HUMORAL IMMUNE RESPONSES AND MICRONUTRIENT LEVELS OF *Ascaris lumbricoides*-INFECTED NIGERIAN CHILDREN ON ANTHELMINTHIC DRUG OR ORAL VACCINATIONS

BY

KAZEEM SANJO AKINWANDE

FIMLS (NIG); M.Sc (Ogun)

MATRIC NO: 183516

A Thesis in the Department of Chemical Pathology,

Submitted to the Faculty of Basic Medical Sciences, College of Medicine

in partial fulfilment of the requirements for the Degree of

DOCTOR OF PHILOSOPHY

of the

UNIVERSITY OF IBADAN

JULY 2019

CERTIFICATION

This is to certify that this project titled **HUMORAL IMMUNE RESPONSES AND MICRONUTRIENT LEVELS OF *Ascaris lumbricoides*-INFECTED NIGERIAN CHILDREN ON ANTHELMINTHIC DRUG OR ORAL VACCINATIONS** by **Kazeem Sanjo Akinwande** was carried out under my supervision in the Department of Chemical Pathology, University of Ibadan.

Signature………………………………………………

Prof. O. G. Arinola

Professor of Immunology and Head,

Department of Chemical Pathology,

Faculty of Basic Medical Sciences,

College of Medicine,

University of Ibadan, Ibadan, Nigeria.

ACKNOWLEDGEMENT

I give all thanks, glory and adoration to Allah swt, the owner and sustainer of my life, for the gift of life, protection in all forms, energy, wisdom and grace bestowed on me to undergo this study. I am most sincerely grateful to my mentor and supervisor, Prof. O. G Arinola, who spared nothing to bring out the best in me, and for supervising this project.

I am most grateful to Prof. E. O. Agbedana, Prof. J. I. Anetor, Dr. Mabel A. Chalres-Davies, Dr. F. M. Abbiyesuku, Dr. K. S. Adedapo, Dr. K. S. Akinlade, Dr. O. M. Akinosun, Dr. I. O. Omotosho, Dr. M. A. Kuti, Dr. A. A. Onifade, Dr. B. E. Orimadegun, Dr. E. B. Bolajoko, Mrs. O. O. Ademola-Aremu and the entire non academic staff of the Department of Chemical Pathology for their encouragement and moral support.

I am very grateful to Dr. Sheu Rahmon for his guidance and support throughout the period of carrying out this project. I am also grateful to Mr. Yakub Surajdeen (Senior) for his encouragement and support. I am greatly indebted to Mr. Edem Fabian, my Ph.D twin brother, for his support, guidance and help in all my statistical analysis. Thank you brother! We are just starting. I also appreciate all the PhD and M.Sc graduates as well as current students of the Department of Chemical Pathology, University of Ibadan that I had course to come across with all through my study. I so much appreciate the family bond that we all share, the encouragements and supports in different forms. I specially appreciate Dr. Kehinde Adigun of the Department of Family Medicine, University College Hospital, Ibadan for his efforts and help during the field of this study.

My sincere appreciation goes to my colleagues in Federal Medical Centre Abeokuta for their understanding, support and covering my back throughout the course of this study. I specially appreciate two of my retired bosses – Mrs. O. O. Oyekanmi, for her support and creating the initial ‘space’ to proceed on my further studies, and Mr. O. A. Oduniyi, a father, friend, and

confidant, for his support in all forms. I am greatly indebted to Alh. R. A. Bakare, Mr. A. A. Awe, Alhaja K. T. Baiyewu, Mr. D. S. Oluwarotimi, Mr. B. M. Onaduja and Mrs. F. A. Olatunji. I also appreciate Mr. B. O. Motayo and all concerned staff of Pathology Department, FMC, Abeokuta for their encouragement throughout the course of this study. Special appreciation goes to Dr. Rufus Akinyemi of the Institute of Advanced Medical Research and Training, College of Medicine, University of Ibadan for his encouragement in all forms.

I thank my lovely wife, Folashade and my lovely children, Mubarak, Maryam, Musodiq and Muhammed for their perseverance and patience throughout the course of my study. I so much appreciate your patients and resilience and most importantly, your understanding when it seemed all chiefs were down. I assure you, I will not forget or take you all for granted. I specially thank my parents, Chief and Mrs. Muhammed Akinwande for their support and prayers. I also appreciate all my siblings and their families, Mr. Biodun Akiniwande, Dr. and Mrs Folake Adebawojo, Mr.& Mrs Funmi Alabi, Mr.& Mrs Lekan Akinwande, Captain & Mrs. Iyabo Akinkunmi, Mr. and Mrs Busayo Balogun, for their supports in all forms. I will also like to thank my childhood friend, Mr. Bola Opadiran for making my Ibadan sojourn 'not all that boring'.

Lastly and once again, all praises, honour and adoration to Almighty Allah (swt) for the strength, provision, safety on roads and in all other forms, and bestowing on me the courage to push this far. *Thee alone I worship, from thee alone, I seek help* (Quran 1vs 4).

To God be the glory!

DEDICATION

I dedicate this project to Allah swt for His mercies over my life,

To my wife and children: Muftihat Folashade, Mubarak, Maryam, Musodiq and Muhammed for keeping on when I was not there for them,

To my parents, Chief and Mrs. Muhammed Akinwande for their support and prayers, and instilling in me the value of education.

ABSTRACT

Intestinal helminth infection is associated with altered immune responses and micronutrient status in infected children. These alterations compromise vaccine efficacy of infected children. There is paucity of information on the interplay between humoral immunity, nutritional status and vaccine response, before and after anthelminthic (Albendazole) treatment or oral vaccination in *Ascaris lumbricoides (Al)*–infected Nigerian children. This study was designed to assess the micronutrient status and immune responses to vaccines in *Al*–infected Nigerian children before and after anthelminthic treatment or oral vaccination.

After ethical approval (UI/EC/13/0331) and informed consent were obtained, a total of 349 children [149 preschool-aged (PSAC) and 200 school-aged children (SAC)] were enrolled into this case-control study. The stool samples were collected and examined for helminth ova using the concentration technique. Twenty three of the *Al*–infected children were Albendazole treated (AT) while twenty three received oral poliovirus vaccine (OPV) and nineteen received oral rotavirus vaccine (ORV). Age and sex-matched helminth free children were randomly selected as controls. Sera were collected before, one and two months after Albendazole treatment or three weeks after oral vaccinations. Serum concentrations of zinc and selenium were determined using Atomic Absorption Spectrophotometry. Vitamins A and C concentrations were determined using HPLC. Interferon–γ (IFN–γ), tumour necrosis factor–α, interleukins (IL)-4, 6, 8 and 10, transferrin, poliovirus-specific IgA and rotavirus-specific IgA concentrations were determined using ELISA. Data were expressed as mean±SEM and analysed using Mann-Whitney *U* test, Wilcoxon Signed Ranks Test and Kruskal Wallis Test, with levels of significance set at $\alpha_{0.05}$.

Eighty three (23.7%) of the children were infected with *A. lumbricoides*. In SAC, serum zinc (139.1±2.5 vs 152.7±2.4μg/dL) and vitamin A (119.3±1.7 vs 153.6±5.5μg/dL) levels were significantly lower while transferrin (178.9±4.1 vs 137.9±4.2mg/dL), selenium (62.1±5.8 vs 35.5±1.6ng/mL), IL-8 (995.2±49.3 vs 562.9±44.0pg/mL), IL-6 (16.6±1.8 vs 4.9±0.4pg/mL), IFN–γ (105.9±9.2 vs 62.9±11.6pg/mL), and IL-4 (210±18.3 vs 106.6±2.5pg/mL) levels were significantly higher in *Al*-infected group compared with controls. In AT group, serum vitamin A levels were significantly higher at one month (203.6±5.4 vs 118.5±2.0μg/dL) and two months (206.2±5.0 vs 118.5±2.0μg/dL) while IL-8 was significantly lower at one month (433.7±85.9vs 619.4±77.4pg/mL) compared with pre-treatment values. In OPV-vaccinated group, post-vaccination serum IL-8 (703.1±41.5 vs 1063.2±69.7pg/mL) and IL-6 (8.1±0.7 vs

14.7±2.5pg/mL) levels were significantly lower compared with pre-vaccination levels. Also, post-vaccination serum poliovirus-specific IgA level was lower in OPV-vaccinated group and higher in controls compared with pre-vaccination levels, but not significant. In ORV-vaccinated group, post-vaccination serum IFN–γ (101.6±18.4 vs 181.1±37.3pg/mL), IL-4 (230.4±55.8 vs 507.1±130.2pg/mL), and IL-8 (545.3±78.9 vs 966.8±159.6pg/mL) levels were significantly lower while IL-10 (0.25±0.04 vs 0.13±0.02ng/mL) level was significantly higher compared with pre-vaccination levels. Also, serum rotavirus-specific IgA level was not significantly lower in ORV-vaccinated group, but was significantly higher (7.9±0.7 vs 6.9±0.4mg/dL) in controls compared with pre-vaccination levels.

Inflammation, deficiencies of zinc, vitamin A and reduced vaccine-specific immunoglobulin A are associated with *Ascaris lumbricoides* infection in Nigerian children but were reversed with anthelminthic treatment. Anthelminthic treatment with micronutrient supplementation will benefit children during vaccination.

Keywords: Albendazole, Cytokines, *Ascaris lumbricoides*, Micronutrients supplementation, Oral vaccination

Word count: 492

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CHAPTER ONE

1.0 INTRODUCTION

Soil-transmitted helminth (STH) infects over one and a half billion people worldwide which correspond to about 24% of population of the worldwith very high prevalences found in China, the Americas, Sub-Saharan Africa and East Asia (WHO, 2016). It was approximated that *Ascaris lunbricoides* infects about 819 million people worldwide while *Trichuris trichuria* and hookworms infect 465 million and 439 million respectively (Pullan *et al.*, 2014). Also, over 270 million and 600 million preschool-aged children and school-aged children respectively, reside in communities where STH are vastly prevalent, and preventive interventions or treatments are required (WHO, 2016). In Nigeria, STH infections are part of the commonest infections (Ekundayo *et al.*, 2007) and their high prevalence, especially *Ascariasis lumbricoides* infection has remained unchanged for over half of a century (Ekundayo *et al.*, 2007). There is therefore the need to confirm this claim by Ekundayo *et al* (2007) about a decade ago. This will give direction of deworming therapy. Moreover, nearly all previous literatures on prevalence of helminth were on adults and school-aged children, neglecting the pre-school aged children. This study therefore is designed to address this knowledge gap.

Morbidity resulting from chronic STH infection are related to growth impairment and malnutrition (Hall *et al.,* 2008). Approximately 40% prevalence of STH was reported among urban slums children in Kenya where anaemia, deficiencies in vitamin A and iron are common (Suchdev *et al.*, 2014). Malnutrition was also associated with STH in children of rural settings in Chad (Bechir *et al.*, 2012). Stunted growth and underweight were

significantly associated with STH in children of orphanage homes in Edo State, Nigeria (Nwaneri and Omuemu, 2013). However, study specifying micronutrient deficiency in STH infected preschool and school aged children in Nigeria, isscarce.

Chronic STH infections are also related to micronutrient malnutrition which results in anaemia and stunted growth. Helminth infection causes iron and protein loss in hosts by feeding on host tissues, especially blood (WHO, 2016). They also cause increased nutrients malabsorption, appetite loss, reduced nutritional intake and physical fitness (WHO, 2016), poor cognitive development, protein-calorie undernutrition and fatigue (Bethony *et al.*, 2006). Study on the anthropometric changes in helminth infected Nigerian children especially at preschool or school-aged stages is therefore necessary to be carried out, in order to emphasize the need for regular deworming programmes in this group of children.

Micronutrients play important roles in immuno-physiologic functions (Failla, 2003) and vaccine efficacy (Sack *et al.*, 2008). Half of children who are between ages 6 months to 5years worldwide, suffer micronutrient deficiency such as zinc, vitamin A, folate iron and iodine, (CDC, 2018) due to inefficient metabolism of ingested micronutrients or due to inadequate intake or STH infection (Rayhan and Khan, 2006). However, studies on micronutrient levels in STH-infected Nigerian children after- or during anthelminthic drug treatment are not encountered in literatures.

Helminth infection classically induces Type 2 immunity and has been linked with modulation of some Type 1 immunity – driven inflammatory diseases (Wang *et al.*, 2008). The infection leads to induction of immuno-regulatory functions through proliferation of regulatory T-cells (Tregs) (Belkaid and Rouse, 2005). Treg cells play major role in immune response regulation and maintainance of homeostasis under disease conditions such as microbial infections ,cancer, autoimmune disease and inflammation (Wang *et al.*, 2008). Down-modulation of the

immune system due to helminth infection has been linked to diminishing responses to bystander antigens (Greene *et al.*, 1983) and routine vaccination (Sabin *et al.*, 1996). Thus, helminth infection is suggested to affect effectiveness of vaccine response in children. Study on vaccine responses during helminth infection and after anthelminthic drug treatment in Nigerian children is lacking.

Rotavirus (RV) causes gastroenteritis in young children and infants. It is a contagious virus that is responsible for about one-third of all diarrhea-related hospital admissions in children and five hundred to six hundred thousand deaths per year, with Asia and sub-Saharan Africa recording the highest death rate among age groups where STH infestation is common (Parashar *et al.,* 2006). RV disease can be prevented through repeated vaccination with a multivalent rotavirus or single vaccine serotype. Mass vaccination against rotavirus in the United States has been reported to protect against rotavirus infection in children but in African countries where rotavirus vaccination trial had been carried out, protection from severe rotavirus-associated acute gastroenteritis is lower (Armah *et al.,* 2010; Madhi *et al.,* 2010) probably as a result of STH infection.

Vaccination against poliovirus infection has been the means of preventing wild poliovirus infection, which can cause paralysis in young children (Ohri and Jonathan, 1999). Live attenuated oral poliovirus vaccine (OPV) is employed for its global eradication. It produces antibodies and protects against poliovirus paralysis through prevention of its spread to the nervous system and producing local immune response in the intestinal mucous lining where poliovirus multiplication takes place (WHO, 2016). The need therefore, to assess vaccine - specific immune status of Nigerian children in this age group where vaccine administration is compulsory and helminth infection is common, became very necessary.

Oral vaccines exhibit Th1 and Th2 responses in vaccinated hosts through mucosal immune responses that take place in the mucous lining of the intestines (Mahon *et al.,* 1995; Azevedo *et al.*, 2006). For instance, initial expression of tumour necrosis factor–α (TNF–α), interleukin -6 (IL-6) and interleukin-12 (IL-12) after rotavirus vaccination signifies the immediate innate immune response and induction of specific immune response to such infection. This is followed by interferon-γ (IFN-γ) and interleukin-10 (IL-10) expression, which depicts Th1/Th2 cytokine balance in the eventual response. IFN-γ with other Th1 cytokines play important role in responses and protection expressed by intestinal immunoglobulin A (IgA) (Azevedo *et al.*, 2006). Also, vaccination against poliovirus induces strong Th1 response through interleukin-2 (IL-2) and IFN-γ secretion in vaccinated hosts indicating that protective immune response against poliovirus infection can be mediated by Th1 cells through their functions in humoral immune response (Mahon *et al.*, 1995). Studies on factors responsible for efficacy of oral vaccines among Nigerian children are not available. However, helminth infection may affect responses to vaccine through the expression of regulatory cytokines (IL-10) or Th1 to Th2 shift, which hitherto down-modulate the effect of Th1 cytokines expressed following vaccination (Sher andCoffman, 1992). Study on cytokine changes in helminth infected children after vaccination or anthelminthic drug treatment is therefore necessary.

1.1 JUSTIFICATION

Sub–Saharan Africa along with China East Africa and the Americas still account for the greatest burden of STH infection. In Nigeria, the number of preschool-aged children with STH infection is unknown, while school-aged children that required preventive drug treatment for STH infection was estimated in 2008 to be about 39 million (WHO, 2008).

Low immunogenic responses to routine vaccinations have been reported in low – income countries when compared with the developed ones (Deming *et al.*, 1997; Levine, 2010; Saleem *et al.*, 2015). Many factors such as maternal trans-plancental antibody titres, micronutrient malnutrition, breastfeeding practices, stomach acidity and interfering gut flora have been reported to be responsible for these observations (Patel *etal.*, 2009). STH infections are also proposed to contribute to malnutrition in children and low intelligent quotient through systemic reduction in digestion and absorption caused by helminths; helminth induced chronic inflammation and loss of nutrients (Northrop-Clewes *et al.*, 2001). Unfortunately, little attention is given to the possible effect of STH infection on vaccine efficacy, on specific micronutrients levels or on specific vaccine immune factors.

1.2 RESEARCH HYPOTHESIS

Intestinal helminths, especially *Ascaris lumbricoides* infection affects micronutrient status and leads to sub-optimal vaccine specific immune response in children.

1.3 AIM

To provide information and establish association between micronutrient status, specific and systemic immune responses to rotavirus and poliovirus vaccines in Nigerian children infected with *Ascaris lumbricoides.*

1.4 OBJECTIVES

1. To assess the burden of STH infection in preschool-aged and school-aged children in selected areas of Ibadan.

2. To determine serum levels of micronutrients [zinc (Zn), iron (Fe), selenium (Se), vitamin A, vitamin C, ferritin, transferrin and haptoglobin] and cytokines (TNF–α, IFN–γ, IL-6, IL-8, IL-4, and IL-10) in children without *Ascaris lumbricoides* infections and children with *Ascaris lumbricoides* infections before anthelminthic drug treatment, 1 month and 2 months after anthelminthic drug treatment.
3. To determine the serum cytokine levels (TNF–α, IFN–γ, IL-6, IL-8, IL-4, and IL-10) in *Ascaris lumbricoides*-infected and *Ascaris lumbricoides*-free preschool-aged or school-aged children before and three weeks after oral rotavirus vaccination or oral poliovirus vaccination respectively.
4. To determine serum levels of rotavirus-specific IgA antibody or poliovirus-specific IgA antibody in *Ascaris lumbricoides*-infected and *Ascaris lumbricoides*-free preschool-aged or school-aged children before and three weeks after oral rotavirus vaccination or oral poliovirus vaccination respectively.

CHAPTER TWO

LITERATURE REVIEW

2.1 Helminthiasis

Helminth infection in humans has been dated back to the pre-historic era. Clinical features that are characteristic of helminth infections were found in the ancient writings of Hippocrates, the Bible and the Egyptian medical papyri. Helminth eggs were also found in mummified human faeces that are dated back to thousands of years (Cox, 2002). Heminthiasis also made impact in world history especially during the Cold War in China, when Mao's troops were forced to abort their amphibious assault on Formosa (as Taiwan was historically known) due to acute schistosomiasis and schistosome was named "the blood-fluke that saved Formosa" (Kernan, 1959).

Helminths are worm-like parasites, classified by the host organ they inhabit and according to their general external shape (Castro, 1996). They are of two major phyla: Platyhelminths and nematodes. Nematodes (roundworms) are filarial worms and intestinal worms (STH) while platyhelminths (flatworms) include the trematodes (flukes), such as tapeworms (cestodes) and schistosomes. (Hotez *et al.*, 2008). The main STH species that humans are infected with are *Ascaris lumbricoides* (roundworm), *Trichuris trichiura* (whipworm) and *Ancylostoma duodenale* or *Necator americanus* (hookworms) (Figure 2.1) (WHO, 2016).

Ascaris lumbricoides
Female
Male
Trichuris trichiura
Female
Male
Hookworm
Female
Male

Figure 2.1:The species of soil-transmitted helminths that infect humans (WHO, 2016)

Highest numbers of STH are harboured by preschool-aged and school-aged children (including adolescents) compared with other age groups (Hotez *et al.,* 2008). This often results in diminished physical fitness, growth stunting, impaired memory and cognition (Nesheim and Crompton, 2002) with adverse consequence of reduced attendance in school and impaired performance in childhood education (Kremer and Miguel, 2003). Helminthiasis can be regarded as the disease of the poor. A third of the about three billion people living on less than two US dollars daily in low – incomecountries of the Americas, Asia and sub-Saharan Africa carry one type of helminth infection or the other. Practically, thousands of impoverished rural villages' inhabitants in subtropics and tropics are usually chronically infected with various types of parasitic worm (Hotez *et al.*, 2007). In Nigeria, practices such as indiscriminate defecation, dumping of excrements at refuse depots, along bush tracks, nearby bushes, river banks, underneath bridges and motor highways, as well as on open fields exacerbate the infection (Adeyeba and Dipeolu, 1984; Arinola and Fawole, 1995). Eating from the same bowl in the open street yard, which is usually practiced in some rural communities, as well as using water for cleaning after defecation, may also account for high STH prevalence (Akogun, 1989). Poor socio-economic status and low level of education of the parents have also been associated with STH infection in children (Ayanwale *et al*., 1982; Arinola and Fawole, 1995). High STH prevalence was also observed in children of petty traders or the unemployed when compared with children of professionals and middle class workers (Adekunle *et al.,* 1986).

Helminth infections also excert effects directly or indirectly on HIV/AIDS and malaria in developing countries. It is not unusual for a person to be co-infected with one or more parasitic worm and malaria (Brooker *et al.,* 2007), or HIV (Gallagher *et al.,* 2005). The high burden of STH infections, along with their co-endemicity with HIV/AIDS and malaria are

cogent reasons for global eradication programmes launch against parasitic infections (Hotez *et al.,* 2007).

