4 CHAPTER FOUR

RESULTS

4.1 Epidemiology of Soil Transmitted Helminth (STH) with co-infection in the middle-belt of Ghana, Africa

4.1.1 Study participants and site description

A total of 1826 participants were contacted and enrolled in the survey over the 12-month period. Out of this, 85.9% (1,569/1826) participants provided all three (stool, urine and blood) samples. The demographic characteristics of these population is presented in Table 4-1. The mean age of the population was 24.1 years and ranged from 1 year to 96 years.

Of the total recruited participants that provided all expected samples, 50.0% (785/1569) were in the target age group for mass drug administration of antihelminth and praziquantel (≤ 16 years old). More than half of the population was educated (57%), with most of them unemployed (60%) and also Christians (68%). The highest level of education was Elementary (High/Middle/Ordinary Level/Advance Level school certificate) among most participants. Only 7.8% (121/1560) of the population had taken antihelminths within three months prior to our visit.

Among children aged up to 16 years, a previous deworming was reported in 8.4% (66/790) compared to 7.3% (57/776) among those aged 16 years and above. The difference between the two proportions was not statistically significant (P=0.458). The mean (standard deviation) of weight and height were 42.3 (19.7) kg and 143 (30.9) cm for males and 43.2 (19.0) kg and 141 (27.2) cm for females respectively.

Considering means of defecation among those that did not have toilet facilities at home, 60.3% (806/1336) used Open Fields, 22.1% (295/1336) used Pit Latrine and 17.1% used Ventilated Improved Pit (KVIP).

Table 4-1: Demographic characteristics of the study population (Field survey 2016)

Age Group (years)		
	Males, n(%)	**Females, n(%)**
<8	**160(50.2)**	**159(49.8)**
8-16	**239(50.7)**	**232(49.3)**
17-30	**121(41.6)**	**170(58.4)**
31-60	**149(39.2)**	**231(60.8)**
61-100	**52(49.5)**	**53(50.5)**
BMI		
		Males	**Females**
	Kg/m^2	**n(%)**	**n(%)**
	Underweight (<18.5)	**340(50.1)**	**339(49.9)**
	Normal weight (18.5-24.9)	**338(47.5)**	**373(52.5)**
	Over weight (>24.9)	**42(24.1)**	**132(75.9)**
Education		
		n(%)
	No education	**568(36.3)**
	Elementary	**898(57.3)**
	Higher education	**100(6.4)**
Occupation		
	Professional	**20(1.3)**
	Trader	**125(8)**
	Farmer	**480(30.9)**
	Unemployed	**931(59.8)**
Religion		
	Muslim	**372(23.8)**
	Christian	**1065(68)**
	Traditional	**36(2.3)**
	None	**93(5.9)**
Bed share		
		Crude proportion
	Yes, n(%)	**1297(82.9)**
	No, n(%)	**268(17.1)**

Table 4-1 continues

Season Reclassified		
		n(%)
	Sep15-Nov15	**438(28.0)**
	Dec15-Feb16	**316(20.2)**
	Mar16-May16	**170(10.9)**
	Jun16-Aug16	**638(40.9)**
Actual Season		
	Nov15-Apr16	**604(48.5)**
	Jun16-Oct16	**642(51.5)**
Footwear		
	Slippers ("Chalewate")	**1030(65.8)**
	Sandals	**191(12.2)**
	Wellingtonboot	**99(6.3)**
	Shoe & "Cambuu"	**129(8.2)**
	None	**117(7.5)**
Toilet		
	Yes, n(%)	**224(14.3)**
	No, n(%)	**1340(85.7)**
Dewormer (last 3-months)		
		Crude
	Yes, n(%)	**123(7.9)**
	No, n(%)	**1443(92.1)**
Scrub Nails before eating		
	Yes, n(%)	**1043(66.6)**
	No, n(%)	**523(33.4)**
Wash hands with soap		
	Yes, n(%)	**1063(67.9)**
	No, n(%)	**503(32.1)**
Water source in House		
	Yes, n(%)	**207(13.2)**
	No, n(%)	**1359(86.8)**
Drinking Water source close to house		
	Pipe-borne	**232(14.8)**
	Well	**256(16.4)**
	River/Stream	**290(18.5)**
	Bore-hole	**710(45.3)**
	Other	**78(5.0)**

Table 4-1 continues

Use Refuse site		
	Yes, n(%)	**1078(68.8)**
	No, n(%)	**488(31.2)**
Animal Reared by Tenant of compound		
	Yes, n(%)	**1182(75.5)**
	No, n(%)	**384(24.5)**
Animal Reared within compound		
	Yes, n(%)	**1174(75.0)**
	No, n(%)	**392(25.0)**
Participants directly involved in Animal Rearing		
	Yes, n(%)	**828(52.9)**
	No, n(%)	**738(47.1)**

4.1.2 Climate and Environmental factors in study area

From September 2015 to August 2016, a mean temperature of 21.6^{o}C and a maximum temperature of 41.2^{o}C occurred in February 2016. Maximum rainfall of 1.27mm was recorded in each of October 2015 and April 2016. The mean Relative Humidity over the period was 65.7% with the maximum of 100% recorded in September 2016. A mean soil moisture of 0.11m^3/m^3 and a maximum of 0.28 m^3/m^3 were recorded in April 2016. These factors have been detailed in Figure 4-1.

A

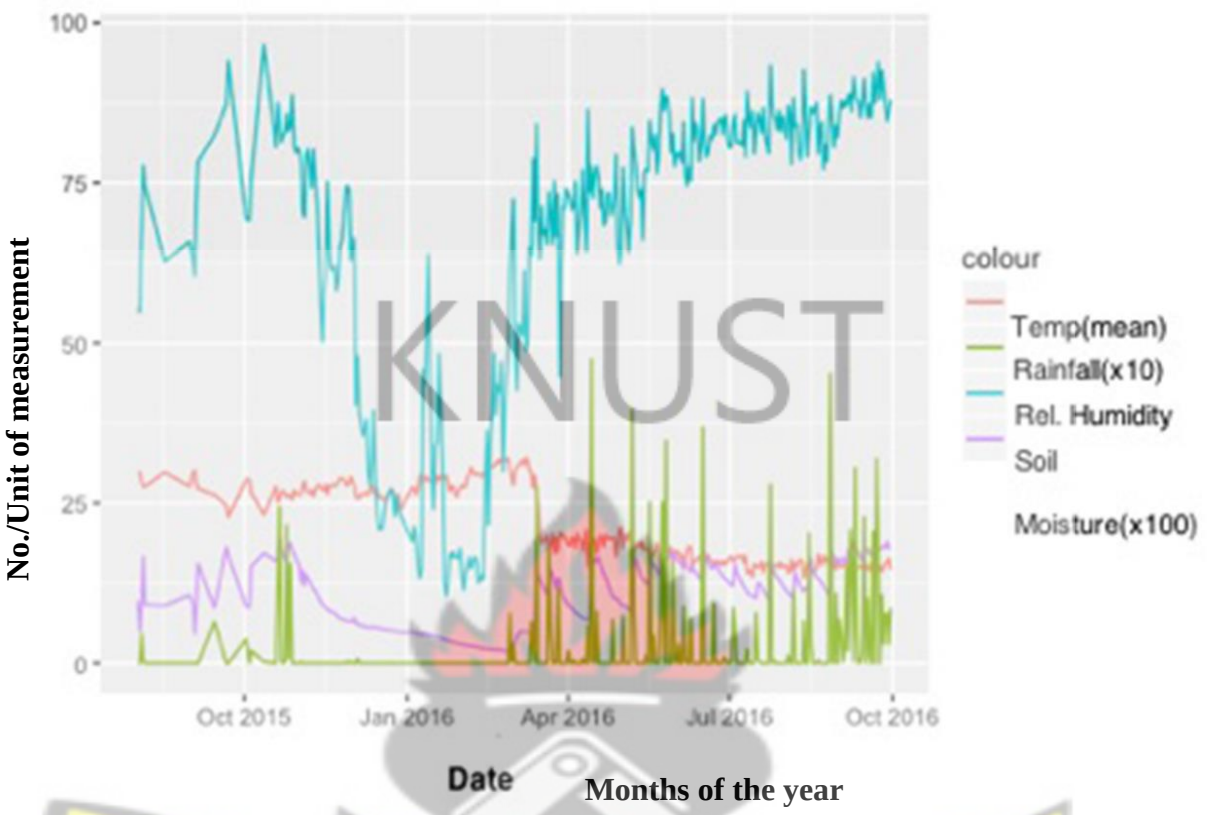
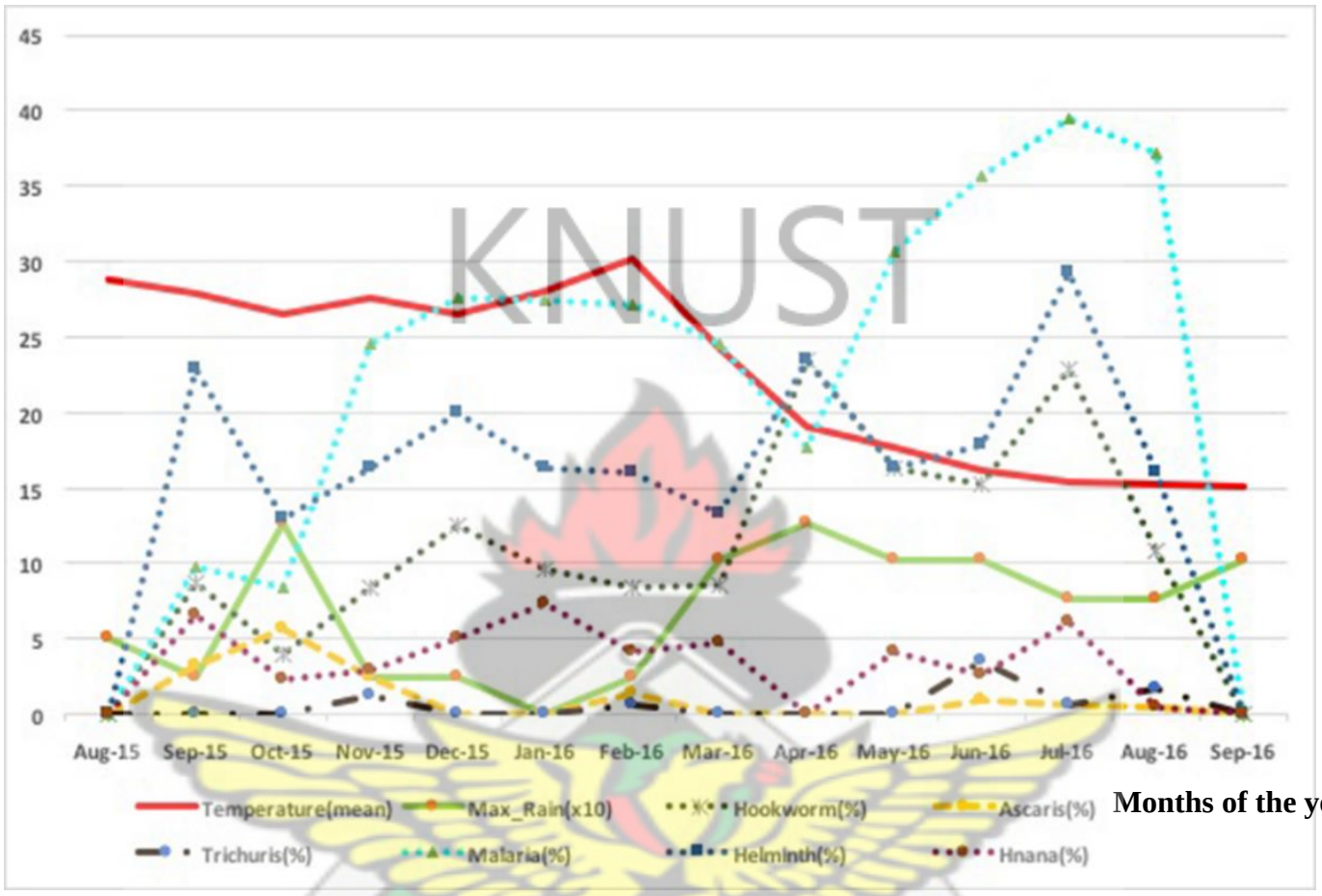


Figure 4-1: Presentation of the climatic conditions(A) and identified infections in the middle belt of Ghana over a 12 month period (B)

Figure 4-1B



No./Unit of measurement

Months of the year

4.1.3 Distribution and Prevalence of parasitic infections

Using the baseline data from the cross-sectional study, an overall prevalence of 19.3% (302/1569) helminth (with at least infection with one of the STH identified) over the one-year period in the middle–belt of Ghana, Africa. The prevalence with respect to Hookworm, *Ascaris lumbricoides*, *Hymenolepis* spp, *Strongyloides stercoralis, Trichuris trichiura, Taenia species* and other infections have been presented in Table 4-2.

Considering polyhelminthiasis, 1.0% of the participants were infected with Hookworm and *Hymenolepis spp* while 4% had Hookworm infection with either of *A. lumbricoides, H. spp, S. stercoralis, Taenia species or Trichuris trichiura*.

When participants who had helminth infection were treated and followed up 14 days post-treatment, 19% remained positive. Suggestive of possible reduction in the potency and effectiveness of the single dose albendazole (400 mg).

Table 4-2: Prevalence of Soil Transmitted Helminth and other parasitic infections among participants

	Infected with intestinal and malaria parasite					
	Total population		≤16 years		>16 years	
Parasite	No. examined	No. infected (%)	No. examined	No. infected (%)	No. examined	No. infected (%)
Helminth	1569	302(19.3)	790	140(17.7)	779	162(20.8)
Hookworms	1569	190(12.1)	790	84(10.6)	779	106(13.6)
Ascaris lumbricoides	1569	23(1.5)	790	9(1.1)	779	14(1.8)
Taenia spp.	1569	23(1.5)	790	12(1.5)	779	11(1.4)
Trichuris trichiura	1569	13(0.8)	790	5(0.6)	779	8(1.0)
Hymenolepis spp	1569	62(4.0)	790	38(4.8)	779	24(3.1)
Strongyloide stercoralis	1569	14(0.9)	790	4(0.5)	779	10(1.3)
Trichostrongyloides	1569	4(0.3)	790	2(0.3)	779	2(0.3)
Dicrocoelium spp.	1569	11(0.7)	790	6(0.8)	779	5(0.6)
Schistosoma mansoni	1569	3(0.2)	790	0	779	3(0.4)
Enterobius vermicularis	1569	2(0.1)	790	0	779	2(0.3)
Schistosoma haematobium	1569	1(0.1)	790	0	779	1(0.1)
Hookworm +						
Strongyloide stercoralis	1369	2(0.2)	704	1(0.1)	665	1(0.2)
Ascaris lumbricoides	1360	2(0.2)	697	0	663	0
Taenia spp.	1362	3(0.2)	698	2(0.3)	664	1(0.2)
Trichuris trichiura	1370	2(0.2)	701	0	669	2(0.3)
Hymenolepis spp	1343	13(1.0)	684	8(1.2)	659	5(0.8)
Protozoa						
Balantidium coli	1569	2(0.1)	790	1(0.1)	779	1(0.1)
Entamoeba coli	1569	0	790	0	779	0
Trichomonas vaginalis	1486	5(0.3)	740	2(0.3)	746	3(0.4)
Intestinal flagellates	1569	116(7.4)	790	47(6.0)	779	69(8.9)

Table 4-2 ***continues***

		Total population		≤16 years		>16 years
Parasite	No. examined	No. infected (%)	No. examined	No. infected (%)	No. examined	No. infected (%)
Helminth and Protozoa						
Intestinal flagellates +						
Hookworm	1289	13(1.0)	663	2(0.3)	626	11(1.8)
Ascaris	1436	3(0.2)	736	1(0.1)	700	2(0.3)
Trichuris trichiura	1442	1(0.1)	738	0	704	0
Strongyloides stercoralis	1443	2(0.1)	739	0	704	2(0.3)
Hymenolepis spp	1405	7(0.5)	715	5(0.7)	690	2(0.3)
Infected with parasites identified in blood						
Malaria, Pf	1569	441(28.1)	790	328(41.5)	779	113(14.5)
Microfilaria	1465	64(4.4)	738	17(2.3)	727	47(6.5)
Malaria, Pf + Microfilaria	1028	23(2.2)	431	11(2.6)	597	12(2.0)
Malaria and Helminth						
malaria +						
Helminth	1060	117(11.0)	492	85(17.3)	568	32(5.6)
Hookworm	1094	78(7.1)	484	53(11.0)	610	25(4.1)
Ascaris	1117	6(0.5)	463	5(1.1)	654	1(0.2)
Trichuris trichiura	1125	5(0.4)	461	2(0.4)	664	3(0.5)
Strongyloides stercoralis	1120	3(0.3)	464	3(0.7)	656	0
***Hymenolepis* spp**	1122	28(2.5)	468	22(4.7)	654	6(0.9)
***Taenia* spp.**	1125	10(0.9)	468	9(1.9)	657	1(0.2)
Intestinal flagellates	1084	36(3.3)	457	21(4.6)	627	15(2.4)

For infection intensity, considering Hookworm, 36.9% had light infection, 7.7% had moderate infection and 55.4% had heavy infection. Intensity among the various age groups and looking at footwear use are provided in Figure 4-2.

With Ascariasis, 83.3% had light infection, 16.7% had moderate infection and none heavily infected. Again, with Trichuriasis, 33.3% had light infection, 22.2% had moderate infection and 44.4% had heavy infection. Using the hookworm egg intensity criteria, with *Hymenolepis* spp, 30% had light infection, 7.5% had moderate infection and 62.5% had heavy infection. Twenty percent (20%) of those infected with *Taenia spp*. had light infection while 80% were classified with heavy infection. The geometric mean count of *Strongyloides stercoralis* larvae in infected individuals was 29 per gram of stool.

Comparing means of egg or larvae count among children with ages less or equal 16 years and those more than 16 years old, difference in hookworm egg count (p=0.505), Ascaris egg count (p=0.118), *Trichuris trichiura* egg count (p=0.281), *Hymenolepis* spp egg count (p=0.404), *Taenia spp*. egg count (p=0.208), *Strongyloides steroralis* larval count (p=0.734) was not significant.

4.1.4 Seasonal influence on helminth infection

The crude prevalence with at least one helminth infection was 17.7% in the Dry Season (November 2015 to April 2016) and 25.1% in the Rainy Season (May 2016 to October 2016). The difference between the prevalence for the two seasons was significantly different (p=0.002).

Considering prevalence of co-infection of hookworm and at least one of the other helminths, the difference in the prevalence in the Rainy (8.4%) and Dry (7.4%) with p value=0.553 was not significant. The influence of some factors on the distribution of helminth can be found in Figure 4-2.

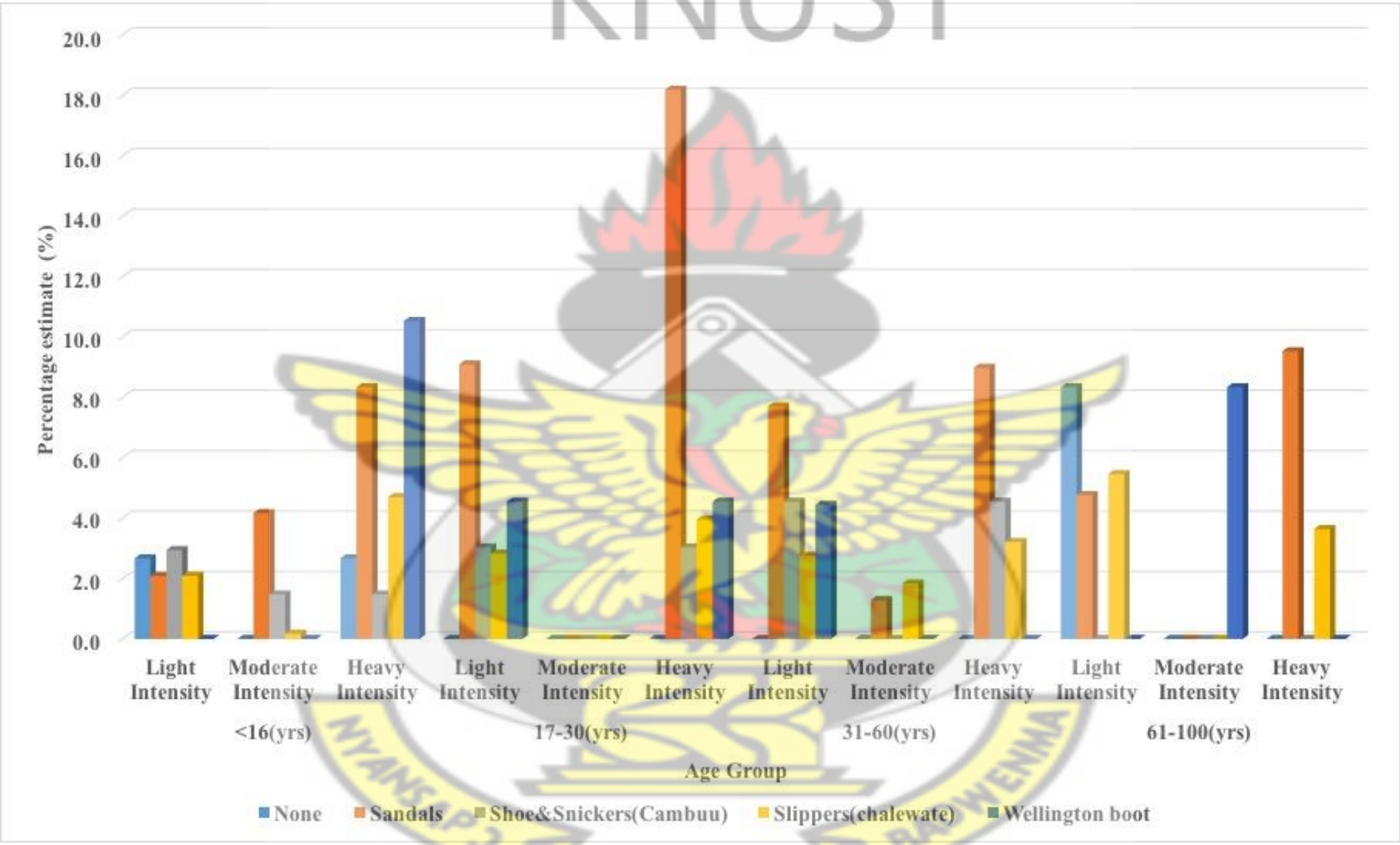


Figure 4-2: Plot of hookworm egg intensity and type of footwear used compared among age groups of the population

4.1.5 Prevalence of Malaria and other parasites

The crude prevalence of asymptomatic malaria parasite infection of 28.1% (441/1569) was recorded in the study with 17.1% in children less than 2 years, 37.9% in those 2 to <8 years and 41.5% among participants that were 8 to 16 years old.

The difference in crude prevalence in the two climatic seasons ($p<0.001$) and across the various age groups ($p<0.001$) were significantly different for malaria parasite infection.

A crude prevalence of infection with Intestinal flagellates was 7.4% and 4.4% prevalence of microfilaria present in their blood films.

With microfilaria, the highest prevalence of 55.6% occurred among 31-60-year-old group, followed by 18.5% prevalence among 17-30-year-old group and the least of 3.7% in children less than 7 years old ($p=0.002$).

Considering infections with Intestinal flagellates, the prevalence was similar across the various age groups ($p=0.186$).

4.1.6 Factors associated with helminth infections

Considering univariate associations between helminth infections, there were significant differences in the odds of being infected with helminth parasite considering the factors in Table 4-3.

However, in a multivariate logistic regression model, significant associations in the odds ratio considering helminth infection were identified with season, footwear use, malaria parasite co-infection, occupation and religion (Table 4-3).

Table 4-3: Helminth distribution and the effects of risk factors assessed in the middle belt of Ghana

	Helminth							
			Univariate			Multivariate		
	No. Cases	No. Controls	OR(SE)	95% CI(L,U)	P-value	OR(SE)	95% CI(L,U)	P-value
Age Group (years)								
<8	48	270	1		0.0803[a],0.2027[b]			
8-16	92	375	1.38	0.94,2.02				
17-30	64	227	1.59	1.05,2.40				
31-60	83	296	1.58	1.06,2.33				
61-100	15	90	0.94	0.50,1.76				
Season Reclassified								
Sep15-Nov15	70	363	1		0.0025[a],0.0011[b]	1		
Dec15-Feb16	53	263	1.05	0.71,1.54		1.27	0.74,2.18	0.381
Mar16-May16	26	144	0.94	0.57,1.53		0.85	0.42,1.72	0.660
Jun16-Aug16	153	488	1.63	1.19,2.23		1.72	1.06,2.80	0.030*
Actual Season								
Dry season (Nov15-Apr16)	94	508	1		0.0003[a,b]	(omitted)		
Rainy season (Jun16-Oct16)	153	488	1.69	1.27,2.26				
Footwear								
Slippers ("Chalewate")	185	840	1		0.0019[a],0.7747[b]	1		
Sandals	56	135	1.88	1.32,2.68		1.60	1.01,2.52	0.044*
Wellingtonboot	23	75	1.39	0.85,2.28		1.36	0.73,2.54	0.330
Shoe & "Cambuu"	18	110	0.74	0.44,1.25		0.93	0.50,1.73	0.817
None	20	97	0.94	0.56,1.55		1.72	0.93,3.18	0.086
Malaria parasite								
No	185	943	1		0.0000[a,b]	1		
Yes	117	322	1.85	1.42,2.42		1.94	1.41,2.67	0.000*
Education								
None	119	448	1		0.3917[a],0.1712[b]			
Elementary/Primary	167	727	0.86	0.66,1.13				
Higher school	16	83	0.73	0.41,1.29				

Table 4-3 continues

Gender								
Male	160	557	1		0.0064[a,b]	1		
Female	142	701	0.71	0.55,0.91		0.76	0.54,1.06	0.107
Toilet								
No	264	1072	1		0.2798[a,b]			
Yes	37	185	0.81	0.55,1.19				
Dewormer (last 3-months)								
No	287	1152	1		0.0436[a,b]	1		
Yes	15	106	0.57	0.33,0.99		0.55	0.26,1.15	0.111
Scrub Nails before eating								
No	109	412	1		0.2728[a,b]			
Yes	193	845	0.86	0.66,1.12				
Wash hands with soap								
No	110	392	1		0.0803[a,b]			
Yes	192	865	0.79	0.61,1.03				
Water source in House								
No	272	1081	1		0.0572[a,b]			
Yes	30	177	0.67	0.44,1.01				
Drinking Water source close to house								
Pipe-borne	28	145	1		0.1362[a],0.1735[b]			
Well	25	130	1.00	0.55,1.80				
River/Stream	67	224	1.55	0.95,2.53				
Bore-hole	150	556	1.40	0.90,2.18				
Other	3	26	0.60	0.17,2.12				
Use Refuse site								
No	105	381	1		0.1400[a,b]			
Yes	197	873	0.82	0.63,1.07				
Share bed								
No	58	207	1		0.2556[a,b]			
Yes	244	1050	0.83	0.60,1.15				

Table 4-3 continues

Occupation								
Unemployed	163	764	1		0.0499[a],0.3756[b]	1		
Farmer	112	368	1.43	1.09,1.87		1.43	1.00,2.04	0.048*
Trader	20	104	0.90	0.54,1.50		0.67	0.28,1.62	0.375
Professional	3	17	0.83	0.24,2.86		0.69	0.08,5.79	0.731
Religion								
Muslim	64	304	1		0.0181[a],0.0865[b]	1		
Christianity	204	860	1.13	0.83,1.54		1.03	0.72,1.48	0.872
Traditional	14	22	3.02	1.45,6.29		3.02	1.35,6.78	0.007*
None	20	72	1.32	0.75,2.32		0.83	0.44,1.59	0.574
Animal Reared by Tenant of compound								
No	73	311	1		0.8422[a,b]			
Yes	229	947	1.03	0.77,1.38				
Animal Reared within compound								
No	75	317	1		0.8958[a,b]			
Yes	227	941	1.02	0.76,1.36				
Participants directly involved in Animal Rearing								
No	130	606	1		0.1200[a,b]			
Yes	171	652	1.22	0.95,1.58				

[a]: Test of homogeneity (equal odds), [b]: Score test for trend of odds

4.1.7 Factors associated with hookworm infections

The univariate logistic regression between hookworm infection and some factors revealed significant differences and this is presented in Table 4-4.

However, in a multivariate logistic regression model, only age, footwear use, malaria parasite co-infection, scrubbing nails during hand washing, type of drinking water close to house, and religion had significant associations in the odds ratio considering hookworm infection (Table 4-4).

Table 4-4: Hookworm distribution and the effects of risk factors assessed in the study area

	Hookworm							
			Univariate			Multivariate		
	No. Cases	No. Controls	OR(SE)	95% CI(L,U)	P-value	OR(SE)	95% CI(L,U)	P-value
Age Group (years)								
<8	23	295	1		0.0511[a],0.0298[b]	1		
8-16	61	406	1.93	1.16,3.19		2.32	1.24,4.34	0.008*
17-30	40	251	2.04	1.19,3.52		2.73	1.23,6.07	0.014*
31-60	53	326	2.09	1.24,3.50		2.21	0.87,5.60	0.095
61-100	13	92	1.81	0.88,3.73		1.73	0.60,5.04	0.311
Season Reclassified								
Sep15-Nov15	29	404	1		<0.000[a,b]	1		
Dec15-Feb16	30	286	1.46	0.86,2.49		0.94	0.43,2.07	0.876
Mar16-May16	21	149	1.96	1.08,3.56		0.80	0.32,1.99	0.643
Jun16-Aug16	110	531	2.89	1.87,4.46		1.38	0.66,2.87	0.393
Actual Season								
Nov15-Apr16	57	545	1		0.0001[a,b]	(omitted)		
Jun16-Oct16	110	531	1.98	1.40,2.79				
BMI								
Underweight	75	598	1		0.032[a],0.990[b]	1		
Normal	102	606	1.34	0.97,1.85		0.91	0.55,1.48	0.692
Over-weight	13	154	0.67	0.36,1.25		0.70	0.31,1.57	0.383
Footwear								
Slippers ("Chalewate")	113	912	1		<0.000[a], 0.2489[b]	1		
Sandals	46	145	2.56	1.74,3.78		1.88	1.10,3.21	0.021*
Wellingtonboot	12	86	1.13	0.60,2.13		1.09	0.49,2.43	0.838
Shoe & "Cambuu"	11	117	0.76	0.40,1.45		0.79	0.37,1.71	0.552
None	8	109	0.59	0.28,1.25		1.62	0.66,3.98	0.292
Malaria parasite								
No	112	1016	1		<0.000[a,b]	1		
Yes	78	361	1.96	1.43,2.69		1.62	1.09,2.40	0.018*

Table 4-4 continues

Education								
None	79	488	1		0.072[a],0.033[b]	1		
Elementary/Primary	105	789	0.82	0.60,1.12		1.07	0.69,1.67	0.763
Higher school	6	93	0.40	0.17,0.94		0.59	0.20,1.74	0.336
Gender								
Male	108	609	1		0.0013[a,b]	1		
Female	82	761	0.61	0.45,0.83		0.72	0.47,1.10	0.124
Toilet								
No	171	1165	1		0.0738[a,b]			
Yes	19	203	0.64	0.39,1.05				
Dewormer (last 3-months)								
No	184	1255	1		0.0115[a,b]	1		
Yes	6	115	0.36	0.15,0.82		0.46	0.17,1.26	0.129
Scrub Nails before eating								
No	78	443	1		0.0173[a,b]	1		
Yes	112	926	0.69	0.50,0.94		0.68	0.47,1.00	0.048*
Wash hands with soap								
No	82	420	1		0.0006[a,b]	1		
Yes	108	949	0.58	0.43,0.80		0.90	0.39,2.08	0.809
Water source in House								
No	177	1176	1		0.0053[a,b]	(omitted)		
Yes	13	194	0.45	0.25,0.80				
Drinking Water source close to house								
Pipe-borne	11	162	1		0.0012[a],0.0003[b]	1		
Well	11	144	1.13	0.47,2.68		1.98	0.69,5.71	0.207
River/Stream	40	251	2.35	1.16,4.73		2.05	0.88,4.73	0.094
Bore-hole	113	593	2.81	1.47,5.36		2.51	1.11,5.68	0.027*
Other	2	27	1.09	0.23,5.22		0.66	0.07,6.06	0.717

Table 4-4 continues

Use Refuse site								
No	75	411	1		0.0089[a,b]	1		
Yes	115	955	0.66	0.48,0.90		1.02	0.68,1.51	0.939
Share bed								
No	39	226	1		0.1672[a,b]	1		
Yes	151	1143	0.77	0.52,1.12		0.84	0.52,1.34	0.458
Occupation								
Unemployed	98	829	1		0.0003[a],0.700[b]	1		
Farmer	82	398	1.74	1.27,2.40		1.33	0.66,2.71	0.427
Trader	7	117	0.51	0.23,1.12		0.55	0.15,2.03	0.366
Professional	1	19	0.45	0.06,3.37		2.25	0.21,24.00	0.501
Religion								
Muslim	39	329	1		0.0001[a],0.0027[b]	1		
Christianity	121	943	1.08	0.74,1.59		1.00	0.63,1.58	0.996
Traditional	12	24	4.21	1.92,9.24		4.36	1.74,10.95	0.002*
None	18	74	2.05	1.11,3.80		0.98	0.47,2.04	0.956
Animal Reared by Tenant of compound								
No	34	350	1		0.0218[a,b]	1		
Yes	156	1020	1.57	1.06,2.33		0.81	0.48,1.36	0.430
Animal Reared within compound								
No	36	356	1		0.0361[a,b]	1		
Yes	154	1014	1.50	1.02,2.20		0.37	0.07,1.96	0.244
Participants directly involved in Animal Rearing								
No	72	664	1		0.0074[a,b]	1		
Yes	117	706	1.53	1.12,2.09		3.16	0.58,17.12	0.182

[a]: Test of homogeneity (equal odds), [b]: Score test for trend of odds

4.1.8 Cluster identification of helminth and *Plasmodium* infection in the study area

Within the one-year study period (September 2015 to August 2016) and considering helminth infections, a total of 281 cases were identified in 71 communities with a population of 130,262. The annual cases were 107.8/100000. In the Purely Spatial analysis, 9 clusters were identified of which HM1 (RR=7.96, p<0.001, radius=7.76 km) – located with a centroid (7.946841 N, 1.625205 W), HM3 (RR=2.56, p<0.001, radius=24.24 km) – located with a centroid (8.134854 N, 1.945655 W), HM4 (RR=16.31, p<0.001, radius=0 km) – located (8.470169 N, 1.575561 W), HM6 (RR=13.58, p=0.002, radius=0 km) – located (8.170988 N, 1.694296 W) and HM7 (RR=7.03, p<0.003, radius=1.73 km) – located with a centroid (8.144346 N, 1.645723 W) had significantly high relative risks (Figure 4-3).