2.2 Soil – Transmitted Helminth Infection (STH)

Soil-transmitted helminth (STH) infections results due to infestation by different types of parasitic worms. The main types that people are infected with are the hookworms, roundworm, and the whipworm (also known as *Necator americanus* or *Ancylostoma duodenale*, *Ascaris lumbricoides* and *Trichuris trichiura*). They are part of the world's commonest infections, affecting the most deprived and poorest communities (WHO, 2016). Morbidity is also related to the worms'intensity. Light infections with the parasites are often symptomless but heavier infections may result in symptoms which include abdominal pain, diarrhoea, weakness or general malaise (WHO, 2016b). Chronic STH infections may contribute to iron-deficiency anaemia and malnutrition, which may negatively affect the childhood mental and physical growth (Stephenson *et al.,* 2000).

2.2.1 Prevalence and Burden of Soil – Transmitted Helminth Infection

Worldwide, soil-transmitted helminths infect about half a billion people (WHO, 2016). A survey estimated that as at 2010, *Ascaris lumbricoides* infected about 819 million people globally, *Trichuris trichiura* infected 464.6 million and 438.9 million people are infected with hookworm and Sub – Saharan Africa accounts for 13.6%, 13.6% and 10.6% of these infections respectively (Pullan *et al.*, 2014). Asia accounts for almost 70% of the global infections. Geostatistical models have also been employed in determing the distribution of STH in sub-Saharan Africa.The proportion by each country in the global infected population and empirical information for all regions are shown in Figure 2.2 (Pullan *et al.*, 2014).

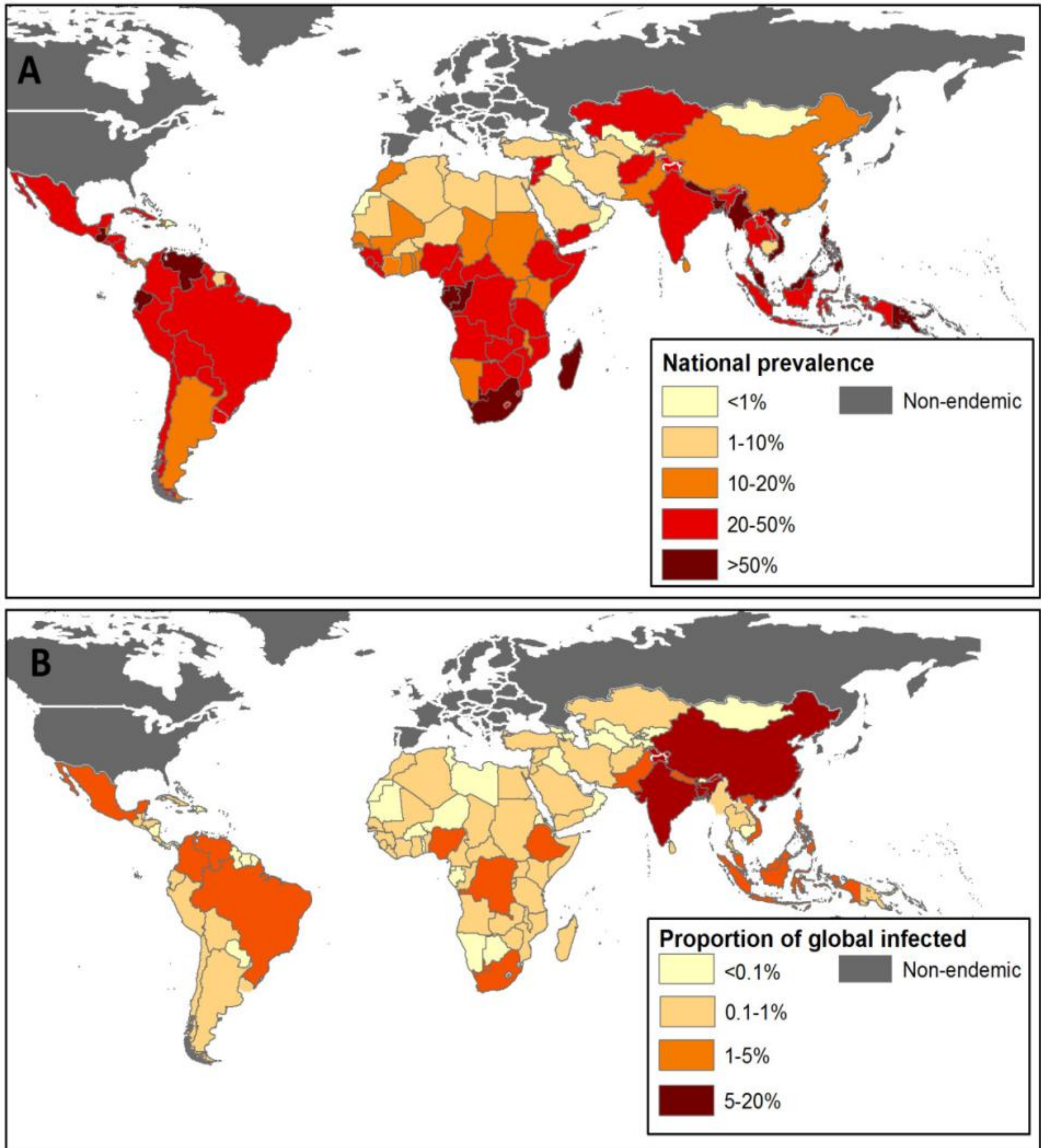


Figure 2.2: Global spread of Soil Transmitted Helminth infection.(A) Prevalence based on geostatistical models and. (B) Each country's proportion,of the infected global population (Pullan *et al.*, 2014).

The Disability –Adjusted Life Year (DALY) framework is employed to measure the STH infection burden globally.This incorporates the years of life lived with disability (YLD) and that lost from premature death (YLL) (Murray *et al.*, 2010). STH globally accounts for 4.98 million YLD in 2010. 22% of this is attributable to *A.lumbricoides,* 65% to hookworm, and 13% to *T. trichiura* where Sub- Saharan Africa records 15.2%, 14.1% and 21.0% respectively (Murray *et al.,* 2010).

The World Health Organisation estimates that about 846 million children worldwide are in need of preventive drug treatment against STH infection (WHO, 2016b) and over 600 million school-aged and 270 million preschool-aged children live in communities where STH are highly transmitted, and require preventive interventions or treatment. A recent study shows that helminth infections are widely spread across Nigeria and about 5.7 million school-aged children carries STH infection. Breakdown showed that 83% of this number is infected with *Ascaris lumbricoides*, 19% with *Trichuris trichuria* and 50% with hookworm infections (Oluwole *et al.*, 2015). Local studies on preschool-aged children revealed prevalence of STH infections in semi – urban communities of Ile Ife, Osun State was 50% (Kirwan *et al.,* 2009) and that of semi-urban communities of Ibadan, Oyo State was 42% (Arinola *et al.,* 2015), with *Ascaris lumbricoides* carrying the greatest burden (47.6% and 88.1% respectively). The spatial distribution of the infections in Nigeria is shown in Figure 2.3.

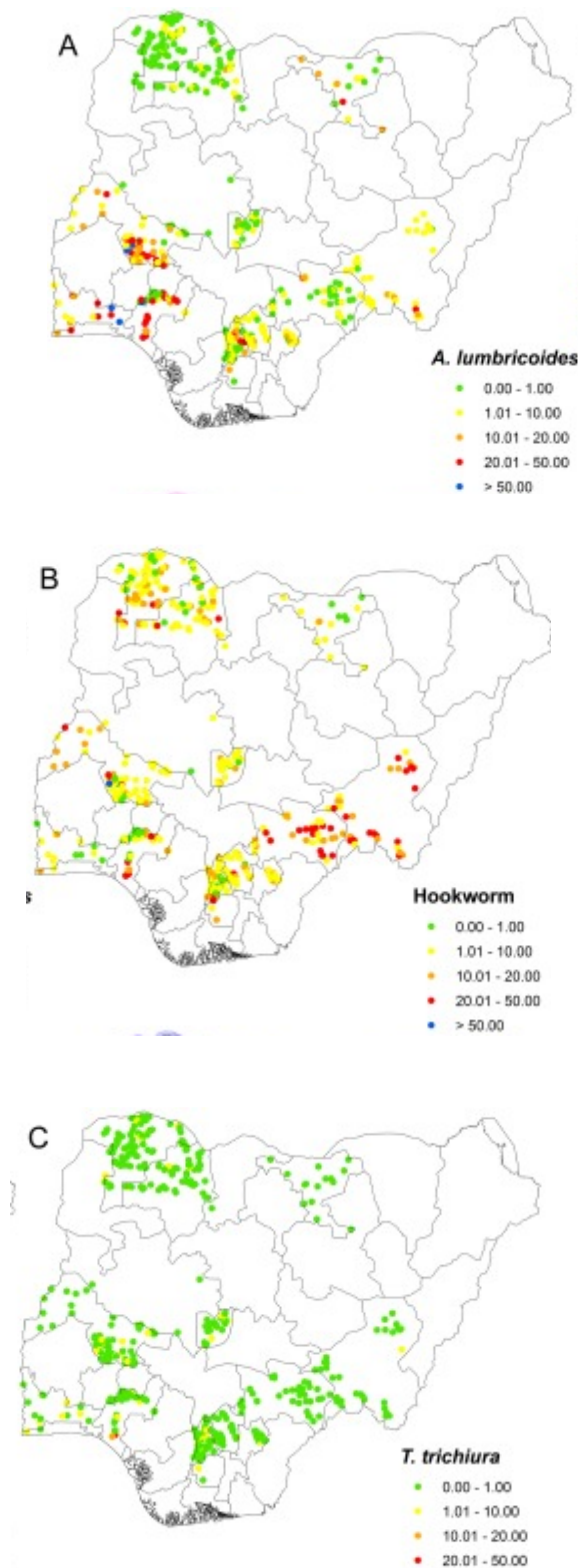


Figure 2.3: Spatial distribution of soil-transmitted helminth infections in Nigeria. (Oluwole *et al.*, 2015)

2.2.2 Transmission and Life Cycles of Soil – Transmitted Helminth Infection

Humans can become infected with STH when infective roundworm (*Ascaris lumbricoides)* or whipworm (*Trichuris trichiura)* eggs are ingested from contaminated hands, utensils or food. Also, infective hookworm larvae (*Necator americanus* and *Ancylostoma duodenale*) in contaminated soil alsoinfect humans by penetrating through the skin (WHO, 2016b). Under favourable environmental conditions, eggs of *Ascaris lumbricoides* become infective after about 18 days or some weeks and the eggs may remain infective for years in the soil. *Trichrus trichuria* eggs develop into infective embryo in 15 to 30 days after passing through an advanced cleavage stage and a two-cell stage in the soil. For hookworms, the eggs hatch into larva in less than 2 days and the released rhabditiform larva grow in the soil and become infective filariform larva after 5 to 10 days.The fliariform larva can survive 3to 4 weeks in condusive conditions (Figure 2.4) (USAID, 2014).

Eggs of *Ascaris lumbricoides* and *Trichrus trichuria* hatch into larva within the infected person's intestinal region and burrow through the intestinal walls, then move via the blood streams to the lungs. In case of hookworms, hatched larva burrow through the skin to the veins, the heart and then to the lungs. They further mature in the lungs (this takes 10 to 14 days in case of *Ascaris lumbricoides)*, burrow through the walls of the alveola, then climb the bronchial tree to reach the throat, after which they are swallowed. Female adult worm of *Ascaris lumbricoides* can grow over 30 cm in length in the small intestine and may hatch up to 200,000 eggs per day.

Adult hookworms attach to the walls of the intestine in the lumen of small intestines, where they live and cause loss of blood, thereby causing anemia and malnutritionin the host.

The thicker part of *Trichuris trichuria* attaches to the intestinal lumen where it reproduces, while the thinner part penetrates into the large intestine. They lay eggs within 60 to 70 days of infection and may shed 3,000 to 20,000 eggs daily. Adult female *Trichuris trichuria* can measure up to 50 mm (2 inches) in length when fully grown, and can live for about 1 to 3 years, (USAID, 2014).

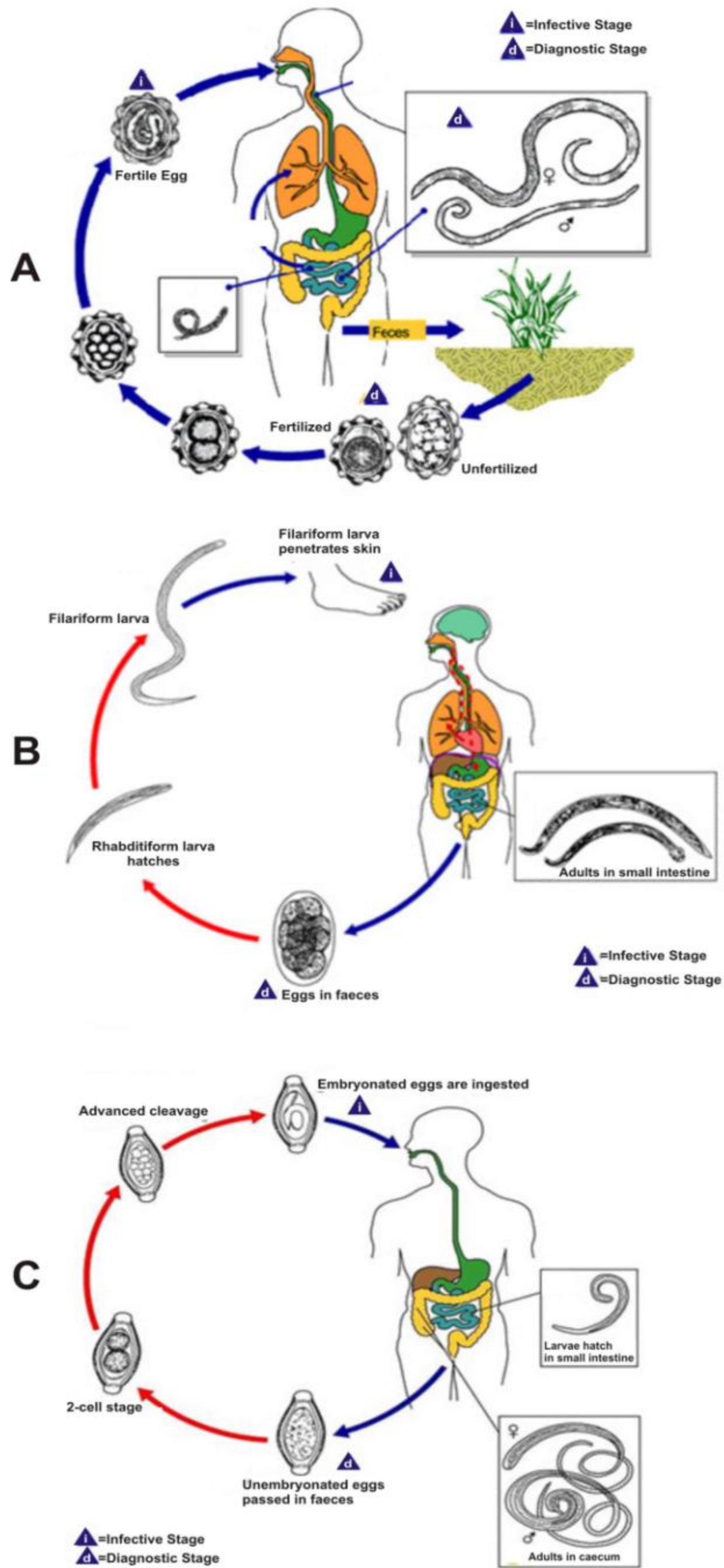


Figure 2.4: Life Cycles and modes of transmission of (A) *Ascaris lumbricoides*, (B) Hookworm and (C) *Trichuris trichuria* (CDC, 2015)

2.2.3 Clinical features of Soil Transmitted Helminthiasis

Infection with STH can be categorized based on the acute or chronic presentations of the clinical features, which results from the migration of the larvae through the skin and viscera or from parasitism by adult helminth worms in the gastrointestinal tract (Bethony *et al.*, 2006).

During migration, STH larvae provoke reactions as they pass across many tissues. For instance, larvae of Ascaris that died while migrating along the liver may induce eosinophilic granulomas (Kaplan *et al.,* 2001). Larval antigens *Ascaris* in the lungs may induce inflammatory reaction consisting of eosinophilic infiltrates which chest radiographs can reveal. Verminous pneumonia that results is usually accompanied by cough, wheezing, fever, dyspnoea, and blood-tinged sputum that results from high intensity infections (Bethony *et al.,* 2006).

Some syndromes generate when the larvae penetrate through the skin. A local papular and erythematous rash resulting in ground itch and pruritis on hands and feet is often associated with repeated exposure to hookworm larvae.The larvae can enter the lungs and cause pneumonitis, although not severe like that caused byascariasis. (Hotez *et al.,* 2004).

Ascaris lumbricoides infection of high intensity can cause abdominal distension and painin the small intestine, as well as causing lactose intolerance, vitamin A and some other micronutrients malabsorption, leading to nutritional and growth failure (Taren *et al.,* 1987). Thier aggregation can lead to partial obstruction of the lumen in children, which could lead to grave consequences which include volvulus, intussusceptions as well as complete obstruction which can cause bowel infarction and intestinal perforation (Khuroo*et al.*, 1990). In the lumen of the appendix, worms' presence can cause gangrene of the appendix tip and appendicular colic, resulting in what is not distinguishable from appendicitis clinically. In

children with high body temperature due to fever, adult worms of *Ascaris lumbricoides* moves due to the increasing unfavourable temperature leading to their ejection through the anusor the nasopharynx. Hepatobiliary and pancreatic ascariasis chould result from adult worms of Ascaris entering and blocking the ampullary orifice of the common bile duct in the duodenum which can lead to cholecystitis, biliary colic or pancreatitis, (Khuroo*et al.,* 1990).

Eosinophilia is also associated with hookworm infection (Maxwell *et al.,* 1987). Hookworms' presence in the small intestine may lead to loss of blood through the intestine, causing iron-deficiency anaemia (Hookworm disease) (Hotez *et al.,* 2004). With just more than 40 adult hookworms, host haemoglobin concentrations can be reduced below 11 g/dL (Lwambo *et al.,* 1992). Heavy infection can also cause hypoproteinaemia and anasarca can result due to chronic protein (Hotez *et al.,* 2004).

Adult *Trichuris trichuria* are found mostly in the caecum, though it can be seen throughout the rectum and the colon in heavy infections. Inflammation that results fom attachment of large numbers of adult *Trichuris trichuria* may cause colitis, which, if prolonged can lead to clinical case that mimcs inflammatory bowel disease, causing abdominal pain, diarrhoea, finger clubbing and anaemia (Bundy and Cooper, 1989). Trichuris dysentery syndrome results due to heavy infection with *Trichuris trichuria*can cause which results in rectal prolapsed or chronic dysentery (Bundy and Cooper, 1989).

2.3 Host Immune Response to Helminth Infection

The extraordinarily high prevalence of helminth infections over the century has undoubtedly reflected their influence in manipulating the host immune system as well as evades any response that could lead to their ejection. The host immune system develops mechanisms to

best balance susceptibility, limit pathology and resistance as well as immune pathogenesis (McSorley and Maizels, 2012). Therefore, responses that allow the infection in preference to completely eliminating the parasites are manifested (Allen and Maizels, 2011).

Generally, exposure to helminths, most especially those with highest diasease burden including schistosomes, gastrointestinal and filarial nematodes could result in three outcomes associated with specific immunity. (Maizels and Yazdandakhsh, 2003; McSorley and Maizels, 2012). The first is characterized by Th-1 immune responses in the blood, resulting in uncontrolled inflammatory (Th-1) disease. In this case, lower IgG4 levels are observed but with evident IgE responses (Arinola *et al.,* 2012). Inflammation of the lymphatic vessels also results, causing pathological cases like *elephantiasis*, which results due to the lymphatic drainage failure. The second is a balanced immune response, showing a balanced Th-1 and Th-2 responses regulated through the activities of T-reg cells. A lesser IgE and IgG4 distribution in the profile of the Th-2 antibody are also observed. The third is refered to as the‘modified T helper 2- cell response’ which results in significant Th-2 responses (expressing significant amount of Interleukin-4) with low level of Th-1 cells (as expressed by IFN-γ) and high level of interleukin-10, indicating the T-Reg cells’involvement as an important mediator of regulation in parasitic infections (Sher and Coffman,1992). A relatively lower IgE together with IgG4 dominates the Th-2 type antibody profiles and individuals with this type of response often clinically have infections which are asymptomatic, but transmissible (Maizels and Yazdandakhsh, 2003).

Characteristic Th-2 immune response is often accompanied with a relatively significant secretion of IL-5, IL-4, IL-13, IL-25, IL-10, and IL-31(Fort*et al.,* 2001; Harnett and Harnett, 2006). Th-2 cells play part in B cells’activation and antibodies production as well as the recruitment and differentiation of eosinophil. Helminth infection is usually characterized by

raised IgE, IgG4 and IgG1 levels, and significant eosinophil and mast cell production which are characteristic of Th-2 response (Wang *et al.,* 2008). Another Th2-inducing cytokine is the thymic stromal lymphopoietin (TSLP). It isproduced by the cells epithelium and can influence the dendritic cells to aid in Th-2 cell proliferation against some helminth infections (Taylor *et al.,* 2009). In conditions such as allergic reactions, Th-2 responses are majorly TSLP dependent. Modified Th-2 cell response is also characterized with IgE dissociation and specific IgG4 responses, accompanied by down-regulation of IgE. Both IgE and IgG4 class switching are promoted by IL-4 (Maizels and Yazdanbakhsh, 2003). IL-10 has been implicated in inhibiting B-cells'switching to IgE while promoting IgG4 proliferation by the same cell (Wang *et al.,* 2008).