Legend: *HM represents Helminth-Malaria infection and Mal represent Malaria

Figure 4-3: Exploration to identify helminth and malaria infection clusters in the study area in the middle belt of Ghana.

4.2 Haematological profile in helminth and malaria parasite co-infection among a rural population in Ghana

4.2.1 Comparing haematological profile of the baseline and follow-up visit

The difference in the mean(±SD) of the haematological parameters from participants at the follow-up visits compared to the baseline levels were significant at the day-14 visit for concentrations of monocytes (7.7(±3.6), P<0.0001) %, neutrophils (32.3(±10), P=0.0158) %, eosinophils (6.5(±5.4), P<0.0001) %, basophils (1.1(±0.5), P=0.0011) %, MCV (79.4(±7.5), P=0.0089) fl, MCH (27.3(±3.2), P=0.0010) pg and MCHC (34.4(±2.2), P=0.0011) g/dL.

For samples collected from participant 3-months post-treatment, the difference compared to baseline haematological parameters were significant with lymphocytes (50.1(±9.5) %, P<0.0001), monocytes (9.6(±2.8), P<0.0001) %, eosinophil (6.6(±5.3), P<0.0001) %, basophil (0.9(±0.3), P=0.0004) %, and platelets (244.8(±85), P=0.0462) x10^9/L. The rest of the parameters were similar.

4.2.2 Comparing blood cell indices and gender

When the blood parameters were compared at baseline among males and females, with the exception of monocyte (6.2(±4.4), P=0.0032) %, neutrophils (34.5(±10.3), p=0.0083) %, eosinophils (4.8(±4.4), P=0.0026) %, RBCs (4.3(±0.6), P<0.0001) x10^12, Hgb (11.9(±1.6), P<0.0001) g/dL, and Hct (34.4(±4.3), P<0.0001) % which were significantly different, the rest were similar.

4.2.3 Changes in blood cell profiles by infection

4.2.3.1 Red blood cell indices and Anaemia

The mean(SD) Hgb concentration at baseline was 12.6 (±2.0) g/dL in males and 12.0 (±1.6) g/dL in females and the difference was significant (p<0.001). Looking at the difference in mean(SD) of Hgb considering gender, it was significant at follow-up 14 days posttreatment

(p=0.003) but similar at follow-up 3-months posttreatment (p=0.450). The lowest mean Hgb concentration of 10.7g/dL was recorded among children <8 years old while the highest of 13.0g/dL was recorded among individuals >16 years.

Using routine haemoglobin cut-off of 11.0g/dl for treatment (Kotepui et al., 2014) to assess anaemia levels, 15.8% (247/1567) of the population had anaemia. Anaemia prevalence was significant in those with malaria (p=0.001) compared to those with helminth and helminth with malaria co-infection. Hookworm (p=0.001) and hookworm with malaria co-infection (p=0.005) significantly caused anaemia when one considers hookworm and malaria parasite respectively (Table 4-5).

Comparing the prevalence of anaemia and the various infections in individuals less than 16 years, prevalence of 25.8% in helminth infection, 26.6% in hookworm infection and 28.9% in malaria parasite infection were obtained. It is important to note that all the infections considered caused "severe" anaemia prevalence of 41.7% among helminth, 47.8% among hookworm and 44.6% among malaria parasite infected individuals (Sant-Rayn et al., 2008, WHO, 2001, WHO, 2014) (Appendix 8.1).

The sensitivity (SE) of using low Hgb to identify malaria was 22.7% (Specificity (SP) of 71.6%) with OR=0.74. Interestingly, with SE (SP) of 10.5% (92.2%), individuals with hookworm-malaria co-infection had odds ratio (OR) of 1.39 in measuring low Hgb. Though about 29% prevalence of high Hgb was recorded considering the infection groups, the predictive diagnostic value had sensitivity ranging from 5.3% to 27.6% with similar OR compared to those with no infections (Table 4-7 to Table 4-10). Low mean cell haemoglobin concentration (MCHC) could predict malaria infection with SE(SP) of 33.3%(71.8%) and OR=1.27. About 48% prevalence of low red cell distribution width (RDW) was found among the infections groups which could predict hookworm-malaria co-infection with SE(SP) of

11%(95%) and OR=2.37 followed by hookworm with 16.8%(905%) and OR=1.92 (Table 4-7 to Table 4-10) .

Table 4-5: Impact of infection on anaemia among participants

	Anaemia		
	Yes , N=1475		
Infection status	n (%), Hb<11 g/dl	Coeff (Std Err)	P-value
No Infection	115 (7.8)	1	
Helminth	1 (0.1)	0.37 (0.25)	0.147
Malaria	62 (4.2)	0.08 (0.02)	0.001
Helminth-Malaria	47 (3.2)	0.02 (0.02)	0.416
	Yes , N=1321		
	n (%), Hb<11 g/dl	Coeff (Std Err)	P-value
No Infection	115 (8.7)	1	
Hookworm	62 (4.7)	0.08 (0.02)	0.001
Malaria	8 (0.6)	-0.05 (0.04)	0.228
Hookworm-Malaria	72 (5.5)	0.13 (0.05)	0.005

4.2.3.2 White blood cell indices

Among the various infection groups, an average of 35% leukocytosis and 24% leukopaenia were reported but none had any diagnostic predictive effect. However absolute eosinophils count predicted helminth infection with SE(SP) of 20.6%(83.2%) and OR=1.28 compared to no infections. Among those with hookworm-malaria co-infection, the odds were about twice with SE(SP) of 9.2%(94.4%) and OR=1.72 (Table 4-7 to Table 4-10). Of importance to note was that low percentage neutrophil count predicted hookworm-malaria co-infection three times the odds of those with no infection (OR=3.25) (Table 4-7 to Table 4-10). Decreased neutrophil-lymphocyte (NL) ratio predicted malaria infection with SE(SP) of 33.1%(73.3) and OR=1.36 while helminth compared to no infection was predicted with OR=1.14. Increased eosinophil-lymphocyte (EL) ratio was predictive of helminth, malaria and hookworm-malaria co-

infection, but increased basophil-lymphocyte (BL) ratio was more predictive of malaria and hookworm-malaria co-infection (Table 4-7 to Table 4-10).

4.2.3.3 Platelets

Less than 3% prevalence of thrombocytopaenia and 30% thrombocytosis were recorded among the various study infection groups. Though small, thrombocytopaenia predicted malaria infection with SE(SP) of 40.0%(71.9%) and OR=1.75 followed by hookworm-malaria co-infection with OR=1.40 (Table 4-7 to Table 4-10).

4.2.4 Infection groups and haematological indices

Considering the baseline haematological indices among the participants, the median values were significantly high for white blood cells (WBCs), monocytes, eosinophils, basophils, EL ratio and BL ratio in helminth-malaria group while the counts of neutrophils, haemoglobin (Hgb), haematocrit (Hct), mean cell volume (MCV), Platelets (Plts) and NL ratio were significantly lower. In malaria group, basophil and ML ratio were higher while mean cell haemoglobin (MCH) concentration was lower significantly. RBCs was the only parameter that was raised in the helminth group (Table 4-6). For in hookworm, WBCs, monocytes, eosinophils, ML ratio, EL ratio and BL ratio were significantly higher while neutrophils, RBCs, Hgb, Hct, MCV, RDW and NL ratio were lower significantly. Only basophils were significantly higher in the malaria group while MCH and Plt were lower (Table 4-6).

Table 4-6: Haematological profile among participants considering their infection groups

						Dunn's Pairwise Comparison test					
	No Infection	Helminth	Malaria	Helminth-Malaria	p-value	NoI vrs HEL	NoI vrs MAL	NoI vrs HELMAL	HEL vrs MAL	HEL vrs HELMAL	MAL vrs HELMAL
	N, Median (IQR)	N, Median (IQR)	N, Median (IQR)	N, Median (IQR)							
WBC, Total x 10^9/L	671 , 6 (5, 7.3)	138 , 5.9 (5, 7)	231 , 6.3 (5.3, 7.7)	89 , 6.8 (5.4, 8.7)	0.0017	0.7221	-2.3363	-3.0771**	-2.2839	-3.0499**	-1.3538
Monocytes %	671 , 6.4 (1.2, 9.3)	138 , 7.55 (1.6, 9.6)	231 , 9 (5.4, 11.7)	89 , 9.1 (6.7, 10.9)	0.0001	-1.9608	-7.3517***	-5.1015***	-3.5092***	-2.8851**	-0.1177
Monocytes x 10^9/L	671 , 0.35 (0.07, 0.58)	138 , 0.445 (0.09, 0.58)	231 , 0.59 (0.31, 0.76)	89 , 0.6 (0.41, 0.82)	0.0001	-1.4604	-7.6501***	-6.1902***	-4.1555***	-4.1325***	-0.9196
Neutrophils %	654 , 33.7 (27.9, 40.8)	136 , 32.25 (26.1, 39.5)	227 , 32.1 (24.7, 39.3)	86 , 30.2 (25.2, 34.4)	0.0002	1.2263	2.661*	3.9238***	0.8246	2.428*	1.9356
Eosinophils %	654 , 3.6 (2, 6.3)	136 , 4.1 (2.45, 7.45)	227 , 3.8 (2.4, 6.8)	86 , 5.8 (3.8, 9.3)	0.0001	-2.2334	-1.247	-5.780***	1.0553	-3.2844**	-4.4773***
Eosinophils x 10^9/L	654 , 0.21 (0.12, 0.38)	136 , 0.255 (0.14, 0.44)	227 , 0.23 (0.14, 0.42)	86 , 0.395 (0.23, 0.69)	0.0001	-1.9264	-1.6688	-6.2657***	0.4888	-3.8989***	-4.6607***
Basophils %	671 , 0.9 (0.7, 1.1)	138 , 0.9 (0.7, 1.1)	229 , 1 (0.8, 1.2)	88 , 1 (0.8, 1.2)	0.0001	0.3666	-3.9236***	-3.0882**	-3.1044**	-2.8178**	-0.3975
Basophils x 10^9/L	671 , 0.05 (0.04, 0.08)	138 , 0.06 (0.04, 0.07)	229 , 0.06 (0.05, 0.08)	88 , 0.065 (0.05, 0.095)	0.0001	0.3107	-3.8943***	-3.4040***	-3.0351***	-3.0420**	-0.701
RBC x10^12/L	671 , 4.42 (4.05, 4.77)	138 , 4.48 (4.19, 4.87)	231 , 4.24 (3.93, 4.58)	89 , 4.25 (3.98, 4.56)	0.0001	-1.996	3.9854***	2.3677*	4.5599***	3.3370**	-0.295
Hgb g/dL	671 , 12.4 (11.3, 13.6)	138 , 12.6 (11.7, 13.6)	231 , 11.9 (10.6, 12.6)	89 , 11.8 (10.7, 12.5)	0.0001	-1.5365	5.3311***	3.5299**	5.1149***	3.9855***	-0.0679
Hct %	671 , 35.5 (32.6, 39.1)	138 , 36.6 (33.9, 38.9)	231 , 34 (31, 36.2)	89 , 33.8 (31.6, 35.9)	0.0001	-2.3282	5.3994***	3.4971***	5.8510***	4.5026***	-0.1393

Continues

MCV fl	671 , 82 (76, 86)	138 , 82 (75, 87)	231 , 80 (75, 85)	89 , 79 (75, 84)	0.0323	-0.4051	2.1938	2.0333	1.9075	1.9658	0.4971
MCH pg	671 , 28.4 (26.5, 30.1)	138 , 28.1 (26.1, 30.3)	231 , 27.6 (26, 29.7)	89 , 27.9 (26, 29.7)	0.04	0.5148	2.4366*	1.931	1.2804	1.2484	0.2562
Plt x 10^9/L	671 , 250 (196, 321)	138 , 239.5 (189, 295)	231 , 236 (186, 292)	89 , 236 (192, 286)	0.0281	1.5178	2.6855*	1.51	0.5855	0.2092	-0.277
NL ratio	654 , 0.64 (0.47, 0.82)	136 , 0.61 (0.44, 0.79)	227 , 0.6 (0.42, 0.81)	86 , 0.58 (0.43, 0.73)	0.1849	0.6667	1.2649	1.9754	0.32	1.189	1.0199
ML ratio	671 , 0.11 (0.02, 0.19)	138 , 0.15 (0.03, 0.2)	231 , 0.18 (0.1, 0.23)	89 , 0.17 (0.13, 0.22)	0.0001	2.0047	-6.6535***	-4.7859***	-2.9760***	-2.5930*	-0.2592
EL ratio	654 , 0.07 (0.04, 0.12)	136 , 0.09 (0.04, 0.15)	227 , 0.07 (0.04, 0.13)	86 , 0.12 (0.07, 0.21)	0.0001	-2.1902	-1.4824	-5.5202***	0.8504	-3.0977**	-4.0987***
BL ratio	671 , 0.02 (0.01, 0.02)	138 , 0.02 (0.01, 0.02)	229 , 0.02 (0.02, 0.02)	88 , 0.02 (0.02, 0.02)	0.0001	-0.0571	-4.6167***	-3.6564***	-3.2291**	-2.9981**	-0.4865

*** : <0.05, **: <0.01, ***: <0.0001**

Table 4-7: Diagnostic performance of haematological indicators in helminth and malaria infection

	Helminth-Malaria				
	Prevalence % (95% CI)	**Sensitivity % (95% CI)**	**Specificity % (95% CI)**	**ROC area**	**OR (95% CI)**
Monocytes<4.9 %	37 (34, 40)	14.8 (11.5, 18.6)	78.9 (75.7, 81.9)	0.47 (0.45, 0.49)	0.65 (0.47, 0.9)
Monocytes>16.3 %	39 (36, 42.2)	17.6 (14.2, 21.4)	78.9 (75.7, 81.9)	0.48 (0.46, 0.51)	0.8 (0.59, 1.08)
Eosinophils>6 %	51 (48, 53.2)	20.9 (18.1, 23.9)	84.1 (81.3, 86.6)	0.53 (0.51, 0.54)	1.4 (1.08, 1.81)
Basophils>2 %	30 (28, 32.2)	17.7 (14.4, 21.5)	81.3 (78.9, 83.6)	0.5 (0.47, 0.52)	0.94 (0.71, 1.24)
RBC<3.22 x10^12	2.2 (1.5, 3.3)	4 (0.101, 20.4)	80.7 (78.2, 83)	0.42 (0.38, 0.46)	0.17 (0, 1.02)
Hgb<8 g/dL	2 (1.2, 2.98)	9.09 (1.12, 29.2)	80.8 (78.3, 83.1)	0.45 (0.39, 0.51)	0.42 (0, 1.63)
MCHC<30 g/dL	0.27 (0.06, 0.8)	0 (0, 70.8)	80.6 (78.1, 82.9)	0.4 (0.39, 0.42)	0 (0, 5.35)
RDW<12.5 %	48 (45, 50.7)	22.5 (19.1, 26.3)	84.2 (81, 87.1)	0.53 (0.51, 0.56)	1.55 (1.15, 2.09)
RDW<21.6 %	43 (40, 46)	16.9 (13.6, 20.8)	84.2 (81, 87.1)	0.51 (0.48, 0.53)	1.09 (0.78, 1.51)
Plt <88 x10^9/L	2.8 (2, 4)	12.5 (3.51, 29)	80.8 (78.3, 83.1)	0.47 (0.41, 0.53)	0.6 (0.22, 1.66)
NL ratio<0.45	33 (30, 36.7)	21.5 (16.8, 26.8)	80.7 (77.2, 83.9)	0.51 (0.48, 0.54)	1.14 (0.8, 1.63)
NL ratio>0.81	57 (55, 60.2)	16.7 (14.1, 19.5)	80.7 (77.2, 83.9)	0.49 (0.47, 0.51)	0.84 (0.63, 1.12)
ML ratio>0.25	37 (35, 39.5)	18.2 (15.2, 21.6)	81.5 (78.9, 83.8)	0.5 (0.48, 0.52)	0.98 (0.75, 1.27)
EL rati<0.04	31 (28, 34.1)	12.4 (8.61, 17)	82.3 (78.9, 85.3)	0.47 (0.45, 0.5)	0.65 (0.43, 1)
EL ratio>0.14	56 (53, 58.4)	21.1 (18.2, 24.3)	82.3 (78.9, 85.3)	0.52 (0.5, 0.54)	1.24 (0.94, 1.64)
BL ratio<0.01	9.4 (7.4, 11.8)	16.2 (8.36, 27.1)	81.6 (78.4, 84.5)	0.49 (0.44, 0.54)	0.86 (0.44, 1.67)
BL ratio>0.02	56 (54, 59)	18.6 (16.1, 21.4)	81.6 (78.4, 84.5)	0.5 (0.48, 0.52)	1.02 (0.78, 1.32)

Table 4-8: Diagnostic performance of haematological indicators in helminth and malaria infection

	Malaria				
	Prevalence % (95% CI)	**Sensitivity % (95% CI)**	**Specificity % (95% CI)**	**ROC area**	**OR (95% CI)**
Monocytes<4.9 %	37 (34, 40)	16.5 (13, 20.4)	65.5 (61.9, 69)	0.41 (0.39, 0.44)	0.38 (0.28, 0.51)
Monocytes>16.3 %	39 (36, 42.2)	28.9 (24.7, 33.3)	65.5 (61.9, 69)	0.47 (0.45, 0.5)	0.77 (0.6, 1)
Eosinophils>6 %	51 (48, 53.2)	30.1 (26.9, 33.4)	73.9 (70.7, 77)	0.52 (0.5, 0.54)	1.22 (0.98, 1.52)
Basophils>2 %	30 (28, 32.2)	28.4 (24.4, 32.7)	72 (69.3, 74.7)	0.5 (0.48, 0.53)	1.02 (0.8, 1.3)
RBC<3.22 x10^12	2.2 (1.5, 3.3)	32 (14.9, 53.5)	71.9 (69.1, 74.6)	0.52 (0.43, 0.61)	1.2 (0.53, 2.76)
Hgb<8 g/dL	2 (1.2, 3.0)	22.7 (7.82, 45.4)	71.6 (68.8, 74.2)	0.47 (0.38, 0.56)	0.74 (0.28, 1.95)
MCHC<30 g/dL	0.27 (0.06, 0.8)	33.3 (0.84, 90.6)	71.8 (69.1, 74.5)	0.53 (0.2, 0.85)	1.27 (0, 9.76)
RDW<12.5 %	48 (45, 50.7)	30.7 (26.8, 34.8)	73.9 (70.1, 77.4)	0.52 (0.5, 0.55)	1.25 (0.97, 1.62)
RDW<21.6 %	43 (40, 46)	27.5 (23.4, 32)	73.9 (70.1, 77.4)	0.51 (0.48, 0.53)	1.07 (0.81, 1.42)
Plt <88 x10^9/L	2.8 (2, 4)	40.6 (23.7, 59.4)	71.9 (69.1, 74.5)	0.562 (0.48, 0.65)	1.75 (0.864, 3.53)
NL ratio<0.45	33 (30, 36.7)	33.1 (27.6, 39)	73.3 (69.4, 76.9)	0.53 (0.5, 0.57)	1.36 (0.99, 1.86)
NL ratio>0.81	57 (55, 60.2)	27.3 (24.1, 30.6)	73.3 (69.4, 76.9)	0.5 (0.48, 0.53)	1.03 (0.8, 1.32)
ML ratio>0.25	37 (35, 39.5)	30.2 (26.5, 34.2)	73.2 (70.3, 75.9)	0.52 (0.49, 0.54)	1.18 (0.94, 1.48)
EL rati<0.04	31 (28, 34.1)	22.4 (17.5, 28)	72.1 (68.3, 75.7)	0.47 (0.44, 0.5)	0.75 (0.53, 1.05)
EL ratio>0.14	56 (53, 58.4)	30.3 (27, 33.8)	72.1 (68.3, 75.7)	0.51 (0.49, 0.54)	1.13 (0.89, 1.43)
BL ratio<0.01	9.4 (7.4, 11.8)	20.6 (11.7, 32.1)	75.8 (72.3, 79)	0.48 (0.43, 0.53)	0.81 (0.44, 1.49)
BL ratio>0.02	56 (54, 59)	31.7 (28.6, 35)	75.8 (72.3, 79)	0.54 (0.52, 0.56)	1.46 (1.16, 1.83)

Table 4-9: Diagnostic performance of haematological indicators in helminth and malaria infection

	Hookworm				
	Prevalence % (95% CI)	**Sensitivity % (95% CI)**	**Specificity % (95% CI)**	**ROC area**	**OR (95% CI)**
Monocytes<4.9 %	37 (34, 40)	6.78 (4.55, 9.65)	83.9 (81, 86.5)	0.45 (0.44, 0.47)	0.38 (0.25, 0.58)
Monocytes>16.3 %	39 (36, 42.2)	10.8 (8.09, 14)	83.9 (81, 86.5)	0.47 (0.45, 0.49)	0.63 (0.44, 0.9)
Eosinophils>6 %	51 (48, 53.2)	14.5 (12.1, 17.1)	90.3 (88, 92.3)	0.52 (0.51, 0.54)	1.58 (1.16, 2.15)
Basophils>2 %	30 (28, 32.2)	10.5 (7.85, 13.6)	87.2 (85.1, 89.1)	0.49 (0.47, 0.51)	0.8 (0.57, 1.12)
RBC<3.22 x10^12	2.2 (1.5, 3.3)	0 (0, 13.7)	86.7 (84.5, 88.6)	0.43 (0.42, 0.44)	0 (0, 1)
Hgb<8 g/dL	2 (1.2, 2.98)	9.09 (1.12, 29.2)	86.9 (84.7, 88.8)	0.48 (0.42, 0.54)	0.66 (0, 2.59)
MCHC<30 g/dL	0.27 (0.06, 0.8)	0 (0, 70.8)	86.8 (84.7, 88.8)	0.43 (0.42, 0.44)	0 (0, 8.5)
RDW<12.5 %	48 (45, 50.7)	16.8 (13.7, 20.2)	90.5 (87.8, 92.7)	0.54 (0.52, 0.56)	1.92 (1.34, 2.73)
RDW<21.6 %	43 (40, 46)	9.93 (7.31, 13.1)	90.5 (87.8, 92.7)	0.5 (0.48, 0.52)	1.05 (0.69, 1.59)
Plt <88 x10^9/L	2.8 (2, 4)	12.5 (3.51, 29)	87 (84.8, 88.9)	0.5 (0.44, 0.56)	0.96 (0.35, 2.65)
NL ratio<0.45	33 (30, 36.7)	13.1 (9.34, 17.7)	86.2 (83, 89)	0.5 (0.47, 0.52)	0.94 (0.62, 1.44)
NL ratio>0.81	57 (55, 60.2)	10.5 (8.38, 12.9)	86.2 (83, 89)	0.48 (0.47, 0.5)	0.73 (0.52, 1.02)
ML ratio>0.25	37 (35, 39.5)	11.9 (9.34, 14.8)	87.7 (85.5, 89.7)	0.5 (0.48, 0.52)	0.96 (0.7, 1.32)
EL rati<0.04	31 (28, 34.1)	5.79 (3.28, 9.37)	88.3 (85.4, 90.8)	0.47 (0.45, 0.49)	0.46 (0.26, 0.82)
EL ratio>0.14	56 (53, 58.4)	14.7 (12.2, 17.5)	88.3 (85.4, 90.8)	0.52 (0.5, 0.53)	1.3 (0.94, 1.8)
BL ratio<0.01	9.4 (7.4, 11.8)	7.35 (2.43, 16.3)	87.7 (85, 90.2)	0.48 (0.44, 0.51)	0.57 (0.23, 1.41)
BL ratio>0.02	56 (54, 59)	12.4 (10.2, 14.8)	87.7 (85, 90.2)	0.5 (0.48, 0.52)	1.01 (0.74, 1.38)

Table 4-10: Diagnostic performance of haematological indicators in helminth and malaria infection

	Hookworm-Malaria				
	Prevalence % (95% CI)	**Sensitivity % (95% CI)**	**Specificity % (95% CI)**	**ROC area**	**OR (95% CI)**
Monocytes<4.9 %	43 (40, 46.6)	2.69 (1.24, 5.04)	89.2 (85.9, 91.9)	0.46 (0.44, 0.48)	0.23 (0.11, 0.46)
Monocytes>16.3 %	42 (38, 45.2)	6.65 (4.16, 9.98)	89.2 (85.9, 91.9)	0.48 (0.46, 0.5)	0.59 (0.35, 1)
Eosinophils>6 %	49 (46, 52.5)	9.24 (6.94, 12)	94.9 (92.8, 96.6)	0.52 (0.51, 0.54)	1.91 (1.19, 3.07)
Basophils>2 %	30 (27, 32.4)	5.86 (3.57, 9.01)	92.3 (90.2, 94.1)	0.49 (0.48, 0.51)	0.75 (0.44, 1.27)
RBC<3.22 x10^12	2.2 (1.3, 3.48)	0 (0, 19.5)	92 (89.8, 93.8)	0.46 (0.45, 0.47)	0 (0, 2.61)
Hgb<8 g/dL	2.5 (1.5, 3.8)	10.5 (1.3, 33.1)	92.2 (90, 94)	0.51 (0.44, 0.59)	1.39 (0, 5.56)
MCHC<30 g/dL	-	-	-	-	-
RDW<12.5 %	46 (43, 49.8)	11 (8.01, 14.7)	95 (92.5, 96.9)	0.53 (0.51, 0.55)	2.37 (1.37, 4.07)
RDW<21.6 %	42 (39, 46.2)	5.47 (3.22, 8.61)	95 (92.5, 96.9)	0.5 (0.49, 0.52)	1.1 (0.58, 2.1)
Plt <88 x10^9/L	2.4 (1.5, 3.78)	10.5 (1.3, 33.1)	92.2 (90.1, 94)	0.51 (0.44, 0.59)	1.4 (0, 5.59)
NL ratio<0.45	31 (27, 34.9)	7.95 (4.42, 13)	91.6 (88.4, 94.1)	0.5 (0.47, 0.52)	0.94 (0.5, 1.79)
NL ratio>0.81	57 (54, 60.4)	5.9 (4.05, 8.28)	91.6 (88.4, 94.1)	0.49 (0.47, 0.51)	0.69 (0.41, 1.13)
ML ratio>0.25	36 (34, 39.4)	7.77 (5.34, 10.8)	93.2 (91.1, 95)	0.51 (0.49, 0.52)	1.16 (0.73, 1.85)
EL rati<0.04	32 (29, 36.3)	1.56 (0.323, 4.5)	93.8 (90.9, 95.9)	0.48 (0.46, 0.49)	0.24 (0.08, 0.75)
EL ratio>0.14	56 (52, 58.8)	9.98 (7.5, 12.9)	93.8 (90.9, 95.9)	0.52 (0.5, 0.54)	1.67 (1.02, 2.74)
BL ratio<0.01	9.7 (7.3, 12.6)	1.96 (0.05, 10.4)	93.7 (91.1, 95.7)	0.48 (0.46, 0.5)	0.3 (0, 1.76)
BL ratio>0.02	54 (51, 57.5)	8.27 (6.14, 10.9)	93.7 (91.1, 95.7)	0.51 (0.49, 0.53)	1.34 (0.84, 2.14)

4.3 Characterization of cell phenotypes involved in hookworm and malaria parasite co-infection in endemic areas in Ghana

4.3.1 Cell population from samples with No stimulation

For samples that were treated with just complete media (RPMI with 10% Heat-Inactivated Human Sera), all the cell phenotypes were not significantly different with respect to infection status and age category at 95% confidence level.

4.3.2 Cellular immune response induced with Pf parasitized RBC (pRBC) stimulation

In exposing cells from participants to *Pf* parasitized RBCs in culture, the cell population expanded significantly for CD4+/IFN-γ+ (p=0.025), CD4+/Foxp3+/IFN-γ+ (p=0.016), CD4+/HLA-DR+ (p=0.019), CD4+/HLA-DR+/IL-4+ (p=0.032) and CD4+/HLA-DR+/IFN-γ+ (p=0.020).

There was a significant increase in CD4+/IFN-γ+ among malaria infected individuals, about twice the increase in the other infection groups. The Th2 phenotype (IL-4) was however similar across the infection groups and also the CD4+/Foxp3+ cells. CD4+ regulatory cells producing IFN-γ+ (CD4+/Foxp3+/IFN-γ+, p=0.016) (Th1) cytokine were significantly higher in malaria infected individuals. Activated CD4+ (CD4+/HLA-DR+) cells were significantly higher in hookworm-malaria co-infected individuals. However, IFN-γ+ and IL-4+ production by activated CD4+ cells were higher in malaria infected individuals (CD4+/HLA-DR+/IFN-γ+ (p=0.020), CD4+/HLA-DR+/IL-4+ (p=0.032)).

For cytotoxic T cells, CD8+/IFN-γ+ (p=0.029) and CD8+/Foxp3+/IFN-γ+ (p=0.026) were significantly different. CD11c+/HLA-DR+/IFN-γ+ (p=0.031) and CD3+/TCR-γδ+ (p=0.026) were significantly different across infections. In addition, the cytokines IL-6 (p=0.017) and IL-4 (p=0.021) were significantly different and higher in hookworm-malaria co-infected individuals. Among the age groups, only CD11c+/HLA-DR+/Foxp3+ and CD11c+/HLA-DR+/IL-4+ were significantly different.

4.3.3 Exploring cellular immune correlates in hookworm and malaria co-infection

Among samples treated with *Pf* parasitized RBCs and at set significance level of ≤0.25 p-value for the purposes of exploring for immune correlates, CD4+, CD4+/IL-4+, CD8+/HLA-DR+, CD11c+/HLA-DR+, CD11c+/HLA-DR+/IL-4+, and CD3+/TCR-γδ+ were significant across the various infection groups (Table 4-14). For CD4+, the percentage mean cell population was significantly lower for samples from hookworm-malaria co-infected compared to no infection group (FH test: 3.9049) using the Fisher-Hayter pairwise comparison test (Table 4-14). Regarding activated cytotoxic T cells (CD8+/HLA-DR+), the significant decrease in the cell population from samples of hookworm-malaria (Coef: 0.239, p=0.014) co-infected individuals in a pairwise comparison analysis revealed no significant difference among the groups. The population of CD11c+/HLA-DR+ (APC/DC) in hookworm compared to no infection group was significantly lower and was confirmed in a pairwise comparison test (Table 4-14). There was marked and significant decrease in the mean cell population of CD3+/TCR-γδ+ cells in hookworm-malaria co-infection compared to no infection (Table 4-14). In a Fisher-Hayter pairwise comparison, the decreases were in hookworm versus hookworm-malaria co-infection (FH test: 4.5401) (Table 4-14). It is suggestive that, percentage mean CD3+/TCR-γδ+ cells in no infection group compared to hookworm-malaria co-infected group are similar. Both CD4+/IL-4+, and CD11c+/HLA-DR+/IL-4+ were upon further comparison analysis similar across the four infection groups.

4.3.4 Analysing cell population from pooled samples

Generally, all the percentage mean cell phenotypes of the CD4+ cells were similar when considered across the infection groups (Table 4-11 to Table 4-14). For cytotoxic T cell lineage, the measured population were significantly different for CD8+ (p=0.028), CD8+/IFN-γ+ (p=0.047) and Activated CD8+ cells (CD8+/HLA-DR+) (p=0.005). Difference in percentage mean of gamma delta T cells (CD3+/TCR-γδ+) (p=0.005) were also significant (Table 4-11).

Considering cytokines from the cultured supernatants, IL-6 (p=0.002), IL-4 (p=0.035), IL-10 (p=0.010) and TNF (p=0.042) were significantly different among infection groups when the samples were pooled together from no media treatment, 1% PHA treatment and *Pf* parasitized RBCs.

4.3.4.1 Pooled samples: Comparing cell population and treatment types

Considering the variables of interest in a univariate regression with significance at ≤0.25 adjusted for cluster over sample treatment type (using media, PHA or *Pf* parasitizedRBC (pRBC)) in culture, CD4+, CD4+/IFN-γ+, CD4+/IL-4+, CD4+/Foxp3+/IL-4+, CD4+/Foxp3+/IFN-γ+, CD4+/HLA-DR+, CD8+, CD8+/IL-4+, CD8+/Foxp3+, CD8+/Foxp3+/IL-4+, CD8+/Foxp3+/IFN-γ+, CD11c+/HLA-DR+, CD11c+/HLA-DR+/IFN-γ+, CD11c+/HLA-DR+/IL-4+, CD11c+/HLA-DR+/Foxp3+ and CD3+/TCR-γδ+ were significantly different.

The percentage mean population of CD4+ were increased among samples treated with 1%_PHA (Coef: 0.219, p<0.001) and pRBC (Coef: 0.171, p<0.001) when each were compared to samples treated with only media. Using the Fisher-Hayter pairwise comparison test a significant difference was observed between media and PHA then media and pRBC respectively (data not shown). Again, CD4+/IFN-γ+ had increased population when cells treated with only media treatment were compared to PHA treatment (Coef: 0.693, p<0.001) and pRBCs treatment (Coef: 0.452, p<0.001). With the exception of CD8+ cell population that did not increase significantly during the stimulation period, all cell phenotypes that were analysed had significant change when compared.