IL-10 is a crucial mediator of regulation in parasitic infection and its role in attenuation of pathogensis has been identified (Sher *et al.,* 1992). High IL-10 levels have been observed in individuals with heavily filariasis (Mahanty *et al*., 1996) and schistosomiasis (King *et al.,* 1996) as well as in individuals with chronic intestinal helminth infections (Figueiredo, *et al.,* 2010). It is a down-modulatory factor in allergic diseases and it is also responsible for asymptomatic phenotypes observed in many parasitic diseases (Mahanty *et al.,* 1996).IL-10 and tumour growth factor-β (TGF-β) are secreted by set of helminth – induced suppressive T cell population referred to as regulatory T-cells (Tregs). Tregs make up about 5% to 10% of $CD4^{+}$T cells in humans; express markers like CTLA-4, CD25, Foxp3, GITR; and usually produce TGF-β and/or IL-10 (Takahashi *et al.,* 2000; Shimizu *et al.,* 2002; Hori *et al.*, 2003).

Tregs through their active immunoregulatory mechanisms, help in controlling morbidity (Belkaid and Rouse, 2005).They also play major roles in immune response regulation and homeostasis maintenance in situations like inflammation, cancer, autoimmune diseases or microbial infections (Bluestone and Abass, 2003).

Apart from the immune-modulatory mechanism initiated by parasites through secretion of IL-10 by Tregs, innate immune cells such as macrophages and Dendritic cells (DC) which direct immune reaction towards the tolerating or activating pathway (Wang *et al.*, 2008) are also manipulated by helminths in the host system. DCs are activated through the expression of high level of Toll –like receptors (TLRs), a pattern recognition receptors (PRRs), (Barton and Medzhitov, 2002) and signals derived from microbial components are translated into stimulus for T-cells, thereby activating T-cell response (Agrawal *et al.*, 2003, de Jong *et al.*, 2002). Helminth – derived molecules also induces DC and this leads to activation and subsequent responses byTh-2 and/or Treg cells (Sher *et al.*, 2003). For instance, the schistosome eggs - derived glycolipid lysophosphatidyserine and carbohydrate determinant lacto-N-fucopentaose may cause activation of TLR4 and TLR2 in dendritic cells respectively (Thomas *et al.,* 2003). Helminthiasis may also cause alteration in expressing TLR4 in mucosal T-cells by producing TGF-β upon stimulation by lipopolyshacharides (Ince *et al.,* 2009). Also, helminthiasis causes dendritic cells' activation and up-regulation of the DC's IL-10 response, which afterwards, could trigger a T-reg and/or Th2 - dominated response (Ilic *et al.*, 2008).

The helminth-induced Th2 cytokines response is also suggested to affect macrophages (Gordon, 2003). While DC's perform crucial roles in initiating and regulating the host immune response, macrophages' contribution is significant at the effector phase (Wang *et al.*, 2008). Macrophages activation by pro-inflammatory cytokines or bacterial products (through TLR engagement) leads to the production of classically – activated macrophages. However, Th2 cytokines induced by helminths, most importantly, IL-4 (Kreider *et al.,* 2007) causes secretion of another macrophage phenotype, called the alternatively activated macrophages (AAM) in chronic cases (Weng *et al.*, 2007; Anthony *et al.*, 2006). A good example of an alternatively activated macrophage (AAM) is the nematode-elicited macrophage (NeMacs) which is found in *Brugia malayi* – infected mice (Allen and McDonald, 1998).

NeMacs morphologically are more multivacuolar and larger compared with other macrophages. They have a distinct gene expression profile and secrete arginine-metabolizing arginase -1 (that compete for nitric oxide synthetase substrate), RELM-α and Chi3L3 (Ym1) (Nair *et al.*, 2003). Ym-1 is a chemotactic factor for eosinophils, which explains the reason NeMac recruitment is accompanied with localized eosinophils infiltration. NeMacs are highly cytostatic, that is, they prevent non-lymphoid cells and T-cells proliferation. They also promote naïve T-cells differentiation to Th2 cells (Allen *et al.*, 1996) and can perhaps, be regarded as type-2 macrophages. The features of a nematode-elicited macrophage are described in Figure 2.5 while the mechanism of the interaction between immune cells in response to helminth infection at acute and chronic stages is shown in Figure 2.6.

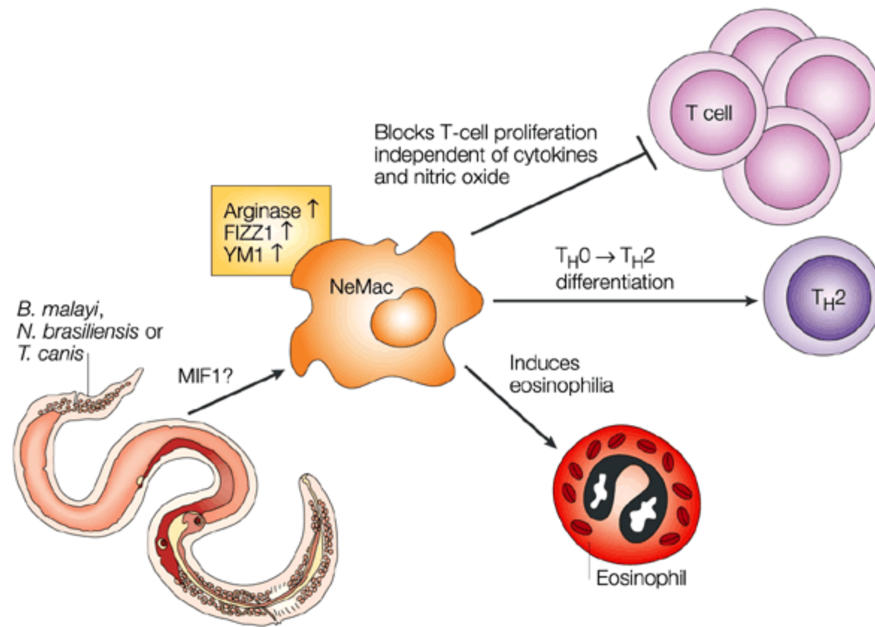


Figure 2.5: Features of Nematode-elicited Macrophage, an example of alternatively activated or type-2 macrophage (AAM) (Maizels and Yazdanbakhsh, 2003).

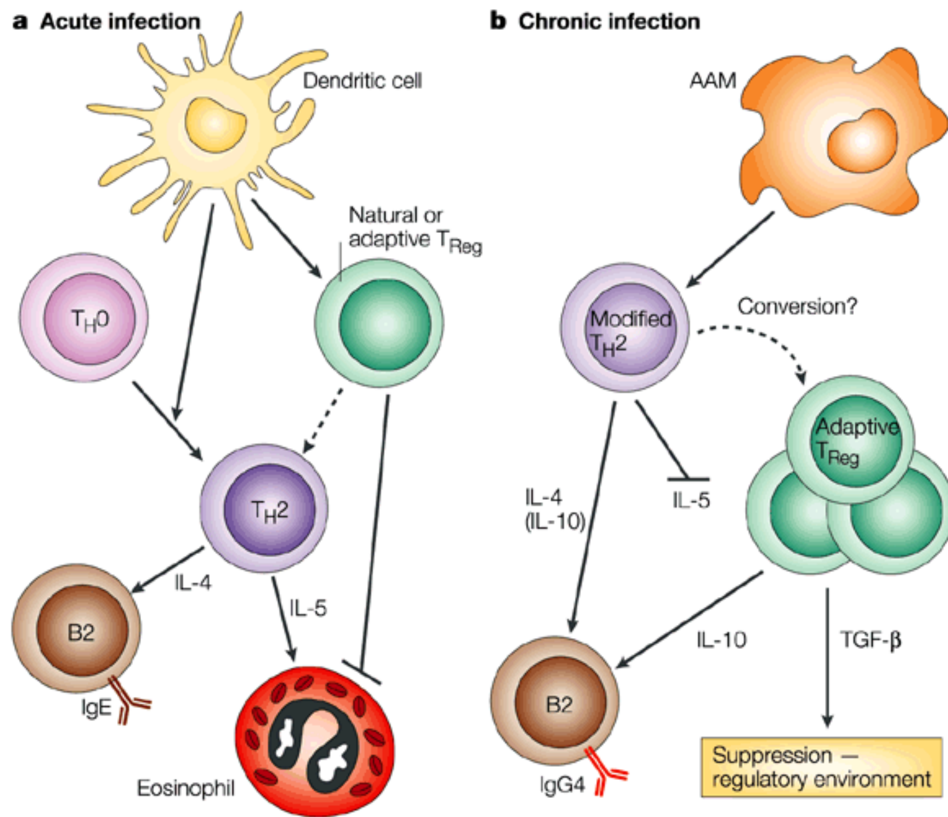


Figure 2.6: Mechanism of interactionbetween immune cells in responding to helminth infection at acute and chronic stages (Maizels and Yazdanbakhsh, 2003).

It is not just through warding off immune attack that soil transmitted helminths survive within the host, they also aggressively subvert the host immune response which in turn, creates opportunities to maximize their successful residence, reproduction was well as feeding within host (Maizels *et al.,* 2004). Soil-transmitted helminths also induce (Th2) immune response through cytokines (IL-10, IL-5, IL-13and IL-4), parasite-specific immunoglobulins and non-specific IgE productions, mast cells', basophils' and eosinophils' mobilisation and expansion (Maizels *et al.,* 1993). Th2 - driven functional effector mechanisms response to STH infections include polyclonal and specific IgE production, eosinophil-mediated killing of the larvae, goblet-cell hyperplasia, degranulation of the mast-cells and increased mucus secretion (Mosmann and Coffman, 1989). Regulatory mechanisms involving IL-10 is also essential for soil-transmitted helminth survival and the down regulatory immuneactions are also beneficial to the infected host through blocking the progression of the immune action to atopic reactions (Yazdanbakhsh *et al.,* 2001).

Secretomes also plays important roles inhelminths' survival. For instance, adult *Necator americanus* secretes natural-killer cell-.binding protein, which binds NK cells as well as induces IFN-γ secretion, which may counteract protective Th2 response that could lead to parasite expulsion (Hsieh *et al.,* 2004). Neutrophil inhibitory factor secreted by dog hookworm, *A caninum,* binds CD11b/CD18 integrins, which then blocks activated human neutrophils' adhesion to vascular endothelial cells as well as release of H_2O_2 from the activated neutrophils (Moyle *et al.,* 1994). A metalloprotease that degrades eotaxin, is also secreted by *N. americanus,* providing strategies to prevent eosinophils activation and recruitment at infection site (Culley *et al.,* 2000).

2.4 Helminth Infection and the Hygiene Hypothesis

Hygiene Hypothesis proposes that the improved living standard and healthcare of developed countries could have resulted in lower childhood infections rates but increased immune dysregulation, which might have deprived these populations of helminth-mediated immunomodulation of the immune response to allergic diseases among susceptible individuals (Liu, 2007; Cooper, 2009). Higher prevalence of allergies like allergic rhinitis, eczema and asthma, are found in developed nations than developing ones (Cooper *et al.,* 2009; Maizels,2005). Focus has therefore shifted to whether infections with intestinal helminth are part of factors wthat counteract allergies in developing countries (Flohr *et al.,* 2009).

Ascaris lumbricoides and *Trichuris trichuria* protect against skin atopy development. (Feary *etal.*, 2011) A study has also shown that helminth infection reverses the usual Th2 response in Nigerian asthmatic patients by increasing the serum concenrations of IL-2 and decreasing that of TGF-β (Arinola *et al.*, 2014). A study also reported lower levels of autoantibody in schistosome-infected individuals than in infection-free subjects and the autoantibodies levels increased in infected subjects after paraziquantel (an antischistosome drug) treatment (Mutapi *et al.*, 2011).

Also, there is aninverse relationship between prevalence of helminth infection and incidence of multiple sclerosis (MS). Gastrointestinal helminths - infected Patients subsequent to diagnosis of MS have been reported to have lower relapse rates compared with uninfected, severity matched patients (Correale and Farez, 2007).

There is also an inverse relationship between STH and Type1 diabetes mellitus (T1D) (Zaccone *et al.,* 2006). Infection of non-obese diabetic mice with helminths resulted in shift from Th1- to a Th2- immune response, which then halted the T1D development (Saunders *et al.,* 2007). Also, administration of IL-10 has alsobeen shown to ameliorate T1D (Raz *et al.,* 2005).

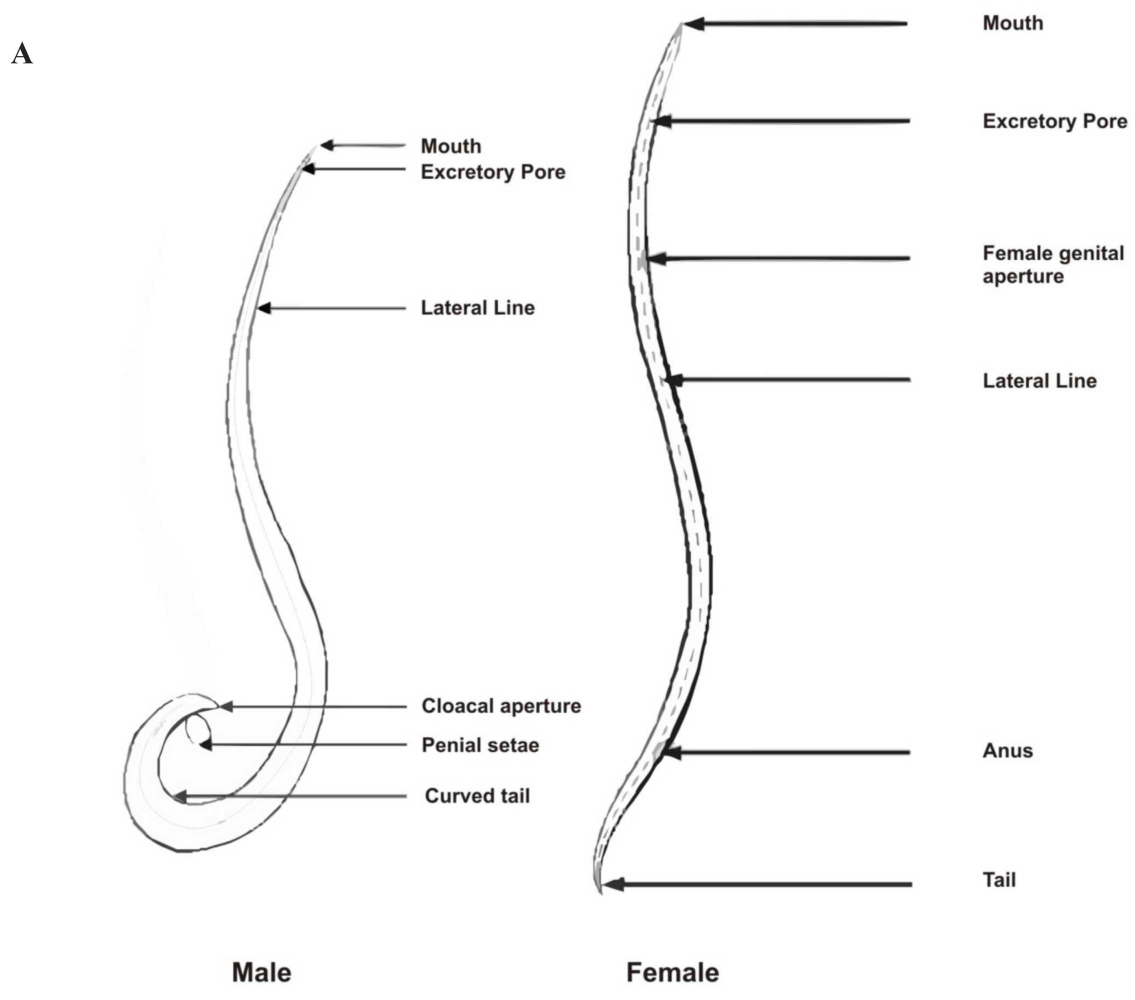
Helminth infection triggers generation of alternatively activated macrophages which protects, instead of exacerbating insulinitis and β-cell destruction (Liu *et al.*, 2010). There is also association between active pulmonary tuberculosis (TB) and intestinal helminth infection (Elias *et al.,* 2006). Anti-helminthic therapy enhanced mycobacterial-specific immuneresponses (Elias *et al.*, 2001). However, helminth infection reactivateslatent tuberculosis infection (Borkow *et al.,* 2001) and weakens BCG vaccine efficacy (Elias *et al.,* 2006).

Studies also revealed that treating pregnant mothers living in hookworm and schistosome diseases - endemic area with anthelminthic drugs showed significantly reduced incidence of atopic eczema in their offsprings (Elliot*,* 2011).

2.5 *Ascaris lumbricoides*

Ascaris lumbricoides, aspecie of Ascaris from phylum nematode, is the largest worm that parasitises the intestine of humans (CDC, 2015b). Usually, the female worm is longer in length (up to 20 – 35 cm) compared with the male (15 to 30 cm.). The male also has a bluntly pointed tail with a ventrally curved posterior end. The female'santerior end has the vulva, that makes up about a third of the worm's body length. The uteri can hold up to 27 million eggs at a time, laying about 200,000 daily (Roberts and Janovy, 2009). Ingested unfertilized eggs may not be infective but ferlilised ones are embryonated and become infective in weeks. Fertilized eggs of Ascaris are oval or round in shape and measure about35–50 µm wide and 45–75 µm long. The unfertilized eggs are usually bigger, measuring about 44 µm wide and 88–94 µm long. The morphological features of adult worms of *Ascaris lumbricoides* and the eggs are shown in Figure 2.7 (Karki, 2017).

A



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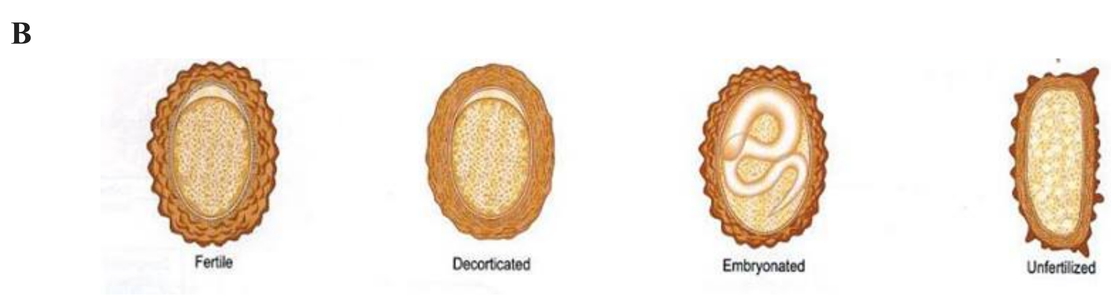


Figure 2.7: Morphological features of (A) the adult worms and (B) eggs of *Ascaris lumbricoides* (Karki, 2017).

2.5.1 Effect of *Ascaris lumbricoides* infection in human host.

Ascaris lumbricoides causes Ascariasis in humans. This is usually associated with the presence of the worm in human intestine. Minor infection with the parasite often gives no symptoms but heavy infection may result in symptoms such as passing of the worms in faeces, abdominal discomfort, bloody sputum, intestinal ulcer, cough and fever (Dold and Holland, 2011). It is the commonest cause of Loeffler's syndrome, which occurs during the migration of the larva through the lungs (Dold and Holland, 2011). Mast cell hyperplasis and eosinolphilia associated with high levels of circulating immunoglobulin E are also characterized with Ascariasis in humans (Jackson et al, 2004).

2.5.2 Immune Responses to *Ascaris lumbricoides* infection in humans

Immunity against Ascariasis in humans is characterized by cellular and antibody responses. The infection stimulates production of all isotypes of antibody, with high serum concentration of total and parasite-specific IgE antibodies (Cooper *et. al.,* 2000; Arinola *et, al.,* 2015). Cellular response is characterized by Th2 response with higher secretion of both IL-4 and IL- 5 in endemic regions. Studies have also demonstrated marked Th-2 cytokine responses in Ascariasis and the roles of interleukins – 4, 5, 9 and 13- associated pathways in the mediation of resistance to the infection as well as expulsion of the parasite (Finkelman, *et a.l,* 1997). Protective immune response may also involve mechanisms mediated by IgE and directed against the invading larvae, but may also be a marker of enhanced Th-2 response (Cooper *et. al.,* 2000). Immunity with *Ascaris lumbricoides* infection also leads to high serum C-reactive protein, ferritin and eosinophil cationic protein levels, which indicates inflammatory or acute phase reactions in infected hosts (McSharry *et. al.,* 1999).

2.6 Micronutrients

Also known as vitamins and minerals, required in minute quantities in humans, micronutrients are dietary components which are very vital to development, well being as well as disease prevention (CDC, 2015b). Micronutrients are derived from the diet since they are produced in the body. Globally, more than 2 billion people, majorly children between ages 6 months and 5 years worldwide experience micronutrient deficiency of one or more forms (Global Report, 2009) accounting for about 7.3% of global burden of disease (Bárány *et al*., 2002). Iron, vitamin A and iodine, deficiencies present serious threat to health and therefore are very important in global health concept, particularly in pregnant women and children in developing countries (WHO, 2016c).

Micronutrients are also important for proper immuno-physiologic functions (Failla, 2003) and vaccine efficacy (Sack *et al.*, 2008). Deficiencies could result from inefficient consumption of available micronutrients because of infection by parasites or due to inadequate intake (Rayhan and Khan, 2006). Generally, malnutrition affects immune response, including the cell-mediated immune responses (Chandra, 1997), cytokines production (Grimble, 1996) and antibody responses (Brussow *et al.,* 1995) particularly those requiring T cell support (Redmond *et al.,* 1995). The functions, immunological importance as well as effect of helminthiasis on some micronutrient minerals and vitamins are hereby described.

2.6.1 Zinc

Zinc is a micronutrient that is very crucial for normal development and functioning of both the innate and adaptive immune cells (Prasad, 2008). Zinc deficiency can result from inadequate intake, which may cause compromised immune responses (Ibs and Rink, 2004).

Deficiency in zinc causes impairment of the complement system, phagocytic functions of neutrophils and macrophages, natural killer cells'cytotoxicity, as well as the ability of immune cells to intitate oxidative killings of invading pathogens (Kruse-Jarres, 1989; Ibs and Rink, 2003). It also compromises adaptive immune response, including lymphocytes' multiplication and functioning (Shankar and Prasad, 1998). B-lymphocytes – linked antibody production is also altered due to depletion in zinc. The relative number of cytotoxic T lymphocytes precursors and CD molecules that cells need for recognition and proliferation of antigens, (for instance, in CD8+CD73+ T lymphocytes), decreases during zinc deficiency. Also, T-cells development is zinc –dependent and its deficiency is implicated in thymic atrophy (Mocchegiani *et al.,* 1995). It is an essential co-factor of the hormone thymulin produced by the thymus. Zinc deficiency also affects the $CD4^{+}$Th cells, leading to impaired production of Th-1 and Th-2 cytokines (Ibs and Rink, 2003).

Some studies reported reduced serum zinc levels in helminth– infected school children (Kongsbak *et al.,* 2006; de Gier *et al.,* 2015). Reduced serum zinc levels has also been reported in helminth – infected preschool – aged, school-aged children and pregnant mothers in Nigeria (Arinola *et al.,* 2015; Ejezie and Nwagha, 2011). The reduced zinc level in the helminth infected children was attributed to low dietary intake (due to loss of appetite), gastrointestinal bleeding, malabsorption, diarrhea, or infection (Arinola *et al.,* 2015).

Zinc deficiency also causes impaired production of IL-4, leads to depressesed IgE, IgG1 and eosinophil productions and impaired antigen-presenting cells (APC) and T cells functions in helminthiasis. This causes systemically disseminated immune response thereby prolonging parasite survival in infected hosts (Scott and Koski, 2000).

2.6.2 Iron

Iron in sufficient quantity is essential for various aspects of the immune functions, including T lymphocytes'proliferation and differentiation, as well as reactive oxygen species (ROS) generation. Conversely, its deficiency impairs immune responses (Doherty, 2007). Ferritin is the stored form of iron in the body while transferrin is the transport protein for iron (Jason *et al.,* 2001). Lower serum ferritin and raised serum transferrin levels are features of iron deficiency anaemia while raised levels of both ferritin and transferrin are observed following inflammatory reactions (Macedo and de Sousa, 2008). Many infectious pathogens require iron for replication and survival. Serum iron levels decrease while ferritin levels increase during acute inflammatory response, suggesting that iron sequestration from infecting organisms is an important form of response to infection by the host (Beard, 2001). Iron overload however, can have advers eeffect on immune function, such as phagocytic function impairments; complement system activation, production of cytokine, as well as T and B lymphocyte function (Doherty, 2007).

Arinola *et al.* (2015) reported reduced serum iron levels in helminth infected pre-school and school-aged children and pregnant mothers in Nigeria. Another study had a similar outcome among children of rural areas of West Malaysia (Ngui *et al.,* 2012). However, studies did not report significant association between *Ascaris lumbricoides* and serum iron as against hookworms and *Trichuris trichuia* which are strongly linked with iron-deficiency anaemia (Rajagopal *et al.,* 2014). Hookworm disease leads to intestinal blood loss thereby resulting in iron-deficiency anaemia. Haemoglobin levels lower than 11g/dl can result with just about 40 adult hookworms in the intestine (Bethony *et al.,* 2006).

2.6.3 Selenium

Selenium is a co-factor for selenoproteins. Selenocysteine for instance is incorporated into the elongating proteins at specific locations during protein synthesis, forming functional selenoproteins (Rayman, 2000) The selenoproteins, glutathione peroxidases (GPx) are cellular antioxidants and redox regulators, that depletes reactive oxygen species, such as lipid hydroperoxides and H_2O_2, to alcohols and water through reduction reaction with glutathione oxidation (Gladyshev, 2006). The implications of these roles are vital role for effective functioning of the immune system and prevention of cancer.

Impairment of some activities of the innate and the adaptive immune response can result due to selenium deficiency (Arthur *et al.,* 2003). Deficiency in selenium enhances the virulence or progression of certain viral infections and plays role in regulating cytokines expression (Baum *et al.,* 2000). Selenium can be toxic at high levels and studies have associated increased selenium level with Type – 2 diabetes mellitus (Bleys *et al.,* 2007).

Reduced serum selenium levels in helminth infected children have been reported in some studies (Thorpe et al, 1990; Amare *et al.,* 2012; Rajagopal *et al.,* 2014). A study in Nigeria conversely showed a raised serum selenium level in children with STH infection compared with helminth-free children (Arinola *et al.,* 2015).

2.6.4 Vitamin A

Vitamin A deficiency is of public health concern the world over, most common in developing countries, where availability of vitamin A-fortified foods is limited. Worldwide, one out of three preschool-aged children as well asone out of six pregnant women suffers vitamin A deficiency because of inadequate dietary intake (CDC, 2015b). Food supplementation with Vitamin A has been aneffective means for reducing mortality for children in developing countries having deficiency in vitamin A as a public health issue (WHO, 2011).

Vitamin A with its metabolites plays important functions in adaptive and innate immune response. In innate immunity, it helps maintaining structural and functional integrity of the skin as well as mucosal cells of the eye, gastrointestinal, genitourinary and respiratory functions which act against infections barrier (Semba, 2004). It is also essential for effective functioning of B and T cells; thereby expressing its necessity in antibody responses to specific antigens (Semba, 2004).

Studies have associated helminthiasis, specifically *Ascaris lumbricoides* infection to vitamin A deficiency (Ahmed *et al.,* 1993; Sommer, 2008; Arinola *et al.,* 2015b). Children with *Ascaris lumbricoides* infection absorb lesser amount vitamin A after oral supplementation which is associated with ability of the intestinal tract to absorb vitamin A (Ahmed *et al.,* 1993). The malabsorption may result due to mucosal changes in the digestion tract and blunting of the intestinal villi (Tripathy *et al.,* 1972). The consequence of vitamin A deficiency such as lower respiratory tract infections and measles (Imdad *et al.,* 2010) makes the association between vitamin A deficiency and *Ascaris lumbricoides* infection to have important consequence for global health, especially since *Ascaris lumbricoides* infection may be one of the commonest chronic infection in childhood globally (Crompton *et al.,* 1989).

2.6.5 Vitamin C

Also known asascorbic acid, Vitamin C is theprimary water-soluble, non-enzymatic antioxidant in plasma and body tissues (Combs and Gerald, 2012). It protects the body's indispensable molecules from ROS damage as well as free radicals from active immune cells during normal metabolism and from toxins and pollutants. Vitamin C is also involved in metabolism of other antioxidants. For instance, vitamin C regenerates the oxidized form of vitamin E (Carr and Frei, 1999).

Impairment in some enzymatic reactions and insufficient synthesis of collagen and catecholamines resulting in conditions such as poor wound healing and lethargy are symptoms of vitamin C deficiency. Research suggests that cholesterol metabolism to bile acids may also have vitamin C involvement, which may affect cholesterol levels and development of gallstones (Simon andHudes, 2000).

The body's cells protection by vitamin C, against ROS generated by immune cells to kill pathogens, makes the vitamin to excert major factor ininnate and adaptive immune response. It stimulates leukocytes' production and function, especially phagocytes, lymphocytes and neutrophils (Anderson *et al.,* 1980; Panush *et al.,* 1982). Phagocytosis (Levy *et al.,* 1996), chemotaxis and cellular motility (Anderson *et al.,* 1980) are all mediated through the involvement of vitamin C. Lymphocytes and neutrophils accumulate vitamin C in high concentrations for protection against oxidative damage from non-specific toxins like hypochlorous acid ("bleach"), peroxynitrite and superoxide radicals, in response to invading microorganisms (Evans *et al.,* 1982). Studies also showed that vitamin C caused increased serum antibodies levels (Feigen *et al.,* 1982) as well as C1q complement proteins in animal models (Haskell and Johnston, 1991).

The association between helminth infection and serum vitamin C concentration in Nigerian children has been presented in some studies. While Arinola *et al.,* (2015) showed no significant difference in serum vitamin C level in preschool-aged and school aged children with or without helminth infection, Airauhi and Idogun (2008) reported decreased levels of vitamin C in children with moderate and heavy Ascaris infections. This is associated with intestinal parasite – induced malabsorption, malnutrition as well as vitaminoses (Airauhi and Idogun, 2008).

2.6.6 Vitamin E

Vitamin E consisit of a family of 8 fat-soluble molecules with antioxidant properties. The α-tocopherol isoform of vitamin E are those that meet the human requirements of vitamin E. It binds α-tocopherol transferprotein (α-TTP) and incorporated into α-tocopherol transport lipoprotein in the blood which delivers it to extrahepatic tissues (Traber, 2012).

Vitamin E protects cell membranes integrity from ROS damage (Traber and Atkinson, 2007). It protects against polyunsaturated atty acids peroxidation, which causes cellular damage, leading to impaired immune responses. Its deficiency also impairs both the cell-mediated and humoral forms of adaptive immune response, including T and B cells function (Moriguchi and Muraga, 2000). Vitamin E also ameliorates age-related immune effects of Tcell proliferation and cytokine production (Wang *et al.,* 1994).

Reduced VitaminE level has also been linked with soil transmitted helminthiasis in children (Airauhi and Idogun, 2008; Lone *et al.,* 2015) and this has also been attributed to helminth induced malabsorption, malnutrition as well as vitaminoses (Airauhi and Idogun, 2008).

2.7 Vaccination

Vaccination is the act of introducing immunogenic material which leads to stimulation ofthe body's immune system to produce immunity against a specific disease, thereby resulting in protection of the body from being infected with the disease (CDC, 2014). The history of vaccination dates back to a millennium, when the Chinese employed inoculation (or "variolation") techniques against smallpox as far back as 900 AD. Experimentation of vaccination protecting against a particular disease, without transmitting the disease itself was first demonstrated by Edward Jenner (Gross and Sepkowitz, 1998). Worldwide, vaccination

has had tremendous impact in disease and death prevention due to infectious diseases, and it has been the most successful public health interventions ever implemented (WHO, 2013). Over 2.5 million deaths are prevented every year due to vaccination and it has offered protection from the threat of vaccine-preventable diseases. Immunized children thrive better and have a better chance of full potential realisation (WHO, 2013).

Vaccinated individuals are rendered immune against the disease they are vaccinated for and the side-effects from the disease- causing organisms are avoided (Zepp, 2010). Protective efficacy of vaccines conferred through inducing antigen-specific antibodies and efficacy of the antibody responses (for example, their avidity and longevity of their protection), requires the vaccine antibodies'persistence and/or the immune memory cells' generation, that are capable of reactivating rapidly and effectively, following subsequent exposure (Siegrist, 2008).

According to Ada (1990), an ideal vaccine should have two sets of requirements: It should be safe, even in immune-compromised people and be highly effective. Secondary requirement is that it should be cost effective, easy to administer, have high thermal stability and might be multivalent to save further on administration cost. It should also be able to confer long memory after single or double doses.

2.7.1 Mechanism of Immunity induced by Vaccination

Life long immunity, which is a feature of adaptive immunity achieved by immunization, results through the persistence of specific immune effectors and/or through induced immune memory cells, reactivated to immune effectors after exposure to pathogen. Basically, antibodies produced by the B lymphocytes, that is capable of binding specifically to a pathogen or toxin are the main vaccine-induced immune effectors (Cooper and Nemerow,

1984; Casadevall, 2004). Other effectors include: $CD8^+$ T-lymphocytes (CTL) which recognise and kill infected cells, or secrete certain antiviral cytokines. Generating and maintaining $CD8^+$ T cell and B cell responses are aided by factors and signals from $CD4^+$T helper (Th) lymphocytes (Igietseme *et al.,* 2004), which are also mediated by regulatory T cells (Treg) that play major role in immune tolerance (Bacchetta *et al.*, 2005).

Antigen presenting cells (APC), such as dendritic cells (DC) which are recruited upon exposure to antigen activates and induces antigen-specific B and Tcell responses (as shown in Figure 2.8). Upon exposure to pathogens, immature dendritic cells (DC) undergo brisk maturation and move towards the lymph nodes, where B and T cell responses take place. The major inertia of responses to vaccine is therefore to provide enough danger signals by the vaccine antigen to initiate inflammatory response by the innate immune cells (Hoebe *et al.,* 2004).

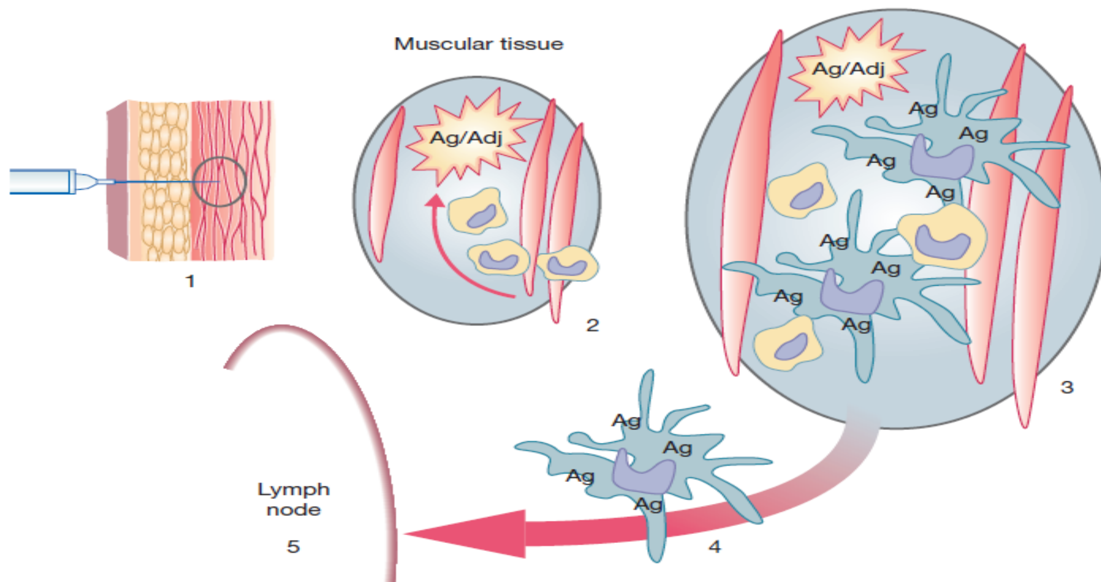


Figure 2.8: Initiation of a vaccine response. (Siegrist, 2008).

After injection (1), the vaccine antigens containing the pathogen's - associated membrane patterns attract dendritic cells, neutrophils and monocytesthat moves round the body (2).Upon the vaccine antigens/adjuvants showing sufficient 'danger signals', the dendritic cells and monocytes are activated. (3) This leads to change in their surface receptors thereby inducing their migration en-route the lymphatic vessels (4), to the draining lymph nodes (5) where the activation of Tand B lymphocytes occurs.

Receptors on the innate immune cells like the Toll-like receptors (Barton and Medzhitov, 2002) play an important role in the modulation of the expression of their surface molecules and produce chemokines and proinflammatory cytokines when triggered by danger signals (Iwasaki and Medzhitov, 2004). This result into the extravasation and attraction of natural killer cells, granulocytes and monocytes, then generates an inflammatory microenvironment where monocytes differentiate into macrophages and immature dendritic cells become activated, triggering their migration towards the draining lymphnodes (Pashine *et al.,* 2005). When danger signals are absent however, dendritic cells remain immature and when they are in contact with naïve T cells, T cells do not differentiate into immune effectors but into regulatory $CD4^{+}$T cells (Tregs) whose function is to maintain immune tolerance (Bacchetta *et al.*, 2005).

The germinal center and extrafollicular responses to vaccine antigens after reaching the lymph nodes or spleen are explained in Figure 2.9. B cells which bind antigens with their surface immunoglobulins undergo brisk activation. In the extrafollicular region, the Bcells rapidly differentiate into plasma cells and produce low-affinity antibodies (IgM $^{+/-}$, IgG/IgA isotypes) which are present at low concentration in the serum within few days after immunization (Siegrist, 2008). Antigen-specific T helper cells activated by antigen-bearing DCs trigger some antigen-specific Bcells to migrate towards follicular dendritic cells (FDCs), thereby initiating reaction at the germinal center (GC). In the GC, B cells receive additional signals from follicular Tcells (Tfh), then undergo massive clonal proliferation, switch from IgM towards IgA, IgG or IgE, undergo affinity maturation and differentiate into plasma cells that secrete large amounts of antibodies which are antigen-specific. At the end of the reaction at the germinal center, a few of the plasma cells exit nodes/spleen and migrate to survival niches in the bone marrow where they are mostly located, and they survive with the aid of signals provided by supporting stromal cells (Siegrist, 2008).

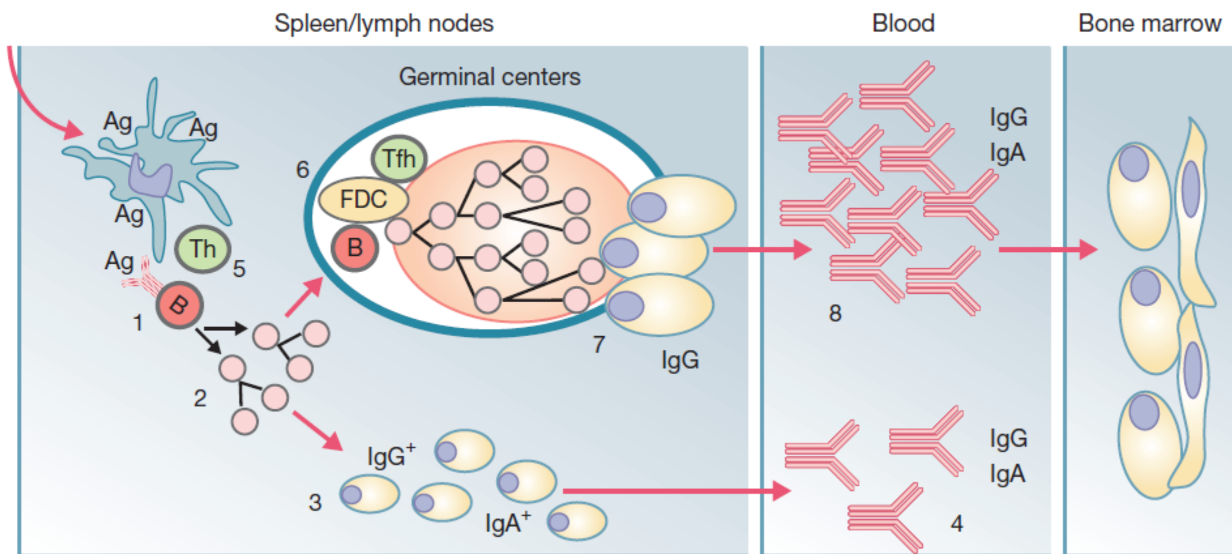


Figure 2.9: Responses at the extrafollicular and germinal center to protein antigens (Siegrist, 2008).

(1) Brisk activation of B cells (2) which rapidly differentiates to plasma cells leading to (3) low-affinity antibodies production (4) Antibodies appear in serum at low concentration (5) B cells' migration towards the follicular dendritic cells inside the (6) Germinal Centre. (7) Further B cells' proliferation and differentiation into antibody-producing plasma cells with the aid of follicular T cells (8) Appearance of antibody in serum in high concentration and migration of few plasma cells into the bone marrow

During the extrafollicular reaction, naïve B cells bind a vaccine antigen with their specific surface IgM, thereby initiating B cell activation and triggering the upregulation of a chemokine receptor, CCR7, which drives the vaccine antigen-specific B cells towards the outer T cell zone of secondary lymphoid tissues, where they are exposed to already activated dendritic cells and T cells and provide B cell activating signals (Reif *et al.,* 2002). The T cell help rapidly drives the differentiation of the B cell into immunoglobulin-secreting plasma cells that produce germline antibodies of low-affinity inthe extrafollicular reaction (MacLennan *et al.,* 2003). Class-switch of immunoglobulin from IgM towards IgA, IgE or IgG occurs during B cells differentiation, through the activation-induced deaminase (AID) enzyme upregulation. During the extrafollicular differentiation pathway, both the $CD4^+$ Th1 and Th2 cells elicit essential helper functions, and the engagement of their CD40L molecules with CD40 on B cells may skew class-switch recombination into particular immunoglobulin classes and subclasses. In rodents for instance, IFN-γ producing Th1 T cells promote a switch towards IgG2a, whereas the generation ofIgG1 and IgE (via IL-4) and IgG2b and IgG3 (via TGF-β) is essentially supported by Th2 cells. In humans, regardless of the polarization of T cellhelp, IgG1 antibodies frequently predominate (Deenick *et al.,* 2010). The extrafollicular reaction is rapid and in a few days after primary immunization, IgM and low concentration of IgG antibodies are present in the blood. The extrafollicular reaction is however short-lived, as most cells die due to apoptosis within a few days. Consequently, it probably plays a minor role in vaccine efficacy (Siegrist, 2008).