4.3.4.2 Pooled samples: Comparing cell population and infection status

At significant level set at ≤0.25 p-value for exploration, difference in population of CD4+, CD4+/IFN-γ+, CD4+/Foxp3+/IFN-γ+, CD4+/HLA-DR+, CD4+/HLA-DR+/IFN-γ+, CD8+/IFN-γ+, CD8+/Foxp3+, CD8+/Foxp3+/IFN-γ+, CD8+/HLA-DR+, CD11c+/HLA-

DR+, CD11c+/HLA-DR+/IFN-γ+ and CD3+/TCR-γδ+ were significant when adjusted for cluster over sample treatment type (media, PHA or parasitized RBC) in the pooled cells.

For CD4+, the percentage mean measured was significantly reduced among hookworm (Coef: 0.137, p=0.026) and hookworm-malaria (Coef: 0.198, p=0.016) co-infected compared to no infection respectively. However, the FH pairwise post-hoc comparison gave no indication of significance among the infection groups (Table 4-13). CD4+/IFN-γ+ was significantly reduced among hookworm infected (Coef: 0.197, p=0.035) compared to the no infection group. Among the three infected groups compared to no infection, CD4+/HLA-DR+ population were significantly reduced in those with malaria (Coef: 0.175, p=0.017).

Considering the Cytotoxic T cell population, CD8+/IFN-γ+ cells were significantly reduced in hookworm-malaria (Coef: 0538, p=0.045) co-infection compared to no infection group. CD8+/HLA-DR+ percentage means were reduced among hookworm (Coef: 0.167, p<0.001), malaria (Coef: 0.038, p=0.002) and hookworm-malaria (Coef: 0.180, p=0.049) compared to no infection group. A pairwise post-hoc comparison confirmed significance with no infection and hookworm and, no infection and hookworm-malaria co-infected (Table 4-13).

Another significant cell population to note were the CD11c+/HLA-DR+. These cells were significantly reduced among malaria (Coef: 0.250, p=0.006) and hookworm-malaria co-infection (Coef: 0.267, p=0.048) groups when compared to the no infection group respectively. However, the observation was not confirmed in a pairwise comparison (Table 4-13).

It is also important to state that CD3+/TCR-γδ+, CD8+/IFN-γ+ and CD8+/Foxp3+/IFN-γ+ were not significantly different among the groups at <0.05 confidence level in the univariate regression model. However, using the set significance level at ≤0.25 to explore the data, and performing the post-hoc analysis, significant difference in the percentage means of the cell population were identified.

This could possibly be due to the sample size used for the subgroup analysis but could also be true since the FH pairwise comparison instead of using significance at 0.05 rule, uses a studentized range critical value which is developed from the least and highest mean values from the comparing groups and could be run on any set of data even with few samples size (Bowers, 2014).

Table 4-11: Summary statistics of significant cell phenotypes and cytokines from cultured pooled samples considering infection status.

		Pooled Samples Infection Status				
		NoI	**HKW**	**MAL**	**HKWMAL**	
		N, Mean, SD	**N, Mean, SD**	**N, Mean, SD**	**N, Mean, SD**	**P-value**
Cytotoxic T Cells	**CD8+**	17, 46.7, 0.4	27, 39, 1	18, 25.9, 0.7	18, 45, 1.2	**0.028**
	CD8+/IFN-γ+	17, 13.8, 2	27, 7.6, 2.8	18, 10.3, 4.5	18, 3.3, 2	**0.047**
	CD8+/HLA-DR+	17, 46.5, 0.6	27, 31.3, 0.5	18, 42.5, 0.5	18, 30.4, 0.5	**0.005**
Gamma-delta cells	**CD3+/TCR-γδ+**	17, 3.8, 0.5	27, 3.7, 0.9	18, 4.3, 0.9	18, 1.8, 0.6	**0.005**
Cytokines	**IL-6**	17, 1.2, 0.8	27, 0.6, 0.8	18, 0.3, 0.6	18, 4.5, 8.8	**0.002**
	IL-4	17, 0.7, 0.6	27, 0.5, 0.6	18, 0.2, 0.4	18, 1.1, 1.4	**0.035**
	IL-10	17, 0.3, 0.5	27, 0.4, 0.6	18, 0.1, 0.3	18, 1.1, 1.6	**0.010**
	TNF	17, 2, 1	27, 1, 1.4	18, 0.6, 0.6	18, 4.5, 13.1	**0.042**

Table 4-12: Summary statistics of significant cell phenotypes and cytokines from pRBC treated samples among infection groups.

		Pf parasitized RBCs Treated				
		Infection Status				
		NoI	HKW	MAL	HKWMAL	
		N, Mean, SD	N, Mean, SD	N, Mean, SD	N, Mean, SD	P-value
T-Helper Cells	CD4+/IFN-γ+	5, 27.2, 0.7	7, 19.9, 0.7	4, 45.8, 0.3	4, 13.3, 0.9	**0.025**
	CD4+/Foxp3+/IFN-γ+	5, 22.2, 0.8	7, 20.7, 0.5	4, 45.7, 0.4	4, 10.3, 1.2	**0.016**
	CD4+/HLA-DR+	5, 75.3, 0	7, 55.6, 0.3	4, 57.3, 0.2	4, 77.1, 0.1	**0.019**
	CD4+/HLA-DR+/IFN-γ+	5, 7.7, 1.1	7, 5.8, 0.8	4, 23.1, 0.8	4, 3.7, 1.2	**0.020**
	CD4+/HLA-DR+/IL-4+	5, 4, 1.1	7, 8, 0.5	4, 17.1, 1.6	4, 3.5, 0.9	**0.032**
Cytotoxic T Cells	CD8+/IFN-γ+	5, 8.2, 1.6	7, 7.6, 1.5	4, 29.5, 0.8	4, 2.9, 1.2	**0.029**
	CD8+/Foxp3+/IFN-γ+	5, 21.4, 1.1	7, 18.2, 0.9	4, 48.7, 0.5	4, 8.8, 1.2	**0.026**
APC/DC	CD8+/HLA-DR+//IFN-γ+	5, 3.2, 1.4	7, 3.3, 1.2	4, 15.6, 0.8	4, 1.9, 1.3	**0.032**
	CD11c+/HLA-DR+/IFN-γ+	5, 13.6, 1.2	7, 13.8, 0.7	4, 41.7, 0.7	4, 8.1, 1.3	**0.031**

Gamma-delta cells	**CD3+/TCR-γδ+**	5, 5.3, 0.7	7, 6.8, 0.6	4, 7, 0.5	4, 2.1, 0.5	**0.026**
Cytokines	**IL-6**	5, 1.1, 0.6	7, 0.8, 0.9	4, 0.5, 0.6	4, 16, 8.3	**0.017**
	IL-4	5, 0.8, 0.5	7, 0.2, 0.5	4, 0.2, 0.5	4, 1.9, 0.5	**0.021**

Table 4-13: Cell phenotypes involved in immune response in hookworm and malaria infections

	HKW	MAL	HKWMAL		NoI vs HKW	NoI vs MAL	NoI vs HKWMAL	HKW vs MAL	HKW vs HKWMAL	MAL vs HKWMAL
	Coef, p-value, 95%CI	Coef, p-value, 95%CI	Coef, p-value, 95%CI	Studentized Critical value	FH-test	FH-test	FH-test	FH-test	FH-test	FH-test
CD4+	-0.137, 0.026 (-0.23,-0.04)	-0.044, 0.314 (-0.19,0.1)	-0.198, 0.016 (-0.31,-0.09)	3.3807583	2.1203	0.6291	2.8147	1.4581	0.9709	2.2174
CD4+/IFN-γ+	-0.197, 0.035 (-0.36,-0.03)	-0.233, 0.403 (-1.18,0.72)	-0.443, 0.07 (-0.98,0.09)	3.3807583	1.4375	1.5524	2.9526	0.2627	1.819	1.4207
CD4+/Foxp3+/IFN-γ+	-0.173, 0.171 (-0.53,0.18)	-0.255, 0.451 (-1.44,0.93)	-0.52, 0.088 (-1.23,0.19)	3.3807583	1.2061	1.6338	3.3249	0.5887	2.4682	1.7157
CD4+/HLA-DR+	-0.047, 0.54 (-0.32,0.23)	-0.175, 0.017 (-0.27,-0.08)	-0.007, 0.599 (-0.06,0.04)	3.3807583	0.7771	2.6768	0.1106	2.1844	0.6677	2.6037
CD8+/Foxp3+	-0.073, 0.111 (-0.19,0.04)	-0.113,0.304(-0.47,0.24)	-0.127,0.202(-0.42,0.16)	3.3807583	1.3483	1.9025	2.1334	0.7427	0.9992	0.2342
CD8+/Foxp3+/IFN-γ+	-0.31, 0.172 (-0.95,0.33)	-0.25,0.513(-1.62,1.12)	-0.54,0.087(-1.27,0.19)	3.3807583	2.4236	1.7934	3.8717*	0.4727	1.8372	2.1086
CD11c+/HLA-DR+	-0.194, 0.136 (-0.54,0.15)	-0.25, 0.006 (-0.33,-0.17)	-0.267,0.048(-0.53,-0.01)	3.3807583	2.2658	2.6721	2.8566	0.6644	0.8695	0.1872
CD11c+/HLA-DR+/IFN-γ+	-0.261, 0.24 (-0.94,0.42)	-0.13,0.687(-1.33,1.07)	-0.501,0.137(-1.39,0.39)	3.3807583	1.8384	0.8391	3.233	0.9379	1.7228	2.4289
CD3+/TCR-γδ+	-0.01, 0.907 (-0.32,0.31)	0.041,0.622(-0.26,0.34)	-0.228,0.053(-0.46,0.01)	3.3807583	0.1851	0.7079	3.9758*	0.9751	4.2306*	4.7521*
CD4+/HLA-DR+/IFN-γ+	-0.204, 0.066 (-0.44,0.03)	-0.184,0.605(-1.49,1.12)	-0.418,0.073(-0.93,0.1)	3.3807583	1.5877	1.3155	2.9822	0.1534	1.699	1.691
CD8+/IFN-γ+	-0.238, 0.173 (-0.73,0.25)	-0.117,0.736(-1.42,1.19)	-0.538,0.045(-1.05,-0.03)	3.3807583	1.866	0.8413	3.8593*	0.9636	2.3907	3.062
CD8+/HLA-DR+	-0.167, 0 (-0.17,-0.16)	-0.038,0.002(-0.04,-0.03)	-0.18,0.049(-0.36,0)	3.3807583	4.1653*	0.8664	4.1192*	3.2751	0.3401	3.3002

The cell population of respective infection groups were compared to NoI. The pairwise comparison was carried out. Tests with * indicates values significantly different from the Studentized Critical value (SCV) of the group.

Table 4-14: Differences in T-cell population and cytokine production considering infection after malaria antigen treatment.

	***Pf* Parasitized Red Blood Cells (pRBC)**									
	Infection Status				POST-HOC ESTIMATES					
	HKW	MAL	HKWMAL	SCV	NoI vs HKW	NoI vs MAL	NoI vs HKWMAL	HKW vs MAL	HKW vs HKWMAL	MAL vs HKWMAL
	Coef, pvalue, 95%CI	Coef, pvalue, 95%CI	Coef, pvalue, 95%CI	FH-test	FH-test	FH-test	FH-test	FH-test	FH-test	FH-test
CD4+	-0.09, 0.247 (-0.25, 0.07)	-0.08, 0.358 (-0.27, 0.1)	-0.24, 0.014 (-0.42,-0.06)	3.649139	1.6989	1.3375	3.9049*	0.1557	2.5922	2.4357
CD8+/HLA-DR+	-0.17, 0.103 (-0.37, 0.04)	-0.04, 0.743 (-0.27, 0.2)	-0.28, 0.021 (-0.52, -0.05)	3.649139	2.4446	0.4716	3.6112	1.779	1.5812	2.9786
CD11c+/HLA-DR+	-0.29, 0.011 (-0.51, -0.08)	-0.24, 0.056 (-0.49, 0.01)	-0.2, 0.102 (-0.45, 0.05)	3.649139	4.0435*	2.9168	2.4545	0.6557	1.1505	0.4386
CD3+/TCR-γδ+	0.09, 0.459 (-0.16, 0.34)	0.1, 0.466 (-0.19, 0.39)	-0.32, 0.033 (-0.6,-0.03)	3.649139	1.0734	1.0571	3.305	0.1286	4.5401*	4.1383*
CD4+/IL-4+	-0.02, 0.528 (-0.11,0.06)	0.05, 0.297 (-0.05,0.14)	0.04,0.406(-0.06,0.13)	3.649139	0.9124	1.5246	1.2066	2.4841	2.1437	0.3017
CD11c+/HLA-DR+/IL-4+	-0.01,0.863(-0.09,0.08)	0.09,0.074(-0.01,0.19)	0.03,0.513(-0.07,0.13)	3.649139	0.2474	2.7018	0.9472	3.1227	1.2448	1.6646

The cell population of respective infection groups were compared to NoI. The pairwise comparison was carried out. Tests with * indicates values significantly different from the Studentized Critical value (SCV) of the group.

4.4 Humoral immune response induced by helminth and malaria parasite co-infection in the middle-belt of Ghana

4.4.1 Summary presentation of concentrations of Plasma Antibodies and Cytokines

Estimation of concentration of IgG to *NF54* antigens (p=0.007) and IgG to hookworm antigens (p=0.018) were significantly different among the infection groups (no infection, helminth (hookworm), malaria, helminth-malaria) in the study. Concentrations for IgG to *Pf 7G8* antigens, IgG1 to *Pf 7G8* antigens, IgM to *Pf 7G8* antigens and IgG to *Trichuris trichiura* antigens were similar (Table 4-15).

Among the age categories, there were statistical difference in IgG to *NF54* antigens (p=0.023), IgM to *Pf* antigens (p=0.021), IgG to hookworm antigens (p=0.019) and IgG to *Trichuris trichiura* antigens (p=0.007).

Concentrations of plasma cytokines TNF-A (p=0.007), IL-4 (p=0.01), IL-5 (p=0.009) and IL-6 (p<0.0001) were significantly different when compared among the infection status of participants (Table 4-15). Higher mean concentrations of these cytokines were measured in malaria infected samples.

4.4.2 Plasma cytokine concentrations among infection groups

The mean TNF-A concentration was significant when considered among the infection groups (p=0.0143) (Table 4-15). Also, TNF-A concentrations among no infection, hookworm, malaria and hookworm-malaria co-infected were significant (p=0.0007) (Table 4-15). The highest mean concentration was measured among malaria infected individuals. In a pairwise comparison using Fisher-Hayter test, the differences were between malaria to helminth (FH test: 4.4989) and malaria to helminth (FH test: 3.5070) were significantly different.

Considering IL-5 production, the difference in a pairwise comparison was significantly different between malaria and helminth (FH test: 3.4135) (Table 4-15). Malaria increased the

production of IL-5 and even in co-infection with helminth but in a pairwise comparison among the infection group, the difference in concentration for IL-5 was not significant. IL-4 was significantly different when concentrations were compared among helminth to malaria (FH test: 4.2743) and malaria to helminth-malaria co-infection (FH test: 3.3959) (Table 4-15).

Concentrations of IL-4 in helminth and helminth-malaria co-infection compared to malaria were significantly lower (Table 4-15). There was however no difference when compared to those with no infection.

Concentrations of IL-6, just like IL-4 and TNF-A, were significantly different when compared to concentrations from helminth infected (FH test: 5.8513) and also when helminth-malaria co-infected (FH test: 4.4184) were compared to malaria infected individuals (Table 4-15).

4.4.3 Antibody concentrations among infection groups

IgG antibody response to *Pf* NF54 antigen was significantly higher when comparing no infection group to helminth (FH test: 3.7492) and to helminth-malaria co-infected (FH test: 5.2243). IgG antibody to hookworm antigens were higher when helminth infected group was compared to no infection (FH test: 3.6222) and malaria (FH test: 3.6166) respectively (Table 4-15).

Antibody to hookworm between no infection and helminth-malaria co-infected were similar. When IgG to *Pf* NF54 antigen were compared among infection groups, a pairwise comparison analysis revealed significant difference between malaria and no infection. Also, for IgG1 to *Pf* 7G8 antigen, the concentration was significantly higher than in hookworm infected compared to no infection (Table 4-15).

Considering IgG to hookworm antigen, the concentration in a pairwise comparison was significantly different between no infection and hookworm as well as between malaria and hookworm (Table 4-15).

Table 4-15: Impact of the significant concentrations of plasma cytokines and antibodies among individuals and their infection status.

	Infection Groups				
	NoI	**HEL**	**MAL**	**HELMAL**	**P-value**
Variables in log scale, N,mean(SD)					
TNF-A	21,0.78(0.86)	**57,0.48(0.88)l**	**26,1.17(1.09)f,l**	**27,0.54(0.87)f**	**0.0143**
IL-5	21,0.23(0.41)	**57,0.24(0.52)l**	**26,0.57(0.76)l**	27,0.29(0.53)	0.0938
IL-4	21,0.44(0.72)	**57,0.34(0.73)l**	**26,0.9(1)f,l**	**27,0.39(0.71)f**	**0.0237**
IL-6	21,0.72(0.74)	**57,0.43(0.77)l**	**26,1.22(0.98)f,l**	**27,0.52(0.78)f**	**0.0007**
IgG (Pf:7G8 antigen)	21,0.21(0.06)	57,0.23(0.05)	26,0.24(0.03)	27,0.23(0.04)	0.357
IgG (Pf:NF54 antigen)	21,0.42(0.09)	**57,0.46(0.07)a**	**26,0.49(0.04)a**	27,0.44(0.07)	**0.0031**
IgG1 (Pf:7G8 antigen)	21,0.05(0.02)	57,0.06(0.03)	26,0.06(0.01)	27,0.05(0.01)	0.2529
IgM (Pf:7G8 antigen)	21,0.06(0.02)	57,0.07(0.04)	26,0.07(0.03)	27,0.07(0.03)	0.3331
IgG (Hookworm antigen)	21,0.17(0.08)	**57,0.24(0.13)a,l**	**26,0.17(0.09)l**	27,0.21(0.12)	**0.0202**
IgG (Trichuris antigen)	21,0.12(0.1)	57,0.12(0.06)	26,0.1(0.05)	27,0.1(0.08)	0.6217

	NoI	**HKW**	**MAL**	**Hnana-Malaria**	**HKWMAL**	**P-value**
Variables in log scale, N,mean(SD)						
IgG (Pf:7G8 antigen)	21,0.21(0.06)	27,0.24(0.04)	26,0.24(0.03)	9,0.22(0.03)	18,0.24(0.04)	0.1636
IgG (Pf:NF54 antigen)	21,0.42(0.09)	27,0.46(0.07)	**26,0.49(0.04)a**	9,0.46(0.06)	18,0.44(0.07)	**0.0049**
IgG1 (Pf:7G8 antigen)	21,0.05(0.02)	**27,0.07(0.04)a**	26,0.06(0.01)	9,0.05(0.01)	18,0.06(0.02)	0.0936
IgM (Pf:7G8 antigen)	21,0.06(0.02)	27,0.08(0.05)	26,0.07(0.03)	9,0.07(0.03)	18,0.07(0.03)	0.4603
IgG (Hookworm antigen)	21,0.17(0.08)	**27,0.26(0.12)a,b**	**26,0.17(0.09)b**	9,0.16(0.05)	18,0.24(0.13)	**0.0037**
IgG (Trichuris antigen)	21,0.12(0.1)	27,0.12(0.06)	26,0.1(0.05)	9,0.11(0.12)	18,0.1(0.05)	0.7203

***Other helminth: *Strongyloides stercoralis*/*Taenia spp*/*Trichuris trichiura*/Hookoworm-Hnana**

Fisher-Hayter pairwise comparisons, significance: < 0.05:a = difference between group and no infection,b = difference between hookworm and malaria infection,c = difference between hookworm and helminth-malaria infection,d = difference between hookworm and Hookworm-Malaria infection,e = difference between hookworm and other intestinal parasite infection,f = difference between malaria and helminth-malaria infection,g = difference between malaria and Hookworm-Malaria infection,h = difference between malaria and other intestinal parasite infection,i = difference between helminth-malaria and Hookworm-Malaria infection,j = difference between helminth-malaria and other intestinal parasite infection,k = difference between Hookworm-Malaria and other intestinal parasite infection,l = difference between indicated infection and other helminth parasite infection

4.4.4 Influence of infection in humoral immunity from cell stimulation and culture supernatant

4.4.4.1 Measures among No Infection group

After stimulation with pRBC for 8 hours, among those with no infection, only IFN-γ and IL-17A (Coeff: 0.888, p=0.044) had a significant correlation coefficient (Figure 4-4).

4.4.4.2 Hookworm versus No Infection group

IL-6 and IL-10 (Coef: 0.922, p=0.003), TNF-A (Coef: 0.763, p=0.046), IFN-γ (Coef: 0.798, p=0.031) and IL-17A (Coef: 0.837, p=0.019) significantly correlated compared to no infection. Among this group, IL-10 had a positive and significant correlation with IFN-γ (Coef: 0.940, p=0.002) and IL-17A (Coef: 0.968, p<0.001). Again, IFN-γ and IL-17A (Coef: 0.911, p=0.004) significantly correlated and this was similar to the observation among those with no infection status (Figure 4-4).

4.4.4.3 Malaria versus No Infection group

IL-6 and IL-2 (Coef: 0.971, p=0.030) correlated significantly just as IL-10 and IL-17A (Coef: 1.000, p<0.001) when compared to no infection. The relationship between IL-4 and IL-10 (Coef: 1.000, p<0.001) as well as IL-4 and IL-17A (Coef: 1.000, p<0.001) were significantly different. Again, IFN-γ and IL-17A concentrations was significantly different in malaria.

4.4.4.4 Hookworm-Malaria versus No Infection

It was only IL-4 and IL-17A (Coef: 0.957, p=0.043) that had a significantly different relationship among samples from hookworm-malaria co-infection (Figure 4-4).

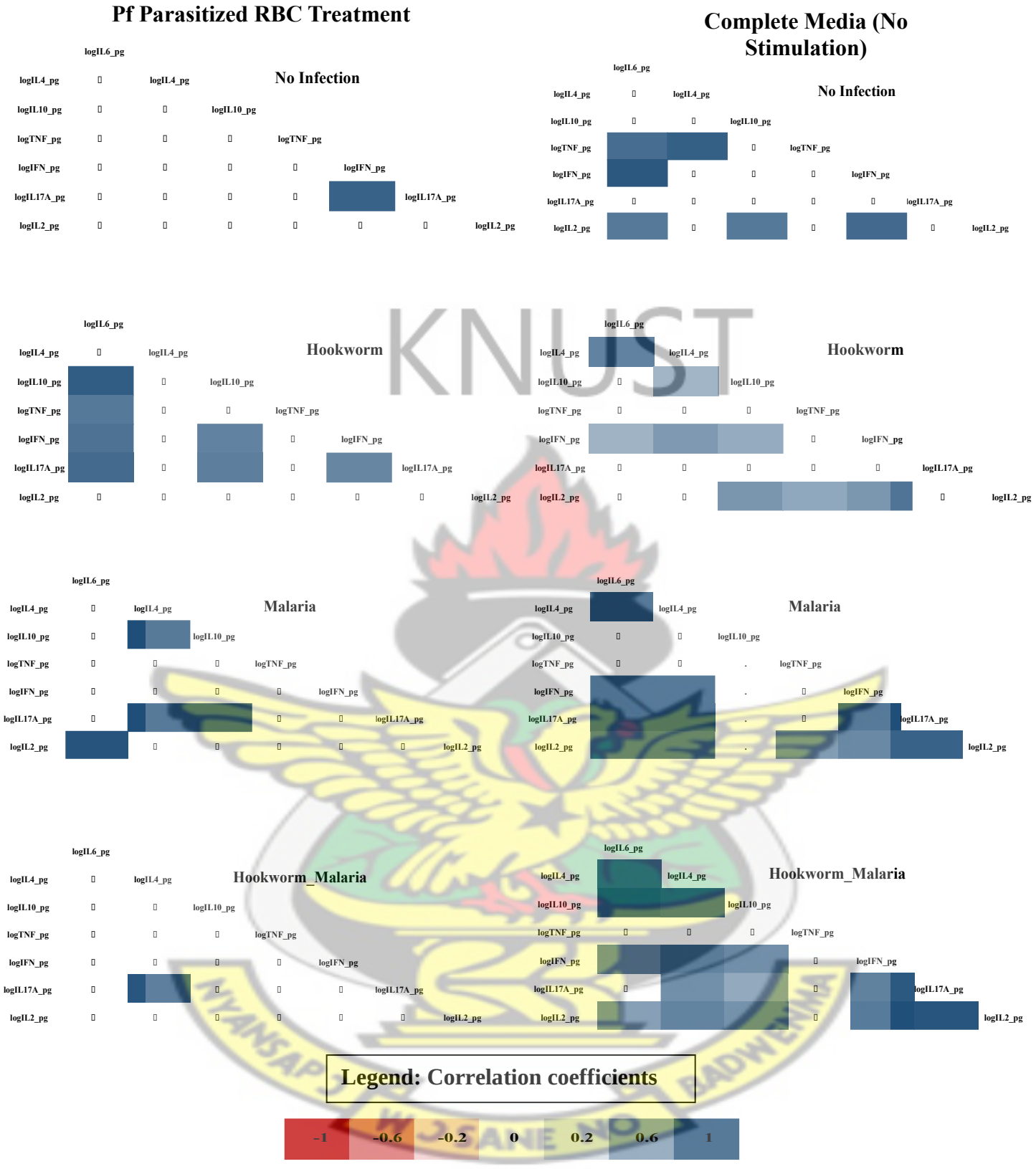


Figure 4-4: Heatmap of significant pairwise correlation coefficients for cytokine production from cell stimulation culture and infection groups.

4.4.5 Exploring factors that influence cytokine production (≤ 0.25 significant level)

4.4.5.1 Using samples treated with Media:

The concentrations of the cytokines involved IL-6, IL-4, IL-10, TNF-A, IFN-γ, IL-17A and IL-2 were not significantly different when considered among the various infections in the study and samples that were not exposed to stimulation.

4.4.5.2 The impact of ccytokines on other cytokines in culture with no treatment:

In the samples treated with no stimulant, IL-6 caused significant increase in the concentrations of IL-10 (Coef: 1.39, p<0.001) and significant decrease in IL-2 (Coef: 0.40, p=0.023). The presence of IL-4 caused a significant increase in concentration of IFN-γ (Coef: 0.40), p=0.019).

IL-10 on the other hand increased the concentration of IL-6 (Coef: 0.33, p<0.001) and IL-2 (Coef: 0.25, p=0.002) and decreased concentration of IL-17A (Coef: 0.53, p=0.002) significantly. TNF-A concentration did not significantly influence the concentrations of the other cytokines.

IFN-γ also caused an increase in the concentration of IL-4 (Coef: 0.38, p=0.019) and IL-2 (Coef: 0.25, p<0.001). IL-17A influenced significantly by increasing IL-2 (Coef: 0.15, p=0.027) concentration and decreasing IL-10 concentration (Coef: 0.35, p=0.01).

4.4.5.3 Using samples treated with pRBC:

IL-6 was significantly different when considered among the infection groups (p=0.017). It was only in hookworm-malaria co-infection (Coef: 0.909, p=0.011) that a significant increase was observed when compared to no infection group. Fisher-Hayter pairwise comparison indicated a significant difference in the concentration of IL-6 among no infection and hookworm-malaria co-infection (FH test: 4.0466), hookworm and hookworm-malaria (FH test: 4.6301) and then malaria and hookworm (FH test: 4.4964) (Table 4-16).

IL-4 concentration was different among the various infection groups (p=0.021). From a pairwise comparison using the Fisher-Hayter test, the difference existed between hookworm and hookworm-malaria (FH test: 4.6065) then malaria and hookworm-malaria co-infection (FH test: 4.1861) (Table 4-16).

IL-10 concentration was similar across the infection groups (p=0.069). Based on its role on pro-inflammatory cytokines, the cytokine in a pairwise comparison revealed that the difference between o infection and hookworm-malaria co-infection (FH test: 3.7157) was significant (Table 4-16).

In exploring TNF-A (p=0.061) across infection groups, a significant difference was seen between hookworm and hookworm-malaria (FH test: 3.9110). The difference between hookworm and hookworm-malaria (Table 4-16) was low. Malaria might increase the production in the presence of hookworm.

Concentrations of IFN-γ, IL-2 and IL-17A were not statistically different when considered among the various infection groups and even at significance level of <0.25 for exploration. Cytokine production was not affected by the age of the participants from which the cells used for the culture were collected (Table 4-16).

4.4.5.4 Influence of cytokines on others in samples treated with pRBC:

Considering samples treated with parasitized RBCs, IL-6 almost doubled the production of IL-10 (Coef: 1.96, p<0.001) and by that same margin decreased the production of IL-17A (Coef: 2.30, p=0.042). IL-4 had no significant influence on the concentration of any of the cytokines.

IL-10 only had significant influence in increasing the concentration of IL-6 (Coef: 0.37, p<0.001). TNF-A did not have any significant influence on the concentrations of the cytokines. But IFN-γ significantly increased the concentrations of IL-17A (Coef: 1.15, p=0.023) and IL-2 (Coef: 0.19, p=0.035).

IL-17A significantly caused a decrease in the concentration of IL-6 (Coef: 0.12, p=0.042) and an increase in IFN-γ (Coef: 0.29, p=0.023). IL-2 had a significant influence on the increase of IFN-γ concentration (Coef: 1.57, p=0.035).

Table 4-16: Differences in cytokine production considering infection after malaria antigen treatment.

	***Pf* Parasitized Red Blood Cells (pRBC)**									
	Infection Status			POST-HOC ESTIMATES						
	HKW	MAL	HKWMAL	Studentized Critical value	NoI vs HKW	NoI vs MAL	NoI vs HKWMAL	HKW vs MAL	HKW vs HKWMAL	MAL vs HKWMAL
	Coef, pvalue, 95%CI	Coef, pvalue, 95%CI	Coef, pvalue, 95%CI		FH-test	FH-test	FH-test	FH-test	FH-test	FH-test
IL-6	-0.06, 0.824 (-0.65,0.53)	-0.16, 0.631 (-0.83,0.52)	0.91, 0.011 (0.24,1.58)	3.649139	0.3203	0.693	4.0466*	0.4425	4.6301*	4.4964*
IL-4	-0.16, 0.153 (-0.38,0.06)	-0.17, 0.184 (-0.42,0.09)	0.21, 0.102 (-0.05,0.46)	3.649139	2.1238	1.9622	2.4503	0.116	4.6065*	4.1861*
IL-10	0.1, 0.516 (-0.22,0.42)	0.01, 0.974 (-0.36,0.37)	0.45, 0.018 (0.09,0.81)	3.649139	0.9383	0.0476	3.7157*	0.8256	3.1002	3.4799
TNF	-0.25, 0.542 (-1.09,0.59)	-0.28, 0.55 (-1.24,0.68)	0.93, 0.058 (-0.04,1.89)	3.649139	0.8802	0.8642	2.886	0.1026	3.9110*	3.5577
IFN-γ	-0.06, 0.738 (-0.41,0.3)	-0.11, 0.562 (-0.52,0.29)	0.15, 0.433 (-0.25,0.56)	3.649139	0.4807	0.8367	1.1376	0.4464	1.6666	1.873
IL-17A	0, 0.986 (-0.2,0.2)	-0.07, 0.519 (-0.3,0.16)	0.09, 0.426 (-0.14,0.32)	3.649139	0.0261	0.9329	1.1555	0.974	1.2611	1.9812
IL-2	-0.04, 0.906 (-0.73,0.65)	-0.22, 0.569 (-1.01,0.57)	0.44, 0.255 (-0.35,1.23)	3.649139	0.1699	0.8233	1.6706	0.7224	1.9466	2.3659

4.5 Co-infection of helminths and malaria parasite in an endemic region: modulation of immune correlates and environmental factors

4.5.1 PCA from population plasma antibodies and plasma cytokines

The variables used in the PCA were transformed prior to using them in the analysis. Variables from the i). demographic characteristics (age, weight, height), ii). blood counts (white blood cells (WBC), Lymphocytes (%), Monocytes (%), Neutrophils (%), Eosinophils (%), Basophils (%), red blood cells (RBC), haemoglobin (Hgb), haematocrit (Hct)) and iii). plasma antibodies and cytokine (IgG (hookworm antigen), IgG (trichuris antigen), IgG (Pf:NF54 antigen), IgG (Pf:7G8 antigen), IgG1 (Pf:7G8 antigen), IgM (Pf:7G8 antigen), IL-6, IL-5, IL-4, TNF-A) were put into the PCA (Table 4-17).

The PCs from all the variables produced 22 components from which APC1 (component 1) and APC2 (component 2) were selected (Figure 4-5). The first two components (APC1 and APC2) explained 38% of that variation and together with the third component (comp3), more than 50% which was the set level used for selecting which variables to use.

Table 4-17: Principal components scores and proportion contributions of variables to explain variations in analysis

Variable	APC1	APC2	Explained (%)	AnCyPC 1	AnCyPC 2	Explained (%)	AnPC 1	AnPC 2	Explained (%)	CyPC 1	CyPC2	Explained (%)
Age	0.293	-0.2062	57.4									
Weight	0.3188	-0.2102	65.7									
Height	0.3224	-0.1989	65.2									
WBC, Total	-0.1709	0.2254	32.3									
Lymphocytes (%)	-0.0507	-0.1122	5.7									
Monocytes (%)	0.0115	0.054	1.1									
Neutrophils (%)	0.029	0.1203	5.5									
Eosinophils (%)	0.0528	-0.0505	2.3									
Basophils (%)	-0.209	0.22	38.6									
RBC	0.0992	-0.1457	12.3									
Hgb	0.2287	-0.2358	45.4									
Hct	0.2132	-0.2579	45.8									
IgG (Hookworm antigen)	0.2438	-0.0527	30.3	0.1954	0.2233	28.3	0.3125	0.5157	57.9			
IgG (Trichuris antigen)	0.161	-0.0345	13.2	0.1306	0.0794	8.6	0.1608	0.7786	74.4			
IgG (Pf:NF54 antigen)	0.1871	-0.0746	19.2	0.1454	0.4585	62.1	0.4386	-0.1306	58.3			
IgG (Pf:7G8 antigen)	0.2578	0.0517	33.8	0.2889	0.4075	76.4	0.5087	-0.0319	76.0			
IgG1 (Pf:7G8 antigen)	0.2134	0.0501	23.4	0.245	0.4398	73.7	0.4997	-0.2137	78.3			
IgM (Pf:7G8 antigen)	0.1686	0.0872	16.7	0.2172	0.3572	51.8	0.4191	-0.253	58.6			
IL-6	0.2276	0.397	81.0	0.4089	-0.2683	86.8				0.4883	0.6945	97.9
IL-5	0.2667	0.3537	79.1	0.4266	-0.2301	88.0				0.4932	-0.6261	97.6
IL-4	0.2683	0.3769	85.5	0.4403	-0.2374	93.7				0.5096	-0.2784	96.3
TNF-A	0.2572	0.3892	85.9	0.4358	-0.2555	94.4				0.5086	0.2193	95.3

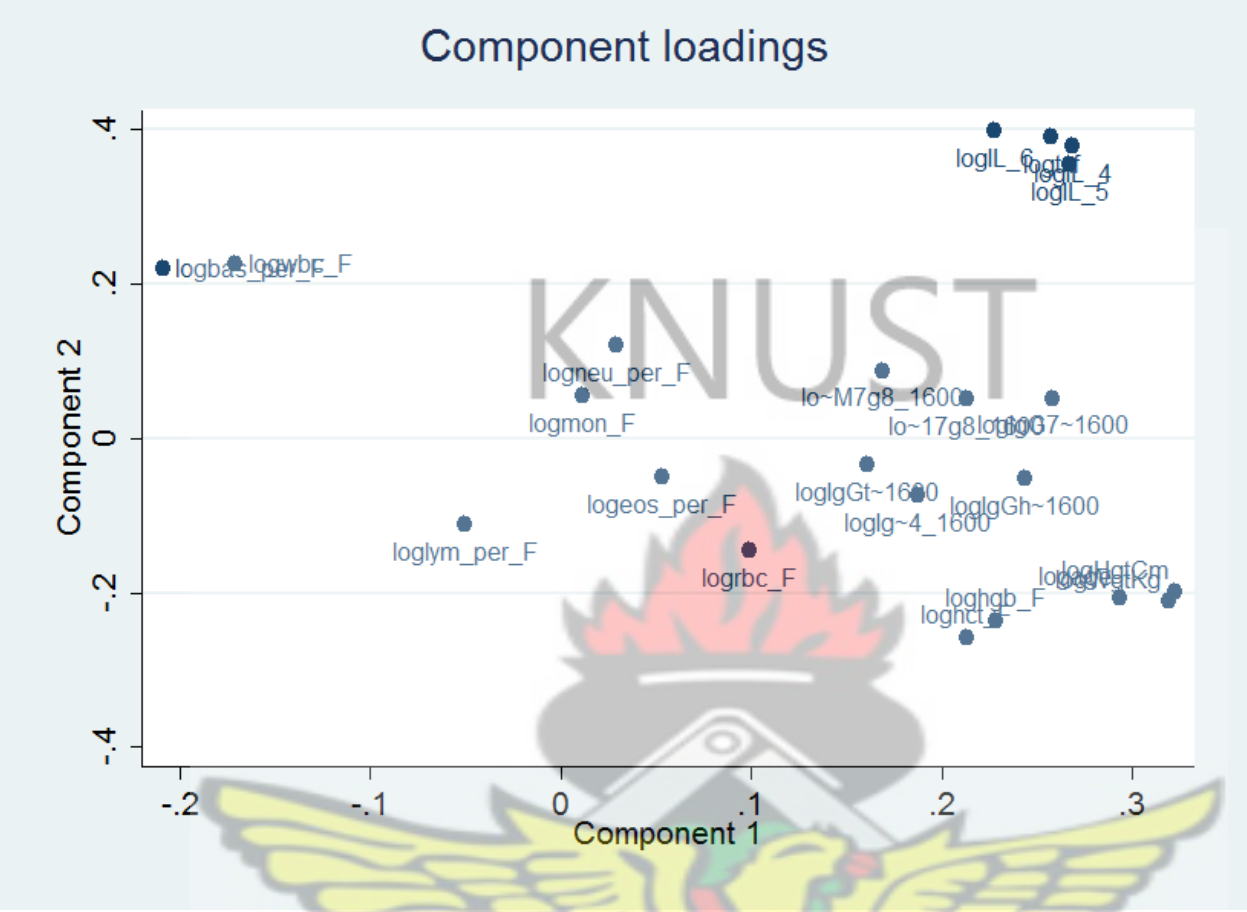


Figure 4-5: Plot of component loading of 22 variables in a PCA using two major components from plasma variables

4.5.1.1 Exploring component loadings by variables

A total of four (4) pairs of two major components were developed from the data obtained from plasma. Mainly, APC1 and APC2 (from demographic, blood cell counts, antibodies measured and cytokine estimation), AnCyPC1 and AnCyPC2 (from antibodies measured and cytokine estimation), AnPC1 and AnPC2 (from antibodies measured), then CyPC1 and CyPC2 (from cytokine estimation) (Table 4-17).

For APC1, all variables positively loaded into it except WBC, lymphocyte and Basophil. However, for APC2, the contributions to the component were by WBC, monocyte, neutrophils, basophils, IgG (*Pf*:NF54 antigen), IgG (*Pf*:7G8 antigen), IgG1 (*Pf*:7G8 antigen), IgM (*Pf*:7G8 antigen) in addition to the cytokines.

AnCyPC1 was positively loaded by all the antibodies measured and cytokines but AnCyPC2 was built by positive loading from only the antibodies. AnPC1 was formed by all the antibodies measured but for AnPC2, only IgG (Hookworm antigen) and IgG (*Trichuris trichiura* antigen) made the component with negative contribution from the others.

Similarly, CyPC1 was positively loaded by all four cytokines but only IL-6 positively influenced CyPC2 as the others provided a negative influence (Table 4-17). Largely, each of the antibody estimates used in the multivariate PC have relatively high contribution to APC1, but only IgG (*Pf*:7G8 antigen), IgG1 (*Pf*:7G8 antigen) and IgM (*Pf*:7G8 antigen) that relate positively to both components unlike IgG (*Pf*:NF54 antigen)), IgG (Hookworm antigen), IgG (*Trichuris trichiura* antigen) which had decreased contribution to the APC2.

The variables age, weight, height, Hgb and Hct had high contributions to APC1 and negative relationship to APC2 as compared to lymphocytes, neutrophils, monocytes, eosinophils and RBCs which fairly remained neutral when compared to the two components (APC1 and

APC2). WBC values and basophil estimation had high contribution to APC2 and negatively to APC1 (Figure 4-5).

APC1 accounted for 23% of the contributions of the components from the 22 variables put into the PCA. Using the first two components (APC1 and APC2) to assess the effect of some risk factors on the component across the population, Infection status (p=0.006), age group (p<0.001), BMI (p<0.001), education (p=0.04), scrubbing of nails during hand wash (p=0.005), bed share (p=0.006) and directly involved with animal rearing (p<0.001) were found to have significant effect (Appendix 8.3). For APC2, Infection status (p<0.001), age group (p=0.001), BMI (p=0.002), education (p=0.03) and use of refuse dump (p=0.04) were found to have significant effect on the component.

4.5.1.2 Exploring PCA from plasma antibodies and cytokines

Using the variables from antibodies and cytokines without the demographic characteristics, two major components could now explain 66.4% of the variation observed from the PCA with 41% attributed to the component 1 (AnCyPC1). Considering the components built from plasma cytokines and antibodies, Infection status (p<0.001), age group (p=0.007), scrubbing of nails during hand wash (p=0.018) and directly involved with animal rearing (p<0.001) had significant effect on AnCyPC1. Continuing with AnCyPC2, occupation (p=0.032), season (p=0.029) and directly involved with animal rearing (p<0.001) had significant effect on the various that made the component.

4.5.1.3 Exploring PCA from plasma antibodies

With only antibodies from plasma, the two major components in the PCA (AnPC1 and AnPC2) accounted for 67% of the variation after analysis. For AnPC1, age group (p=0.003), occupation (p=0.006), scrubbing of nails during hand wash (p=0.034) and directly involved in animal rearing (p<0.001) had significant effect. Also, on AnPC2, occupation (p=0.041), means of

defaecation (p=0.014), source of drinking water (p=0.024) and animal reared in compound (p=0.018) had significant effect.

4.5.1.4 Exploring PCA from only plasma cytokines

Considering only plasma cytokine variables, the two components; CyPC1 and CyPC2, were responsible for 96.8% of the variation seen in the PCA. Looking at the effects of some factors on the components, only Infection status (p<0.001) had significant effect with no observed significant effect by the factors on the second component CyPC2.

Table 4-18: Assessing the effects of hookworm and malaria parasitic infections on the principal components using Mann-Whitney test

Variables from		HKW	NoI		MAL	NoI		HKWMAL	NoI	
		medan(IQR)	medan(IQR)	p-value	medan(IQR)	medan(IQR)	p-value	medan(IQR)	medan(IQR)	p-value
PLASMA	APC1	21, -0.12 (3.7)	27, 1.07 (2.2)	0.047	21, -0.12 (3.7)	26, 0.09 (2.6)	0.480	21, -0.12 (3.7)	18, 0.48 (2.51)	0.430
	APC2	21, -0.19 (2.86)	27, -0.28 (2.23)	0.926	21, -0.19 (2.86)	26, 1.72 (3.32)	0.025	21, -0.19 (2.86)	18, 0.29 (2.95)	0.311
	AnCyPC 1	21, -0.5 (2.67)	27, -0.27 (3.81)	0.072	21, -0.5 (2.67)	26, 1.12 (3.32)	0.036	21, -0.5 (2.67)	18, -0.29 (3.43)	0.367
	AnCyPC 2	21, -0.61 (1.83)	27, 0.68 (2.25)	0.066	21, -0.61 (1.83)	26, -0.17 (1.85)	0.4284	21, -0.61 (1.83)	18, -0.35 (2.03)	0.447
	AnPC1	21, 0.18 (2.54)	27, 0.88 (1.92)	0.043	21, 0.18 (2.54)	26, 0.22 (1.17)	0.1233	21, 0.18 (2.54)	18, 0.33 (1.07)	0.226
	AnPC2	21, 0.04 (0.69)	27, 0.15 (1.51)	0.486	21, 0.04 (0.69)	26, -0.38 (0.88)	0.049	21, 0.04 (0.69)	18, -0.18 (1.35)	0.632
	CyPC1	21, -0.68 (2.26)	27, -1.24 (3.88)	0.747	21, -0.68 (2.26)	26, 1 (3.95)	0.127	21, -0.68 (2.26)	18, -0.86 (3.71)	0.7645
	CyPC2	21, -0.16 (0.78)	27, -0.16 (0.13)	0.085	21, -0.16 (0.78)	26, -0.16 (0.6)	0.611	21, -0.16 (0.78)	18, -0.16 (0.12)	0.100

		MAL	HKW		HKWMAL	HKW		HKWMAL	MAL	
		medan(IQR)	medan(IQR)	p-value	medan(IQR)	medan(IQR)	p-value	medan(IQR)	medan(IQR)	p-value
PLASMA	APC1	27, 1.07 (2.2)	26, 0.09 (2.6)	0.113	27, 1.07 (2.2)	18, 0.48 (2.51)	0.247	26, 0.09 (2.6)	18, 0.48 (2.51)	0.650
	APC2	27, -0.28 (2.23)	26, 1.72 (3.32)	0.017	27, -0.28 (2.23)	18, 0.29 (2.95)	0.211	26, 1.72 (3.32)	18, 0.29 (2.95)	0.283
	AnCyPC1	27, -0.27 (3.81)	26, 1.12 (3.32)	0.656	27, -0.27 (3.81)	18, -0.29 (3.43)	0.517	26, 1.12 (3.32)	18, -0.29 (3.43)	0.328
	AnCyPC2	27, 0.68 (2.25)	26, -0.17 (1.85)	0.233	27, 0.68 (2.25)	18, -0.35 (2.03)	0.297	26, -0.17 (1.85)	18, -0.35 (2.03)	0.886
	AnPC1	27, 0.88 (1.92)	26, 0.22 (1.17)	0.4655	27, 0.88 (1.92)	18, 0.33 (1.07)	0.417	26, 0.22 (1.17)	18, 0.33 (1.07)	0.962
	AnPC2	27, 0.15 (1.51)	26, -0.38 (0.88)	0.020	27, 0.15 (1.51)	18, -0.18 (1.35)	0.319	26, -0.38 (0.88)	18, -0.18 (1.35)	0.352
	CyPC1	27, -1.24 (3.88)	26, 1 (3.95)	0.250	27, -1.24 (3.88)	18, -0.86 (3.71)	0.921	26, 1 (3.95)	18, -0.86 (3.71)	0.271
	CyPC2	27, -0.16 (0.13)	26, -0.16 (0.6)	0.299	27, -0.16 (0.13)	18, -0.16 (0.12)	0.980	26, -0.16 (0.6)	18, -0.16 (0.12)	0.407

Table 4-19: Assessing the effects of helminth and malaria infections on the principal components using Mann-Whitney test

		HEL	NoI		HEL	MAL	
		medan(IQR)	medan(IQR)	p-value	medan(IQR)	medan(IQR)	p-value
PLASMA	APC1	21, -0.12 (3.7)	30, 0.01 (2.36)	0.909	30, 0.01 (2.36)	26, 0.09 (2.6)	0.440
	APC2	21, -0.19 (2.86)	30, -1.36 (2.31)	0.082	30, -1.36 (2.31)	26, 1.72 (3.32)	<0.001
	AnCyPC1	21, -0.5 (2.67)	30, -0.88 (1.19)	0.310	30, -0.88 (1.19)	26, 1.12 (3.32)	<0.001
	AnCyPC2	21, -0.61 (1.83)	30, 0.84 (1.91)	0.017	30, 0.84 (1.91)	26, -0.17 (1.85)	0.053
	AnPC1	21, 0.18 (2.54)	30, 0.41 (2.64)	0.267	30, 0.41 (2.64)	26, 0.22 (1.17)	0.522
	AnPC2	21, 0.04 (0.69)	30, -0.12 (1.14)	0.619	30, -0.12 (1.14)	26, -0.38 (0.88)	0.224
	CyPC1	21, -0.68 (2.26)	30, -1.24 (0)	<0.001	30, -1.24 (0)	26, 1 (3.95)	<0.001
	CyPC2	21, -0.16 (0.78)	30, -0.16 (0)	0.007	30, -0.16 (0)	26, -0.16 (0.6)	0.137

4.5.1.5 Assessing the effect of infection on principal components with Mann-Whitney Test

4.5.1.5.1 Effect of infection on plasma variables in PCA

Using the Mann-Whitney test, the contribution of the variables from individuals with hookworm were significantly reduced compared to no infection group (APC1:p=0.047 and AnPC1:p=0.043). There was also a significant reduction in the median component score among malaria and no infection for APC2 (p=0.025), AnCyPC1 (p=0.036) and AnPC2 (p=0.049). Considering effect on component among malaria and hookworm, a significant difference was observed for APC2 (p=0.017) and AnPC2 (p=0.020). From a broader perspective, considering helminth and no infection, a significant difference was observed for AnCyPC2 (p=0.017), CyPC1 (p<0.001) and CyPC2 (p=0.007). But for helminth and malaria, apart from a significant effect on CyPC1 (p<0.001) just as in helminth and no infection, the differences were for APC2 (p<0.001) and AnCyPC1 (p<0.001) (Table 4-18 and Figure 4-6).

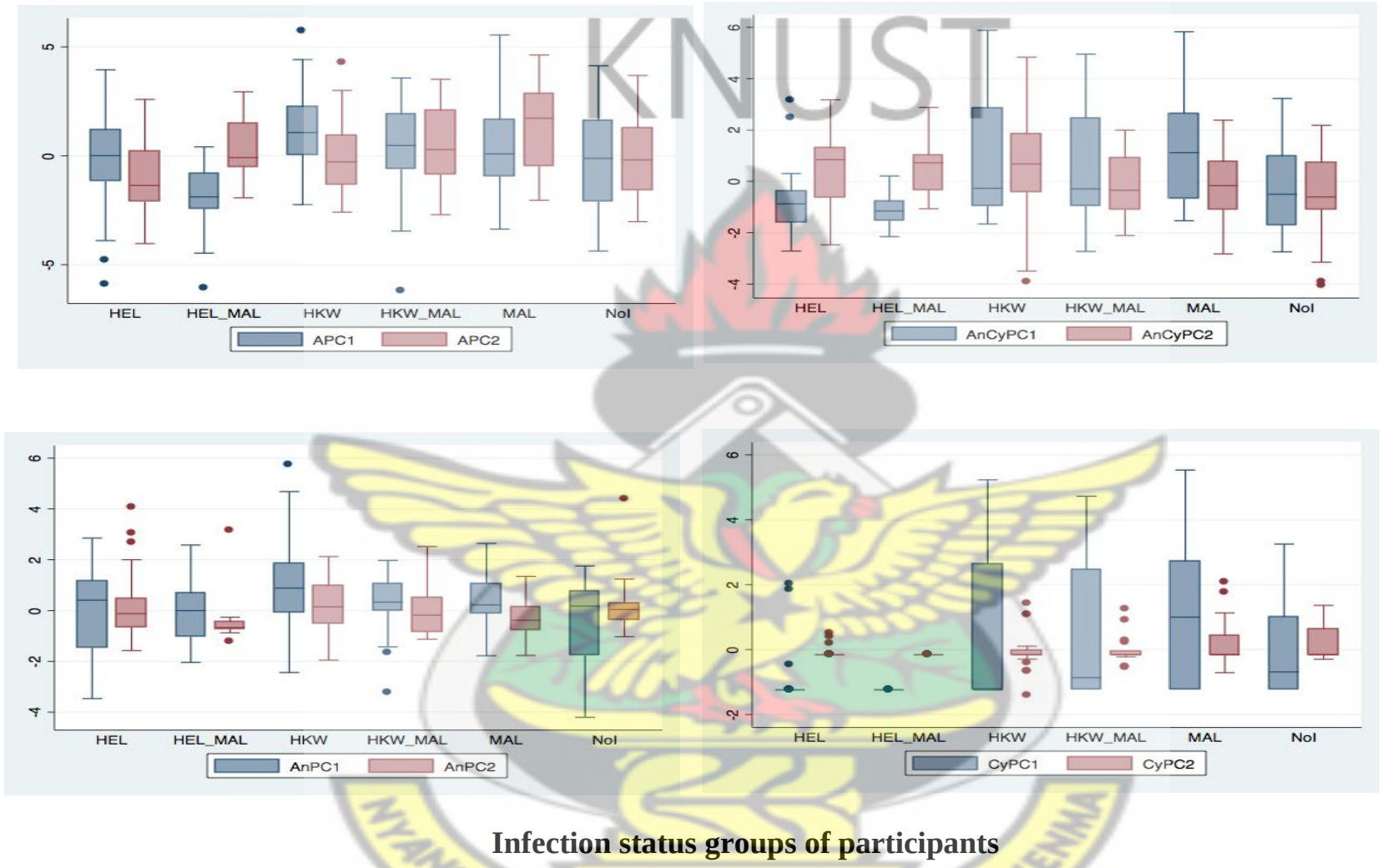


Figure 4-6: Impact of helminth and malaria infection status on principal component derived from plasma antibodies and cytokines

4.5.1.5.2 Cell Phenotypes and Cytokines from Cell culture

When effect of infection on PC1 and PC2 were assessed considering Th1/Th2/Th17 paths, there was no significant difference in the median component scores for Th1. For Th2/PC2 and Th17/PC2, significant measures were observed among hookworm and malaria (p=0.034) respectively.

When the cells were treated with pRBCs, significant effects were seen in comparisons of hookworm-malaria co-infection and hookworm (p=0.038), hookworm-malaria co-infection and malaria (p=0.021) for ApPC2. Also, Malaria and Hookworm (p=0.038) for CePC1, hookworm-malaria co-infection and no infection (p=0.028), hookworm-malaria co-infection and hookworm (p=0.023) and hookworm-malaria co-infection and malaria (p=0.021) for CePC2. Only hookworm-malaria co-infection and malaria (p=0.043) showed a significant effect on CyPC1 (Figure 4-7).

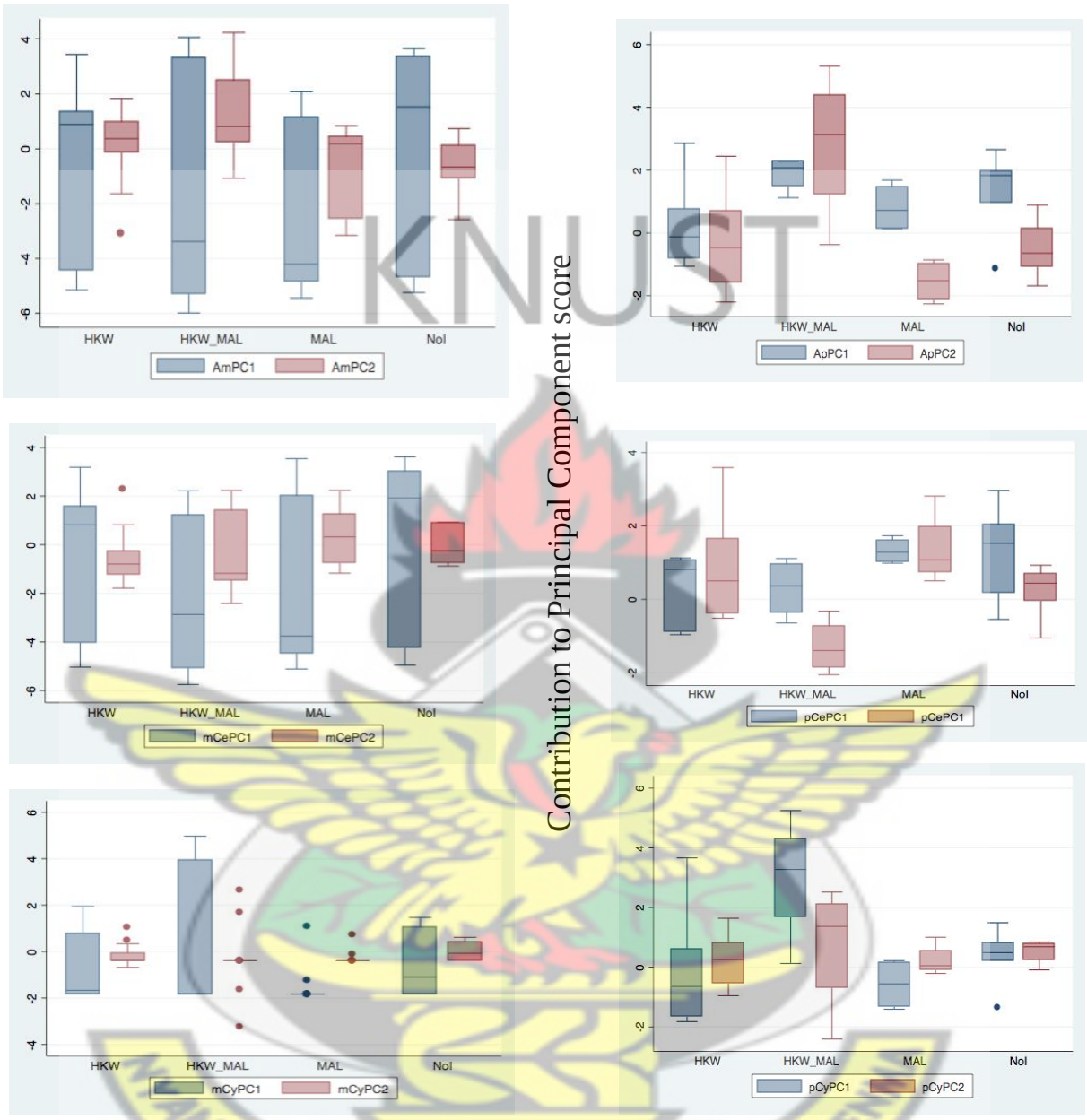


Figure 4-7: Effects of infection status on the level of principal components derived from culture of cells from participants

5 CHAPTER FIVE

DISCUSSIONS

5.1 Epidemiology of Soil Transmitted Helminth (STH) with co-infection in the middle-belt of Ghana, Africa

A total prevalence of 19.3% of helminth infection estimated was low in the middle-belt of Ghana (De Silva et al., 2003, Humphries et al., 2011, Yelifari et al., 2005, Campbell et al., 2016a, Mangali et al., 1994, Toma et al., 1999) within a period when two successive Mass Drug Administration (MDA) exercises with anthelminths (albendazole and praziquantel) have been carried out. The attributed prevalence of 12.1% to hookworm compared to 45% in earlier reports by Humphries et al, 2011 was lower (Humphries et al., 2011). A reduction from the report of 45% hookworm prevalence could be suggestive of the success of the control measures adopted over the period (Salam et al., 2015).

Interestingly, the study reports of a prevalence of 1.5% *Ascaris lumbricoides*, 0.9% *Strongyloides stercoralis* and 0.8% *Trichuris trichiura* in the study area but these are lower compared to what was documented elsewhere (Kounnavong et al., 2011). Of importance to note is the increased prevalence of 4.0% *Hymenolepis nana* and 1.5% *Taenia species* (Humphries et al., 2011) infections despite the high coverage of the MDA with albendazole in the study area. Could it be as a result of the parasites developing resistance to the drugs?

With polyhelminthiasis, the estimate of 1.0% prevalence of hookworm and *Hymenolepis* spp was reported (Kounnavong et al., 2011).

Evidence of improved sanitation (increased use of community refuse dumping site), personal hygiene (washing of hands with soap, scrubbing nails during handwashing) and available good sources of drinking water recorded in this study could have contributed to the reduction of helminth infection in the study area (Efunshile et al., 2015, Campbell et al., 2016a,

Kounnavong et al., 2011). Homes with toilet facilities (14%) were few and that increased "Open Field" defecation among participants (Kounnavong et al., 2011).

Rearing of animals (Yelifari et al., 2005) within compounds and direct animal keeping by study participants were high and these are known to contribute largely to helminth transmission and infection. Fortunately, these were not found to significantly increase the prevalence of helminths infection, probably because of the positive mitigating factors mentioned earlier. The observation is not different from what Toma et al, 1999 (Toma et al., 1999) reported. This presupposes that an improvement in the provision of toilet facilities across the study area could add up to an effective helminth control measure.

The highest hookworm prevalence of about 30% was found in the 8-16 year group. Children in lower age groups have increased odds of being infected with hookworm (Table 4-4). Again, hookworm transmission and infection are known to be associated with poor sanitation and personal hygiene (Toma et al., 1999, Kounnavong et al., 2011, Campbell et al., 2016a). Generally, adults keep high personal hygiene and sanitized environment than children. Children are highly expected to contract hookworm infection than adults especially when they are frequently in touch with the soil.

Infections with hookworm, *Ascaris l., Trichuris trichiura, H.* spp, *Taenia* spp *and Strongyloides stercoralis* were recorded among children less than 8 years of age. That suggest that policy makers should review the MDA programme to target children within this age bracket. The continuous target of only school going groups (mostly 8-16 years) might reserve and maintain helminth transmission in the lower age group making the control and elimination of the parasites difficult.

The study reports of 26.6% and 46.9% helminth and malaria parasite co-infections and 19.2% and 48.7% hookworm and malaria parasite co-infections in the age groups less than 8 years

and 8-16 years respectively, a situation that was found to be significantly associated with decreasing age. This also suggests why parasitic control measures should focus on children. Particularly, for hookworm, about 49% of the co-infections with malaria occurred among children in the 8-16 year old group, followed by 19% among those in the less than 8 year old group. Since the two parasitic infections occur endemic in most developing and poor countries (Toma et al., 1999, Bethony et al., 2006, Brooker et al., 2006, Degarege et al., 2010, Humphries et al., 2011, Kounnavong et al., 2011, Ojurongbe et al., 2011, Adegnika and Kremsner, 2012, Campbell et al., 2016a, Phongluxa et al., 2013), a more targeted approach to reduce their burden would be a step in the right direction and appropriate. A combination of the morbidity associated with hookworm infection and mortality from malaria parasite infections in children in the school going age would be burdensome if not curbed.

Provision of good drinking water is known to reduce the risk of helminth transmission and infection (Salam et al., 2015, Campbell et al., 2016a, Humphries et al., 2013). When assessed, the contribution of drinking water commonly used by those who did not have source of water in their compounds, majority used well and then bore-hole. These sources of water compared to pipe-borne water increased the odds of hookworm infection though only bore-hole (OR=2.51, p=0.027) remained significant in the multivariate model. Bore-holes are considered safe drinking water and one would wonder its contribution to increasing hookworm infection. Individuals who go and fetch from the bore-holes could contaminate the water and make it unsafe and possible source to transmit hookworm infection. It would be advisable to educate individuals who collect water from other sources out of their houses to at least boil to make the water safe before use.

Animal rearing increases the risk of helminth infection particularly hookworm (Campbell et al., 2016a, Humphries et al., 2011, Humphries et al., 2013) but this was not the case in this study. Though 75% of the population had animals reared in their compounds with 70% directly

involved in keeping the animals, the odds of helminth (OR=1.22, p=0.120), hookworm (OR=3.16, p=0.182), *Trichuris trichiura* (OR=2.02, p=0.233), *Ascaris lumbricoides* (OR=0.57, p=0.186) and *H.* spp. (OR=0.63, p=0.223) infections considering rearing of animals as a risk factor were not significant.

Helminth infections were found to cluster with significant relative risks in places within the study area in the middle-belt of Ghana (Figure 4-3). These are location that are largely surrounded by water bodies which might be contributing factor to helminth infection transmission in such zones. Interesting to note and suggestive of places to target in case of specific control measures, malaria parasite infection cluster zones with significant relative risks were found intersecting some of the helminth infection risk zones. This exploratory analysis provides a target of communities that could be used for piloting helminth and malaria control programmes in the middle-belt of Ghana.

.

5.2 Haematological profile in helminth and malaria parasite co-infection among a rural population in Ghana

Generally, the haematological indices of the population presented at baseline were all in the normal ranges when compared to the reference ranges established by Dosoo et al, 2012 (Dosoo et al., 2012). This is expected since none of the participants had symptomatic presentation of any of the infections considered in this study. Particularly for helminths in chronic state, the haematological profile tend to be normal (Roberts, 2016, Taylor-Robinson et al., 2012).

The study describes the changes that occur in the haematological profile of helminth, malaria and their co-infections. Helminth; especially hookworm, and malaria infections frequently cause phenomenal changes in the haematological profiles of their human host (Adu-Gyasi et al., 2012, Sanou et al., 2012). The extent of changes one could observe are dependent on the

acute or chronic nature of the infections or even in co-infections. The rate at which the body responds to the pathological agents and tries to clear and replace reactive cells cleared from circulation also affect the blood cell balance (Bethony et al., 2006, Crompton et al., 1990, Hoffman et al., 2013).

A prevalence of 15.8% of anaemia in the population was recorded in this study. The anaemia prevalence when considered in children <16 years and infected with other helminth, hookworm or malaria parasites was "severe". This observation was consistent with other studies on anaemia in endemic places of the parasites (Hoffman et al., 2013, Kenneth et al., 2016, Roberts, 2016).

Prevalence of 19.3% helminth including 12.1% hookworm and 28.1% of *Pf* among the participants were established in this study. A marked association of malaria parasite infection with anaemia is expected due to the blood loss through haemolysis and mopping of infected RBCs from peripheral circulation in the spleen. With hookworm, the sucking of blood in the lumen of the intestines of their hosts is also observed to contribute to anaemia (Kinung'hi et al., 2014, Njua-Yafi et al., 2016, Sant-Rayn et al., 2008). But contrary to the expectations that hookworm infection influences anaemia, only malaria had significant contribution ($p<0.001$) compared to helminth and hookworm mono-infections (Abanyie et al., 2013, Njunda et al., 2015).

Another important point to note is that, in those with helminth and malaria co-infection and, hookworm and malaria co-infections, anaemia was markedly observed (David and William, 2006, Ridley, 2012, Kotepui et al., 2014). In response to blood loss, haemopoetic activities are increased to restore the deraignment of the blood parameters as a result of the destructions by the body in fighting infection (Kinung'hi et al., 2014, Njua-Yafi et al., 2016). It is also important to note that using only Hgb and the RBC as the only indicators to estimate anaemia might not give accurate account.

In relating hookworm to anaemia in participants, there was no significant difference when Hgb was used. This is similar to the observation made by Pasricha et al, 2008 but in their study, hookworm was found to correlate with iron deficiency which is vital in anaemia (Kinung'hi et al., 2014, Njua-Yafi et al., 2016, Sant-Rayn et al., 2008). A suggestion that anaemia is better explained using Hgb together with other parameters like the cell morphology, ferritin, vitamins, transferrin receptor, alpha-thalassemia and haemoglobin E (Kenneth et al., 2016, Hoffman et al., 2013) is the way to go if resources are available. Sometimes, it takes blood film commenting to mostly reveal the state of anaemia that might be associated with hookworm infection especially when only RBC and Hgb are used without other indices such as the MCHC, MCV and RDW which could also suggest the nature of red cell volume distribution (Pasricha et al., 2008).

Leukopaenia was much associated with helminth while leukocytosis was frequently found among malaria infected individuals contrary to what others reported in endemic regions for the two infections (Kenneth et al., 2016, Pasricha et al., 2008, Salam et al., 2015, Yatich et al., 2009). As has been reported in this study, significant monocytosis, eosinophilia and basophilia compared to no infection group were found among those with helminth, malaria, and helminth-malaria co-infection. This explains that, helminth and malaria infections both induce monocytes, eosinophils and basophils probably due to the increased haemopoiesis in response to the infections associated blood loss. Pairwise comparison revealed hookworm mono-infection compared to malaria parasite mono-infection had significantly increased eosinophils (Lichtman et al., 2017, Schmaier and Lazarus, 2011).