In the germinal centers (GCs), antigen-specific B cells which are activated by specific T cells, proliferate and differentiate into plasma cells following up-regulation of the CXCR5 chemokine expression. They migrate towards B cell follicles after attraction by CXCL13 chemokine - expressing follicular dendritic cells (FDCs). Follicular dendritic cells (FDCs)

play an important role in B cell responses as they attract antigen-specific B and T cells and capture or retain the antigen for extended periods. B cells undergo massive clonal proliferation after receiving additional activation and survival signals from both FDCs and follicular T cells (Vinuesa *et al.,* 2005). This intense proliferation is also associated to immunoglobulin class-switch recombination from IgM towards IgA, IgE or IgG and maturation of the B cells affinity for their specific antigen. This results in production of more antibodies with higher antigen binding capacity. B cells process these vaccine antigens into small peptides which are displayed on their surface through MHC class II molecules, and become available for binding to specific subset of $CD4^+$ T cells and follicular helper T cells (Tfh) (Vinuesa *et al.,* 2005). These CXCR5-expressing follicular helper T cells migrate towards the CXCL13-expressing FDCs. The cellular interactions between antigen bearing FDCs, antigen-specific GC B cells and antigen-specific Tfh result in the selection, survival and proliferation of B cells with the highest possible antigen-specific affinity. They also provide the required signal for the subsequent differentiation of germinal centre B cells either towards memory B cells or towards plasma cells secreting large amounts of specific antibodies (Vinuesa *et al.,* 2005).

Vaccine responses in T cell are also elicited in parallel to responses in B cell, via interactions with activated DCs. Immature DCs take up vaccine antigens and provide the necessary signals required for their migration to draining lymph nodes. During migration, maturation of DCs occurs and there are changes in their surface expression of molecules (Randolph *et al., 2005*). At the same time, processing of antigens into small fragments occurs, and they are displayed at the cell surface in the grooves of MHC molecules. MHC class I molecules present peptides from antigens that are produced within infected cells, whereas MHC class II molecules display phagocytosed antigens (Groothuis *et al.,* 2005). Thus, mature dendritic

cells reaching the T cell zone of lymph nodes display MHC-peptide complexes and high levels of costimulation molecules at their surface (Kapsenberg, 2003). Antigenic peptides displayed by class II MHC molecules are recognized by the $CD4^+$ T cells, whereas CD8 T cells bind to class I MHC-peptide complexes. Their recognition is restricted to short peptides (10–18 [CD4+] or 8–11 [CD8+] amino acids) displayed on specific MHC class II or I molecules respectively (Krogsgaard and Davis, 2005).

Activated $CD4^+$ T cells provide supportive functions through signals, for dendritic cells (CD40L, etc.), resulting in B cells and for $CD8^+$ cytotoxic T cells further activation. Activation of $CD4^+$ T cell by dendritic cells triggers their differentiation along two mutually exclusive and distinct differentiation pathways: Th1-type $CD4^+$ T cells basically produce IFN-γ and TNF-α, which participates in intracellular pathogens'elimination both directly (cytokine responses) and indirectly through their support to activation of macrophage and differentiation of $CD8^+$ T cells (Kapsenberg, 2003; O'Garra and Robinson, 2004). Th2-type $CD4^+$ T cells basically produce IL-13, IL-5 and IL-4 which are directly implicated in the defense against extracellular pathogens such as helminths (Stetson *et al.,* 2004). Both Th1 and Th2 cells support activation and differentiation of B cells during extrafollicular responses, whereas follicular (Tfh) $CD4^+$ helper T cells provide help to the germinal center B cells (Vinuesa *et al.,* 2005).

2.8 Determinants of Primary Vaccine Antibody Responses

The intensity of vaccine induced GC, and thus of peak antibody responses, is modulated by numerous determinants. The main determinants are the vaccine antigen nature and its intrinsic immunogenicity. Live vaccines generally elicit the strongest antibody responses, which activate innate reactions better and thus better support the adaptive immune effectors induction. For instance, live viral vaccines trigger efficiently, the innate immune system

activation, in a pattern similar to that occurring after a natural infection including the initial mucosal stage of replication for vaccines administered through the nasal/oral routes. Live bacterial vaccines like BCG multiply both at the injection site, where they generate the induction of a prolonged inflammatory reaction and at distance with preference for local draining lymph nodes. Non-live vaccines, whether containing polysaccharides, proteins, inactivated microorganisms or glycoconjugates may contain pathogen-related patterns recognition receptors that are capable of initiating innate responses (van Duin *et al.,* 2006). However, vaccine-induced activation remains more limited, both in time and space in the absence of microbial replication. Non-live vaccines basically activate innate responses at their injection site (van Duin *et al.,* 2006).

T-independent responses are triggered by bacterial polysaccharide antigen (of *C. pneumoniae, N. meningitidis, H. influenzae, S. typhi*). This is characterized by the absence of immune memory and induction of slightly increased antibodies titers with low-affinity.The germinal centres are not induced by the polysaccharide antigens. Repeat exposure to the same polysaccharide antigens subsequently, results in a repeat primary response which follows the same process as in naïve individuals. Repeat vaccination with other strains of bacterial polysaccharide antigens may produce lower antibody responses compared with the first immunization. This is known as hyporesponsiveness (Granoff *et al.,* 1998; Richmond *et al.,* 2000).

Also of importance are the site and route of administration of vaccines. The high number of dendritic cells in the derma allows a marked (up to 10-fold) reduction of the dose of the antigen in intradermal immunization, but it generally results in lower vaccine antibody responses (Chen and Gluud, 2005), which might be associated with the preferential induction of Th1 responses by skin dendritic cells. Patrolling dendritic cells are also numerous in the

well-vascularized muscles, which is the preferred injection route for most vaccines. They are however fewer in adipose tissues, such that subcutaneous injections may not be as effective as intramuscular injections under conditions of limited immunogenicity (de Lalla *et al.,* 1988). Despite many efforts, few live vaccines are currently designed for immunization through the mucosal route. The extreme difficulty in producing non-live mucosal vaccines suggests the need to overcome a large number of physical, chemical and immunological barriers that require the use of strong adjuvants (Lavelle and O'Hagan, 2006).

Vaccine antibody responses in healthy individuals are also influenced by their genetic determinants (Newport *et al,* 2004). Apart from MHC restriction, few genetic determinants of vaccine antibody responses have yet been identified. Vaccine antibody responses are also affected by immune competence, which are limited at the two extremes of life, by acute or chronic stress,by acute or chronic diseases and by different factors that affects innate and/or B and T cell immunity (Hohler *et al.,* 2005).

The magnitude of primary vaccine antibody response is also determined by the optimal dose of the vaccine antigen, which is determined experimentally. Higher primary antibody responses are elicited by higher doses of non-live antigens up to a certain threshold. (Benhamou *et al,* 1984). Primary antibody responses may be restricted by limiting the dose of vaccine antigen, but increased B cell competition for follicular dendritic cells-associated antigens. This results in a more stringent selection of higher affinity germinal centre B cells and into stronger secondary responses. Very few non-live vaccines after a single vaccine dose, induce high and sustained antibody responses, even in healthy adults. Therefore, primary immunization schedules usually include at least two vaccine doses, which are repeated at a minimal interval of 3–4 weeks in order to generate successive waves of B cell and germinal centre responses. Occasionally, these priming doses may be combined into a

single ‘double’ dose, as used in hepatitis A or B immunizations (Van Der Wielen *et al.,* 2000; Cassidy, 2001).

Adjuvant functions to overcome the poor immunogenicity of subunit vaccines through improving pathogen recognition and eliciting response similar to the natural innate immunity. Using purified sub-unit antigen, an effective adjuvant can increase the breadth and durability of the achievable response. In 1932, Aluminium was introduced as adjuvants in human vaccines and was the only licensed vaccine adjuvant in use for about 70 years (Marrack, *et al.,* 2009). There is focus on overcoming the pathogen and population-related challenges facing 21st century vaccines in designing modern adjuvants and as such, the potential to help prevent infectious diseases of global significance are considered and provided in producing the present day sophisticated adjuvants, for which successful vaccines have not been possible using traditional technologies (Reed *et al.,* 2013).

Adjuvants formulation includes danger signals which could trigger initiation of the innate immune system sufficiently is required for most non-live vaccines. These adjuvants in two categories: immune modulators that provide more activation signals and differentiation to monocytes and dendritic cells; as well as delivery system that prolongs the deposit of antigen at injection site, thereby recruiting more dendritic cells into the reaction (Pashine *et al.,* 2005). Aluminium adjuvants primarily functions in increasing antibody production thereby suitable for vaccine units that targets pathogens that are inactivated by antibodies. It however could not be used in preventing infections caused by intracellular pathogens. Rapid advances in vaccine development have been achieved through expansion of the understanding of the potential role of adjuvants. New adjuvenated vaccines under development target challenging pathogens such as cytomegalovirus infection, dengue fever and HIV, malignanies such as

lung cancer, melanoma, and immunologically challenged populations such as the elderly (Stanberry and Strugnell, 2011).

2.9 Rotavirus Infection and Vaccination

Rotavirus is a contagious virus which causes gastroenteritis (inflammation of the intestines and stomach) especially in young children and infants. Symptoms usually include fever, severe diarrhea, abdominal pain vomiting and which can result in severe dehydration and even death if not quickly treated (CDC, 2014)**.**Worldwide, Group A rotavirus (RV) strains are the major causative agent of acute gastroenteritis (AGE) in young children and infants (Bresee *et al.*, 2004). RV disease accounts for between 500,000 to 600,000 deaths annually, and is responsible for more than a third of hospitalizations that are diarrhea-related withmost deaths occurring in sub-Saharan Africa and Asia (Parashar *et al.,* 2006). RV targets mature enterocytes in small intestines and its replication leads to increase in intracellular calcium ion level affected by its non-structural proteins, increased chloride secretion, and shutting-off of the synthesis of host cell protein, thereby resulting in acute secretory and osmotic diarrhea (Estes and Kapikan, 2007).

Primary rotavirus infection typically results in acute gastroenteritis (AGE), but resistance against subsequent infectionsis developed afterwards, thereby lowering the risk of the disease. (Velazquez *et al.,* 1996).Acute gastroenteritis of rotavirus origin can be serotype-specific and related to the levels of the neutrialsing antibodies (NT) against the virus. A NT level of $\geq$1/128 confers protection against rotavirus disease. (Chiba *et al.,* 1986), which suggests that humoral immunity against rotavirus is correlated with protection (Velazquez *et al.*, 1996). Serum high levels of Rotavirus-specific IgA antibodies in the body correlates with protection from RV (Coulson *et al*, 1992).

In children, the majority of blood circulating rotavirus-specific B-cells express gut-specific homing-receptor α4β7 (Jaimes *et al.,* 2004), suggestive of local protective action. Protection against rotavirus infection does not often times correlate with the serotype-specific neutralising antibodies presence, despite the importance of the rotavirus type–specific immune responses (Ward *et al.,* 1992). RV is relatively a poor inducer of virus-specific $CD8^+$ cells (Jaimes *et al.*, 2004). During the convalescent phase, circulating rotavirus-specific Th-cells are detected in infants' blood samples. Rotavirus-infected dendritic cells in-vitro can also stimulate rotavirus-specific T cells to produce Th1 cytokines, mainly IFN-γ, following infection with rhesus rotavirus (Offit *et al.,* 1991) but are less efficient in antigen presentation in young children and infants than in adults (Jaimes *et al*., 2004).

Rotavirus disease prevention can be achieved through repeated vaccination with a single rotavirus serotype. However, multivalent vaccines are required in case the production of strong neutralizing antibody responses are required, which would contain virus strains carrying VP7 and VP4 molecules that are representative of the main co-circulating wild-type human rotavirus strains. The RotaShield (Wyeth-Lederle Vaccines) and RotaTeq (Merck) vaccines are produced based on this principle, with RotaTeq containing proteins of the serotypes P1A, G1, G2, G3, G4 and their encoding RNAs. By contrast, the monovalent vaccine of serotype G1P1A (Rotarix; GlaxoSmithKline Biologicals), that are derived from human isolates was produced based on the observation that cross-protection (ie, a heterologous immune response) develops during the course of successive natural infections (Velazquez e*t al.,* 1996).

In the United States, mass vaccination against Rotavirus is reported to lead to delay in onset of rotavirus infection in children, with titres significantly lower compared with previous seasons (Payne and Parashar, 2018). The efficacy of the rotavirus vaccine is not affected by

the nutritional status of the infants. Phase III trials with Rotarix in South Africa and Malawi have shown that protection against severe acute gastroenteritis due to rotavirus is lower in Malawi (50%; mean, 60%) and South Africa (70%) (Madhi *et al.,* 2012). With RotaTeq however, protection rate of 51% in Asia and 64% in Africa were reported (SAGE, 2009). Due to the high mortality associated with rotavirus disease across the regions, an apparent benefit in terms of prevented deaths, even at relatively reduced vaccine efficacy, was observed (Madhi *et al.*, 2012). The Strategic Advisory Group of Experts on Immunization, of the World Health Organization has recomended worldwide incorporation of rotavirus vaccination into all national immunization programmes provided it can be funded and organized (SAGE, 2009).

2.10 Poliomyelitis and Oral Poliovirus Vaccination

Poliomyelitis results from infection with poliovirus (PV), a member of the genus *Enterovirus* which are RNA-viruses that colonize mainly the intestine and oropharynx of the gastrointestinal tract (Cohen, 2014). The virus has an incubation period of between 3 days and 35 days, but commonly, span of 6 days to 20 days are reported (Atkinson *et al.,* 2009). Poliovirus infects and causes poliomyelitis in humans alone (Ryan and Ray, 2004) and they are of 3 serotypes —poliovirus type 1 (PV1), type 2 (PV2), and type 3 (PV3)—with the slight difference between each, having to do with the make-up of their capsid protein (Katz *et al.,* 2004). The three serotypes are extremely virulent and produce the same symptoms of the disease (Ryan and Ray, 2004). The PV1 serotype is the most commonly encountered, and the one closely associated with paralysis (Orhi and Jonathan, 1999).

Vaccination against poliovirus infection came about in 1955 and 1961 with the introduction of live-attenuated oral polio vaccine (OPV) and inactivated (killed) polio vaccine (IPV)

(Sabin types 1, 2 and 3) respectively. OPV has however been the choice vaccine for the global eradication programme based on its action on mucosal immunity, very low cost and the ease of oral administration of the vaccine (WHO, 2015). OPV produces antibodies to all the three poliovirus strains in the blood and protect individuals against polio paralysis in cases of infection, through prevention of the spread of the virus to the nervous system and also by producing a local immune response in the mucous lining of the intestine, which is the primary poliovirus multiplication site (WHO, 2016). Vaccination against poliovirus induces strong Th1 response through secretion of IFN-γ and IL-2 indicating mediation by Th1 cells, but this may prove defective in neonates vaccinated much earlier in life (Vekemans *et al.,* 2002).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Selection of Study Participants

A total of three hundred and forty nine (349) children aged between 6 months and 15 years were recruited into this prospective case-control study. They included one hundred and forty nine (149) pre-school aged children, aged between 6 months and 60 months and two hundred (200) school-aged children, between the ages of 5 to 15 years. The children were recruited at Gbada-Ajia Community in Ona-Ara Local Government Area of Oyo State, as well as Alabata and Laleye communities of Akinyele Local Government Area of Oyo State. All of the sites are semi – urban communities. Each of the communities is about 10km and 15km from the State capital (Ibadan), with an average population of about 500 inhabitants who are mainly petty traders and farmers. Semi-structured questionnaire was administered to every participant to determine socio-demographic featuresof each participant.

3.2 Grouping of Study Participants

The study participants were grouped into preschool-aged and school aged children based on their ages: 6 months to 60 months as pre-school aged and 5 years to 15 years as school aged. School attendance was also used to verify the status of the school aged children. After general screening for helminths, *Ascaris lumbricoides*-infected children were selected for participation in this study. Their sera were employed to determine levels of serum cytokines, micronutrients and the vaccine-specific IgA antibodies before and after vaccination. Study on the determination of the effect of *Ascaris lumbricoides*- infection on vaccination in pre–school aged children was carried out using rotavirus vaccine because the vaccine is usually administered to pre-school aged children. Oral poliovirus vaccine was used for the study in

school aged children. Study on micronutrients and cytokines response before and after anthelminthic treatment was also carried out on the school aged children. Specified numbers of controls for each group were selected through random sampling from the pool of the serum samples obtained from the helminth – free children. Figure 3.1 describes the cohort of preschool-aged and school-aged children as engaged for different aspects of the study.

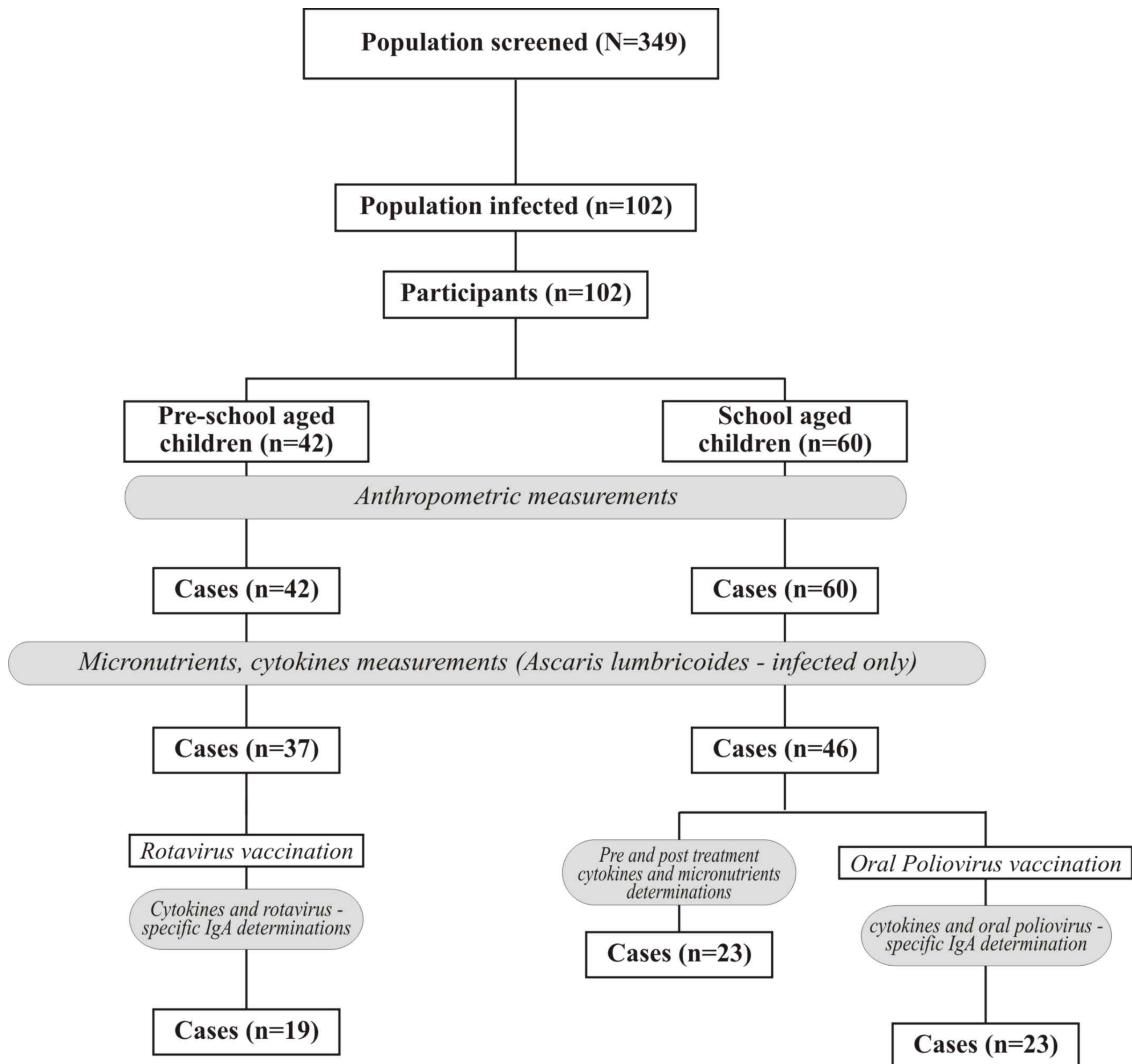


Figure 3.1: Cohort of pre-school aged and school aged children with and without intestinal helminth infection as engaged for different aspects of the study

3.2 Ethical Clearance

Ethical clearance was received from the University College Hospital / University of Ibadan Joint Ethics Committee and the Oyo State Ministry of Health, Ibadan, Oyo State, Nigeria. Participants were enrolled following town-hall and the Parents – Teachers Association's health awareness meetings carried out in the communities and schools respectively. Those whose parents consented to participate were recruited into the study. Massive deworming exercises were carried out in all the communities at the end of the study.

3.3 Inclusion Criteria

Participants in the study were recruited based on the following:

- Children assented and whose parents gave consent to their children participating in the study.
- Absence of hepatic disease after clinical examination.
- Absence of sickle cell anaemia or any congenital abnormalities or deformities.
- Children with no history of any form of allergic diseases or reactions.