Excretory Secretory (ES) products from helminths have been found to inhibit eosinophils and other cell infiltration into the lungs upon exposure to house dust mites (HDM). This could support the claim of eosinophilia in this study, because eosinophils produced due to the hypersensitivity induced by helminths are restricted to circulation in the peripheral blood

(Lichtman et al., 2017, Loukas and Prociv, 2001, Kotepui et al., 2014). It was also clear that both hookworm and malaria infections had similar effect on the concentrations of basophils contrary to an expected increase among hookworm individuals alone (Loukas and Prociv, 2001, Zaiss et al., 2015).

Malaria was generally found to cause a significant decrease in RBC compared to other groups, as was observed in estimating the MCV and MCH (Smith and Maizels, 2013, Zaiss et al., 2015). Though, Hgb concentration in helminth was similar to what was measured compared to no Infection group, those with malaria and helminth co-infection had significantly reduced Hgb concentration. This is a confirmation of the assertion that malaria contributes to significant reduction in Hgb compared to helminth and even hookworm. Thrombocytopaenia was significantly associated with malaria and also had a diagnostic predictive value (Smallwood et al., 2017). However, this was not the case in malaria and helminth confection. It is possible that some immune mechanisms in response to helminth infection counteracts the increased clearing or used up of platelets from circulation, recruitment to the tissue level, and platelet production which might be advantageous in co-infection with malaria. Since WBCs are regularly involved in inflammation and immune response to get rid of foreign substances and infectious agents, the increase or decrease in WBC counts cannot be specifically associated with particular infectious disease when all possible confounders were not assessed to a large extent.

It was clear from this study that WBC did not predict any diagnostic value in relation to helminth, malaria or the co-infections of the parasites. Exploring further, neutropenia and eosinophilia were more suggestive of helminth and hookworm infections with increased odd ratios as was reported by Kotepui et al, 2014 (Kenneth et al., 2016, Roberts, 2016, Kotepui et al., 2014). This is consistent with the natural response elicited using the Th2 arm of the immune

system to helminth infection and IL-5 cytokine produced by Th2 CD4 T-cells, further induce the production of eosinophils (Mangla et al., 2017).

Diagnostically, low Hgb was predictive of hookworm-malaria co-infection (OR=1.39). This could be as a result of the cumulative effect of malaria and hookworm parasites on blood loss in their hosts (Kotepui et al., 2014). Another marker confirmed in this study to be of high diagnostic values is the RDW. Individuals with hookworm-malaria co-infection had low RDW about twice the odds of having no infection (OR=2.37). The point of interest is why hookworm-malaria co-infected individuals have RBCs with uniform sizes? Hookworm and malaria induce blood cell production. This rapid erythropoesis is expected to present with cells of varying sizes. This then is supposed to mean that participants with hookworm-malaria co-infection had chronic infection and the host cells were not responding aggressively as expected hence the observed RDW as suggested by others (Abanyie et al., 2013, Amoako et al., 2014).

5.3 Characterization of cell phenotypes involved in hookworm and malaria parasite co-infection in endemic areas in Ghana

Hookworm and largely helminths induce a Th2 response which is characterized by increases in regulatory T cell (Treg) counts and IL-10 levels to create high anti-inflammatory environment (Smith and Maizels, 2013), a cytokine very necessary to minimize mortality and morbidity as a result of helminth infection (Helmby, 2015, Kenneth et al., 2016, Kepha et al., 2017).

In this study, there was no difference in the CD4+ cell phenotype counts and cytokines measured in all the four infection groups of participants before treatment. This is what happened when cells were exposed to no stimulant during culture and was consistent with what Doetze et al. 2000, found and suggested that in asymptomatic infections of helminth, malaria or their co-infections, there is no difference in the cell count and even the cytokines being produced (Doetze et al., 2000, Ojurongbe et al., 2011, Smith and Maizels, 2013, Suchard et al.,

2010). As a proxy, what was measured among samples without treatment represented the immune environment of the participants with asymptomatic infection of hookworm, malaria and their co-infections.

Among hookworm infected individuals, the $CD4^+$ T-cells had significant increase in IL-4 as compared to IFN-γ+ cells as suggested (Perez-Mazliah and Langhorne, 2015, Loukas and Prociv, 2001). It was clear that most of the CD4+/Foxp3+ cells were responsible for the IFN-γ production together with CD4+/HLA-DR+/IFN-γ+ cells. Is hookworm suppressing the immune system to avoid the worms from being expelled? This was as expected since malaria generally is responded to with the Th1 (IFN-γ) phenotype of the immune system.

Regulatory cytotoxic T-cells producing IFN-γ (CD8+Foxp3+IFN-γ+) and CD8+IFN-γ+ cells were significantly decreased among those who had asymptomatic hookworm-malaria co-infection compared to no infection which is similar to reports published (Li et al., 2015, Smith and Maizels, 2013, Suchard et al., 2010).Since cytotoxic cells are much more involved in malaria parasite elimination than helminth, the high counts of the cytotoxic regulatory cells in malaria infected individuals was imminent.

When the cells were exposed to *Pf* parasitized RBCs, over a period, activated CD8+ (CD8+/HLA-DR+) among hookworm-malaria co-infected, dendritic cells (DC) in hookworm and *TCR-γδ* in hookworm-malaria co-infected were significantly decreased comparable to conclusions by others (Li et al., 2015, Loukas and Prociv, 2001, Perez-Mazliah and Langhorne, 2015, Smith and Maizels, 2013, Suchard et al., 2010). Meanwhile, there was a significant increase in the population of CD11c+/HLA-DR+ cells between hookworm-malaria and no infection, then hookworm and hookworm-malaria co-infected. This is an indication that helminth actually drive the Th2 immune response and this characterization is also contributed by IL-4 production from APCs (CD11c+/HLA-DR+) (Helmby, 2015, Sanou et al., 2012, Smith and Maizels, 2013).

Of note is that 1) regulatory T cells and other T-cell population could be increased at the tissue level and thereby depleting those in circulation to present with the apparent imbalance in the cell phenotypes considering the various infection groups, also 2), the crosstalk between helminth and other parasitic infections with the microbiota might disturb the cellular and cytokine homeostasis during infection with helminths in particular (Adkinson et al., 2014, Helmby, 2015, Osborne et al., 2014, Zaiss et al., 2015).

It is observed that intense hookworm larvae in immunological analysis demonstrated reduced IFN-γ and IL-17A cytokines (Helmby, 2015) but in this study, the cytokine environment (for example IFN-γ and IL-17A) among the asymptomatic participants looking at the infection status were not different as also was observed and reviewed in (Loukas and Prociv, 2001) (Loukas et al., 2006, Loukas and Prociv, 2001). However, IL-6 was significantly higher when hookworm-malaria co-infected and no infection was compared as well as among hookworm and hookworm-malaria co-infected. As expected, IL-10 to play its regulatory functions, were significantly higher in those who had hookworm co-infection than no infection. This gives an indication that, the suppressive and regulatory role of IL-10 are higher among individuals with hookworm-malaria co-infection (Li et al., 2015, Lichtman et al., 2017, Loukas and Prociv, 2001).

Treatment with *Trichuris suis* in a therapy showed an increase in IL-4 and IL-10. But in this study the increase was only in IL-4 concentration among those who had helminth and malaria co-infection and this was significant compared to those who had no infection. Concentration of IL-10 was similar when considered among participants and their infection status (Smith and Maizels, 2013, Fleming et al., 2011). The difference in cytokine concentration observed could simply be because of the species of helminths, whether infections was systemic or localized, whether the dose was light or heavy and whether the infection was of acute or chronic duration (Smith and Maizels, 2013, Christine, 2010).

5.4 Humoral immune response induced by helminth and malaria parasite co-infection in the middle-belt of Ghana

Cells activation by infectious agent antigens further lead to the production of antibodies by activated B-cells. Depending on the effector T cells involved, the necessary antibody class switching to the B-cell determine the antibodies produced. Concentration of IgG to *Pf* antigen *NF54* when considered among individuals with no infection, helminth or malaria were significant but similar to those with co-infections.

Immune response to malaria antigens is enhanced in the presence of helminth parasite or there is synergy in the processes leading to the production of IgG specific antigens in helminth or malaria infections. This process is suppressed in co-infected individuals as seen in this study with the reduced antibody concentration among those with helminth-malaria co-infection (Allen and Maizels, 2011, Hewitson et al., 2009, Loukas and Prociv, 2001). These considerations among hookworm, malaria or their co-infections give a different and supporting picture in the events leading to antibody productions to hookworm antigens.

Concentrations of IgG to hookworm antigens revealed a significant difference between no infection and hookworm infected as well as between hookworm and malaria infected individuals. It was only among malaria infected individuals that concentration of IgG to *Pf NF54* when compared to no infection was significantly different.

With IgG1; a protective antibody together with IgG3 in malaria, the antibody production was significantly higher among hookworm infected individuals rather than those who had malaria and this gave a clear indication that antibody production to hookworm is complimentary to the protection against malaria (Actor, 2014, Njua-Yafi et al., 2016, Righetti et al., 2012, Salazar-Castañon et al., 2014, Hartgers and Yazdanbakhsh, 2006, Hewitson et al., 2009, Selzer and Caffrey, 2012). IgG1, IgG3 and IgG4 have been implicated as protective antibody sub-classes while simultaneously the presence of IgG3 and IgG4 relate to hookworm chronicity and current

infections respectively (Hartgers and Yazdanbakhsh, 2006, Hewitson et al., 2009, Salazar-Castañon et al., 2014, Selzer and Caffrey, 2012). Could it be that the IgG1, which is an indication of Th2/IL-4 environment, produced in hookworm have stronger affinity for malaria antigens (Lamb, 2012, Stanisic et al., 2009) and also be a similar type of antibody produced during malaria infection? This could be worth exploring to the development of control and preventive measures in malaria.

The school of thought that propagate hookworm infection to be beneficial in raising protection to malaria parasites with this information could advocate for considerations in screening for simultaneous infections with helminth in populations that will be receiving malaria vaccines either on trial or even routine delivery (Quinnell et al., 2004, Smallwood et al., 2017, Adkinson et al., 2014). This also brings to mind what effect anthelminthics might have on host response to malaria parasite antigens and largely malaria vaccines (Bartsch et al., 2016, Hotez et al., 2014, Moorthy et al., 2013). If these drugs could reverse the antibody production processes, then one could be right in saying that anthelminthics could have detrimental effects on response to protective antibodies to malaria vaccines (Kepha et al., 2017, Taylor-Robinson et al., 2012). When people who are without infection were exposed to malaria parasites, the antigens induced a positive correlation between IL-17A and IFN-gamma and these are the cytokines that are largely at play in similar infections. Such is consistent compared to other studies which support the claim that malaria infection activate the Th1 phenotype immunity (Loukas et al., 2006, Loukas and Prociv, 2001, Stanisic et al., 2009).

In hookworm infected individuals exposed to malaria antigens, IL-17A assumes a significant positive correlation with IL-6, IL-10 and IFN-gamma. In the same vain, a significant relationship is observed between TNF-A and IL-10 each with IL-6. This clearly indicated the suppression effect of the Th2 phenotype (IL-4) in the presence of malaria antigens to hookworm (Hotez et al., 2013, Lamb, 2012, Loukas and Prociv, 2001).

Of high significance and to note for effective control measures was the activation of IL-4 and IL-10 when individuals with malaria parasite infection were exposed to parasitized RBCs. One would have expected a boost in the development of the Th1 (IFN-gamma) phenotype but that was not the case. This adds to the unpredictable nature of the immune response induced in malaria infection and also with helminths in endemic areas (Boef et al., 2013, Damania and Dittmer, 2014). It was however clear that in hookworm and malaria co-infection, immunity to hookworm dominates when the individual is further exposed to malaria antigens. This calls for attention to look at the dire effect in the search for malaria vaccines using endemic regions for vaccine trials.

5.5 Co-infection of helminths and malaria parasite in an endemic region: modulation of immune correlates and environmental factors

Immune response to parasitic infection is modulated by several factors including personal hygiene, nutritional, immunological and environmental in endemic places (Campbell et al., 2016a, Esrey et al., 1991, Strunz et al., 2014). Previous exposure to infectious agents in the body and those from the environment such as house dust mite influence the immune *milieu* (innate, cellular or humoral response) and kind of response that will be elicited to current infections (Adkinson et al., 2014, Bousquet and Michel, 1993).

Rather than assessing individual immunological markers and profiles to ascertain the immune response to helminth, malaria and their co-infection, PCA was used to study the pattern of cytokines and antibodies production together with individual and environmental factors interactions (Mori et al., 2016, Ateba-Ngoa et al., 2015).

From the major components built from demographic variables, blood cell counts, antibodies measured and cytokine estimation in PCA the kind of infection, age group, BMI, education level, scrubbing of nails during hand wash, individuals who shared bed and those who were directly involved with animal rearing were found to have contributed significantly to the

principal component (APC1). The interplay of several factors in the PC mimics what usually happens in immune modulation accounting for poverty, lack of adequate drinking water, environmental, nutritional and risk factors such as personal hygiene and poor sanitation (Campbell et al., 2016a, Lemaitre et al., 2014).

Ultimately, when it comes to immune response (cytokine and antibody production) in human, infection status, age, personal hygiene practice such as scrubbing of nails during hand wash and animal rearing had significant influence. These effects agree with what was described by Campbell et al., 2016 (Campbell et al., 2016a, Campbell et al., 2016b).

Among individuals with helminth infection compared to those with no infection, antibody and cytokine contributions to the PC score was significantly higher but lower when compared to those with malaria. This is indicative of the massive cytokine production to malaria infection compared to helminths. However, the cytokines (IL-6, IL-5, IL-4 and TNF-A) induction from hookworm infected individuals compared to malaria was higher and this difference was significant. There were no detectable antibodies to the *Trichuris trichiura* antigens among those with malaria. An indication that not every individual in an endemic region gets exposed to the tropical parasites of endemicity.

In this study, helminth infection induced synergistically IL-4 and IL-5 as well as IL-6 and TNF-A. The Th2 phenotype (IL-4 and IL-5) acted antagonistically to IL-6 and TNF-A unlike what happened in those with malaria where both Th2 phenotype and the pro-inflammatory cytokines loaded positively into the principal component similarly. This observation is consistent with other studies that described the immune response induced by helminth and malaria infections (Ateba-Ngoa et al., 2015, Allen and Maizels, 2011).

Established in this study was the fact that the immune *milieu* in those with helminth and malaria co-infection compared to those without infection were similar (Allen and Maizels, 2011, Damania and Dittmer, 2014). With Th2 response, malaria parasite is able to suppress immunity

to hookworm in co-infected individuals. Whereas hookworm boost immunity to malaria, the contrary is true that immunity to helminth is suppressed in malaria co-infections. Again, IL-17A which is one of the cytokines for mucosal surface immunity was significantly high in median component score when PBMCs were exposed to malaria parasite antigens in culture.

It was evident that, previously hookworm mono-infection or hookworm-malaria co-infected individuals stimulate TH17 response in the presence of malaria antigens (Loukas et al., 2006, Luckheeram et al., 2012, Nacher, 2011, Perez-Mazliah and Langhorne, 2015), a pathway that needs to be evaluated for negative effects on the success of malaria therapy, management and prevention for instance with vaccines. Activation of the Th17 effector cells inhibit immunity to malaria (Th1) and in vaccination, one might not attain the needed immune response to maintain protection in the presence of hookworm mono-infection or hookworm and malaria co-infection.

It was evident in this study that monocytes, neutrophils and eosinophils acted synergistically and in opposition to the response from lymphocytes and basophils. This action was significantly less in hookworm infected individuals when compared to those without infection. In hookworm infection the antagonistic effect of lymphocytes and eosinophils to monocytes, neutrophils and basophils was higher than a similar effect in malaria. However, the observation in malaria was larger than in those with helminths. The reduced aggression to hookworm infection as seen supports the statement that hookworm as a master regulator down modulates the immune system just as in individuals without infection and hence their ability to evade and live longer in infected people (Anderson and May, 1985, Fleming et al., 2006, Loukas et al., 2006, Loukas and Prociv, 2001).

It is apparent that the biological influence of Infection status, age and BMI could have direct effect on the immunological response to hookworm but little could have been suspected of education, scrubbing of nails during hand wash, bed share and direct involvement in animal

rearing (Adkinson et al., 2014, Campbell et al., 2016a, Hotez et al., 2006). This is probably due to their indirect influence on the biology involved in eliciting immune response. For instance, education and scrubbing of nails bother on personal hygiene which in the poverty of the state could dispose individuals to filth which would contribute to increased helminth infection (Adkinson et al., 2014, Campbell et al., 2016a, Hotez et al., 2006). Again, poor sanitation and animal rearing predispose individuals to allergens that might enable them develop formidable immune protection on the basis of the "hygiene hypothesis" (Campbell et al., 2016a, Crompton et al., 1990, Zaiss et al., 2015) and these need further exploration in structured studies to identify their direct effect on immunity build-up.

6 CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Study conclusions

Hookworm infection was significantly increased in younger age groups which covered the school-age and among those who did not have safe drinking water. Proper sanitation, protective footwear, religion and good personal hygiene practices were found to influence helminth and hookworm infection in the area. Children less than the age targeted for MDA had helminth infections. Proper hand washing while scrubbing the finger nails was a protective attitude that needs to be encouraged when hookworm infection and transmission is considered. Individuals who were traditionalists had significant increase in the odds of contracting hookworm infection.

Malaria infection still remains about a 2-fold contributor of contracting helminth infection and for that matter, the two infections must be targeted together in control measures in places where the parasites are endemic.

Anaemia prevalence was moderate. It should be noted that in chronic helminth or hookworm infection, anaemia is not easily identifiable using Hgb measurement. With other RBC indices like MCHC and MCV unlike in malaria parasite infection which shows marked effect on anaemia (low Hgb). Low RDW among individuals with hookworm and malaria co-infection suggested chronic infection because of the uniformly distributed RBC volume. It must also be noted that leukocytosis and eosinophilia were associated with hookworm infection compared to malaria parasite infection which rather had significant influence on basophilia, monocytosis and neutrophilia.

Regulatory T-cells population and cytokine environment induced in the asymptomatic hookworm infection, malaria parasite infection and in their co-infected states were not

significantly different. Hookworm infected individuals with malaria parasites co-infection significantly produced IL-4 producing CD4+ T-cells (CD4+IL-4+) and IFN-γ producing regulatory cytotoxic T-cells (CD8+Foxp3+IFN-γ+). In hookworm infections, the presence of malaria parasites largely stimulates the production of Th2 effector cells with significant cytotoxic T-cells production. When cells were activated with *Pf* parasites, activated cytotoxic T-cells (CD8+HLA-DR+) population were decreased among hookworm and malaria co-infected individuals. Dendritic cell (CD11c+HLA-DR+) population also decreased in hookworm infection but in hookworm and malaria co-infection *TCR-γδ* cells rather decreased. There was also a significant increase in IL-4 producing CD11c+HLA-DR+ cells associated with hookworm infection. IL-4; a Th2 cytokine, IL-6; a pro-inflammatory cytokine, and IL-10; a regulatory cytokine, were significantly increased in those with hookworm and malaria co-infection when stimulated with malaria parasites. It is also important to note that hookworm infections were also associated with significant increase in IL-17A cytokine responsible for mucosal immunity.

Individuals with hookworm infection had significant increase in the concentrations of IgG1 antibodies to malaria parasites than even those with *Pf* infection. This suggests possible crosstalk in the IgG1 antibody production to hookworm and *Pf* parasites. Exposure to malaria parasites antigens induce a positive correlation between IL-17A and IFN-gamma. In hookworm infected individuals IL-17A positively correlate with IL-6, IL-10 and IFN-gamma. Also, proinflammatory cytokines positively correlate in hookworm infection (TNF-A and IL-6). Among individuals infected with malaria, upon exposure to malaria antigens, they unpredictably induce IL-4 cytokine.

Helminth induce Th2 (IL-4 and IL-5) phenotype response which acts antagonistically to IL-6 and TNF-A unlike in malaria where all the cytokines acted positively and in one direction. Also, hookworm down modulates the immune system just as in individuals without infection

to evade and live longer in infected people. Infection status, age, BMI, education, scrubbing of nails during hand wash, bed share and direct involvement in animal rearing had significant influence on the immune response among participants just as on their infection status.

6.2 Limitations of study

The few numbers recorded for some specific parasites made some subgroup analysis inadequate. That depicts the picture of helminth prevalence in the study area for the one year period.

Inability due to financial constraints to analyse samples for all participants for the immunological evaluations is considered a limitation. However, the selected samples are deemed adequate to answer the necessary questions. Again, the random selection of the subset of samples used for the immunological analysis made the results representative.

ELISA set up for IgE and subclass- IgG1 using helminth antigens did not work out to give desirable results. This led to the inability of the study to consider outputs for IgE and IgG1 concentrations to crude helminth antigens and for that matter could not discuss matters in relation to IgE antibody production and its relationship in helminth, hookworm or malaria parasite infections.

Cytokine concentrations in plasma were considered for IL-2, IL-4, IL-6, TNF, IL-5 and IFN-γ. Estimates were successful for IL-4, IL-6, TNF and IL-5. Detectable results were not obtained for IL-2 and IFN-γ due to the inability to calibrate the two parameters with the software for data analysis from the CBA kit. Due to scarce resources, sample processing could not be repeated. However, the results were presented as obtained and validated with standards anticipating that any error or bias that would be found in the analysis would be uniformly distributed but not only affect a selected group.

Stimulation assays were done on PBMCs that had challenges with viability due to changes in the unstable cryostorage conditions. Due to this challenge, a viability component stain was added to each tube for analysis to ensure that only viable cells were used in cell phenotyping.

6.3 Recommendations

Education to improve personal hygiene and sanitation together with provision of proper sources of water for living should be considered dearly. Particularly, health education to individuals to boil water from sources such as bore-hole and well should be encouraged. This will make the water safe before use and possible contamination from those who carry the water might be mitigated.

With the high rate of "open defaecation" in the community, the study recommends policies to get adequate toilet facilities to minimize if not halt open defaecation.

The MDA programme is implemented by giving the drug to all school going children, meaning those that are below the school age will not be covered. With the prevalence of helminth recorded among children age less than 8 years old (for example hookworm (7.2%)), the study recommends to policy makers to review the MDA programme to include children below the school going age in the respective places of implementation. This the study believes would require targeting children at homes rather than waiting for them at school.

An active surveillance system needs to be put in place by programme implementers to evaluate the effectiveness of the anthelminths used in the control programmes to be able to pick up the development of resistance to the selected drugs. Also, they should assess the impact of helminth therapy on immunity to helminth in their hosts.

Using Hgb alone as an indicator to monitor anaemia is not enough in chronic helminth infection. Other indicators (for example, MCV, MCHC measurements) should be added to be able to pick up other forms of anaemia including iron-deficiency.

It is important to note that protective immune response to hookworm and malaria have a crosstalk relationship and for that matter, control measures to malaria. the study recommends studying the impact of hookworm infection on malaria immunity in vaccine trials.

6.4 Further studies

- Studies to evaluate the mass drug administration programme and to improve coordination for reducing transmission and strengthening control of helminth infections.
- A longitudinal study to evaluate the impact of water, sanitation, hygiene, poverty and the population immunology on the effectiveness of control programmes on neglected tropical diseases targeting soil transmitted helminths in endemic areas.
- Studies to monitor and screen to identify albendazole resistant markers in mass drug administration programmes in helminth endemic areas.
- Studies to search for new therapies for the treatment of helminth infections.
- Since helminth resides in the gut lumen, the study suggests human studies in endemic populations to identify the diagnostic effectiveness of mucosal IgA in helminths infection and their importance for vaccine development.
- The role of gamma delta and regulatory T cells in the immune response to hookworm and malaria parasite co-infection and the impact of antihelminth therapy on cell function.
- Identification and validation of affordable, improved screening and diagnostic tools for helminths in endemic tropical regions.

REFERENCES

Abanyie, F., McCracken, C., Kirwan, P., Molloy, S., Asaolu, S., Holland, C., Gutman, J. & Lamb, T. 2013. Ascaris co-infection does not alter malaria-induced anaemia in a cohort of Nigerian preschool children. *Malar J*, 12.

Abbas, A., Lichtman, A. & Pillai, S. 2014. *Basic immunology: functions and disorders of the immune system*, Elsevier Saunders, USA.

Abbas, A., Lichtman, A. & Pillai, S. 2015. *Cellular and Molecular Immunology*, Elsevier Saunders, USA.

Actor, J. 2012. *Elsevier's Integrated Review Immunology And Microbiology, Second Edition,* China, Saunders Elsevier Inc.

Actor, J. 2014. *Introductory Immunology: Basic Concepts for Interdisciplinary Applications*, Academic Press, UK.

Adegnika, A. & Kremsner, P. 2012. Epidemiology of malaria and helminth interaction: a review from 2001 to 2011. *Curr Opin HIV AIDS*, 7**,** 221-4.

Adkinson, F., Stephen, H., Bruce, B., Robert, F., Wesley, B., Robyn, E. & William, W. 2014. *Allergy Principles and Practice, ed. Middleton. Eighth Edition. Elsevier Saunders, USA*.

Adu-Gyasi, D., Adams, M., Amoako, S., Mahama, E., Nsoh, M., Amenga-Etego, S., Baiden, F., Asante, K., Newton, S. & Owusu-Agyei, S. 2012. Estimating malaria parasite density: assumed white blood cell count of 10,000/µl of blood is appropriate measure in Central Ghana. *Malaria journal*, 11**,** 238.

Adu-Gyasi, D., Asante, K., Newton, S., Amoako, S., Dosoo, D., Ankrah, L., Adjei, G., Amenga-Etego, S. & Owusu-Agyei, S. 2015a. Malaria parasite density estimated with white blood cells count reference value agrees with density estimated with absolute in children less than 5 years in central Ghana. *Malar Res Treat.*, 2015**,** 8.

Adu-Gyasi, D., Asante, K., Newton, S., Dosoo, D., Amoako, S., Adjei, G., Amoako, N., Ankrah, L., Tchum, S., Mahama, E., Agyemang, V., Kayan, K. & Owusu-Agyei, S. 2015b. Evaluation of the diagnostic accuracy of CareStart G6PD deficiency Rapid Diagnostic Test (RDT) in a malaria endemic area in Ghana, Africa. *PloS one*, 10**,** e0125796.

Adu-Gyasi, D., Asante, K. P., Amoako, S., Amoako, N., Ankrah, L., Dosoo, D., Tchum, S. K., Adjei, G., Agyei, O., Amenga-Etego, S. & Owusu-Agyei, S. 2018. Assessing the performance of only HRP2 and HRP2 with pLDH based rapid diagnostic tests for the diagnosis of malaria in middle Ghana, Africa. *PLOS ONE*, 13**,** e0203524.

Adu-Gyasi, D., Kye-Amoah, K. & Sorvor, E. 2010. Prevalence of urinary schistosomiasis in Offinso-South Municipality in Ashanti Region-Ghana. *International Journal of Infectious Diseases*, 14**,** e270.

Agrawal, M. 2012. Parasitological Diagnosis. *Schistosomes and Schistosomiasis in South Asia.* Springer.

Allen, J. & Maizels, R. 2011. Diversity and dialogue in immunity to helminths. *Nature Reviews Immunology*, 11**,** 375-388.

Alonso, P. L., Brown, G., Arevalo-Herrera, M., Binka, F., Chitnis, C., Collins, F., Doumbo, O. K., Greenwood, B., Hall, B. F. & Levine, M. M. 2011. A research agenda to underpin malaria eradication. *PLoS medicine*, 8**,** e1000406.

Amoako, N., Asante, K., Adjei, G., Awandare, G., Bimi, L. & Owusu-Agyei, S. 2014. Associations between red cell polymorphisms and Plasmodium falciparum infection in the middle belt of Ghana. *PloS one*, 9**,** e112868.

Anderson, R. & May, R. 1982. Population dynamics of human helminth infections: control by chemotherapy. *Nature,* 297**,** 557-563.

Anderson, R. & May, R. 1985. Herd immunity to helminth infection and implications for parasite control. *Nature,* 315**,** 493-496.

Anderson, R. & Medley, G. 1985. Community control of helminth infections of man by mass and selective chemotherapy. *Parasitology,* 90**,** 629-660.

Asante, K., Abokyi, L., Zandoh, C., Owusu, R., Awini, E., Sulemana, A., Amenga-Etego, S., Adda, R., Boahen, O. & Segbaya, S. 2010. Community perceptions of malaria and malaria treatment behaviour in a rural district of Ghana: implications for artemisinin combination therapy. *BMC public health,* 10**,** 409.

Ateba-Ngoa, U., Adegnika, A., Zinsou, J., Kassa, R., Smits, H., Massinga-Loembe, M., Mordmüller, B., Kremsner, P. & Yazdanbakhsh, M. 2015. Cytokine and chemokine profile of the innate and adaptive immune response of schistosoma haematobium and plasmodium falciparum single and co-infected school-aged children from an endemic area of Lambaréné, Gabon. *Malaria journal,* 14**,** 94.

Ateba-Ngoa, U., Zinsou, J., Kassa, R., Feugap, E., Honkpehedji, Y., Massinga-Loembe, M., Moundounga, H., Mouima, A., Mbenkep, L. & Wammes, L. 2014. Assessment of the effect of Schistosoma haematobium co infection on malaria parasites and immune responses in rural populations in Gabon: study protocol. *SpringerPlus,* 3**,** 388.

Ayeh-Kumi, P. F., Obeng-Nkrumah, N., Baidoo, D., Teye, J. & Asmah, R. H. 2015. High levels of urinary schistosomiasis among children in Bunuso, a rural community in Ghana: an urgent call for increased surveillance and control programs. *J Parasit Dis,* 39**,** 613-23.

Barda, B., Rinaldi, L., Ianniello, D., Zepherine, H., Salvo, F., Sadutshang, T., Cringoli, G., Clementi, M. & Albonico, M. 2013. Mini-FLOTAC, an innovative direct diagnostic technique for intestinal parasitic infections: experience from the field. *PLoS Negl Trop Dis,* 7**,** e2344.

Bartsch, S., Hotez, P., Asti, L., Zapf, K., Bottazzi, M., Diemert, D. & Lee, B. 2016. The Global Economic and Health Burden of Human Hookworm Infection. *PLoS Neglected Tropical Diseases,* 10**,** e0004922.

Beech, R., Skuce, P., Bartley, D., Martin, R., Prichard, R. & Gilleard, J. 2011. Anthelmintic resistance: markers for resistance, or susceptibility? *Parasitology,* 138**,** 160-174.

Behnke, J. 2008. Structure in parasite component communities in wild rodents: predictability, stability, associations and interactions.... or pure randomness? *Parasitology,* 135**,** 751-766.

Behnke, J., Lowe, A., Clifford, S. & Wakelin, D. 2003. Cellular and serological responses in resistant and susceptible mice exposed to repeated infection with Heligmosomoides polygyrus bakeri. *Parasite immunology,* 25**,** 333-340.

Beldomenico, P. & Begon, M. 2010. Disease spread, susceptibility and infection intensity: vicious circles? *Trends in Ecology & Evolution,* 25**,** 21-27.

Belkaid, Y. 2007. Regulatory T cells and infection: a dangerous necessity. *Nature Reviews Immunology,* 7**,** 875-888.

Bentwich, Z. 2000. Good worms or bad worms: do worm infections affect the epidemiological patterns of other diseases? *Parasitology today (Personal ed.),* 16**,** 312.

Bereczky, S., Montgomery, S., Troye-Blomberg, M., Rooth, I., Marie-Anne, S. & Färnert, A. 2004. Elevated anti-malarial IgE in asymptomatic individuals is associated with reduced risk for subsequent clinical malaria. *International journal for parasitology,* 34**,** 935-942.

Bethony, J., Brooker, S., Albonico, M., Geiger, S., Loukas, A., Diemert, D. & Hotez, P. 2006. Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. *The Lancet,* 367**,** 1521-1532.

Bhattarai, A., Ali, A. S., Kachur, S. P., Mårtensson, A., Abbas, A. K., Khatib, R., Al-Mafazy, A.-w., Ramsan, M., Rotllant, G. & Gerstenmaier, J. F. 2007. Impact of artemisinin-based combination therapy and insecticide-treated nets on malaria burden in Zanzibar. *PLoS medicine,* 4**,** e309.

Boef, A., May, L., van Bodegom, D., van Lieshout, L., Verweij, J., Maier, A., Westendorp, R. & Eriksson, U. 2013. Parasitic infections and immune function: effect of helminth infections in a malaria endemic area. *Immunobiology,* 218**,** 706-11.

Boel, M., Carrara, V., Rijken, M., Proux, S., Nacher, M., Pimanpanarak, M., Paw, M., Moo, O., Gay, H., Bailey, W., Singhasivanon, P., White, N., Nosten, F. & McGready, R. 2010. Complex interactions between soil-transmitted helminths and malaria in pregnant women on the Thai-Burmese border. *PLoS Negl Trop Dis,* 4**,** e887.

Bousquet, J. & Michel, F. 1993. *In Allergy Principles and Practice, ed. Middleton, Reed, Adkinson, Yunginges, Busse, pp. 1059–1076; 1305–1307, 1663, Mosby Year Book, St Louis*.

Bowers, D. 2014. *Medical statistics from scratch: an introduction for health professionals. Third Edition*, John Wiley & Sons.

Briand, V., Watier, L., Le Hesran, J., Garcia, A. & Cot, M. 2005. Coinfection with Plasmodium falciparum and Schistosoma haematobium: protective effect of schistosomiasis on malaria in Senegalese children. *Am J Trop Med Hyg,* 72**,** 702-707.

Brooker, S., Akhwale, W., Pullan, R., Estambale, B., Clarke, S., Snow, R. & Hotez, P. 2007. Epidemiology of plasmodium-helminth co-infection in Africa: populations at risk, potential impact on anemia, and prospects for combining control. *The American journal of tropical medicine and hygiene,* 77**,** 88-98.