3.4 Exclusion Criteria

Participants with the following conditions were excluded from the study:

- Children that their parents gave no consent or withdrew from the study during the period of the study.
- Children with clinical features of gastrointestinal disease like gastroenteritis, etc
- Children with clinical features of infectious disease such as HIV, malaria, hepatitis, or any form of communicable diseases, etc.

3.5 Sample Size Calculation

The sample size was calculated and determined using the formula :

$$Sample\ size\ (S) = \frac{2(Z\alpha + Z\beta)^2 X m(1-m)}{(P_1 - P_0)^2}$$

Where:

$m = (P_1 – P_0) / 2$

S = minimum sample size of each group (study group and controls)

Zα = standard normal deviation corresponding to 2 sided α level of 5% = 1.96

Zβ = standard normal deviation, corresponding to a β error of 10% (power of 90%) = 1.28

P_0 = Intestinal helminth infection prevalence in Ibadan = 28.1% (0.281)

(Ogunkambi and Sowemimo, 2014)

P_1 = Expected intestinal helminth infection prevalence in general population to difference = 50% (0.5) (arbitrary estimate and assumption)

m= (0.5 – 0.208) /2 = 0.146

$$S = \frac{2(1.96 + 1.28)^2 X 0.146 (1- 0.146)}{(0.5 – 0.281)^2} = 30.7$$

A minimum of 40 participants were recruited into each group of preschool-aged school aged children with *Ascaris lumbricoides* and children who are helminth - free.

3.6 Anthropometric Measurements and Determination of Anthropometric Indices

The height, weight and mid upper arm circumference of all the children were measured using standard methods as described by Rosalind(1993). The body weight was taken using a digital scale which had earlier been calibrated and validated. Each child was weighed without shoes or heavy clothing worn. Height was also taken using a heightometer. The length of children who were not yet walking was measured while lying down using a tape. The mid-upper arm

circumference (MUAC) was measured using a standard flexible measuring tape. The measurement was taken at the upper arm midpoint on the left arm of each child. The body weight and height were measured and reported to the nearest kilogramme (kg) and centimeter (cm) respectively, while MUAC was taken to the nearest 0.1cm.

The children's anthropometric indices were calculated using the WHO AnthroPlus software for assessing growth of the world's children and adolescents (WHO, 2007). Nutritional survey module was employed to compute the age, sex, body weight and height of each child. This derived the mean (± SD) z-scores for weight-for-age z-score (WAZ), length or height-for-age z-score (L/HAZ) and BMI-for-age z-score (BAZ) (indicated as weight for age, length/height for age and BMI for age respectively). The z-scores were also used in determining the percentage of children that are underweight (WAZ <2SD), stunted (L/HAZ<2SD), wasted (BAZ <2SD) and with possible risk of overweight (BAZ >1SD).However, the weight-for-age, height for age and BMI for age reference data are only available for children with ages less than 10 years old, as the indicator on AnthroPlus does not differentiate between height and body mass,during the age period when children undergo pubertal growth spurt which may appear as having excessive weight (by weight-for-age) (WHO, 2007).

3.7 Stool Specimen Collection and Processing

Fecal specimen was scooped using spatula, put into a labeled screw cap polystyrene bottle and tightly screwed. The stool specimens were examined microscopically within 12 hours of collection using the Concentration Technique (CDC, 2016). The magnifications of X10 and X40 were used, respectively, to identify characteristic ova of the intestinal helminth.

3.7.1 Examination of Stool Specimen using Concentration Technique

Principle:

Sedimentation Concentration Technique involves the use of Formol Ethyl Acetate solution, which is of lower specific gravity compared with the parasite eggs, thereby concentrating the eggs in the sediment.

Procedure:

This was performed as previously described (Arinola *et al,* 2014). Ten mililitres of Formol Ether Acetate Solution was added to a heap stool sample in a centrifuge tube. It was covered with plastic seal and then mixed vigorously. The mixture was centrifuged lightly at 1000g for two minutes. The supernatant was discarded and the sediment was re-suspended, then observed under X10 and X40 objective of compound microscope for characteristic helminths.

3.8 Blood Sample Collection and Processing

Five millilitres (ml) of venous blood was obtained from each child and dispensed into plainpolysterene bottle. The blood samples were put in upright position unill they are clotted, they were then retracted and spun at 4000rpm for 10 minutes. The sera were removed into plain sterile cryo- precipitate tube and frozen at -20°C until analysis.

3.9 Oral Vaccination and Follow -up

Nineteen (19) helminth – infected and nineteen (19) helminth – free (controls) pre-school aged children who assented and whose parents gave consents and willingness to participate throughout the study were selected. They were orally administered with rotavirus vaccine after pre-vaccination blood sample had been collected. Blood sample collection was repeated at three weeks post vaccination. Also, twenty three (23) helminth – infected and twenty three (23) helminth – free school aged children were selected and administered with oral poliovirus

vaccine after pre-vaccination blood sample collection. Blood sample collection was repeated at three weeks post vaccination.

3.9.1 Procedure for Oral Rotavirus Vaccination

One dose (1.5ml) of Rotarix™ (GlaxoSmithkilne) contains live attenuated human rotavirus RIX4414 strain in a plunger (oral applicator). The vaccine was kept at 4°C inside a thermos flask but allowed to reach normal temperature before administration. The protective tip cap was removed from the oral applicator. Each child was seated in a reclining position and the entire content of the plunger (the vaccine) was emptied into the child's mouth towards the inner cheek. Each child was allowed to resume normal sitting position observed for about 10 minutes after the administration of the vaccine, to ensure it was not vomited.

3.9.2 Procedure for Oral Poliovirus Vaccination

The oral poliovirus vaccine (Sabin, GlaxoSmithkline) was supplied in glass vials with dropper and stored at 4°C inside a thermos flask. It was allowed to reach normal temperature before administration. Each child was held firmly lying on his/her back. The child's mouth was opened by squeezing the cheeks gently between fingers to make the child's lips point outward. The dropper was held over the child's mouth at an angle of about 45 degrees and two drops of the vaccine was dropped onto the rear part of the child's tongue. Each child was allowed to resume normal sitting position observed for about 10 minutes after the administration of the vaccine, to ensure it was not vomited.

3.10 Anthelminthic Drug Treatment among the School aged Children

Twenty three helminth – infected school aged children were administered with single dose of 400mg Albendazole (Glaxosmithkline) immediately after pre-anthelminthic drug treatment

blood sample was collected. Sample collection was repeated at one and two months after anthelminthic drug treatment.

3.10.1 Procedure for Anthelminthic Drug Treatment

400mg single dose of Albendazole tablet (Gloxosmithkline) was orally administered by each child, with the aid of water provided. Each child was observed for about 10 minutes after the administration of the vaccine, to ensure it was not vomited.

3.11 Laboratory Estimation of Cytokines (IFN–γ, TNF–α, IL-4, IL-8 IL-6, and IL-10), Ferritin, Transferrin, Haptoglobin, Rotavirus-Specific IgA and Poliovirus-Specific IgA using Enzyme Liked Immunosorbent Assay

The serum levels of IFN–γ, TNF–α, IL-4, IL-8, IL-6, IL-10, ferritin, transferrin, haptoglobin, rotavirus-specific IgA and poliovirus-specific IgA were determined using Enzyme Linked Immunosorbent Assay (ELISA) as described by the manufacturers (Abcam, MA, USA, AssayPro, MO, USA and Calbiotech, USA, Sunlong Biotech, Hangzou, China).

Principle:

The enzyme linked immunorsorbent assay (ELISA) is based on antigen-antibody interaction. Protein antigens present in patient's sample are allowed to bind in wells pre-coated with antibodies. The plate is washed after a period of incubation, to remove remaining sample component to reduce interference. To this plate, a corresponding second enzyme linked antibody is added which catalyzes the conversion of a suitable substrate to produce a colour. The colour produced is measured as a function of antigens present in the sample.

Assay Procedure:

The ELISA was performed as previously described (Arinola *etal.,* 2014). All the reagents, sample and standards were allowed to attain working room temperature prior to commencement of the assay. Stock standard solution was diluted to varying concentration used for the standard curve. 50µl of standards and samples were added to each immunoplate well, covered and allowed to stay at room temperature for 2 hours. The immunoplate was washed 5 times using a plate washer (TECAN, Mannedorf, Switzerland). 50µl of Biotinlyated Human antibody to the cytokines and other analytes as applicable was added to each well. It was then allowed to stay at room temperature for 2 hours, after which the wash was repeated 5 times. 50µl of Streptavidin –Preoxidase Conjugate was added to each well, incubated for 30 minutes at room temperature and wash was repeated 5 times. 50µl of Chromogen Substrate was added to each well, incubated for 15 minutes inside a dark cupboard to allow the blue colour to develop. 50µl of Stop Solution was then added to bring the reaction to a stop, changing the colour from blue to yellow. The absorbance of each well was read at 450nm with the aid of a microplate reader (SpectraMax 384 Plus (Molecular Devices, USA)). Using a four-parameter logistic curve-fit, the unknown sample concentration was extrapolated from the standard curve.

3.12 Estimation of Trace Metals

The serum concentrations of Zinc (Zn), Selenium (Se) and Iron (Fe) were determined using Atomic absorption spectrophotometry (AAS).

Principle:

Atomic Absorption Spectrophotometry (AAS) involves generation of a gaseous population of free atoms through heating a sample in flame and then passing a narrow band-width light at

a defined wavelength through the atoms in the flame. This results in the absorption of radiation that is selective for the particular element under analysis.

Assay Procedure:

Samples were thawed and allowed to attain room temperature and 1:20 dilution was made for each sample. Working standard solutions were prepared by diluting the stock standard with deonised water and the required standardization for each corresponding trace elements was established. Each sample was aspirated into AAS for analysis. Atomic Absorption Absorbance measurement of serum trace element concentration was performed on the Spectrometer as described below:

Flame was ignited and lamp switched on and equipment was allowed to warm up while aspirating deionised water into the flame and adjusted to zero absorbance.The cathode lamp of each particular element was inserted and turned up. This generated the specific energy level at particular wavelength characteristic of the element of interest and their concentrations determined. Standard (corresponding to analyte of interest) was aspirated into the equipment for standardization. This process was repeated periodically to check for any drift in sensitivity. Each diluted sample was aspirated into the equipment and results were displayed digitally and recorded. The Operating conditions for the Atomic Absorption Spectrometric analysis of each element is given in Table 3.1.

Table 3.1: Operating Conditions for the Atomic Absorption Spectrometric Analysis of Each Element

Operational Conditions	Zn	Fe	Se
Wavelength	213.8	248.3	196
Slit	0.7	0.7	0.7
Burner Height	Low	Low	Low
Gas Mixture	Acetylene	Acetylene	Acetylene
Air Pressure (psi)	30	30	30
Acetylene Pressure (psi)	10	10	10
Atomising Air Flow	83	83	83
Lamp Current (mA)	30	30	30
Scale Expansion	3	3	3
Noise Suppression	2	2	2
Dilution per aspiration	1:20	1:20	1:20
Standard	4	4	1

3.13 Estimation of Serum Concentrations of Vitamins A and C Using High Performance Liquid Chromatography (HPLC)

Serum vitamin A and vitamin C concentrations were determined using High Performance Liquid Chromatography (HPLC) as described by Bates (1997).

Principle:

HPLC is a chromatographic technique used in separating, identifying and quantifying components in a mixture. It involves passing a liquid sample over a solid adsorbent material that is packed into a column using a flow of liquid solvent. Analytes in samples interact in a slightly different manner with the adsorbent material, and thereby slowing down the flow of the analytes differently. If the interaction is weak, the analytes passes through and flow off the column within a short period oftime, but if the interaction is strong, the elution time will be longer. For the determination of vitamins A and C, the isocratic separation via HPLC at 30°C uses a "reversed phase" column. Each run for vitamins A and C last for 15 and 12 minutes respectively. A UV-detector records the chromatograms at two different wavelengths (Vitamin A at 325 nm, Vitamin C 300 nm). The quantification is carried out using plasma calibrator for each analyte and concentration is calculated using "internal standard method" via integration of the peak heights and respective peak areas.

Assay Procedure:

250µl of standard, controls and sample were added to 50µl internal standard, and 250µl of precipitating reagent in a 1.5ml precipitation tube. The mixture was briefly mixed using a vortex mixer and left for 30 minutes between 2-8°C, then centrifuged at 10,000g for two minutes (for vitamin A) or 10 minutes (for vitamin C).100µl of the supernatant was picked and injected into the HPLC-system and the chromatograms are detected through the UV detector. The chromatographic features of each of the analytes are given in Table 3.2.

Table 3.2: The chromatographic features for vitamin A and vitamin C in HPLC analysis

Features	Vitamin A	Vitamin C
Column material	Nucleosil® C_{18}, 10 µm	Bischoff Prontosil AQ, 5 µm
Column dimension	12 mm x 4mm	125mm x 4mm
Flow rate	0.8ml/min	0.75ml/min
UV-detection	325nm	25 nm
Injection volume	100µl	20µl
Running time	15min	1 min
Temperature	30°C	30°C

Calculation

For vitamin A:

$$\text{Conc of sample} = \frac{\text{peak area (sample) x conc. Calibrator}}{\text{Peak area (calibrator)}} \times F$$

$$F = \frac{\text{peak area Internal standard of calibrator}}{\text{Peak area of calibrator}}$$

For vitamin C:

$$\text{Conc of sample} = \frac{\text{peak area (sample) x conc. Calibrator}}{\text{Peak area (calibrator)}}$$

3.14 Statistical Analysis

Statistical data evaluation was carried out using the Statistical Package for Social Sciences (SPSS) version 21.0. Serum levels of micronutrients were summarized and expressed as mean±standard deviation and the differences in mean values were compared using the paired Student's t-test. Analysis of Variance (ANOVA) was employed to compare the serum levels of micronutrients before and different times after anti-helminth treatment. Serum levels of cytokines were expressed as median (interquartile range). Mann-Whitney U test was used to compare differences in levels of serum cytokines between helminth positive and helminth negative subjects. Wilcoxon Signed Ranks test was employed to compare between pre-vaccinated cytokines and post-vaccinated cytokines levels. Kruskal Wallis test was employed to compare the levels of serum cytokines before and different times after anti-helminth treatment. Level of statistical significance was set at $\alpha_{0.05}$.

CHAPTER FOUR

4.0 RESULTS

Figure 4.1a shows the prevalence of helminth infection among all children recruited for the study. Of the three hundred and forty nine (349) children recruited, a total of one hundred and two [102 (29.20 %)] children were infected with different species of helminths. Figure 4.1b shows percentage distribution of helminth species in the infected children. *Ascaris lumbricoides* has the highest prevalence (81.37%) followed by hookworms (7.84%) and multiple infections of *Ascaris lumbricoides* and hookworms (5.88%). The overall prevalence of *Ascaris lumbricoides* infection in the children was 23.7%.

Figure 4.2a shows the prevalence of helminth infection in pre-school aged children. Of the one hundred and forty nine (149) pre-school aged children recruited, forty two [42 (28.2%)] were infected with helminths. The percentage distribution of helminths species in the infected children (Figure 4.2b) shows that *Ascaris lumbricoides* has the highest prevalence (88%) followed by hookworms (7%) and *Fasciola hepatica* (3%).

Figure 4.3a shows the prevalence of helminth infection in school aged children. Of the two hundred (200) school aged children recruited, sixty [60 (30%)] were infected with helminths. Figure 4.3b shows percentage distribution of helminthspecies in the infected children as follows: *Ascaris lumbricoides* has the highest prevalence (77%) followed by multiple infections of *Ascaris lumbricoides* and hookworms (10%) and hookworms (8%).

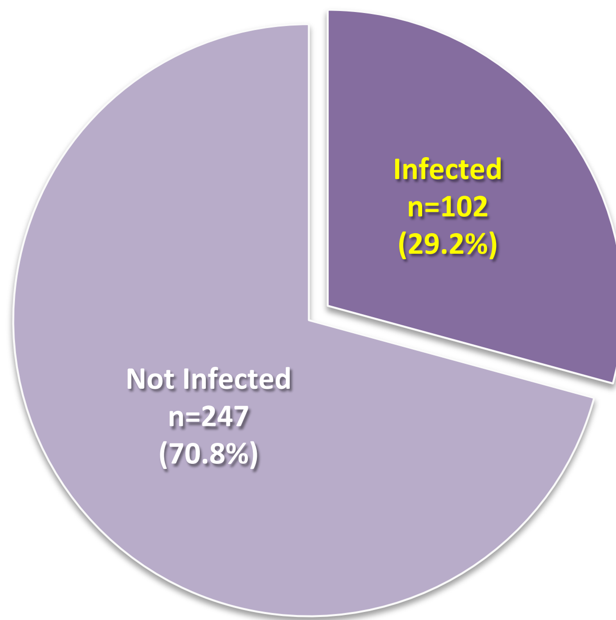


Figure 4.1a: Prevalence of intestinal helminth infection in all children recruited for the study (N=349)

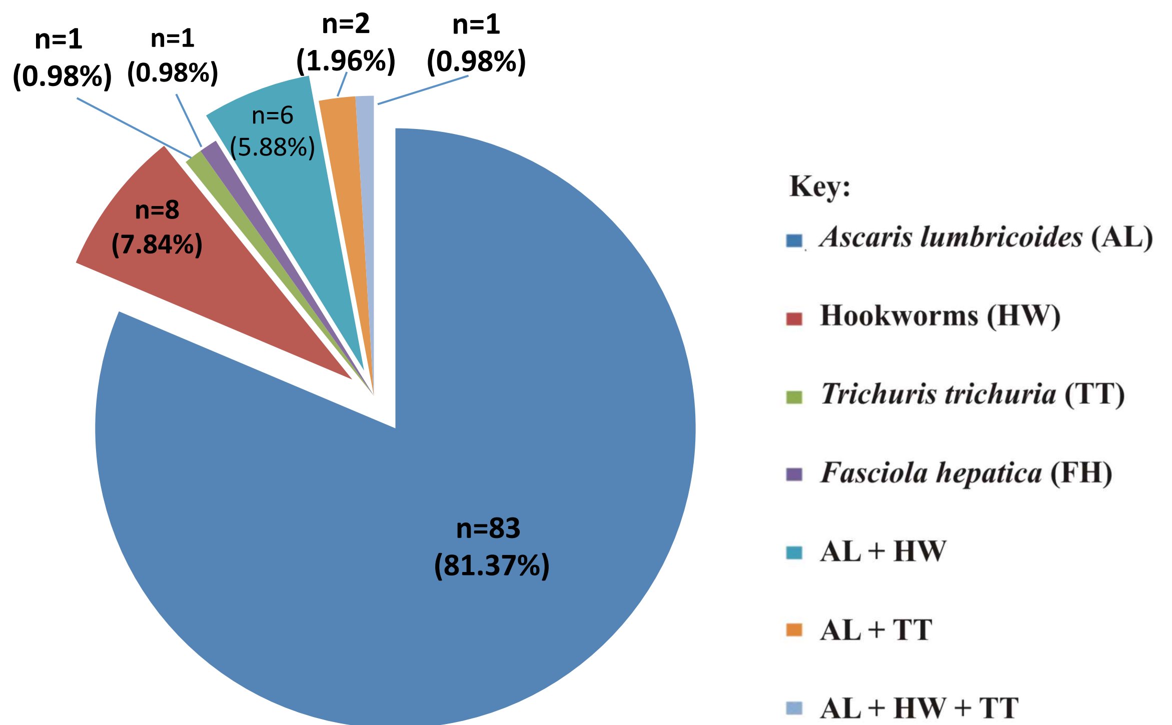


Figure 4.1b: Distribution of intestinal helminth species among infected children

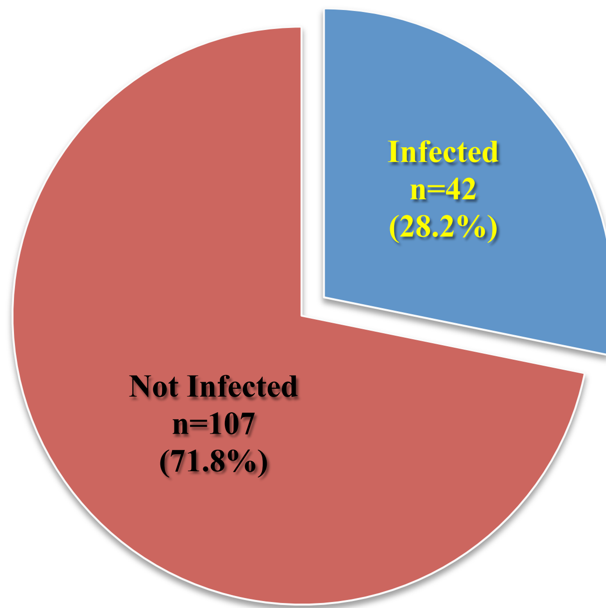


Figure 4.2a: Prevalence of intestinal helminth infection in pre-school aged children (n=149)

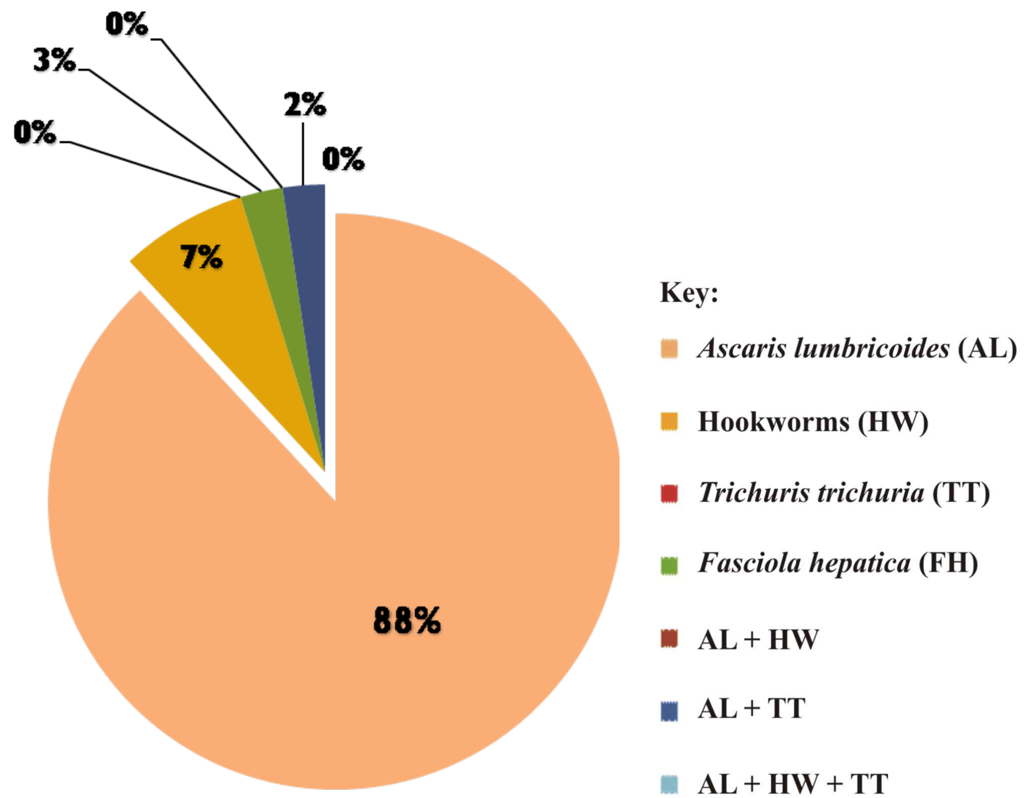


Figure 4.2b: Distribution of intestinal helminth species in pre-school aged children

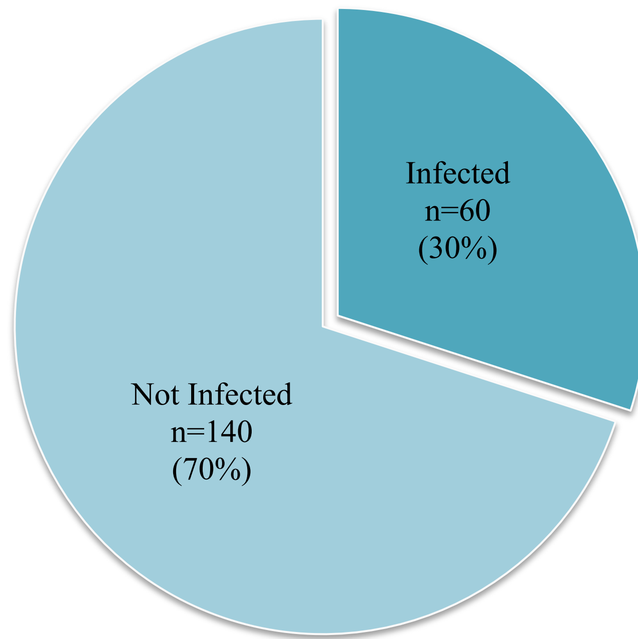


Figure 4.3a: Prevalence of intestinal helminth infection in school aged children (n=200)

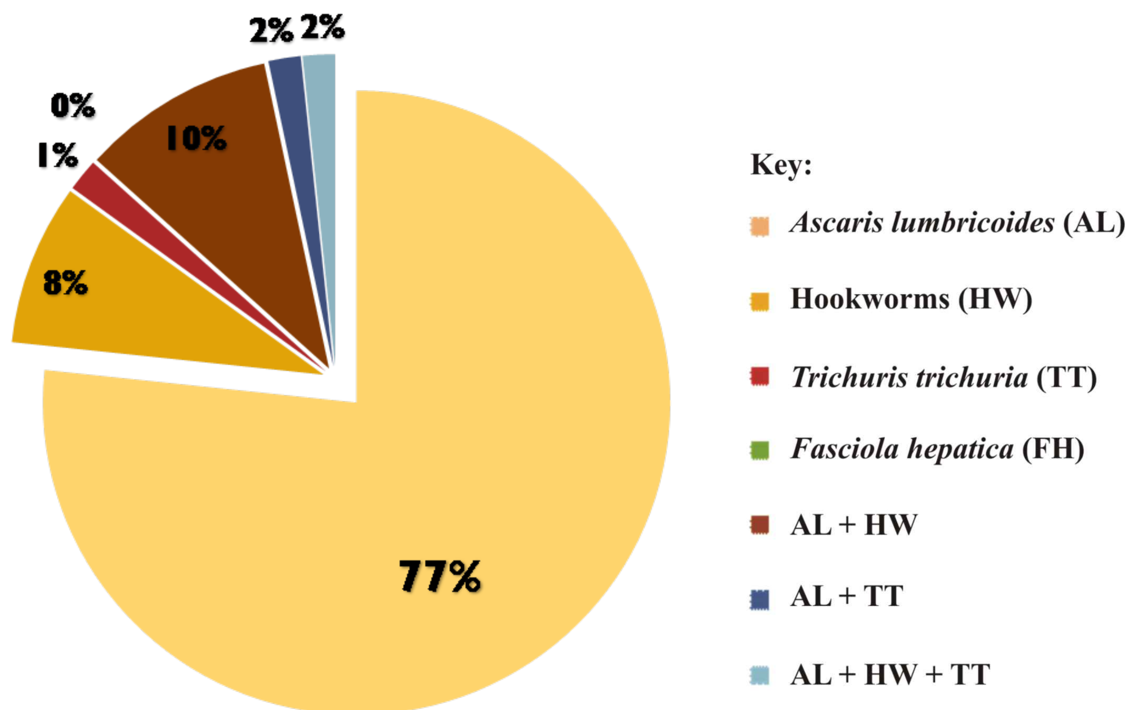


Figure 4.3b: Distribution of intestinal helminth species among infected school aged children

Table 4.1 shows the age and sex distribution of intestinal helminth infected children compared with uninfected children. There were no significant differences in the age groups and gender distribution when helminth infected groups were compared with controls. Neonates (1-12 months) were least infected with helminth among pre-school aged children while adolescents (16-19 years) were least infected among the school aged children.

Table 4.2 shows the relationship between the socio-demographic statuses of parents of intestinal helminth infected children compared with parents of uninfected children. There were significant associations between the helminth status of the children with parents' duration in the community (X^2 =10.987, p=0.019) and toilet types (X^2=6.883, p=0.045). Children whose parents' used bush toilet and had stayed more than 4 years in the community had high prevalence of helminth infection. There were no significant associations between the levels of parents' education, occupation, animal ownership, water sources for domestic use, cooking method, solid waste disposal and the helminth status of the children.

Table 4.1: Age and gender distribution of intestinal helminth infected children compared with uninfected children

Age group		Helminth Positive (%) (n = 102)	Helminth Negative (%) (n = 247)	Chi-Square	Fischer's Exact Test	p-value
Pre-school aged children	1 – 12months	2 (2.0)	19 (7.7)	NA	8.763	0.170
	13- 24months	14 (13.7)	29 (11.7)			
	25-36months	14 (13.7)	25 (10.1)			
	37-48months	12 (11.8)	34 (13.8)			
School Aged Children	5 – 9 years	33 (32.4)	67 (27.1)	NA	6.215	0.214
	10 – 15 years	26 (25.5)	73 (29.6)			
	16 – 19 years	1 (1.0)	0 (0)			
Gender Distribution						
Pre-school aged children	Male	22 (14.8)	55 (36.9)	0.012	NA	0.914
	Female	20 (13.4)	52(34.9)			
School Aged Children	Male	33(16.5)	66 (33.0)	1.037	NA	0.309
	Female	27 (13.5)	74(37.0)			

n = Number in each group
NA = Not Applicable

Table 4.2: Socio-demographic status of parents of intestinal helminth infected children compared with those of uninfected children

		Helminth Positive (%) (n = 102)	Helminth Negative (%) (n = 247)	Chi-Square	Fischer's Exact Test	p-value
Level of Parents' Education	No Formal Education	38 (37.2)	68 (27.5)	NA	2.302	0.519
	Primary	42 (41.2)	124 (50.2)			
	Secondary	21 (20.6)	51 (20.6)			
	Tertiary	1 (1.0)	4 (1.6)			
Level of Parents' Occupation	Farming	54 (52.9)	126 (51.0)	NA	1.259	0.550
	Business	44 (43.1)	103 (41.7)			
	Professional	4 (3.9)	18 (7.3)			
Animal ownership by parents	None	44 (43.1)	111 (44.9)	NA	3.299	0.879
	Cat	8 (7.8)	15 (6.1)			
	Cat and Dog	4 (3.9)	8 (3.2)			
	Dog	19 (18.6)	44 (17.8)			
	Goat	14 (13.7)	35 (14.2)			
	Fowl	8 (7.8)	17 (6.9)			
	Goat and Fowl	5 (4.9)	17 (6.9)			
Water sources for domestic use	Well	63 (61.8)	144 (58.3)	1.152	NA	0.762
	Well / Rain	12 (11.8)	40 (16.2)			
	Borehole	21 (20.6)	50 (20.2)			
	Stream	6 (5.9)	13 (5.3)			
Cooking Method	Firewood	82 (80.4)	178 (72.1)	NA	4.132	0.229
	Firewood and Kerosine	10 (9.8)	24 (9.7)			
	Kerosine Stove	10 (9.8)	44 (17.8)			
	Gas Stove	0 (0)	1 (0.4)			
Toilet Types	Bush	91 (89.2)	197 (79.8)	NA	6.883	0.045*
	Pit	11 (10.8)	45(18.2)			
	Water Closet	0 (0)	5 (2.0)			
Duration in the Community	< 1 year	3 (2.9)	21 (8.5)	NA	10.987	0.019*
	2 years	3 (2.9)	9 (3.6)			
	3 years	9 (8.8)	42 (17.0)			
	>4 years	87 (85.3)	175 (70.9)			
Solid Waste Disposal	Refuse Heaps	82 (80.4)	192 (77.7)	NA	4.807	0.154
	Burning	4 (3.9)	26 (10.5)			
	Bush	16 (15.7)	29 (11.7)			

*Statistically significant
NA – Not Applicable

Table 4.3 compares the anthropometric indices of intestinal helminth infected pre-school aged children and uninfected pre-school aged children. There were no statistically significant differences in the mean age, weight, height, body mass index and mid upper arm circumference between the pre-school aged children with helminth infection compared with children without helminth infection. Also, there were no significant differences in the weight-for-age (0.44 ± 1.02 vs 0.31 ± 1.49, p=0.604), length or height-for-age (2.26 ± 1.39 vs 1.77 ± 2.04, p=0.155) and BMI-for-age z-scores (1.50 ± 1.36 vs 1.01 ± 1.52, p=0.071) of pre-school aged children with helminth infection compared with children without helminth infection. Highest percentages of stunting (57.5%) and overweight (66.7%) were observed amongst the helminth infected pre-school aged children

Table 4.4 compares the anthropometric indices of intestinal helminth infected school aged children and uninfected school aged children. There were no statistically significant differences in the mean age, weight, height, body mass index and mid upper arm circumference between the school aged children with helminth infection compared with children without helminth infection. Also, there were no significant differences in the weight-for-age (0.54 ± 0.98 vs 0.64 ± 1.19 p=0.677), length or height-for-age (0.87 ± 1.44 vs 1.08 ± 1.56 p=0.373) and BMI-for-age z-scores (0.62 ± 1.00 vs 0.64 ± 0.98, p=0.898) of school aged children with helminth infection compared with children without helminth infection.

Table 4.3: Anthropometric indices of intestinal helminth infected pre-school aged children compared with uninfected pre-school aged children.

	Helminth Positive (n = 42)	Helminth Negative (n = 107)	t-test	p-value
Age (months)	31.45 ±12.84	29.29 ±14.74	0.719	0.902
Body Weight (kg)	13.05 ± 2.88	12.62 ± 4.15	0.617	0.538
Height (cm)	83.52 ±10.57	82.49 ±13.74	0.441	0.660
BMI (kg/m^2)	18.88 ± 4.07	18.46 ± 4.00	0.585	0.559
MUAC (cm)	15.00 ± 1.43	14.96 ± 1.60	0.142	0.887
Weight for Age	0.44 ± 1.02	0.31 ± 1.49	0.519	0.604
Length/ Height for Age	2.26 ± 1.39	1.77 ± 2.04	1.430	0.155
BMI for Age	1.50 ± 1.36	1.01 ± 1.52	1.821	0.071
			X^2	**p-value**
% Underweight	2.6	11.8	3.381	0.067
% Stunted	57.5	42.7	2.245	0.119
% Overweight	66.7	51.5	3.307	0.653
% Wasted	0	2		

n = number of children in each group
BMI = Body Mass Index
MUAC = Mid Upper Arm Circumference
X^2 = Chi square
% = Percentage

Table 4.4: Anthropometric indices of intestinal helminth infected school aged children compared with uninfected school aged children.

	Helminth Positive (n = 60)	Helminth Negative (n = 140)	t-test,	p-value
Age (months)	107.15 ±34.6	110.23 ±32.3	0.605	0.546
Body Weight (kg)	25.12 ± 6.89	25.28 ± 6.75	0.154	0.887
Height (cm)	126.53 ±14.8	126.90 ±14.4	0.164	0.870
BMI (kg/m^2)	15.42 ± 1.29	15.45 ± 1.77	0.158	0.875
MUAC (cm)	17.55 ± 1.86	17.68 ± 2.01	0.424	0.672
	(n = 33)	(n = 67)		
Weight for Age	0.54 ± 0.98	0.64 ± 1.19	0.418	0.677
Length/ Height for Age	0.87 ± 1.44	1.08 ± 1.56	0.892	0.373
BMI for Age	0.62 ± 1.00	0.64 ± 0.98	0.132	0.894
			X^2	
% Underweight	6.1	10.4	0.520	0.471
% Stunted	30	29.3	0.002	0.963
% Overweight	0	4.3	1.625	0.898
%Wasted	5	7.2		

n = number of children in each group
BMI = Body Mass Index
MUAC = Mid Upper Arm Circumference
X^2 = Chi square
% = Percentage

Figure 4.4a shows the serum micronutrient levels of pre-school aged children with *Ascaris lumbricoides* infection compared with pre-school aged children without the infection. There were significantly lower serum levels of zinc (96.1 ± 20.0 vs 140.9 ± 22.5µg/dl, p=0.000), vitamin A (92. 3± 21.8 vs 122.6 ± 27.2µg/dl, p=0.004), iron (111.5 ± 43.3 vs 160.3 ± 34.6µg/dl, p=0.003), ferritin (80.0 ± 29.8 vs 112.7 ± 19.0ng/ml, p=0.002), transferrin (124.5 ± 37.3 vs 173.2 ± 41.8mg/dl, p=0.004) and haptoglobin (49.6 ± 35.3 vs 93.2 ± 32.8µg/dl, p=0.003) in *Ascaris lumbricoides*-infected pre-school aged children compared with uninfected pre-school aged children. However, there was significantly higher level of selenium (68.9 ± 30.9 vs 39.0 ± 29.6ng/ml, p=0.020) in *Ascaris lumbricoides*-infected pre-school aged children compared with uninfected pre-school aged children. There was no significant difference in the serum levels of vitamin C (2.41 ± 1.14 vs 3.04 ± 0.56mg/dl, p=0.071) in *Ascaris lumbricoides*-infected pre-school aged children compared with uninfected pre-school aged children.

Figure 4.4b shows the serum micronutrient levels of school aged children with *Ascaris lumbricoides* infection compared with school aged children without the infection. There were significantly lower serum levels of zinc (139.1 ± 16.9 vs 152.7 ± 16.2µg/dl, p=0.044) and vitamin A (119.3 ± 11.5 vs 153.6 ± 37.5µg/dl, p=0.002) in *Ascaris lumbricoides*-infected school aged children compared with uninfected school aged children. Also, there was significantly higher serum level of selenium (62.1 ± 39.3 vs 35.5 ± 11.0ng/ml, p=0.032) and transferrin (178.9 ± 27.5 vs 137.9 ± 28.8mg/dl, p=0.001) in *Ascaris lumbricoides*-infected school aged children compared with uninfected school aged children. The lower serum levels of vitamin C (2.66 ± 0.49 vs 3.05 ± 1.55mg/dl, p=0.371) and higher serum levels of iron (170.5 ± 30.9 vs 162.4 ± 24.2µg/dl, p=0.467), ferritin (113.6 ± 14.8 vs 106.0 ± 11.6ng/ml, p=0.157) and haptoglobin (150.7 ± 71.9 vs 125.3 ± 16.1µg/dl, p=0.244) in *Ascaris lumbricoides*-infected school aged children compared with uninfected school aged children were not statistically significant.

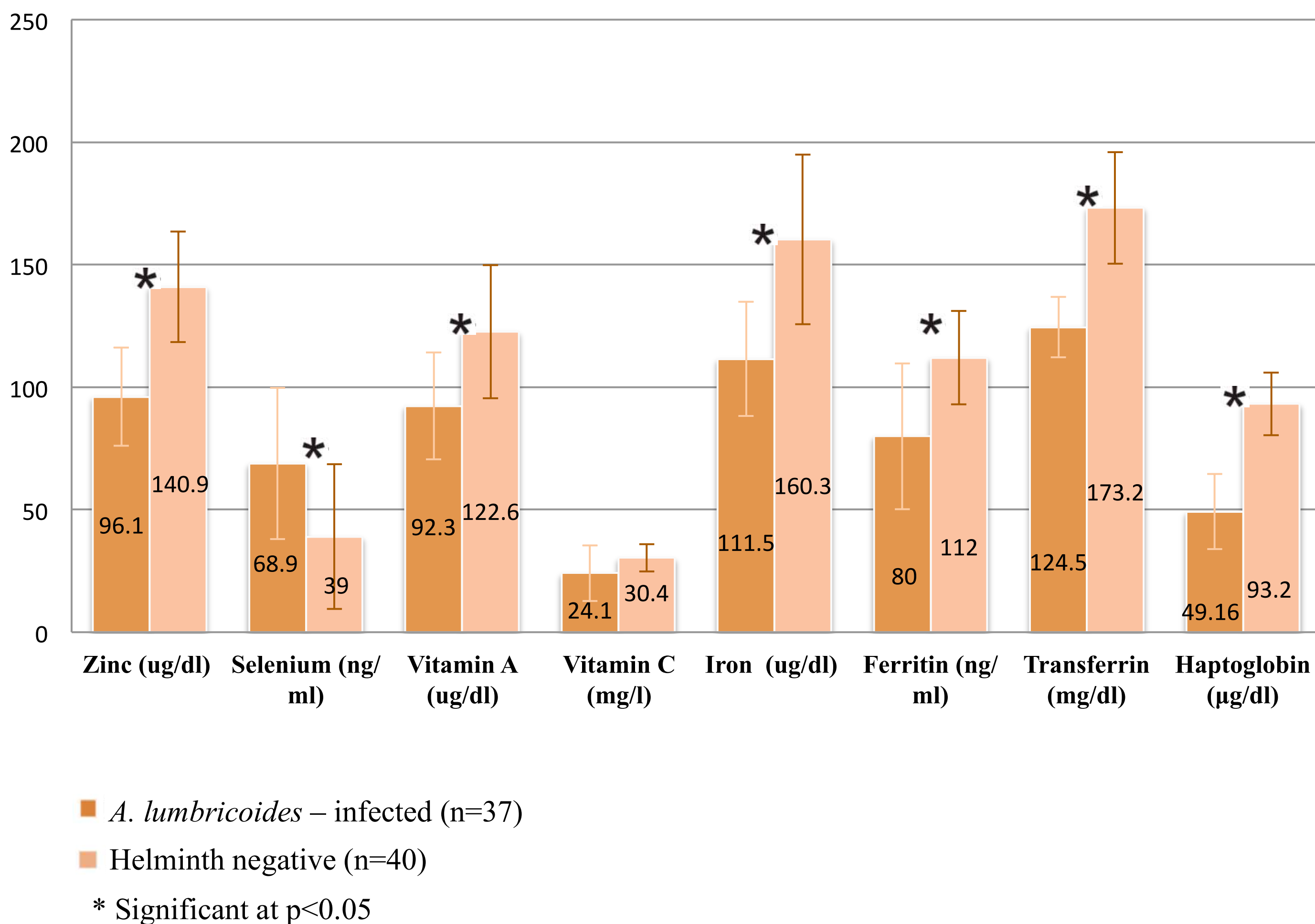
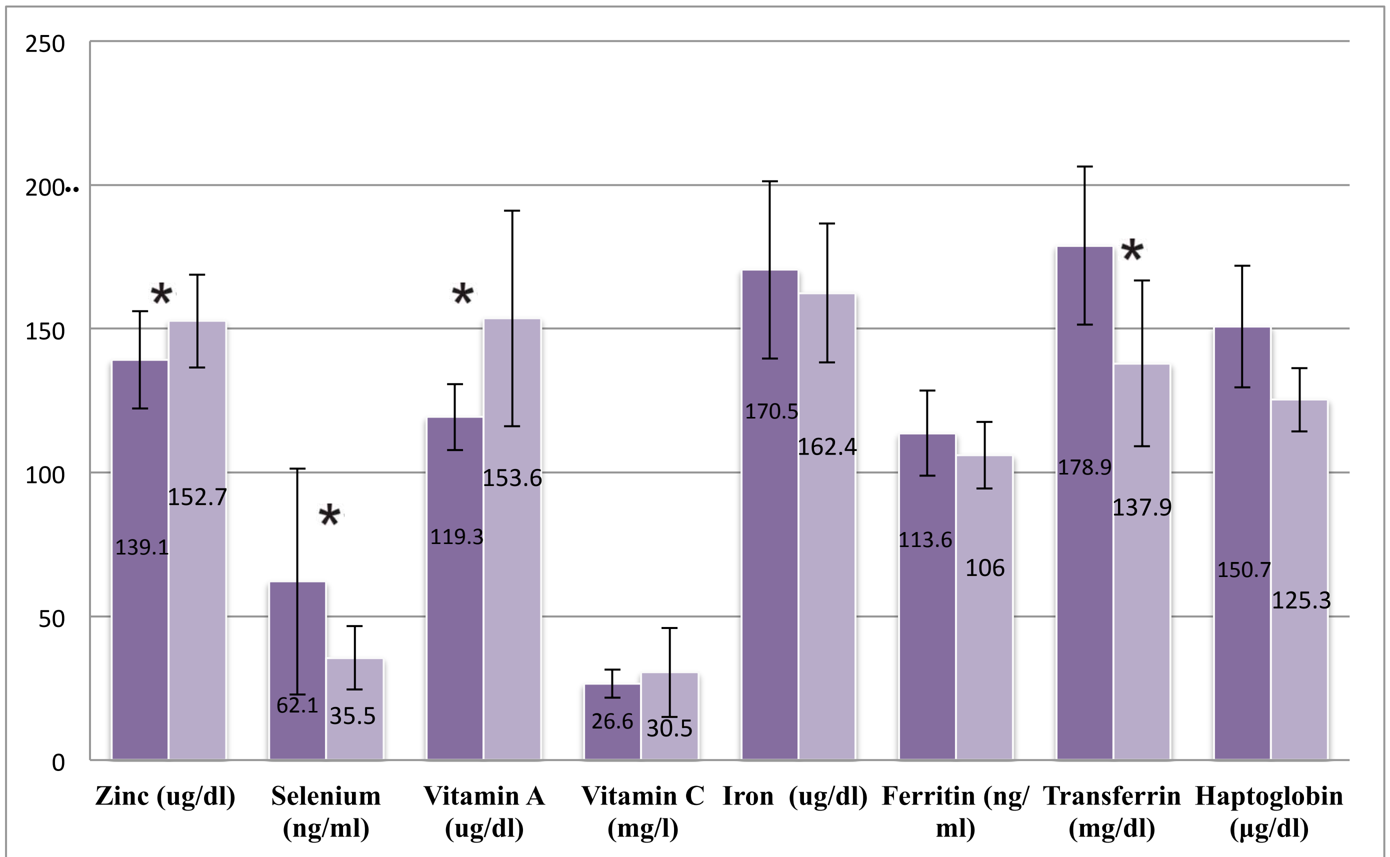


Figure 4.4a: Serum micronutrient levels of pre-school aged children with *Ascaris lumbricoides* infection compared with children without the infection.