Brooker, S., Clements, A. & Bundy, D. 2006. Global epidemiology, ecology and control of soil-transmitted helminth infections. *Advances in parasitology,* 62**,** 221-261.

Brooker, S., Nikolay, B., Balabanova, D. & Pullan, R. 2015. Global feasibility assessment of interrupting the transmission of soil-transmitted helminths: a statistical modelling study. *Lancet Infect Dis,* 15**,** 941-50.

Bustinduy, A., Sutherland, L., Chang-Cojulun, A., Malhotra, I., DuVall, A., Fairley, J., Mungai, P., Muchiri, E., Mutuku, F., Kitron, U. & King, C. 2015. Age-Stratified Profiles of Serum IL-6, IL-10, and TNF-alpha Cytokines Among Kenyan Children with Schistosoma haematobium, Plasmodium falciparum, and Other Chronic Parasitic Co-Infections. *Am J Trop Med Hyg,* 92**,** 945-51.

Butterworth, A., Fulford, A., Dunne, D., Ouma, J., Sturrock, R. & Bundy, D. 1988. Longitudinal Studies on Human Schistosomiasis [and Discussion]. *Philosophical Transactions of the Royal Society of London B: Biological Sciences,* 321**,** 495-511.

Campbell, S., Nery, S., D'Este, C., Gray, D., McCarthy, J., Traub, R., Andrews, R., Llewellyn, S., Vallely, A. & Williams, G. 2016a. Water, sanitation and hygiene related risk factors for soil-transmitted helminth and Giardia duodenalis infections in rural communities in Timor-Leste. *International Journal for Parasitology,* 46**,** 771-779.

Campbell, S., Nery, S., Doi, S., Gray, D., Soares Magalhães, R., McCarthy, J., Traub, R., Andrews, R. & Clements, A. 2016b. Complexities and Perplexities: A Critical Appraisal of the Evidence for Soil-Transmitted Helminth Infection-Related Morbidity. *PLoS Neglected Tropical Diseases,* 10**,** e0004566.

CDC, C. f. D. C. a. P. 2012. Hymenolepiasis. Global Health – Division of Parasitic Diseases. https://www.cdc.gov/parasites/hymenolepis/biology.html. Accessed: 27th May 2017.

Chan, M. 1997. The global burden of intestinal nematode infections - fifty years on. *Parasitology Today,* 13**,** 438–43.

Cheesbrough, M. 2006. District Laboratory Practice in Tropical Country. Part 2. Second Edition. Cambridge University Press, UK.

Cheesbrough, M. 2009. District Laboratory Practice in Tropical Countries. Part 1 ,Second Edition. Cambridge University Press, UK. Cambridge.

Chen, W., Jin, W. & Hardegen, J. 2003. Conversion of peripheral CD4+CD25-naive T cells to CD4+CD25+ regulatory T cells by TGF-β induction of transcription factor Foxp3. *Journal of Experimental Medicine,* 198**,** 1875– 1886.

Christine, D. 2010. *Clinical Immunology and Serology: A Laboratory Perspective. Third Edition. F. A. Davis Company, USA.*

Conrad, D. 1990. FcεRII /CD23: The Low Affinity Receptor for IgE1 *Annu. Rev. Immunol,* 8**,** 623-5.

Corrêa-Oliveira, R., Golgher, D., Oliveira, G., Carvalho, O., Massara, C., Caldas, I., Colley, D. & Gazzinelli, G. 2002. Infection with Schistosoma mansoni correlates with altered immune responses to Ascaris lumbricoides and hookworm. *Acta tropica,* 83**,** 123-132.

Courtin, D., DJILALI-SAÏAH, A., Milet, J., Soulard, V., Gaye, O., MIGOT-NABIAS, F., Sauerwein, R., Garcia, A. & Luty, A. 2011. Schistosoma haematobium infection affects Plasmodium falciparum-specific IgG responses associated with protection against malaria. *Parasite immunology,* 33**,** 124-131.

Crompton, D., Stephenson, L., Schad, G. & Warren, K. 1990. Hookworm infection, nutritional status and productivity. *Hookworm disease-current status and new directions.***,** 231-264.

Damania, B. & Dittmer, D. P. 2014. What Lies Within: Coinfections and Immunity. *Cell host & microbe,* 16**,** 145-147.

Danso-Appiah, A., Stolk, W. A., Bosompem, K. M., Otchere, J., Looman, C. W. N., Habbema, J. D. F. & de Vlas, S. J. 2010. Health Seeking Behaviour and Utilization of Health Facilities for Schistosomiasis-Related Symptoms in Ghana. *PLOS Neglected Tropical Diseases,* 4**,** e867.

David, J. T. & William, P. A. J. 2006. *Markell and Voge's Medical Parasitology,* Saunders Elsevier.

De Silva, N. R., Brooker, S., Hotez, P. J., Montresor, A., Engels, D. & Savioli, L. 2003. Soil-transmitted helminth infections: updating the global picture. *Trends in parasitology,* 19**,** 547-551.

Degarege, A., Animut, A., Legesse, M. & Erko, B. 2010. Malaria and helminth co-infections in outpatients of Alaba Kulito Health Center, southern Ethiopia: a cross sectional study. *BMC research notes,* 3**,** 143.

Degarege, A., Legesse, M., Medhin, G., Animut, A. & Erko, B. 2012. Malaria and related outcomes in patients with intestinal helminths: a cross-sectional study. *BMC infectious diseases,* 12**,** 291.

Delves, P. J., Martin, S. J., Burton, D. R. & Roitt, I. M. 2011. *Roitt's essential immunology,* John Wiley & Sons.

Deribe, K., Meribo, K., Gebre, T., Hailu, A., Ali, A., Aseffa, A. & Davey, G. 2012. The burden of neglected tropical diseases in Ethiopia, and opportunities for integrated control and elimination. *J Parasites vectors,* 5**,** 240.

Dev, V., Hira, C. & Rajkhowa, M. 2001. Malaria-attributable morbidity in Assam, north-eastern India. *Annals of tropical medicine and parasitology,* 95**,** 789-796.

Diallo, T. O., Remoue, F., Gaayeb, L., Schacht, A.-M., Charrier, N., De Clerck, D., Dompnier, J.-P., Pillet, S., Garraud, O. & N'Diaye, A. A. 2010. Schistosomiasis coinfection in children influences acquired immune response against Plasmodium falciparum malaria antigens. *PLoS One,* 5**,** e12764.

Diallo, T. O., Remoué, F., Schacht, A. M., Charrier, N., Dompnier, J. P., Pillet, S., Garraud, O., N'diaye, A., Capron, A. & Capron, M. 2004. Schistosomiasis co-infection in

humans influences inflammatory markers in uncomplicated Plasmodium falciparum malaria. *Parasite immunology,* 26**,** 365-369.

Dieli, F., Troye-Blomberg, M., Farouk, S., Sireci, G. & Salerno, A. 2001. Biology of gammadelta T cells in tuberculosis and malaria. *Curr Mol Med,* 1**,** 437-446.

Doetze, A., Satoguina, J., Burchard, G., Rau, T., Löliger, C., Fleischer, B. & Hoerauf, A. 2000. Antigen-specific cellular hyporesponsiveness in a chronic human helminth infection is mediated by Th3/Tr1-type cytokines IL-10 and transforming growth factor-β but not by a Th1 to Th2 shift. *International Immunology,* 12**,** 623-630.

Dosoo, D. K., Kayan, K., Adu-Gyasi, D., Kwara, E., Ocran, J., Osei-Kwakye, K., Mahama, E., Amenga-Etego, S., Bilson, P. & Asante, K. P. 2012. Haematological and biochemical reference values for healthy adults in the middle belt of Ghana. *PloS one,* 7**,** e36308.

Duarte, J., Herbert, F., Guiyedi, V., Franetich, J.-F., Roland, J., Cazenave, P.-A., Mazier, D., Kombila, M., Fesel, C. & Pied, S. 2012. High levels of IgE autoantibody to 14-3-3 epsilon protein correlate with protection against severe Plasmodium falciparum malaria. *Journal of Infectious Diseases***,** jis595.

Dunne, D. & Mountford, A. 2001. Resistance to infection in humans and animal models. *Imperial College Press, London, UK***,** 133-212.

Efunshile, A. M., Olawale, T., Stensvold, C. R., Kurtzhals, J. A. & Konig, B. 2015. Epidemiological study of the association between malaria and helminth infections in Nigeria. *Am J Trop Med Hyg,* 92**,** 578-82.

Elkins, D., Haswell-Elkins, M. & Anderson, R. 1986. The epidemiology and control of intestinal helminths in the Pulicat Lake region of Southern India. I. Study design and pre- and post- treatment observations on Ascaris lumbricoides infection. *Trans R Soc Trop Med Hyg,* 80**,** 774–792. .

Esrey, S. A., Potash, J. B., Roberts, L. & Shiff, C. 1991. Effects of improved water supply and sanitation on ascariasis, diarrhoea, dracunculiasis, hookworm infection, schistosomiasis, and trachoma. *Bulletin of the World Health organization,* 69**,** 609.

Farouk, S., Dolo, A., Bereczky, S., Kouriba, B., Maiga, B., Farnert, A., Perlmann, H., Hayano, M., Montgomery, S. & Doumbo, O. 2005. Different antibody- and cytokine-mediated responses to Plasmodium falciparum parasite in two sympatric ethnic tribes living in Mali. *Microbes Infect,* 7**,** 110-117.

Feasey, N., Wansbrough-Jones, M., Mabey, D. & Solomon, A. 2010. Neglected tropical diseases. *British Med Bulletin,* 93**,** 179-200.

Fleming, F. M., Brooker, S., Geiger, S. M., Caldas, I. R., Correa-Oliveira, R., Hotez, P. J. & Bethony, J. M. 2006. Synergistic associations between hookworm and other helminth species in a rural community in Brazil. *Tropical Medicine & International Health,* 11**,** 56-64.

Fleming, J., Isaak, A., Lee, J., Luzzio, C., Carrithers, M. & Cook, T. 2011. Probiotic helminth administration in relapsing-remitting multiple sclerosis: a phase 1 study. *Mult Scler.,* 17**,** 743–54.

Fosu, G. B. 1981. Disease classification in rural Ghana: framework and implications for health behaviour. *Social Science & Medicine. Part B: Medical Anthropology,* 15**,** 471-482.

Freeman, M. C., Ogden, S., Jacobson, J., Abbott, D., Addiss, D. G., Amnie, A. G., Beckwith, C., Cairncross, S., Callejas, R. & Colford Jr, J. M. 2013. Integration of water, sanitation, and hygiene for the prevention and control of neglected tropical diseases: a rationale for inter-sectoral collaboration. *PLoS Negl Trop Dis,* 7**,** e2439.

Geiger, S. M., Massara, C. L., Bethony, J., Soboslay, P. T., Carvalho, O. S. & Corrêa-Oliveira, R. 2002. Cellular responses and cytokine profiles in Ascaris lumbricoides and Trichuris trichiura infected patients. *Parasite immunology,* 24**,** 499-509.

Gonçalves, M. L. C., Araújo, A. & Ferreira, L. F. 2003. Human intestinal parasites in the past: new findings and a review. *Memórias do Instituto Oswaldo Cruz,* 98**,** 103-118.

Grencis, R. 1997. Th2–mediated host protective immunity to intestinal nematode infections. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences,* 352**,** 1377-1384.

Grogan, J. L., Kremsner, P. G., Deelder, A. M. & Yazdanbakhsh, M. 1998. Antigen-specific proliferation and interferon-γ and interleukin-5 production are down-regulated during Schistosoma haematobium infection. *Journal of Infectious Diseases,* 177**,** 1433-1437.

Gyapong, M., Nartey, A., Oti, E. & Page, S. 2016. The Social and Economic Impact of Neglected Tropical Diseases in Sub-Saharan Africa. *Neglected Tropical Diseases-Sub-Saharan Africa.* Springer.

Habtamu, K., Abdissa, B. & Zeleke, M. 2015. Prevalence of *Necator americanus* infection and risk factors among school-age children in Mirab

Abaya District, South Ethiopia *Asian Pacific Journal of Tropical Disease,* 5**,** 363-368.

Hagel, I., Cabrera, M., Puccio, F., Santaella, C., Buvat, E., Infante, B., Zabala, M., Cordero, R. & Di Prisco, M. 2011. Co-infection with Ascaris lumbricoides modulates protective immune responses against Giardia duodenalis in school Venezuelan rural children. *Acta Trop* 117**,** 189–195.

Haider, B. A., Humayun, Q. & Bhutta, Z. A. 2009. Effect of administration of antihelminthics for soil transmitted helminths during pregnancy. *Cochrane Database Syst Rev***,** Cd005547.

Harbir, S. 2015. Hymenolepiasis. Medscape. http://emedicine.medscape.com/article/998498-overview#a6. Accessed 27th May 2017.

Hartgers, F. & Yazdanbakhsh, M. 2006. Co-infection of helminths and malaria: modulation of the immune responses to malaria. *Parasite immunology,* 28**,** 497-506.

Hawdon, J. M. 2014. Controlling soil-transmitted helminths: time to think inside the box? *The Journal of parasitology,* 100**,** 166-188.

Helmby, H. 2015. Human helminth therapy to treat inflammatory disorders- where do we stand? *BMC Immunology,* 16**,** 12.

Hewitson, J., Grainger, J. & Maizels, R. 2009. Helminth immunoregulation: The role of parasite secreted proteins in modulating host immunity. *Molecular and Biochemical Parasitology,* 167**,** 1-11.

Hirata, T., Nakamura, H., Kinjo, N., Hokama, A., Kinjo, F., Yamane, N. & Fujita, J. 2007. Increased Detection Rate of Strongyloides stercoralis by Repeated Stool Examinations Using the Agar Plate Culture Method. *The American journal of tropical medicine and hygiene,* 77**,** 683-684.

Hoffman, R., Benz Jr, E. J., Silberstein, L. E., Heslop, H., Weitz, J. & Anastasi, J. 2013. *Hematology: Basic Principles and Practice, Expert Consult Premium Edition-Enhanced Online Features,* Elsevier Saunders, Canada.

Hotez, P. 2008. Hookworm and poverty. *Annals of the New York Academy of Sciences,* 1136**,** 38-44.

Hotez, P., Fenwick, A., Savioli, L. & Molyneux, D. 2009. Rescuing the bottom billion through control of neglected tropical diseases. *Lancet,* 373**,** 1570-5.

Hotez, P. J., Alvarado, M., Basáñez, M.-G., Bolliger, I., Bourne, R., Boussinesq, M., Brooker, S. J., Brown, A. S., Buckle, G. & Budke, C. M. 2014. The global burden of disease study 2010: interpretation and implications for the neglected tropical diseases. *PLoS Negl Trop Dis,* 8**,** e2865.

Hotez, P. J., Bethony, J. M., Oliveira, S. C., Brindley, P. J. & Loukas, A. 2008. Multivalent anthelminthic vaccine to prevent hookworm and schistosomiasis. *Expert Rev Vaccines,* 7**,** 745-52.

Hotez, P. J., Diemert, D., Bacon, K. M., Beaumier, C., Bethony, J. M., Bottazzi, M. E., Brooker, S., Couto, A. R., da Silva Freire, M. & Homma, A. 2013. The human hookworm vaccine. *Vaccine,* 31**,** B227-B232.

Hotez, P. J. & Ehrenberg, J. P. 2010. Escalating the global fight against neglected tropical diseases through interventions in the Asia Pacific region. *Advances in parasitology,* 72**,** 31-53.

Hotez, P. J. & Kamath, A. 2009. Neglected tropical diseases in sub-Saharan Africa: review of their prevalence, distribution, and disease burden. *PLoS neglected tropical diseases,* 3**,** e412.

Hotez, P. J., Molyneux, D. H., Fenwick, A., Ottesen, E., Ehrlich Sachs, S. & Sachs, J. D. 2006. Incorporating a Rapid-Impact Package for Neglected Tropical Diseases with Programs for HIV/AIDS, Tuberculosis, and Malaria. *PLOS Medicine,* 3**,** e102.

Humphries, D., Mosites, E., Otchere, J., Twum, W. A., Woo, L., Jones-Sanpei, H., Harrison, L. M., Bungiro, R. D., Benham-Pyle, B. & Bimi, L. 2011. Epidemiology of hookworm infection in Kintampo North Municipality, Ghana: patterns of malaria coinfection, anemia, and albendazole treatment failure. *The American journal of tropical medicine and hygiene,* 84**,** 792-800.

Humphries, D., Simms, B. T., Davey, D., Otchere, J., Quagraine, J., Terryah, S., Newton, S., Berg, E., Harrison, L. M., Boakye, D., Wilson, M. & Cappello, M. 2013. Hookworm infection among school age children in Kintampo north municipality, Ghana: nutritional risk factors and response to albendazole treatment. *Am J Trop Med Hyg,* 89**,** 540-8.

Imoukhuede, E. B., Ventura, R., Imbault, N., van Schooten, H. & Leroy, O. 2010. European Malaria Vaccine Initiative: portfolio and perspectives for the future. *Human vaccines,* 6**,** 146-150.

Jeandron, A., Abdyldaieva, G., Usubalieva, J., Ensink, J. H. J., Cox, J., Matthys, B., Rinaldi, L., Cringoli, G. & Utzinger, J. 2010. Accuracy of the Kato-Katz, adhesive tape and FLOTAC techniques for helminth diagnosis among children in Kyrgyzstan. *Acta tropica,* 116**,** 185-192.

Jongsuksuntigul, P., Intapan, P. M., Wongsaroj, T., Nilpan, S., Singthong, S., Veerakul, S. & Maleewong, W. 2003. Prevalence of Strongyloides stercoralis infection in northeastern Thailand (agar plate culture detection). *Journal of the Medical Association of Thailand= Chotmaihet thangphaet,* 86**,** 737-741.

Katie, E., Julius, M., Marielle, H., Marguerite, M. L., Maria, Y., Magali, M., Edwin, Q., Tom, O., Peter, K., Adrian, H., Hermelijn, S. & Akim, A. 2014a. Co-infection with Schistosoma haematobium modulates the gene expression profile of malaria infection in schoolchildren in Gabon. *Mal J,* 13**,** 30.

Katie, E., Julius, M., Marielle, H., Marguerite, M. L., Maria, Y., Magali, M., Edwin, Q., Tom, O., Peter, K., Adrian, H., Hermelijn, S. & Akim, A. 2014b. Co-infection with *Schistosoma haematobium* modulates the gene expression profile of malaria infection in schoolchildren in Gabon. *Malar J,* 13**,** 30.

Keegan, A., Snapper, M., Van- Dusen, R., Paul, W. & Conrad, D. 1989. Superinduction of the murine B cell FcεRII by T helper cell clones. Role of interleukin-4. *J. Immu-nolo* 142**,** 3868-74.

Keiser, J. & Utzinger, J. 2008. Efficacy of current drugs against soil-transmitted helminth infections: systematic review and meta-analysis. *Jama,* 299**,** 1937-1948.

Kenneth, K., Marshall, A., Josef, T., Marcel, L., Oliver, W., Linda, J. & Michael, A. 2016. *Williams Hematology. Ninth Edition*, McGraw-Hill Education, New York.

Kepha, S., Mwandawiro, C., Anderson, R., Pullan, R., Nuwaha, F., Cano, J., Njenga, S., Odiere, M., Allen, E., Brooker, S. & Nikolay, B. 2017. Impact of single annual

treatment and four-monthly treatment for hookworm and Ascaris lumbricoides, and factors associated with residual infection among Kenyan school children. *Infectious Diseases of Poverty,* 6**,** 30.

KHRC 2010. Kintampo Health Research Centre Annual Report.

Kinung'hi, S. M., Magnussen, P., Kaatano, G. M., Kishamawe, C. & Vennervald, B. J. 2014. Malaria and Helminth Co-Infections in School and Preschool Children: A Cross-Sectional Study in Magu District, North-Western Tanzania. *PLoS ONE,* 9**,** e86510.

Kirwan, P., Jackson, A., Asaolu, S., Molloy, S., Abiona, T., Bruce, M., Ranford-Cartwright, L., SM, O. & Holland, C. 2010. Impact of repeated four-monthly anthelmintic treatment on Plasmodium infection in preschool children: a double-blind placebo-controlled randomized trial. *BMC Infect Dis,* 10**,** 277.

KMHD 2015. Kintampo Municipal Health Diretorate. Yearly updates.

Knopp, S., Steinmann, P., Keiser, J. & Utzinger, J. 2012. Nematode infections: soil-transmitted helminths and Trichinella. *Infectious disease clinics of North America,* 26**,** 341-358.

Koga, K., Kasuya, S., Khamboonruang, C., Sukhavat, K., Ieda, M., Takatsuka, N., Kita, K. & Ohtomo, H. 1991. A modified agar plate method for detection of Strongyloides stercoralis. *The American journal of tropical medicine and hygiene,* 45**,** 518.

Kotepui, M., Phunphuech, B., Phiwklam, N., Chupeerach, C. & Duangmano, S. 2014. Effect of malarial infection on haematological parameters in population near Thailand-Myanmar border. *Malaria journal,* 13**,** 218.

Kotze, A. C., Clifford, S., O'grady, J., Behnke, J. M. & Mccarthy, J. S. 2004. An in vitro larval motility assay to determine anthelmintic sensitivity for human hookworm and strongyloides species. *The American journal of tropical medicine and hygiene,* 71**,** 608-616.

Kounnavong, S., Vonglokham, M., Houamboun, K., Odermatt, P. & Boupha, B. 2011. Soil-transmitted helminth infections and risk factors in preschool children in southern rural Lao People's Democratic Republic. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 105**,** 160-166.

Kouyos, R. D., Metcalf, C. J. E., Birger, R., Klein, E. Y., Abel zur Wiesch, P., Ankomah, P., Arinaminpathy, N., Bogich, T. L., Bonhoeffer, S., Brower, C., Chi-Johnston, G., Cohen, T., Day, T., Greenhouse, B., Huijben, S., Metlay, J., Mideo, N., Pollitt, L. C., Read, A. F., Smith, D. L., Standley, C., Wale, N. & Grenfell, B. 2014. The path of least resistance: aggressive or moderate treatment? *Proceedings of the Royal Society B: Biological Sciences,* 281**,** 20140566.

Kwarteng, A., Asante, K., Abokyi, L., Gyaase, S., Febir, L., Mahama, E., Konadu, D., Tawiah, T., Adu-Gyasi, D., Dosoo, D., Amenga-Etego, S., Ogutu, B. & Owusu-Agyei, S. 2015. Provider compliance to artemisinin-based combination therapy at primary health care facilities in the middle belt of Ghana. *Malaria Journal,* 14**,** 361.

Lamb, T. 2012. *Immunity to parasitic infection,* John Wiley & Sons.

Lemaitre, M., Watier, L., Briand, V., Garcia, A., Le Hesran, J. Y. & Cot, M. 2014. Coinfection with Plasmodium falciparum and Schistosoma haematobium: additional evidence of the protective effect of Schistosomiasis on malaria in Senegalese children. *The American journal of tropical medicine and hygiene,* 90**,** 329-334.

Lertanekawattana, S., Wichatrong, T., Chaisari, K., Uchikawa, R. & Arizono, N. 2005. Immunological characteristics of patients infected with common intestinal helminths: results of a study based on reverse-transcriptase PCR. *Annals of tropical medicine and parasitology,* 99**,** 71-80.

Levecke, B., Behnke, J., Ajjampur, S., Albonico, M., Ame, S., Charlier, J., Geiger, S., Hoa, N., Kamwa Ngassam, R., Kotze, A., McCarthy, J., Montresor, A., Periago, M., Roy, S., Tchuem Tchuenté, L., Thach, D. & Vercruysse, J. 2011. A comparison of the sensitivity

and fecal egg counts of the McMaster egg counting and Kato-Katz thick smear methods for soil-transmitted helminths. . *PLoS Negl Trop Dis,* 5.

Li, X., Chen, J., Wang, L., Sun, J., Chen, S., Chen, J., Zhang, X. & Zhou, X. N. 2015. Profiling B and T cell immune responses to co-infection of Mycobacterium tuberculosis and hookworm in humans. *Infect Dis Poverty,* 4**,** 20.

Lichtman, M., Kaushansky, K., Prchal, J., Levi, M., Burns, L. & Armitage, J. 2017. *Williams Manual of Hematology*, McGraw Hill Professional.

Loukas, A., Bethony, J., Brooker, S. & Hotez, P. 2006. Hookworm vaccines: past, present, and future. *The Lancet infectious diseases,* 6**,** 733-741.

Loukas, A. & Prociv, P. 2001. Immune Responses in Hookworm Infections. *Clinical Microbiology Reviews,* 14**,** 689-703.

Luckheeram, R., Zhou, R., Verma, A. & Xia, B. 2012. CD4+T Cells: Differentiation and Functions. *Clinical and developmental immunology*, 2012.

Lyke, K., Dicko, A., Dabo, A., Sangare, L., Kone, A., D, C., Guindo, A., Traore, K., Daou, M., Diarra, I., Sztein, M., Plowe, C. & Doumbo, O. 2005. Association of Schistosoma haematobium infection with protection against acute Plasmodium falciparum malaria in Malian children. *Am J Trop Med Hyg.*, 73**,** 1124-30.

Magalhães, R. J. S., Biritwum, N.-K., Gyapong, J. O., Brooker, S., Zhang, Y., Blair, L., Fenwick, A. & Clements, A. C. 2011. Mapping helminth co-infection and co-intensity: geostatistical prediction in Ghana. *PLoS neglected tropical diseases,* 5**,** e1200.

Maizels, R. M. & Yazdanbakhsh, M. 2003. Immune regulation by helminth parasites: cellular and molecular mechanisms. *Nature Reviews Immunology,* 3**,** 733-744.

Mangali, A., Sasabone, P., Syafruddin, Abadi, K., Hasegawa, H., Toma, T., Kamimura, K., Hasan, M., Miyagi, I. & Mogi, M. 1994. Prevalence of intestinal helminthic infections in Kao District, north Halmahera, Indonesia. *Southeast Asian J Trop Med Public Health,* 25**,** 737-44.

Mangla, A., Goel, A. & Singh, T. 2017. Changes in hematological manifestations in children with vivax malaria. *International Journal of Contemporary Pediatrics,* 2**,** 141-144.

Mayer, K. H., Karp, C. L. & Auwaerter, P. G. 2007. Coinfection with HIV and tropical infectious diseases. II. Helminthic, fungal, bacterial, and viral pathogens. *Clinical Infectious Diseases,* 45**,** 1214-1220.

Mazigo, H. D., Amuasi, J. H., Osei, I. & Kinung'hi, S. M. 2018. Integrating use of point-of-care circulating cathodic antigen rapid diagnostic tests by community health workers during mass drug administration campaigns to improve uptake of praziquantel treatment among the adult population at Kome Island, North-Western Tanzania: a cluster randomized community trial. *J BMC public health,* 18**,** 840.

McKee, A. S. & Pearce, E. J. 2004. CD25+ CD4+ cells contribute to Th2 polarization during helminth infection by suppressing Th1 response development. *The Journal of Immunology*, 173**,** 1224-1231.

McSorley, H. J. & Maizels, R. M. 2012. Helminth infections and host immune regulation. *Clinical microbiology reviews,* 25**,** 585-608.

Milner, T., Reilly, L., Nausch, N., Midzi, N., Mduluza, T., Maizels, R. & Mutapi, F. 2010. Circulating cytokine levels and antibody responses to human Schistosoma haematobium: IL-5 and IL-10 levels depend upon age and infection status. *Parasite immunology*, 32**,** 710-721.

MoH, M. o. H. G. H. S. G. 2010 Standard Treatment Guidelines:Introduction. 6th Edition 2010.

Mohrs, M., Shinkai, K., Mohrs, K. & Locksley, R. M. 2001. Analysis of type 2 immunity in vivo with a bicistronic IL-4 reporter. *Immunity,* 15**,** 303-311.

Moorthy, V. S., Newman, R. D. & Okwo-Bele, J.-M. 2013. Malaria vaccine technology roadmap. *The Lancet*, 382**,** 1700-1701.

Mori, Y., Kuroda, M. & Makino, N. 2016. *Nonlinear Principal Component Analysis and Its Applications*, Springer.

Mulu, A., Kassu, A., Legesse, M., Erko, B., Nigussie, D., Shimelis, T., Belyhun, Y., Moges, B., Ota, F. & Elias, D. 2014. Helminths and malaria co-infections are associated with elevated serum IgE. *Parasites & vectors,* 7**,** 240.

Nacher, M. 2011. Interactions between worms and malaria: good worms or bad worms? *Malaria journal,* 10**,** 259.

Nacher, M., Gay, F., Singhasivanon, P., Krudsood, S., Treeprasertsuk, S., Mazier, D., Vouldoukis, I. & Looareesuwan, S. 2000. Ascaris lumbricoides infection is associated with protection from cerebral malaria. *Parasite immunology,* 22**,** 107-113.

Nacher, M., Singhasivanon, P., Silachamroon, U., Treeprasertsuk, S., Vannaphan, S., Traore, B., Gay, F. & Looareesuwan, S. 2001. Helminth infections are associated with protection from malaria-related acute renal failure and jaundice in Thailand *AmJTrop Med Hyg,* 65**,** 834–836.

Nacher, M., Singhasivanon, P., Yimsamran, S., Manibunyong, W., Thanyavanich, N., Wuthisen, P. & Looareesuwan, S. 2002. Intestinal helminth infections are associated with increased incidence of Plasmodium falciparum malaria in Thailand. *J Parasitol.,* 88**,** 55-8.

Naing, C., Whittaker, M. A., Nyunt-Wai, V., Reid, S. A., Wong, S. F., Mak, J. W. & Tanner, M. 2013. Malaria and soil-transmitted intestinal helminth co-infection and its effect on anemia: a meta-analysis. *Transactions of The Royal Society of Tropical Medicine and Hygiene,* 107**,** 672-683.

Nakayamada, S., Takahashi, H., Kanno, Y. & O'Shea, J. 2012. Helper T cell diversity and plasticity. *Curr Opin Immunol,* 24**,** 297-302.

Nebie, I., Diarra, A., Ouedraogo, A., Soulama, I., Bougouma, E., Tiono, A., Konate, A., Chilengi, R., Theisen, M. & Dodoo, D. 2008. Humoral responses to Plasmodium falciparum blood-stage antigens and association with incidence of clinical malaria in children living in an area of seasonal malaria transmission in Burkina Faso, West Africa. *Infection and immunity,* 76**,** 759-766.

Nikolay, B., Brooker, S. & Pullan, R. 2014. Sensitivity of diagnostic tests for human soil-transmitted helminth infections: a meta-analysis in the absence of a true gold standard. *International journal for parasitology,* 44**,** 765-774.

Njua-Yafi, C., Achidi, E., Anchang-Kimbi, J., Apinjoh, T., Mugri, R., Chi, H., Tata, R., Njumkeng, C., Nkock, E. & Nkuo-Akenji, T. 2016. Malaria, helminths, co-infection and anaemia in a cohort of children from Mutengene, south western Cameroon. *Malaria Journal,* 15**,** 69.

Njunda, A. L., Fon, S. G., Assob, J. C. N., Nsagha, D. S., Kwenti, T. D. B. & Kwenti, T. E. 2015. Coinfection with malaria and intestinal parasites, and its association with anaemia in children in Cameroon. *Infectious diseases of poverty,* 4**,** 1-7.

Nkuo-Akenji, T. K., Chi, P. C., Cho, J. F., Ndamukong, K. K. & Sumbele, I. 2006. Malaria and helminth co-infection in children living in a malaria endemic setting of mount Cameroon and predictors of anemia. *Journal of Parasitology,* 92**,** 1191-1195.

O'Brien, J. 2009. 12th Annual Conference on Vaccine Research.

Ojurongbe, O., Adegbayi, A. M., Bolaji, O. S., Akindele, A. A., Adefioye, O. A. & Adeyeba, O. A. 2011. Asymptomatic falciparum malaria and intestinal helminths co-infection among school children in Osogbo, Nigeria. *Journal of research in medical sciences: the official journal of Isfahan University of Medical Sciences,* 16**,** 680.

Ortu, G., Assoum, M., Wittmann, U., Knowles, S., Clements, M., Ndayishimiye, O., Basanez, M. G., Lau, C., Clements, A., Fenwick, A. & Magalhaes, R. J. 2016. The impact of an

8-year mass drug administration programme on prevalence, intensity and co-infections of soil-transmitted helminthiases in Burundi. *Parasit Vectors,* 9**,** 513.

Osborne, L. C., Monticelli, L. A., Nice, T. J., Sutherland, T. E., Siracusa, M. C., Hepworth, M. R., Tomov, V. T., Kobuley, D., Tran, S. V., Bittinger, K., Bailey, A. G., Laughlin, A. L., Boucher, J.-L., Wherry, E. J., Bushman, F. D., Allen, J. E., Virgin, H. W. & Artis, D. 2014. Virus-helminth coinfection reveals a microbiota-independent mechanism of immunomodulation. *Science,* 345**,** 578-582.

Owusu-Agyei, S., Asante, K., Adjuik, M., Adjei, G., Awini, E., Adams, M., Newton, S., Dosoo, D., Dery, D., Agyeman-Budu, A., Gyapong, J., Greenwood, B. & Chandramohan, D. 2009. Epidemiology of malaria in the forest-savanna transitional zone of Ghana. *Malar J,* 8.

Owusu-Agyei, S., Nettey, O. E. A., Zandoh, C., Sulemana, A., Adda, R., Amenga-Etego, S. & Mbacke, C. 2012. Demographic patterns and trends in Central Ghana: baseline indicators from the Kintampo Health and Demographic Surveillance System. *Global health action,* 5.

Pasricha, S.-R., Caruana, S. R., Phuc, T. Q., Casey, G. J., Jolley, D., Kingsland, S., Tien, N. T., MacGregor, L., Montresor, A. & Biggs, B.-A. 2008. Anemia, iron deficiency, meat consumption, and hookworm infection in women of reproductive age in northwest Vietnam. *The American journal of tropical medicine and hygiene,* 78**,** 375-381.

Perera, M., Carter, R., Goonewardene, R. & Mendis, K. 1994. Transient increase in circulating gamma/delta T cells during Plasmodium vivax malarial paroxysms. *Journal of Experimental Medicine,* 179**,** 311-315.

Perez-Mazliah, D. & Langhorne, J. 2015. CD4T-cell subsets in malaria: TH1/TH2 revisited. *CD4+ T cell differentiation in infection: amendments to the Th1/Th2 axiom***,** 50.

Phongluxa, K., Xayaseng, V., Vonghachack, Y., Akkhavong, K., van Eeuwijk, P. & Odermatt, P. 2013. Helminth infection in southern Laos: high prevalence and low awareness. *Parasites & vectors,* 6**,** 328.

Pritchard, D., Hooi, D., Brown, A., Bockarie, M., Caddick, R. & Quinnell, R. 2007. Basophil competence during hookworm (Necator americanus) infection. *The American journal of tropical medicine and hygiene,* 77**,** 860-865.

Pullan, R. L., Smith, J. L., Jasrasaria, R. & Brooker, S. J. 2014. Global numbers of infection and disease burden of soil transmitted helminth infections in 2010. *J Parasites Vectors,* 7**,** 37.

Quinnell, R., Bethony, J. & Pritchard, D. 2004. The immunoepidemiology of human hookworm infection. *Parasite immunology,* 26**,** 443-454.

Ricci, N. D., Fiúza, J. A., Bueno, L. L., Cançado, G. G. L., Gazzinelli-Guimarães, P. H., Martins, V. G., Matoso, L. F., de Miranda, R. R. C., Geiger, S. M. & Correa-Oliveira, R. 2011. Induction of CD4+ CD25+ FOXP3+ regulatory T cells during human hookworm infection modulates antigen-mediated lymphocyte proliferation. *PLoS Negl Trop Dis,* 5**,** e1383.

Ridley, J. W. 2012. *Parasitology for Medical and Clinical Laboratory Professionals,* USA, DELMAR CENGAGE Learning.

Righetti, A. A., Glinz, D., Adiossan, L. G., Koua, A.-Y. G., Niamké, S., Hurrell, R. F., Wegmüller, R., N'Goran, E. K. & Utzinger, J. 2012. Interactions and Potential Implications of Plasmodium falciparum-Hookworm Coinfection in Different Age Groups in South-Central Côte d'Ivoire. *PLoS Neglected Tropical Diseases,* 6**,** e1889.

Riley, E. M. 1999. Is T-cell priming required for initiation of pathology in malaria infections? *Immunology today,* 20**,** 228-233.

Roberts, D. 2016. Hematologic Changes Associated with Specific Infections in the Tropics. *Hematology/oncology clinics of North America,* 30**,** 395-415.

Roussilhon, C., Brasseur, P., Agnamey, P., Pérignon, J.-L. & Druilhe, P. 2010. Understanding human-Plasmodium falciparum immune interactions uncovers the immunological role of worms. *PLoS One,* 5**,** e9309.

Salam, R., Haider, B., Humayun, Q. & Bhutta, Z. 2015. Effect of administration of antihelminthics for soil-transmitted helminths during pregnancy. *Cochrane Database Syst Rev***,** Cd005547.

Salazar-Castañon, V., Legorreta-Herrera, M. & Rodriguez-Sosa, M. 2014. Helminth Parasites Alter Protection against Plasmodium Infection. *BioMed research international,* 2014.

Sanchez-Arcila, J., Perce-da-Silva, D., Vasconcelos, M., Rodrigues-da-Silva, R., Pereira, V., Aprigio, C., Lima, C., Fonseca e Fonseca, B., Banic, D., Lima-Junior, J. & Oliveira-Ferreira, J. 2014. Intestinal parasites coinfection does not alter plasma cytokines profile elicited in acute malaria in subjects from endemic area of Brazil. *Mediators Inflamm,* 2014**,** 857245.

Sangster, N. 1999. Anthelmintic resistance: past, preent and future. *Int J Parasitol,* 29**,** 115-124.

Sanou, S., Régis, W., André, L., Amidou, D., Alphonse, O., Jean-Baptiste, Y., Espérance, O., Federica, V., Charlotte, B., Marita, T.-B., David, M., Amagana, D., Maria, G., Yves, T., Sodiomon, B. & Issa, N. 2012. Haematological parameters, natural regulatory CD4+CD25+FOXP3+ T cells and gδ T cells among two sympatric ethnic groups having different susceptibility to malaria in Burkina Faso Guillaume. *BMC Research Notes,* 5.

Sant-Rayn, P., Caruana, S., Phuc, T., Casey, G., Jolley, D., Kingsland, S., Tien, N., MacGregor, L., Montresor, A. & Beverley-Ann, B. 2008. Anemia, iron deficiency, meat consumption, and hookworm infection in women of reproductive age in northwest Vietnam. *The American journal of tropical medicine and hygiene,* 78**,** 375-381.

Schmaier, A. & Lazarus, H. 2011. *Concise guide to hematology,* John Wiley & Sons.

Schmidt, G. & Roberts, L. 2009. *Foundations of Parasitology,* McGraw-Hill, New York.

Selzer, P. & Caffrey, C. 2012. *Parasitic helminths: Targets, screens, drugs and vaccines*, John Wiley & Sons.

Sen, G., Boatin, B. & Habbema, J. 2002. Can ivermectin mass treatments eliminate onchocerciasis in Africa? *Bull World Health Organ,* 80**,** 384-391.

Shapiro, A., Tukahebwa, E., Kasten, J., Clarke, S., Magnussen, P., Olsen, A., Kabatereine, N., Ndyomugyenyi, R. & Brooker, S. 2005. Epidemiology of helminth infections and their relationship to clinical malaria in southwest Uganda. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 99**,** 18-24.

Shiba, K. R., Shoji, U., Nobumasa, K. & Takeo, M. 1996. *Atlas of Medical Parasitology. First Edition*, Kyokuseisya Co.,Ltd, Japan.

Smallwood, T., Giacomin, P., Loukas, A., Mulvenna, J., Clark, R. & Miles, J. 2017. Helminth Immunomodulation in Autoimmune Disease. *Frontiers in Immunology,* 8**,** 453.

Smith, J. & Brooker, S. 2010. Impact of hookworm infection and deworming on anaemia in non-pregnant populations: a systematic review. *Tropical Medicine & International Health,* 15**,** 776-795.

Smith, K. & Maizels, R. 2013. Nematodes and Regulatory T Cells. *Parasitic Nematodes: Molecular Biology, Biochemistry and Immunology***,** 67.

Sokal, R. & Rohlf, F. 1995. Biometry: the principles and practice ofstatistics in biological research. *State University of New Yorkat Stony Brook, New York, NY*.

Sokhna, C., Le Hesran, J.-Y., Mbaye, P., Akiana, J., Camara, P., Diop, M., Ly, A. & Druilhe, P. 2004. Increase of malaria attacks among children presenting concomitant infection by Schistosoma mansoni in Senegal. *Malaria Journal,* 3**,** 43.

Stanisic, D., Richards, J., McCallum, F., Michon, P., King, C., Schoepflin, S., Gilson, P., Murphy, V., Anders, R. & Mueller, I. 2009. Immunoglobulin G subclass-specific responses against Plasmodium falciparum merozoite antigens are associated with control of parasitemia and protection from symptomatic illness. *Infection and immunity,* 77**,** 1165-1174.

Strunz, E. C., Addiss, D. G., Stocks, M. E., Ogden, S., Utzinger, J. & Freeman, M. C. 2014. Water, Sanitation, Hygiene, and Soil-Transmitted Helminth Infection: A Systematic Review and Meta-Analysis. *PLoS Medicine,* 11**,** e1001620.

Suchard, M. S., Mayne, E., Green, V. A., Shalekoff, S., Donninger, S. L., Stevens, W. S., Gray, C. M. & Tiemessen, C. T. 2010. FOXP3 expression is upregulated in CD4+ T cells in progressive HIV-1 infection and is a marker of disease severity.

Tay, S. C., Nani, E. A. & Walana, W. 2017. Parasitic infections and maternal anaemia among expectant mothers in the Dangme East District of Ghana. *BMC Res Notes,* 10**,** 3.

Taylor-Robinson, A. 2010. Regulation of immunity to Plasmodium: Implications from mouse models for blood stage malaria vaccine design. *Experimental parasitology,* 126**,** 406-414.

Taylor-Robinson, D., Maayan, N., Soares-Weiser, K., Donegan, S. & Garner, P. 2012. Deworming drugs for soil-transmitted intestinal worms in children: effects on nutritional indicators, haemoglobin and school performance (Review). *The Cochrane Library*.

Toma, A., Miyagi, I., Kamimura, K., Tokuyama, Y., Hasegawa, H., Selomo, M., Dahlan, D., Majid, I., Hasanuddi, I., Ngatimin, R., Mogi, M. & Kuwabara, N. 1999. Questionnaire survey and prevalence of intestinal helminthic infections in Barru, Sulawesi, Indonesia. *Southeast Asian J Trop Med Public Health,* 30**,** 68-77.

Tshikuka, J., Scott, M., Gray-Donald, K. & Kalumba, O. 1996. Multiple infection with plasmodium and helminths in communities of low and relatively high socio-economic status. *Ann Trop Med Parasitol.,* 90**,** 277-93.

Urban, J. F., Noben-Trauth, N., Donaldson, D. D., Madden, K. B., Morris, S. C., Collins, M. & Finkelman, F. D. 1998. IL-13, IL-4Rα, and Stat6 are required for the expulsion of the gastrointestinal nematode parasite Nippostrongylus brasiliensis. *Immunity,* 8**,** 255-264.

Verweij, J. J., Canales, M., Polman, K., Ziem, J., Brienen, E. A., Polderman, A. M. & van Lieshout, L. 2009. Molecular diagnosis of Strongyloides stercoralis in faecal samples using real-time PCR. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 103**,** 342-346.

Villegas-Mendez, A., Greig, R., Shaw, T., De Souza, J., Gwyer, F. & Stumhofer, J. 2012. IFN-g-producing CD4+ T cells promote experimental cerebral malaria by modulating CD8+ T cell accumulation within the brain. *J Immunol,* 189**,** 968-79.

Voehringer, D., Reese, T. A., Huang, X., Shinkai, K. & Locksley, R. M. 2006. Type 2 immunity is controlled by IL-4/IL-13 expression in hematopoietic non-eosinophil cells of the innate immune system. *The Journal of experimental medicine,* 203**,** 1435-1446.

Voeks, R. A. & Sercombe, P. 2000. The scope of hunter–gatherer ethnomedicine. *Social Science & Medicine,* 51**,** 679-690.

Walther, M., Jeffries, D., Finney, O. C., Njie, M., Ebonyi, A., Deininger, S., Lawrence, E., Ngwa-Amambua, A., Jayasooriya, S. & Cheeseman, I. H. 2009. Distinct roles for FOXP3+ and FOXP3− CD4+ T cells in regulating cellular immunity to uncomplicated and severe Plasmodium falciparum malaria. *PLoS Pathog,* 5**,** e1000364.

WHO, UNICEF 2001. UNU. *Iron deficiency anaemia: assessment, prevention and control, a guide for programme managers. Geneva: World Health Organization*.

WHO, Word Health Organization 2012. *Bench Aids for the diagnosis of intestinal parasitic infections. WHO: Geneva*.

WHO, World Health Organization 1991. *Basic laboratory methods in medical parasitology*, WHO.

WHO, World Health Organization 2008. *World health statistics 2008*, World Health Organization.

WHO, World Health Organization 2014. World malaria report 2014. Geneva: World Health Organisation; 2014. http://www.who.int/malaria/publications/world_malaria_report_2014/wmr-2014-no-profiles.pdf. . World Health Organization.

WHO, World Health Organization 2015. *Guidelines for the treatment of malaria*, World Health Organization.

Wilson, R. 1993. Immunity and immunoregulation in helminth infections. *Current opinion in immunology*, 5**,** 538-547.

Xu, B., Gordon, C., Hu, W., McManus, D., Chen, H.-G., Gray, D., Ju, C., Zeng, X.-J., Gobert, G. & Ge, J. 2012. A Novel Procedure for Precise Quantification of Schistosoma japonicum Eggs in Bovine Feces. *PLoS neglected tropical diseases*, 6**,** e1885.

Xu, L., Kitani, A., Fuss, I. & Strober, W. 2007. Cutting edge: regulatory T cells induce CD4+CD25 -Foxp3- T cells or are self-induced to become Th17 cells in the absence of exogenous TGF-β. *Journal of Immunology*, 178**,** 6725–6729.

Yatich, N. J., Yi, J., Agbenyega, T., Turpin, A., Rayner, J. C., Stiles, J. K., Ellis, W. O., Funkhouser, E., Ehiri, J. E. & Williams, J. H. 2009. Malaria and intestinal helminth co-infection among pregnant women in Ghana: prevalence and risk factors. *The American journal of tropical medicine and hygiene*, 80**,** 896-901.

Yelifari, L., Bloch, P., Magnussen, P., Lieshout, L. v., Dery, G., Anemana, S., Agongo, E. & Polderman, A. 2005. Distribution of human Oesophagostomum bifurcum, hookworm and Strongyloides stercoralis infections in northern Ghana. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 99**,** 32-38.

Yoshida, A., Maruyama, H., Yabu, Y., Amano, T., Kobayakawa, T. & Ohta, N. 1999. Immune responses against protozoal and nematodal infection in mice with underlying Schistosoma mansoni infection. *Parasitology international*, 48**,** 73-79.

Zaiss, M., Rapin, A., Lebon, L., Dubey, L., Mosconi, I., Sarter, K., Piersigilli, A., Menin, L., Walker, A., Rougemont, J., Paerewijck, O., Geldhof, P., McCoy, K., Macpherson, A., Croese, J., Giacomin, P., Loukas, A., Junt, T., Marsland, B. & Harris, N. 2015. The Intestinal Microbiota Contributes to the Ability of Helminths to Modulate Allergic Inflammation. *Immunity*, 43**,** 998-1010.

Zhu, J., Min, B., Hu-Li, J., Watson, C. J., Grinberg, A., Wang, Q., Killeen, N., Urban, J. F., Guo, L. & Paul, W. E. 2004. Conditional deletion of Gata3 shows its essential function in TH1-TH2 responses. *Nature immunology*, 5**,** 1157-1165.

Ziem, J. B., Magnussen, P., Olsen, A., Horton, J., Asigri, V. L. & Polderman, A. M. 2006. Impact of repeated mass treatment on human Oesophagostomum and hookworm infections in northern Ghana. *J Tropical Medicine International Health*, 11**,** 1764-1772.

Zuur, A., Ieno, E. & Smith, G. 2007. *Analyzing ecological data*, Springer Science & Business Media.

APPENDICES

Appendix 1: Standard Operating Procedure for PBMCs Isolation

Separation, counting and freezing of PBMC

Introduction

The separation of PBMC (peripheral blood mononuclear cells) is essential for all subsequent analyses in immune monitoring. Without an effective and careful isolation, freezing and thawing of the cells, immune monitoring will not be successful and will not provide reliable results.

For separation of mononuclear cells from whole blood the density gradient centrifugation is the most widely used robust method. The separation of PBMCs using Leucosep® tubes containing a porous polyethylene barrier is described. This very efficient, user friendly and reproducible method is especially suitable for multicenter studies with a central immune monitoring facility.

Reagents

Leucosep® tubes, 50 ml, Mr. Frosty cryocontainers, RPMI, Penicilli/Streptomycin, L-Glutamine, FCS or FBS (fetal calf/bovine serum) (Heat-inactivated, filtered), 0.22 μm Filters for serum PVDF membrane, Na Heparin tubes (10 mL), Falcon Tubes (50 mL), Falcon Tubes (15 mL), RBC lysis solution, Pipette tips 1000ul, Isopropyl alcohol, 70% ethanol for disinfection, DMSO Dimethyl Sulfoxide, Serological pipette (10 mL), Serological pipette (5 mL), Lymphoprep, Collection needle, Storage Boxes (81-places), Zip lock bags, pipetting aid for 5-25 ml graded serological pipettes, 5, 10 and 25 ml graded serological pipettes, Nitrile Glove(Powder free)medium size, small tip permanent marker, cryotubes (1.8 ml)

RESPONSIBLITY

This SOP shall be performed by all trained staff at the immunology Lab - KHRC.

A day before sampling (or advance preparation).

1. Prepare Leucosep tubes in MSC. Pipette 15 ml of Lymphoprep into a 50 ml Leucosep tubes and centrifuge at 1000 g for 1 min to get the Lymphoprep below the porous filter disc. Label tubes and lids with subject number and store in the dark.
2. Label all cryovials for plasma collection.
3. Place Mr Frostys for cryovials in the fridge (1 per 3 volunteers).
4. Make up R0 (500 ml RPMI + 5 ml Pen/strep + 5 ml L-glutamine) and R10 (500 ml RPMI + 5 ml Pen/strep + 5 ml L-glutamine + 50 ml filtered FCS).

CAUTION:

Samples must be processed in a biosafety cabinet recorded in laboratory log books.

Processing blood

1. Pour 15-30 mL heparinised blood into a 50 ml Leucosep tube and centrifuge at 1000 g for 13 min at room temperature (RT) without brake (see centrifuge manual). Record the time on the sample record sheet.

2. Take samples of the plasma fraction and place in labelled cryovials. Store at -80°C and record location in log books.

3. Aspirate excess plasma containing the PBMC from each tube into a new, labelled 50ml falcon tube. This should amount to approximately 10-20 ml of cells from and adult.

4. Top the tubes up to 40 ml with R0, and spin at 1800 rpm for 5min at RT.

5. Aspirate the supernatant and flick the bottom of the falcon tube to resuspend cell pellets. Pool the 2 tubes of cells from one donor together (if applicable); top up with R0 to the 40 ml mark and spin at 1800 rpm for 5 min at RT.

6. If the pellet shows significant contamination with red blood cells, then pour off the supernatant and resuspend the pellet in 5ml of RBC Lysis Solution. Rest the cells for maximum 5min before topping up to 30 mL with R0. Spin at 1800 rpm for 5 min at RT.

7. Pour off the supernatant, flick the pellet, and resuspend in 10 ml R10 for counting.

Counting cells

1. Prepare the Haematology Analysers by running phosphate buffered saline (PBS) solution four times through the chambers.

2. Place 100 µL of each cell suspension from the 10 ml cell suspension into a labelled and sterile 1ml eppendorf tubes and diluted with 100ul of PBS. Run the cell suspension as per the SOP of running a full blood count and record the number of cells counted.

3. If the analyser reports very high counts, then the above step (2) should be repeated this time diluting further with 200 µl of PBS and mixing well by pipetting up and down.

Freezing cells

1. Resuspend the remaining cells up to a 0.5 ml of FCS per vial to be frozen and always have at least 5 million cells per vial. i.e 10 vials will have 5 ml of FCS, 8 vials will have 4ml of FCS etc.

2. Place the cells in the fridge for 30 min.

3. Add an equal volume of ice-cold 20% DMSO in FCS (5 ml for 10 vials, 4 ml for 8vials etc) to the cells and mix by pipetting gently up and down. Record the time of adding DMSO-FCS to the Standard record sheet.

4. Aliquot the cell suspensions to labelled cryovials (1 ml per vial) and immediately place them in the Mr Frosty containers and placed in the –80°C freezer.
5. After an overnight (or over weekend if frozen on a Friday) incubation in the –80°C, transfer the frozen cells into liquid nitrogen and record location.

Appendix 2: Standard Operating Procedure for stool processing

1. ROUTINE WET MOUNT (NORMAL SALINE)

Procedure:

a) Mark the number of the specimen on the slide.

b) Put two drops of normal saline in the middle of the slide.

c) Using an applicator, take a small portion of the stool from inside and from the surface of the specimen.

d) Mix the sample with the drops of saline solution on the slide. Add drops of saline when the film is thick to make the wet film translucent. Or blot excess saline when too wet.

e) Place a coverslip over the mixture.

f) Examine the preparation under the microscope use the 10x and 40x objectives.

Reduce the amount of light by lowering the condenser to increase the contrast.

Normal Saline preparation:

Dissolve 8.5g sodium chloride (NaCl) in one litre of distilled water.

2. WET MOUNT (LUGOL'S IODINE)

Procedure:

a) Mark the number of the specimen on the slide.

b) Put two drops of working iodine (Lugol Iodine) in the middle of the slide.

c) Using an applicator, take a small portion of the stool from inside and from the surface of the specimen.

d) Mix the sample with the drops of iodine solution on the slide. Add drops of the iodine when the film is thick to make the wet film translucent. Or blot excess reagent when too wet.

e) Place a coverslip over the mixture.

f) Examine the preparation under the microscope use the 10x and 40x objectives.

Reduce the amount of light by lowering the condenser to increase the contrast.

Lugol Iodine (stock):

Iodine 1 g

Potassium Iodide (KI) 2 g

Distilled water (DW) 100 ml

Dissolve the KI in about 30 ml D.W., add the iodine and mix until dissolved,

complete to 100 ml with D.W. and store in a brown bottle.

Working Iodine Solution:

Dilute 5 times the stock iodine with D.W.

3. FORMOL ETHYL ACETATE CONCENTRATION

Procedure:

a) Add 5 ml of 10% formalin to about 1g of faeces in 15 ml centrifuge tube.

b) Mix until you get a suspension. Top up with volumes of 10% formalin (up to 10 ml) till you have slightly cloudy suspension.

c) Filter the suspension through a gauze filter (40–60 mesh) into a clean centrifuge tube (falcon tube). Discard the gauze filter with residue.

d) Add 2 ml of ethyl acetate and mix well for at least one minute.

e) Centrifuge for ten (10) minute at 500 g.

f) Loosen the fatty plug at the top with a stick applicator, pour away the supernatant by inverting the tube.

g) Repeat steps b), d) and e) if there still remain large amount of debris

l) Mix the sediment at the bottom of the tube.

m) Transfer entire sediment to a slide for examination covering with a cover glass.

n) Use the X10 and X40 objectives to examine the whole area under the coverslip for ova, cysts or larvae.

COUNTING

Detect and count all eggs of the parasite in 10 g of faeces.

4. KATO KATZ TECHNIQUE

The kato katz kit was purchased from the Mahidol University, Thailand with plastic template donated from the Noguchi Memorial Institute for Medical Research, Department of Parasitology, Ghana

Materials and reagents

Flat-sided applicator stick, wooden, Screen, stainless steel, nylon or plastic, 60–105 mesh, Template, stainless steel, plastic or cardboard, Microscope, Microscope slides, Cellophane, Flat-bottomed jar, Forceps, Toilet paper or absorbent tissue, Scrap paper (e.g. newspaper), Glycerol–malachite green solution

Procedure:

Important: Care must be taken to avoid contamination during collection of stool specimens. Always wear gloves.

1. Soak the cellophane strips in the glycerol–malachite green (or methylene blue) solution for at least 24 hours before use.

2. Transfer a small amount (approximately 0.5 g) of faeces on to a piece of scrap paper (newspaper is ideal).

3. Press the screen on top of the faecal sample.

4. Using the applicator stick, scrape across the upper surface of the screen to sieve the faecal sample.

5. Place the template on a clean microscope slide. Transfer the sieved faecal material into the hole of the template and level with the applicator stick.

6. Remove the template carefully so that all the faecal material is left on the slide and none is left sticking to the template.

7. Cover the faecal sample on the slide with a glycerol-soaked cellophane strip.

8. If any glycerol is present on the upper surface of the cellophane, wipe it off with a small piece of absorbent tissue.

9. Invert the microscope slide and press the faecal sample against the cellophane on a smooth surface (a piece of tile or flat stone is ideal) to spread the sample evenly.

10. Do not lift the slide straight up or it may separate from the cellophane. Gently slide the microscope slide sideways while holding the cellophane.

11. Preparation of the slide is now complete. Wipe off any excess glycerol with a piece of absorbent tissue to ensure that the cellophane stays fixed.

12. Use the X10 and X40 objectives to examine the whole area under the coverslip parasites.

Calculation

Approximately 41.7 mg of faeces was used for each slide. Slides should be allowed to clear for 20 min prior to microscopic examination. Calculate the egg count of each slide and express the intensity of infection as Egg Per Gram (EPG) of faeces.

Appendix 3: SOP for antibody estimates by ELISA

STANDARD OPERATING PROCEDURE

FIO-CIL-BR-IM-EN-SOP-080-R01

for

Clinical Immunology Laboratory

1. Procedures

- All procedures performed and equipment used must be recorded on forms A and B of this SOP.
- Activities outlined in steps 9.1-9.4 are performed by Operator.

1.1. Plate Layout and Coding

*All other components are: Antigen, DBB, 2nd Ab[b], Streptavidin-HRP, OPD.

Table 1: Template for anti-IgG, anti-IgG1 and anti-IgM estimation in samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	TS1 @ 1:200											
B	TS1@ 1:400											
C	TS1@ 1:800											
D	TS1@ 1:1600											
E												
F												
G												
H												

Table 2: Template for anti-IgE estimation in samples

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Assay Procedure for Anti-IgE

1.2. For each assay (Day 1) – Basically Coating your plates

1.2.1. Determine the number of plates to be coated according to the number of samples to be assayed; each plate can contain a maximum of 11 samples.

1.2.2. Dilute the antigen to the appropriate working concentration in PBS 1X. Stir solution for 5±2 min.

1.2.3. Dilute the selected analyte to the appropriate working concentration in PBS 1X. Vortex for 20 (±10) seconds.

1.2.4. Add 100 µl/well of the antigen at the appropriate concentration to all wells using the appropriate MAXISORP® or POLYSORP® microtiter ELISA plates.

1.2.5. Add 100 µl/well of the selected analyte at the appropriate working dilution to positive control wells (Tables 1 and 2).

1.2.6. Add 100 µl/well of PBS 1X to wells for Blanks (Table 1).

1.2.7. Cover plates with sealing film and then incubate ON at 2 – 8 °C.

1.3. For each run continued (Day 2) – Basically Adding the diluted samples

1.3.1. Prepare DBB which is 1% BSA (this is made in-house).

1.3.2. Wash plates 3 times with wash buffer PBS 1X in 0.5% Tween (PBST20).

1.3.3. Decant and blot plates 5 times on paper towels.

1.3.4. Add 300 µl/well of DBB to all wells, cover with sealing film and incubate for 2 hours (± 15 min) at RT to block plates.

1.3.5. Decant and blot plates 5 times on paper towels.

1.3.6. Add test samples (100 µl) and appropriate positive controls and blank to the plates in their respective dilutions.

1.3.7. Cover plates with sealing film and incubate ON at 2 – 8 °C.

1.4. For each run continued (Day 3) – Basically, developing the plates for reading

1.4.1. Wash plates 5 times with PBST20 (prepared in-house).

1.4.2. Decant and blot plates 5 times on paper towels.

1.4.3. Using DBB as a diluent, prepare the appropriate working dilution of the biotin-conjugated anti-IgE (2^{nd} Ab^{b}). Mix several times by gentle inversion by hand. The appropriate working dilution for each 2^{nd} Ab^{b} is determined by previous optimization and developmental protocols for each combination of antigen versus immunoglobulin versus NRP or the selected analyte. These experiments are recorded in laboratory notebooks and should be referenced in the worksheet.

1.4.4. Add 100 µl/well of 2^{nd} Ab^{b} at the desired dilution to all wells.

1.4.5. Cover plates with sealing film and incubate for 2 hours ± 15 min at RT.

1.4.6. After incubation, wash plates 5 times with PBST20 as described above.

1.4.7. Dilute the Streptavidin-HRP. Avoiding direct light, prepare a 1:1000 working solution of Streptavidin-HRP in BB. Mix by vortexing for 30 ± 10 seconds.

1.4.8. Decant and blot on paper towel.

1.4.9. Add 100 µl of the diluted Streptavidin-HRP to each of the wells and incubate plates with a sealer or a lid for 1 hour (± 15 min) at RT.

1.4.10. Prepare OPD substrate according to manufacturer's instructions.

1.4.11. Wash plates 5 times with PBST20 as described above.

1.4.12. Decant and blot plates 5 times on paper towels.

1.4.13. Add 100 µl/well of mixed OPD substrate to all wells.

1.4.14. Incubate plates at RT in the dark for 30 ± 5 min.

1.4.15. Immediately read plates at the wavelength of 650 nm on BIO-RAD xMARK microplate reader/ Microplate Manager 6.3 or any available plate reader.

1.4.16. Add 50 µl/well of 1M H_2SO_4 to all wells to stop the reaction.

1.4.17. Immediately read plates at the wavelength of 450 nm on BIO-RAD xMARK microplate reader/ Microplate Manager 6.3 or any available plate reader.

1.4.18. Save raw data and record the storage.

1.4.19. If another assay is to be performed, return to step 10.2 and proceed until all assays have been completed.

Assay Procedure for Anti-IgG, IgM, IgG1, IgA

1.5. For each assay (Day 1) – Basically Coating your plates

1.5.1. Determine the number of plates to be coated according to the amount of samples to be assayed; each plate can contain a maximum of 11 samples.

1.5.2. Dilute the antigen to the appropriate working concentration in PBS 1X. Stir solution for 5±2 min.

1.5.3. Dilute the selected analyte to the appropriate working concentration in PBS 1X. Vortex for 20 (±10) seconds.

1.5.4. Add 100 µl/well of the antigen at the appropriate concentration to all wells using the appropriate MAXISORP® or POLYSORP® microtiter ELISA plates.

1.5.5. Add 100 µl/well of the selected analyte at the appropriate working dilution to positive control wells (Tables 1 and 2).

1.5.6. Add 100 µl/well of PBS 1X to wells for Blanks (Table 1).

1.5.7. Cover plates with sealing film and then incubate ON at 2 – 8 °C.

1.6. For each run continued (Day 2)

1.6.1. Prepare DBB which is 1% BSA (this is made in-house) for biotinylated secondary antibodies and 5% Skimmed Milk for HRP conjugated secondary antibody.

1.6.2. Wash plates 3 times with wash buffer PBS 1X in 0.5% Tween (PBST20).

1.6.3. Decant and blot plates 5 times on paper towels.

1.6.4. Add 300 µl/well of DBB to all wells, cover with sealing film and incubate for 2 hours (± 15 min) at RT to block plates.

1.6.5. Decant and blot plates 5 times on paper towels.

1.6.6. Add test samples (100 µl) and appropriate positive controls and blank to the plates in their respective dilutions.

1.6.7. Cover plates with sealing film and incubate for 1 hour at 2 – 8 °C.

1.6.8. Wash plates 5 times with PBST20 (prepared in-house).

1.6.9. Decant and blot plates 5 times on paper towels.

1.6.10. Using DBB as a diluent, prepare the appropriate working dilution of the biotin-conjugated anti-IgX (2^{nd} Ab^b). Mix several times by gentle inversion by hand. The appropriate working dilution for each 2^{nd} Ab^b is determined by previous optimization and developmental protocols for each combination of antigen versus immunoglobulin versus NRP or the selected analyte. These experiments are recorded in laboratory notebooks and should be referenced in the worksheet.

1.6.11. Add 100 µl/well of 2^{nd} Ab^b at the desired dilution to all wells.

1.6.12. Cover plates with sealing film and incubate for 1 hour ± 15 min at RT.

1.6.13. After incubation, wash plates 5 times with PBST20 as described above.

1.6.14. Dilute the Streptavidin-HRP. Avoiding direct light, prepare a 1:1000 working solution of Streptavidin-HRP in BB. Mix by vortexing for 30 ± 10 seconds.

1.6.15. Decant and blot on paper towel.

1.6.16. Add 100 µl of the diluted Streptavidin-HRP to each of the wells and incubate plates with a sealer or a lid for 1 hour (± 15 min) at RT.

NB: For HRP conjugated secondary antibodies, start developing the assay without the Streptavidin step.

1.6.17. Prepare OPD substrate according to manufacturer's instructions.

1.6.18. Wash plates 5 times with PBST20 as described above.

1.6.19. Decant and blot plates 5 times on paper towels.

1.6.20. Add 100 µl/well of mixed OPD substrate to all wells.

1.6.21. Incubate plates at RT in the dark for 30 ± 5 min.

1.6.22. Immediately read plates at the wavelength of 650 nm on BIO-RAD xMARK microplate reader/ Microplate Manager 6.3 or any available plate reader.

1.6.23. Add 50 µl/well of 1M H_2SO_4 to all wells to stop the reaction.

1.6.24. Immediately read plates at the wavelength of 450 nm on BIO-RAD xMARK microplate reader/ Microplate Manager 6.3 or any available plate reader.

1.6.25. Save raw data and record the storage.

If another assay is to be performed, return to step 10.2 and proceed until all assays have been completed.

2. Calculations

Subtract the measured optical densities of the blank from all TS.

Difference between the OD obtained for the positive sample and what was obtained on each plate should not be more than 15% to accept results from an assay.

OD for TS = Measure the arithmetic mean of the OD for each duplicate of samples at each dilution.

Arbitrary Units could be used if the setup has samples with established concentration through calibration Test Samples according to the procedures specified in the appropriate **Qualification Protocol.**

References

- John R. Crowther, (2001) The ELISA Guide book Methods in Molecular Biology. Volume 149, Humana Press.
- Ministério da Saúde FIOCRUZ Centro de Pesquisa René Rachou
- Laboratory of Vaccines for the Developing World, Institute for Glycomics, Griffith University, Australia
- James Cook's University, Australia

Appendix 4: Cytokine Bead Array Protocol

Work out the number of samples to run including 10 standards.
Make up 2 spares to allow for loss during aliquoting Standards
Reconstitute standards with 200 ul assay diluents, freeze in 20 ul lots (10xconc)
Use 1 aliquot 20 ul add 180 ul assay diluents
using 10 eppendorf tubes add 100 ul assay diluents into 9 tubes.
First tube has 200 ul, mix well transfer 100 ul to the next tube mix. serial dilutions continue to tube 9 tube. 10 will have ONLY diluent

Procedure

Vortex each capture bead suspension

Use 2 ul of each capture bead to make a master mix for the amount of samples needed (including the 2 spares)

Add equal volume of phycoerythrin (PE) detection reagent (equal to the total volume of beads)
Vortex
Transfer to V well microtitre plate and add 24 ul per sample of Inflammation kit Or 20 ul for Th1/Th2 kit
Add 10 ul of standards and sample to appropriate wells Incubate 2 hrs at room temp PROTECT FROM LIGHT!
Add 150 ul wash buffer to each well
Centrifuge plate 200 g (~1000rpm) for 3 minutes, brake off, aspirate off supernatant Resuspend in 200 ul wash buffer Ready to read.

Appendix 5: SOP for PBMC processing, stimulation and staining for flow cytometry

Kintampo Health Research Centre/ Griffith University, Institute for Glycomics and West African Centre for Cell Biology and Infectious Disease Programme

Standard Operating Procedure

Number: 01

Effective: 10th March 2016

I. I. Purpose

This SOP outlines the procedures for performing surface and intracellular staining for enumerating lymphocytes in a population with helminth and malaria infection, co-infections and controls.

II. Scope

This SOP applies specifically to acquisition and analyses of surface and intracellular stained lymphocytes from culture of cryopreserved PBMCs collected from a population with helminth and malaria infection, co-infections and controls.

Reagent Preparation

RPMI-1640

- RPMI-1640
- 10% heat inactivated human serum,
- 2 nM L-glutamine,
- 100 U/ml penicillin and
- 100 mg/ml streptomycin sulphate

Complete Culture Media

For each 500 ml of RPMI-1640 with glutamine, add

- 50 ml of Heat inactivated human serum
- ** volume of absolute PenStrep Concentration

III. Safety

In order to use the instrument, personnel must wear laboratory coats, disposable gloves, googles and have a documented vaccination against hepatitis B certificate. Consumables must be handled according to the institutional guidelines, manufacturer' instructions and reagent data sheets.

IV. Thawing and Cell Culture

1. Remove cells from liquid nitrogen. For each assay, select an RC negative control for use as single stain controls (eg RC13).
2. Thaw 2 vials at a time. Gently agitate in 37 ^{o}C water bath until thawed
3. Dilute 1:10 by adding complete culture medium dropwise
    a. Media should be pre-warm to 37 ^{o}C in the water bath before use (RPMI-1640 containing 10% heat inactivated human serum, 2 nM L-glutamine, 100 U/ml penicillin and 100 mg/ml streptomycin sulphate).
4. Wash PBMCs (2X) twice with complete medium
    a. Conditions for centrifuge: 1500 rpm, 4min, @ RT, Acce 7, Dec 2
5. Count using Trypan Blue. (Spin down completely and resuspend in 1ml of media.
    a. Take 10 µl into 90 µl of Trypan Blue and count the number of cells.
    b. Calculate the number of cells as follows;

i. No. of cells counted x dilution factor (10 for 10:90) x 10^4/ml
ii. Dilute or concentrate to get 2 x 10^6 cells/ml

6. Resuspend cells in complete medium at a concentration of 2 x 10^6 cells/ml.
7. Add 100 µl of cell suspension to the requisite wells of a 96 well round bottom plate.
   a. This yields 2 x 10^5 cells/well (200000 cells in each 100 µl of the suspension)
8. Add 100 µl of the relevant treatments (eg 1%_PHA in complete media) to the requisite wells of a 96 well round bottom plate. Ideally, a minimum of 2 wells should be assigned for each treatment.
9. Ensure the outer wells of the plate are filled with incomplete medium ((RPMI-1640 containing 2 nM L-glutamine, 100 U/ml penicillin and 100 mg/ml streptomycin sulphate)
10. Incubate at 37 ^oC, 5% $C0_2$ for 7 days.
    NB: if performing intracellular cytokine staining, CBA assays on cell supernatants or examining cellular proliferation by tritiated thymidine uptake, perform the necessary procedures on Day06 of culture) (PART VII - STOPPING INTRACELLULAR SECRETION).

V. STOPPING INTRACELLULAR SECRETION

1. Bring plate out of incubator into the hood
2. To the wells designated for the INTRACELLULAR STAINING, add 0.4 µl of GolgiStop.
3. Incubate for 4 hours
4. Start the staining process.

VI. Flow cytometry Prepare cells

1. Remove plate from incubator.
2. Spin at 1200 rpm for 4 minutes.
3. Carefully remove the majority of the supernatant from each well.

- Supernatant from the wells for IC staining that did not receive the BFA should be separated for cytokine analysis using BD CBA kit

4. Resuspend each well in 100 µl of PBS 1X
5. Pool duplicate wells for each treatment on a new 96 well round bottomed plate or tubes.
6. Distribute 100 µl into respective tubes

- Cells + Media
- Cells + PHA
- Cells + pRBC.

7. Spin at 1200 rpm for 4 minutes.
8. Carefully remove the majority of the supernatant from each well.
9. Add 100 µl of PBS 1X to each well/tube
10. Add 1ul of reconstituted viability dye to each well and mix well.

- Preparation of Viability dye (live/dead cells): This must be done in Sodium azide free buffer

  Add 1 µl of viability dye (diluted in DMSO) to 9 µl of PBS 1X.

  This becomes the working reconstituted viability dye for staining

11. Incubate at room temperature in the dark for 10 mins
12. Spin at 1200 rpm for 4 minutes.
13. Carefully remove the majority of the supernatant from each well.
14. Add 10 µl of Brilliant Stain Buffer to each tube
15. Add 90 µl of Stain Buffer (FBS) to each tube
16. Add TCR-gamma delta cell surface marker stains for first staining step.
17. Place on ice in the dark for 25 mins.
18. Spin at 1200 rpm for 4 minutes.
19. Carefully remove the majority of the supernatant from each well.
20. Add 10 µl of Brilliant Stain Buffer to each tube
21. Add 90 µl of Stain Buffer (FBS) to each tube
22. Add stains for second step (CD4, CD8, CD3, HLA-DR and CD11c cell surface markers) in Stain Buffer.
23. Place on ice in the dark for 25 minutes.
24. Add 100 µl of Stain buffer to each well.
25. Spin at 1200 rpm for 4 minutes.
26. Carefully remove the majority of the supernatant from each well.
27. Add 100 µl of 1X Fix/Perm Buffer to each well.
28. Incubate at 2 ^{o}C -8 ^{o}C for 35 mins away from light
29. Add 100 µl of Perm/Wash buffer to each tube after incubation
30. Spin at 1200 rpm for 4 minutes.
31. Carefully remove the majority of the supernatant from each well.
32. Add 100 µl of Perm/Wash Buffer to each well.
33. Add the antibodies for the intracellular proteins (FoxP3, IL-4 and IFN-gamma) in 1X Perm/Wash.
34. Incubate at 2 ^{o}C -8 ^{o}C for 35 mins.
35. Add 100 µl of Perm/Wash Buffer to the cell suspension
36. Spin at 1200 rpm for 4 minutes.
37. Carefully remove the majority of the supernatant from each well.
38. Resuspend cells in 200 µl of Stain Buffer (FBS) for acquisition on the Flow cytometer.

Appendix 6: Questionnaire

Helminths and Malaria INFECTIONS WITHIN KHDSS

KINTAMPO HEALTH RESEARCH CENTRE (KHRC)

Helminths_Malaria INFECTION FORM (2014)

FORM NO FORMNO

BATCH NO. BATCHNO

1.0 PARTICIPANT IDENTIFICATION

1.1. Study identification number..... H M STDYID

1.2. HRB code: ..... HRB

1.3. Current Household ID..... HOUSEID

1.4. Name of Respondent ..... RNAME

1.5. Participant's Permanent ID PERMID

1.6. Date of visit: (DD/MMM/YYYY)..... DATEVISIT

1.7. Fieldworker/Supervisor: ..... FW

1.8. GIS coordinate of residential compound

Longitude (X) ..... LONG

Latitude (Y)..... LATI

1.9. Number of people in the household HSEDNO

2.0 GENERAL AND DEMOGRAPHIC QUESTIONS:

2.1 Date of Birth (**88/888/8888** if not known) DOB

2.2 Age (yrs)..... AGE

2.3 Sex..... 1. Male 2. Female GENDER

2.4 Weight (Kg)..... WgtKg

2.5 Height (cm)..... HgtCm

2.6 What is the highest level of education you have completed? SCHOOL

1. No school	2. Elementary	3. High school	4. College
5. Higher education (professional or post-graduate)		6. Religious schooling only	7. Literacy classes only

3. BEHAVIOURAL CHARACTERISTICS

3.1 Do you have toilet in your house? 1. Yes 2. No TOILET

3.2 By what means does participant mostly defecate (choose one)?

1. Open Fields	2. Pit Latrine	3. Water Closet	4. KVIP	DEFMEN

3.3 Do you scrub your nails before meals? 1. Yes 2. No NAILS

3.4 Do you wash your hands with soap before eating? 1. Yes 2. No HDWAS

3.5 Have you dewormed (taken anti-helminthic) in the last three months? | 1.Yes | 2.No | DWORM

3.6 What type of drugs do you use to treat or deworm? (multiple responses allow)

1.Herbal (Traditional)	2.Pharmaceutical drugs	9. NA	TYPEDE

3.7 IF your answer to 3.6 is 2 then which of the following anti-helminthic drugs do you usually buy whenever you need one? (Not Applicable-NA; Not Known-NK)

1. Vermox	2. Zentel	3. Mebendazole	4. Ivermectin		USETYP
5. Praziquantel	6. Albendazole	7. Pripsen	8. NK	9. NA	

3.8 Is there a source of water in your house?..... | 1. Yes | 2. No | HWATA

3.9 What is your source of water in your house?

1. Pipe borne water	2. Well	3. River/Stream	4.Bore-hole	HTYWA
5. Other (Specify.....				

3.10 IF Question 3.8 is NO, is there a source of water close to your house?..... | 1. Yes | 2. No | 9. NA | CWATA

3.11 What is your source of water that is close to your house?

1. Pipe borne water	2. Well	3. River/Stream	4.Bore-hole	CTYWA
5. Other (Specify.....			9. NA	

3.12 What is your source of **drinking water** in your house?

1. Pipe borne water	2. Well	3. River/Stream	4.Bore-hole	DRWAT
5. Sachet water	6. Bottle water	Other (Specify.....		

3.13 Do you use the refuse dumping site in the community?..... | 1. Yes | 2. No | REFUS

3.14 Which of the following do you wear for protection when going to farm or working in the soil?

1. Slippers (chalewate)	2. Sandals	3. Wellington boot	PRSHOE
4. Snickers (Cambuu)	5. Shoe	6. None	

3.15 Do you sleep in a mosquito net?..... | 1. Yes | 2. No | NET

3.16 When do you use the net when sleeping?

1. Only at night	2. All times	9. NOT APPLICABLE	NETIME

3.17 How often do you wash you mosquito net?

1. Every six months	2. Once in a year	3. Does not wash	9. NA	NETWAS
4. Other				

3.18 Do you share bed with any of your siblings?..... | 1. Yes | 2. No | BEDSHR

4. SOCIAL CHARACTERISTIC

4.1 Occupation

11.Professional: teacher, nurse, accounts, administrator, etc	12.Clerical/Secretarial	13.Trader/businessman/driver with own car etc	OCCUP
14.Employed tradesman, driver without own car, builder, etc	15.Farmer/labourer/domestic worker	16.Other	
17. No	99. NA		

4.2 Religion.....	1. Muslim	2. Christian	3.Traditional		RELIGN
	4. None	5. Other (specify)	8. NK	9. NA	

4.3 Are you pregnant? (NA if male and female less than 12years).....	1.Yes	2.No	9.NA	PREGNT
4.4 Have you lost pregnancy before? (NA if male and female less than 12years).....	1. Yes	2. No	9.NA	PREGLOS

4.5 Are animals being reared by anybody in the selected household?..	1. Yes	2. No	ANIREA
4.6 Are the animals reared within the compound?	1. Yes	2. No	ANICMP
4.7 Are you directly involved in the feeding, cleaning and keeping of the reared animals?	1. Yes	2. No	ANIKEEP
4.8 Is there any health facility within the community?	1. Yes	2. No	HOSPI

5.0 KAP and MASS DRUG ADMINISTRATION (school-aged children) Cross-out if Adult

5.1. Have you ever been given a de-wormer at school?		1. Yes	2. No	DRUGSC
5.2. Do you have toilet at school?		1. Yes	2. No	TSCH
5.3. Do you have provision at school to wash your hands after toilet?		1. Yes	2. No	WASHSC
5.4. Does the provision include soap?	1. Yes	2. No	9.NA	SOAPSC
5.5. Do you wash your hands after visiting the toilet at school?		1. Yes	2. No	WSHAFT
5.6. If yes to 5.5, Do you wash as a person with soap at school after visiting the toilet?	1. Yes	2. No	9.NA	SOAPAF
5.7. a). Do you wash your hands before eating at school?		1. Yes	2. No	WASHEA
5.8. If yes to 5.7, Do you wash your hands with soap before eating?	1. Yes	2. No	9.NA	SOAPEA

6.0 KAP and MASS DRUG ADMINISTRATION (All respondent)

6.1. What are the perceived causes of worm infection? (multiple responses allow)

1. Drinking dirty water	2. Bathing in dirty water	3. Open defecation in water			PCAUSE
4. Malnutrition	5. Consumption of dirty fruits	6. Not wearing shoes	8. NK	9. NA	
7. Poor hygiene					

6.2. Which of the following are signs and symptoms of worm infection? (multiple responses allow) SIGNS

1. Blood in stool	2. Blood in urine	3. Abdominal pain
4. Vomiting	5. Diarrhoea	6. mucoid in stool and slippery
8. NK	9. NA	

6.3 How can you prevent getting worm infection? (multiple response allowed)

1. Avoid drinking dirty water	2. Avoid bathing in dirty water	3. Avoid open defecation in water		PREVEN
4. avoid eating unwashed fruits	5. Taking worm drugs from time to time	8. NK	9. NA	

6.4 Where did you get your information on worms and parasite infection

1. Hospital	2. Health worker	3. Radio	4. Television	5. Family	INFO
6. School	7. Traditional healer		8. NK	9.NA	

Appendix 7: Ethical Approval Letters

Kintampo Health Research Centre (KHRC) Institutional Ethics Committee (IEC)
P.O Box 200
Kintampo, B/A
Ghana, West Africa

Tel: +233(3520)92037 (Ext 117)
E-mail: fred.kanyoke@kintampo-hrc.org

FULL ETHICAL APPROVAL CERTIFICATE

Dennis Adu-Gyasi
Kintampo Health Research Centre
Box 200
Kintampo, B/A
Ghana, West Africa

Date: 24th November 2014

Study File Number: 2014-20

Title of study: Epidemiology and Immunology of Helminths and Plasmodium species infections in the middle belt of Ghana.

Principal Investigator(s): Dennis Adu-Gyasi

Co-Investigator(s): David Dosoo, Nicholas Amoako, Dr. Seth Owusu-Agyei, Dr. Kwaku Poku Asante, Prof. Ben Gyan, Prof Daniel Dodoo, Dr. Bright Adu

Type of Review: Full Board Review

Approval Date: 24th November, 2014

Expiration Date: 24th November, 2015

1. The Kintampo Health Research Centre Institutional Ethics Committee (IEC) is constituted and operates in conformance with requirements of 45 CFR 46, 21 CFR 50, 21 CFR 56 and section 3 of the International Council on Harmonization Guidelines. The OHRP Federal wide Assurance number for the committee is 00011103; the IRB registration number is 0004854.

1. The study in title was reviewed by the IEC on 23rd September 2014 and a Conditional Ethical Approval (CEA) was granted to the study.

2. The IEC subsequently granted Full Ethical Approval (FEA) for implementation of the study after condition/concerns were addressed in a revised protocol.

3. The following documents were reviewed and approved:
   3.1 Epidemiology and Immunology of Helminths and Plasmodium species infections in the middle belt of Ghana, Version 17, dated 2nd July, 2014
   3.2 Epidemiology and Immunology of Helminths and Plasmodium species infections in the middle belt of Ghana, Version 23, dated 21Nov2014.
   3.3 Informed consent form, Version 4, dated 2nd July 2014
   3.4 Informed consent form, Version 8, dated 2Sep2014

File number: 2014-20

THE CHAIRMAN, KINTAMPO
HEALTH RESEARCH CENTRE
INSTITUTIONAL ETHICS
COMMITTEE

Kintampo Health Research Centre (KHRC) Institutional Ethics Committee (IEC)
P.O Box 200
Kintampo, B/A
Ghana, West Africa
Tel: +233(3520)92037 (Ext 117)
E-mail: fred.kanyoke@kintampo-hrc.org

3.5 Informed assent form, Version 5, dated 2nd July 2014
3.6 Informed assent form, Version 8, dated 2Sep2014
3.7 Study Questionnaire, Version 5, dated 2nd July 2014
3.8 Study Questionnaire, Version 8, dated 2Sep2014
3.9 Study Budget
3.10 Curriculum Vitae of Investigators

4. During study implementation, the IEC must be informed within 72 hours by the principal investigator (PI) of learning of any (a) unexpected, serious, study related adverse events; (b) disclosed adverse events, or (c) unanticipated problems with the study which may pose risk to study participants or others.

5. Changes or modifications to this research activity must be submitted and approved by the IEC before they are implemented.

6. PI(s) would be required to submit application for renewal of this approval certificate (if necessary) plus a progress report.

7. PI(s) is required to notify the IEC of study completion (end of data collection/last follow-up) or early termination of the research project.

8. Submit final report of the study three months after approval certificate expires (study closure)

9. Before conduct of the study, submit original/final copy of your informed consent and assent forms for an **authentication** stamp before making photocopies for your consent process.

10. Regulated study records, including IEC approvals and signed consent forms, must be securely maintained by PI(s) and available for audits for three years after the study is closed with the IEC.

Sincerely,

THE CHAIRMAN, KINTAMPO HEALTH RESEARCH CENTRE INSTITUTIONAL ETHICS COMMITTEE

Nana Franklin Fei
(Voting member)
For: Chair
Institutional Ethics Committee
Kintampo Health Research Centre

File number: 2014-20

Kintampo Health Research Centre (KHRC) Institutional Ethics Committee (IEC)
P.O Box 200
Kintampo, B/A
Ghana, West Africa

Tel: +233(3520)97604 (Ext 117)
E-mail: iec.sec@kintampo-hrc.org

RENEWAL APPROVAL CERTIFICATE

Dennis Adu-Gyasi
Kintampo Health Research Centre
Box 200
Kintampo, B/A
Ghana, West Africa

Date: 21st October 2015

Study File Number: 2014-20/Renewal

Title of study: Epidemiology and Immunology of Helminths and Plasmodium species infections in the middle belt of Ghana.

Principal Investigator(s): Dennis Adu-Gyasi

Co-Investigator(s): David Dosoo, Nicholas Amoah, Dr. Seth Owusu-Agyei, Dr. Kwaku Poku Asante, Prof. Ben Gyan, Prof Daniel Dodoo, Dr. Bright Adu

Type of Review: Full Board Review

Approval Date: 20th October 2015

Expiration Date: 20th October 2016

1. The Kintampo Health Research Centre Institutional Ethics Committee (IEC) is constituted and operates in conformance with requirements of 45 CFR 46, 21 CFR 50, 21 CFR 56 and section 3 of the International Council on Harmonization Guidelines. The OHRP Federal wide Assurance number for the committee is 00011103; the IRB registration number is 0004854.
2. The above study's progress report and extension request were reviewed and approved by the IEC on 20th October 2015.
3. The study's approval certificate has therefore been renewed till 20th October, 2016.
4. The IEC must be informed within 72 hours by the principal investigator (PI) of learning of any (a) unexpected, serious, study related adverse events; (b) disclosed adverse events, or (c) unanticipated problems with the study which may pose risk to study participants or others.
5. All safety monitory reports, including DSMB summaries and reports, must be submitted to the IEC as soon as they become available to PI(s).

File number: 2014-20/Renewal 1

THE CHAIRMAN, KINTAMPO HEALTH RESEARCH CENTRE INSTITUTIONAL ETHICS COMMITTEE

Kintampo Health Research Centre (KHRC) Institutional Ethics Committee (IEC)
P.O Box 200
Kintampo, B/A
Ghana, West Africa
Tel: +233(3520)97604 (Ext 117)
E-mail: iec.sec@kintampo-hrc.org

6. Changes or modifications to this research activity must be submitted and approved by the IEC before they are implemented.

7. PI(s) would be required to submit application for renewal of this approval certificate (if necessary) plus a progress report.

8. PI(s) is required to notify the IEC of study completion (end of data collection/last follow-up) or early termination of the research project.

9. Submit final report of the study three months after approval certificate expires (study closure)

10. Submit original/final copy of your informed consent forms for an **authentication stamp** before making photocopies for your consent process.

11. Regulated study records, including IEC approvals and signed consent forms, must be securely maintained by PI(s) and available for audits for three years after the study is closed with the IEC.

Sincerely,

THE CHAIRMAN - INSTITUTIONAL
HEALTH RESEARCH CENTRE
INSTITUTIONAL ETHICS
COMMITTEE

Rev. Dr. Joe Eyison
Chair
Institutional Ethics Committee
Kintampo Health Research Centre

NOGUCHI MEMORIAL INSTITUTE FOR MEDICAL RESEARCH

Established 1979 A Constituent of the College of Health Sciences

University of Ghana

Phone: +233-302-916438 (Direct)
+233-289-522574
Fax: +233-21-502182/513202
E-mail: nirb@noguchi.mimcom.org

NMIMR-IRB
P. O. Box LG 581
Legon, Accra
Ghana

My Reference: DF 22

November 13, 2015
Dennis Adu-Gyasi, MSc
KHRC
P. O. Box 200
Kintampo

RE: Our Study # 033/14-15 **At:** NOGUCHI MEMORIAL INSTITUTE FOR MEDICAL RESEARCH-IRB

Dear Dennis Adu-Gyasi, MSc:

Meeting Date: 11/4/2015 **At:** NOGUCHI MEMORIAL INSTITUTE FOR MEDICAL RESEARCH-IRB

Protocol Title:

Epidemiology and immunology of helminth and Plasmodium species Scale document down elt of Ghana

This is to advise you that the above referenced Study has been presented to the Institutional Review Board, and the following action taken subject to the conditions and explanation provided below.

Internal #: 1149
Expiration Date: 11/3/2016
On Agenda For: Renewal
Reason 1: Progress Report **Reason 2:**
Description:
IRB ACTION: Renewed
Condition 1:
Action
Explanation:

Yours Sincerely,

NMIMR-IRB
IRB Administrator
cc: Ben Adu Gyan , Bright Adu, PhD , NMIMR, David Dosoo, MSc. , KHRC, Dr. Kwaku Poku Asante, , KHRC, Research and Epidemiology, Nicholas Amoako, Mphil , Prof. Daniel Dodoo , NMIMR, Immunology Department, Seth Owusu Agyei, PhD, Kintampo Health Research Centre, Ghana Health Service, O. O. Box 200

NOGUCHI MEMORIAL INSTITUTE FOR MEDICAL RESEARCH
Established 1979 A Constituent of the College of Health Sciences
University of Ghana

Phone: +233-302-916438 (Direct)
+233-289-522574
Fax: +233-21-502182/513202
E-mail: nirb@noguchi.mimcom.org

NMIMR-IRB
P. O. Box LG 581
Legon, Accra
Ghana

My Reference: DF 22

November 13, 2015
Dennis Adu-Gyasi, MSc
KHRC
P. O. Box 200
Kintampo

RE: Our Study # 033/14-15 **At:** NOGUCHI MEMORIAL INSTITUTE FOR MEDICAL RESEARCH-IRB

Dear Dennis Adu-Gyasi, MSc:

Meeting Date: 11/4/2015 **At:** NOGUCHI MEMORIAL INSTITUTE FOR MEDICAL RESEARCH-IRB

Protocol Title:

Epidemiology and immunology of helminth and Plasmodium species infections in the middle belt of Ghana

This is to advise you that the above referenced Study has been presented to the Institutional Review Board, and the following action taken subject to the conditions and explanation provided below.

Internal #: 1149
Expiration Date: 11/3/2016
On Agenda For: Renewal
Reason 1: Progress Report **Reason 2:**
Description:
IRB ACTION: Renewed
Condition 1:
Action
Explanation:

Yours Sincerely,

NMIMR-IRB
IRB Administrator
cc: Ben Adu Gyan , Bright Adu, PhD , NMIMR, David Dosoo, MSc. , KHRC, Dr. Kwaku Poku Asante, , KHRC, Research and Epidemiology, Nicholas Amoako, Mphil , Prof. Daniel Dodoo , NMIMR, Immunology Department, Seth Owusu Agyei, PhD, Kintampo Health Research Centre, Ghana Health Service, O. O. Box 200

Appendix 8: Tables presenting categorizations of infections and data used for analysis and discussions

Appendix 8.1: Classification of public health significance of anaemia in populations on the basis of prevalence estimated from blood levels of haemoglobin. Department of Nutrition for Health and Development (NHD) World Health Organization, 2001.

Category of public health significance	Prevalence of anaemai (%)
Severe	40 or higher
Moderate	20.0 - 39.9
Mild	5.0 - 19.9
Normal	4.9 or lower

Appendix 8.2: Criteria for classification of worm intensity (Sant-Rayn et al., 2008, WHO, 2012)

	Intensity of worm infection (egg per gram of stool)			
Parasite	Nil	Light Intensity	Moderate Intensity	Heavy Intensity
Hookworm	0	1 - 1999	2000 - 4000	>4000
Ascaris lumbricoides	0	1 - 4999	5000 - 50000	>50000
Trichuris trichiura	0	1 - 999	1000 - 10000	>10000
Taenia species*	0	1 - 1999	2000 - 4000	>4000
H. nana/ H. dimunita*	0	1 - 1999	2000 - 4000	>4000

**** adopted the criteria for hookworm***

Appendix 8.3: Effects of parasitic infections and factors on the principal components from variables of plasma antibodies and cytokines

Component Analysed		APC1		APC2		AnCyPC1		AnCyPC2		AnPC1		AnPC2		CyPC1		CyPC2	
	n	Rank Sum	p-value	Rank Sum	p-value	Rank Sum	p-value	Rank Sum	p-value	Rank Sum	p-value	Rank Sum	p-value	Rank Sum	p-value	Rank Sum	p-value
Infection Status																	
No Infection	21	1276	0.006	1330	<0.001	1253	<0.001	1070	0.113	1108	0.371	1535	0.131	1479	<0.001	1692	0.172
Hookworm	27	2269		1717		2151		1959		2058		2096		1963		1672	
Malaria	26	1761		2283		2183		1525		1819		1402		2195.5		1891.5	
Hookworm-Malaria	18	1287		1378		1297		1082		1214		1176		1304.5		1097.5	
Helminth	30	1795		1296		1424		2321		1935		2025		1348.5		1802.5	
Helminth-Malaria	9	258		642		338		689		512		412		355		490	
No Infection	21	1276	0.39	1330	<0.001	1253	0.061	1070	0.056	1108	0.312	1535	0.117	1479	0.008	1692	0.058
Helminth	57	4064		3013		3575		4280		3993		4121		3311.5		3474.5	
Malaria	26	1761		2283		2183		1525		1819		1402		2195.5		1891.5	
Helminth-Malaria	27	1545		2020		1635		1771		1726		1588		1660		1588	
Age Group																	
<8	50	2535	<0.001	3503	0.001	3100	0.007	2974	0.238	2944	0.003	3110	0.117	3178	0.302	3297	0.115
8-16	15	233		1272		530		748		535		640		761.5		935.5	
17-30	21	1580		1329		1462		1323		1445		1398		1444.5		1138.5	
31-60	34	2997		1462		2304		2422		2485		2453		2074.5		2024.5	
61-100	5	530		309		479		408		466		274		416.5		479.5	
Gender																	
Male	58	3744	0.656	3329	0.108	3440	0.289	3599	0.785	3451	0.315	3780	0.533	3522	0.4658	3588	0.719
Female	67	4131		4546		4435		4276		4424		4095		4354		4286	
occupation																	
Farmer	25	2179	<0.001	1214	<0.001	1815	0.209	1975	0.032	2014	0.006	1447	0.041	1577	0.829	1554	0.826
Professional	1	47		30		17		49		27		42		36		51	
Trader	18	1581		740		1196		1254		1323		1524		1071		1020	
Unemployed	80	3943		5766		4722		4472		4386		4737		5066		5125	

Component Analysed		APC1		APC2		AnCyPC1		AnCyPC2		AnPC1		AnPC2		CyPC1		CyPC2	
	n	Rank Sum	p-value	Rank Sum	p-value	Rank Sum	p-value	Rank Sum	p-value	Rank Sum	p-value	Rank Sum	p-value	Rank Sum	p-value	Rank Sum	p-value
religion																	
Christian	82	5187	0.509	4723	0.151	4946	0.58	5093	0.671	5068	0.971	5231	0.376	4964	0.404	5055	0.874
Muslim	38	2242		2683		2473		2248		2323		2112		2467		2348	
Traditional	2	74		97		84		162		112		160		72		100	
BMI																	
Underweight	56	2588	<0.001	4221	0.002	3444	0.161	3369	0.724	3301	0.315	3089	0.087	3577	0.298	3953	0.064
Normal weight	61	4768		3298		4097		3998		4142		4195		3932.5		3466.5	
Over weight	8	519		356		334		508		432		591		365.5		455.5	
Education																	
No education	40	2902	0.04	2523	0.03	2817	0.207	2749	0.432	2865	0.185	2437	0.549	2647	0.284	2337	0.156
Elementary	78	4423		5155		4717		4662		4615		4898		4913		5214	
Higher education	7	550		197		341		464		395		540		315		324	
Toilet																	
Yes	25	1533	0.796	1275	0.064	1313	0.106	1370	0.206	1316	0.11	1723	0.361	1417	0.277	1597.5	0.877
No	100	6342		6600		6562		6505		6559		6152		6459		6277.5	
Means of defacation																	
Open Field	46	2779	0.91	3358	0.105	3152	0.541	3093	0.628	3172	0.351	2341	0.014	3033	0.789	2937	0.988
Pit latrine	33	2166		1872		1954		2118		2089		2063		2025		2104	
Water closet	8	478		389		419		425		377		594		433		491	
KVIP	38	2452		2256		2350		2239		2237		2877		2384		2343	
Scrub Nails before eating																	
Yes	87	6002	0.005	5442	0.834	5922	0.018	5693	0.255	5876	0.034	5665	0.323	5683	0.228	5493	0.943
No	38	1873		2433		1953		2182		1999		2210		2192		2382	
Wash hands with soap																	
Yes	92	5917	0.498	5717	0.658	5805	0.96	5808	0.946	5863	0.708	5875	0.658	5830	0.832	5904	0.501
No	33	1958		2158		2070		2067		2012		2000		2045		1971	

	Component Analysed		APC1		APC2		AnCyPC1		AnCyPC2		AnPC1		AnPC2		CyPC1		CyPC2	
		n	Rank Sum	p-value	Rank Sum	p-value	Rank Sum	p-value	Rank Sum	p-value	Rank Sum	p-value	Rank Sum	p-value	Rank Sum	p-value	Rank Sum	p-value
Dewormer (last 3-months)																		
	Yes	13	609	0.089	814	0.968	647	0.164	742	0.533	670	0.228	919	0.419	743	0.494	860	0.712
	No	112	7266		7061		7228		7133		7205		6956		7132		7015	
Use Refuse site																		
	Yes	111	7000	0.956	6731	0.04	6886	0.402	7140	0.25	7032	0.76	7052	0.644	6788	0.074	6949.5	0.705
	No	14	875		1144		989		735		843		823		1088		925.5	
bed share																		
	Yes	98	5711	0.006	6271	0.561	5927	0.138	6150	0.886	5982	0.249	6263	0.593	6037	0.359	6288.5	0.445
	No	27	2164		1604		1948		1725		1893		1612		1839		1586.5	
Water source in House																		
	Yes	8	159	0.796	128	0.376	145	0.796	143	0.74	147	0.854	196	0.105	158	0.807	172	0.414
	No	29	544		575		558		560		556		507		545		531	
Drinking Water source close to house																		
	Pipe-borne	24	1081	0.972	933	0.133	1040	0.209	1047	0.4	1002	0.175	1192	0.024	1018	0.898	988	0.943
	Well	15	670		518		510		527		494		825		623.5		673.5	
	River/Stream	29	1234		1487		1458		1409		1459		969		1336		1292	
	Bore-hole	18	756		803		733		758		786		755		763.5		787.5	
Footwear																		
	Yes	110	6980	0.704	6880	0.704	6928	0.988	6884	0.727	6889	0.755	6933	0.982	6971	0.729	7032	0.389
	None	15	895		995		947		991		986		942		904		843	

	Component Analysed		APC1		APC2		AnCyPC1		AnCyPC2		AnPC1		AnPC2		CyPC1		CyPC2	
		n	Rank Sum	p-value	Rank Sum	p-value	Rank Sum	p-value	Rank Sum	p-value	Rank Sum	p-value	Rank Sum	p-value	Rank Sum	p-value	Rank Sum	p-value
Actual Season																		
	Nov15-Apr16	86	5431	0.425	4971	0.074	5250	0.827	5678	0.029	5537	0.164	5264	0.888	5112	0.272	5305	0.921
	Jun16-Oct16	36	2072		2532		2253		1825		1966		2239		2391		2198	
Animal Reared by Tenant of compound																		
	Yes	84	5198	0.621	5551	0.173	5488	0.303	5513	0.245	5572	0.141	4843	0.018	5363	0.678	4994	0.081
	No	41	2677		2324		2387		2362		2303		3032		2512		2881	
Animal Reared within compound																		
	Yes	86	5423	0.979	5635	0.248	5657	0.203	5659	0.199	5752	0.075	5087	0.078	5501	0.625	5147.5	0.109
	No	39	2452		2240		2218		2216		2123		2788		2375		2727.5	
Participants directly involved in Animal Rearing																		
	Yes	52	3969	<0.001	3175	0.613	3942	<0.001	3992	<0.001	4224	<0.001	2921	0.075	3398	0.499	2935.5	0.058
	No	73	3906		4700		3933		3883		3651		4954		4478		4939.5	