■ *A. lumbricoides* – infected (n=46)

■ Helminth negative (n=40)

* Significant at p<0.05

Figure 4.4b: Serum micronutrient levels of school aged children with *Ascaris lumbricoides* infection compared with children without the infection.

Figure 4.5a shows the serum cytokine levels of pre-school aged children with *Ascaris lumbricoides (AL)* infection compared with pre-school aged children without the infection.Serum levels of interleukin-8 (1122.3 [IQR 262.2-1447.6] vs 501.3 [IQR 250.6-705.5] pg/ml, p=0.021) and interleukin-6 (14.60 [IQR 8.18-38.34] vs 9.57 [IQR 5.76-11.22] pg/ml, p=0.041) were significantly higher in pre-school aged childrenwith *Ascaris lumbricoides* infection compared with pre-school aged children without the infection. The higher serum levels of IFN –γ, (170.15 [IQR74.75-246.05] vs 95.92 [IQR 74.01-156.25] pg/ml, p=0.210) interleukin-4 (365.1 [IQR 156..8-732.3] vs 217.4 [IQR 128.7-269.8] pg/ml, p=0.065) and interleukin-10 (0.11 [IQR 0.07-0.21] vs 0.09 [IQR 0.07-0.13] ng/ml, p=0.372) as well as lower serum level of TNF–α(41.43 [IQR 35.56-57.70] vs 48.08 [IQR 39.26-57.39] pg/ml, p=0.391) in pre-school aged children with *Ascaris lumbricoides* infection compared with pre-school aged children without the infection were not statistically significant.

Figure 4.5b shows the serum cytokine levels of school aged children with *Ascaris lumbricoides* infection compared with school aged children without the infection.Serum levels of IFN –γ(108.21 [IQR 75.18-137.18] vs 64.14 [IQR 25.68-88.07] pg/ml, p=0.014), interleukin-4 (204.4 [IQR 139.2-299.3] vs 89.3 [IQR 65.9-134.1] pg/ml, p=0.001), interleukin-8 (1193.9 [IQR 659.6-1321.9] vs 765.3 [IQR 218.7-802.8] pg/ml, p=0.014) and interleukin-6 (16.29 [IQR 9.92-34.79] vs 4.92 [IQR 2.69-6.52] pg/ml, p=0.000) were significantly higher in school aged childrenwith *Ascaris lumbricoides* infection compared with school aged children without the infection. The higher serum level of TNF–α(51.30 [IQR 40.89-61.45] vs 43.36 [IQR 36.72-52.59] pg/ml, p=0.145) and lower level of IL-10 (0.12 [IQR 0.07-0.52] vs 0.14 [IQR 0.13-0.36] ng/ml, p=0.724) in school aged children with *Ascaris lumbricoides* infection compared with school aged children without the infection were not statistically significant.

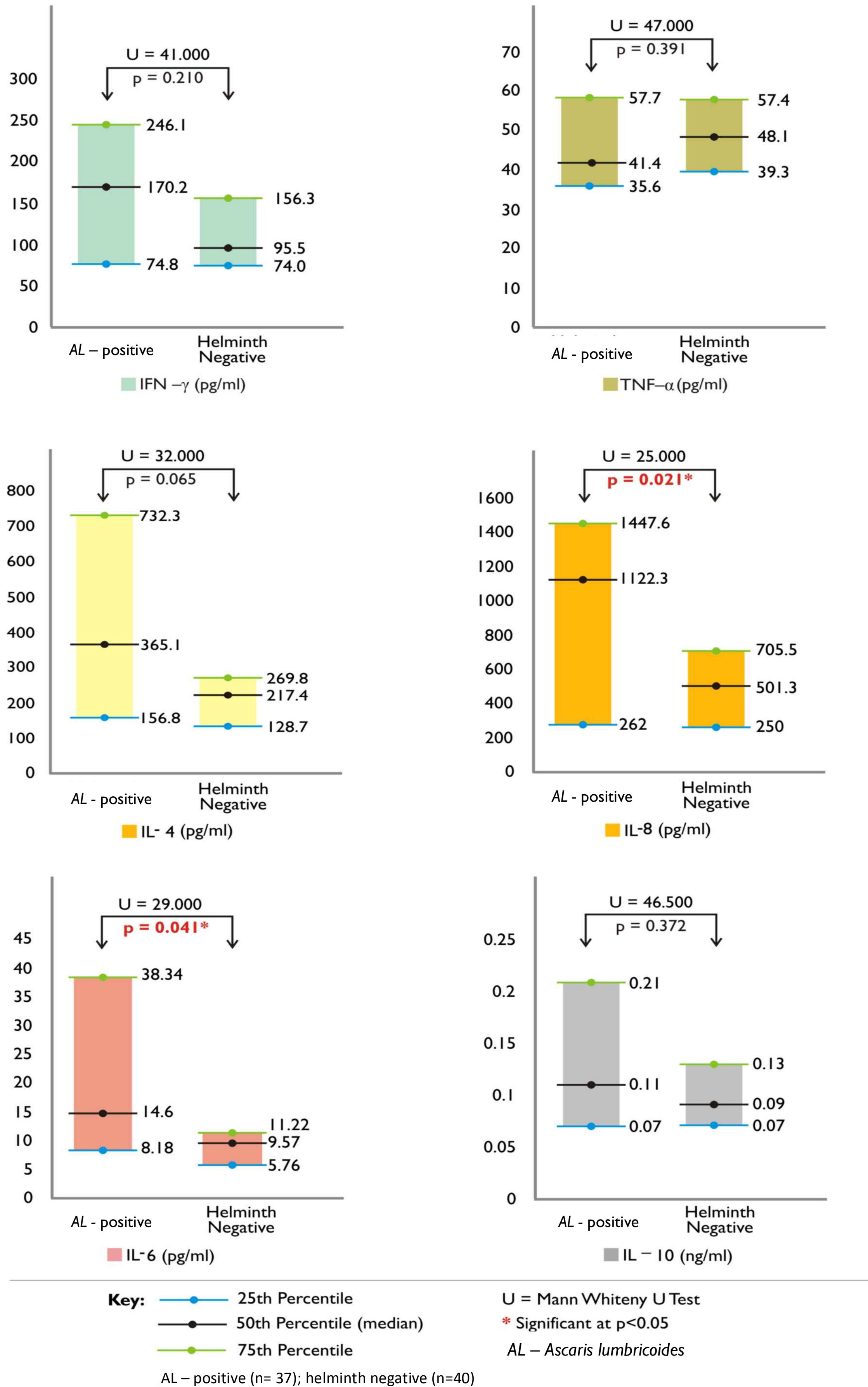


Figure 4.5a: Serum cytokine levels of pre-school aged children with *Ascaris lumbricoides* infection compared with children without the infection

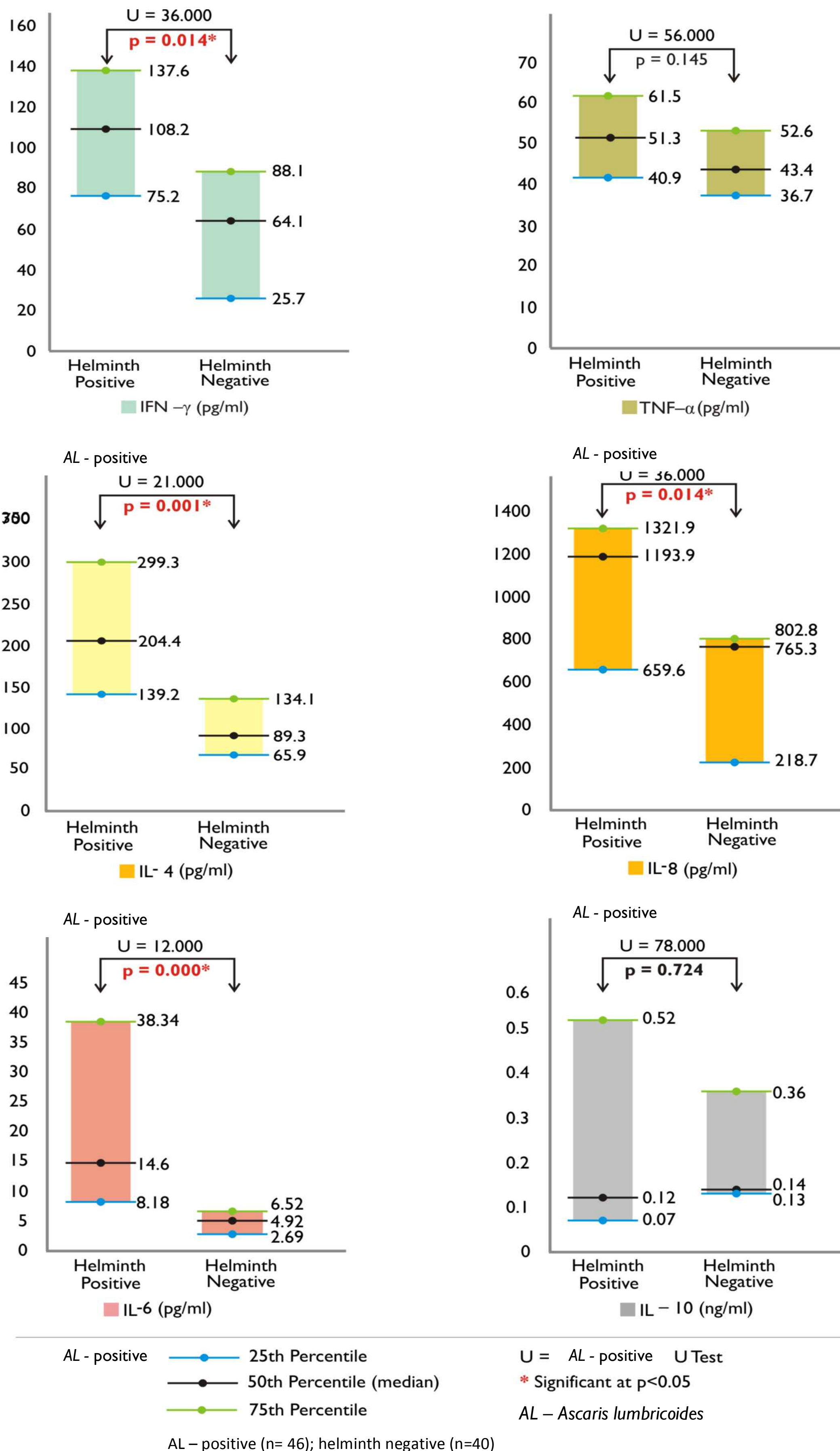


Figure 4.5b: Serum cytokine levels of school aged children with *Ascaris lumbricoides* infection compared with children without the infection

Table 4.5 compares the serum micronutrient levels of school aged children with*Ascaris lumbricoides* infection beforeanthelminthicdrug treatment, one and two months after anthelminthic drug treatment. Serum vitamin A levels were significantly higher at 1 month (203.60 ± 25.90vs 118.53 ± 9.74µg/dl, p=0.0001) and 2 months post-treatment (206.21 ± 24.11 vs 118.53 ± 9.74µg/dl, p=0.0001) compared with the pre-treatment level. Serum levels of zinc (137.93±22.90 vs 145.04±34.89 vs 136.73 ± 17.78, p=0.705), selenium (0.64 ± 0.11 vs 0.71 ± 0.17 vs 0.63 ± 0.41, p=0.723) and transferrin (188.01±31.22 vs 197.73±47.57 vs 185.85 ± 24.25, p=0.678) were insignificantly higher post-treatment compared with their pre-treatmentlevels.

Table 4.6 compares the serum cytokine levels of school aged children with *Ascaris lumbricoides* infection before anthelminthic drug treatment, one and two months after anthelminthic treatment. Median serum levels of IL-8 were significantly lower at one month post-anthelminthic treatment (270.1 [IQR 167.9-566.2] vs 542.3 [IQR 320.7-935.3]pg/ml, p=0.030) but insignificantly lower at two months post-anthelminthic treatment (13.1 [IQR 5.8-793.9] vs 542.3 [IQR 320.7-935.3]pg/ml, p=0.158) compared with the level before anthelminthic treatment. Median serum levels of IL-10 and IL-6 were insignificantly lower one month post- anthelminthic treatment (0.07 [IQR 0.06-0.11]ng/ml and 8.10 [IQR 6.84-11.47]pg/ml vs 0.09[IQR 0.07-0.13]ng/ml and 8.99 [IQR 7.01-12.11]pg/ml respectively, p=0.552, p=0.510) and insignificantly higher at two months post-anthelminthic treatment (0.10 [IQR 0.07-0.18]ng/ml and 10.45 [IQR 5.68-11.66]pg/ml vs 0.09[IQR 0.07-0.13]ng/ml and 8.99 [IQR 7.01-12.11]pg/ml p=0.177 and 0.875 respectively,) compared with the levels before anthelminthic treatment.

Median serum levels of IFN–γ and IL-4 were insignificantly higher at one month post-anthelminthic treatment (92.36 [IQR 57.18-97.04]ng/ml and 139.6 [IQR 79.6-171.9]pg/ml vs 81.59[IQR 15.64-141.39]ng/ml and 105.5 [IQR 71.9-142.9]pg/ml, p=0.875, and 0.245

respectively) and insignificantly lower at two months post-anthelminthic treatment (76.29[IQR 51.07-125.05]ng/ml ng/ml and 80.0 [IQR 32.8-159.6]pg/ml vs 81.59[IQR 15.64-141.39]ng/ml and 105.5 [IQR 71.9-142.9]pg/ml, p=0.642, and 0.778 respectively) compared with the levels before anthelminthic treatment. Median serum level of TNF–α was insignificantly higher at one and two months post-anthelminthic treatment (28.09 [IQR 22.34-41.23] vs 27.30 [IQR 23.82-33.86] vs 25.61 [IQR 21.47-30.05]pg/ml, p=0.892) compared with the level before anthelminthic treatment.

Table 4.5: Serum micronutrient levels of school-aged children with *Ascaris lumbricoides* infection before and after anthelminthic treatment.

	Pre-Treatment (*Al* – infected) (n=23)	**1 Month Post-Treatment (n=23)**	**2 Months Post-Treatment (n=23)**	**F-value**	**p-value**
Zinc (ug/dl)	136.73 ± 17.78	145.04± 34.89	137.93± 22.90	0.353	0.705
Selenium (ug/dl)	0.63 ± 0.41	0.71 ± 0.17	0.64 ± 0.11	0.327	0.723
Vit. A (ug/dl)	118.53 ± 9.74	203.60 ± 25.90[#]	206.21 ± 24.11[#]	66.512	0.000*
Transferrin (mg/dl)	185.85 ± 24.25	197.73 ± 47.57	188.01 ± 31.22	0.393	0.678

*Significant at $p<0.05$
[#]Significant compared with pre-treatment
Al – Ascaris lumbricoides

Table 4.6: Serum cytokine levels of school-aged children with *Ascaris lumbricoides* infection before and after anthelminthic treatment.

	Pre-Treatment (*Al* – infected) (n=23)	1 Month Post-Treatment (n=23)	2 Months Post-Treatment (n=23)	X^2	p-value
IFN–γ (pg/ml)	81.59 (15.64-141.39)	92.36 (57-18-97.04)	76.29 (51.07-125.05)	0.229	0.892
TNF–α(pg/ml)	25.61 (21.47-30.05)	27.30 (23.82-33.86)	28.09 (22.34-41.23)	2.382	0.304
IL–4 (pg/ml)	105.0 (71.9-142.9)	139.6 (79.6-171.9)	80.0 (32.8-159.6)	3.145	0.208
IL–10(ng/ml)	0.09 (0.07-0.13)	0.07 (0.06-0.11)	0.10 (0.07-0.18)	2.874	0.238
IL-8(pg/ml)	542.3 (320.7-935.3)	270.1 (167.9-566.2) #	13.1 (5.8-793.9)	6.945	0.031*
IL-6(pg/ml)	8.99 (7.01-12.11)	8.10 (6.84-11.47)	10.45 (5,68-11.66)	0.519	0.771

*Significant at p<0.05

#Significant compared with pre-treatment (Wilcoxon Signed Ranks Test)

X^2- Kruskal Wallis Test

Al – Ascaris lumbricoides

Table 4.7a shows the serum cytokine levels in *Ascaris lumbricoides*–infected and uninfected pre-school aged children before oral rotavirus vaccination. Pre-vaccination serum levels of IL-8 and IL-6 were significantly higher in *Ascaris lumbricoides*–infectedpre-school aged children compared with pre-vaccination levels in helminth uninfected pre-school aged children (1122.3 [IQR 262.6-1447.6]pg/ml and 14.60 [IQR 8.18-38.34]pg/ml vs 501.3 [IQR 250.6-705.5.]pg/ml and 9.57 [IQR 5.76-11.22]pg/ml, p=0.021 and 0.041 respectively). The higher pre-vaccination serum levels of IFN –γ (170.15 [IQR 74.75-246.05] vs 95.92 [IQR 74.01-156.25]pg/ml, p=0.210,)TNF–α(41.43 [IQR 35.56-57.71] vs 48.08 [IQR 39.26-57.39]pg/ml, p=0.391), IL-4 (365.1 [IQR 156.8-732.1] vs 217.4 [IQR 128.7-269.8]pg/ml, p=0.065) and IL-10 (0.11 [IQR 0.07-0.21] vs 0.09 [IQR 0.07-0.13]ng/ml, p=0.372) in *Ascaris lumbricoides*–infected pre-school aged children compared with pre-vaccination serum levels in helminth uninfected pre-school aged children were not statistically significant.

Table 4.7b shows the serum cytokine levels in *Ascaris lumbricoides*–infected and uninfected pre-school aged children after oral rotavirus vaccination. Post vaccination serum levels of IL-8 was significantly higher in *Ascaris lumbricoides*–infected pre-school aged children compared with post-vaccination serum level in helminth uninfected pre-school aged children (539.2 [IQR 266.1-808.0] vs 321.5 [IQR 171.5-451.7]pg/ml, p=0.056). The lower post-vaccination serum levels of IFN–γ (96.52 [IQR 46.03-151.06] vs 99.61 [IQR 67.90-130.42]pg/ml, p=0.692),TNF–α(39.69 [IQR 31.63-45.18] vs 44.21 [IQR 32.78-49.40]pg/ml, p=0.644), IL-4 (169.2 [IQR 73.30-378.2] vs 184.1 [IQR 129.6-321.9]pg/ml, p=0.817) and the higher post-vaccination serum levels of IL-6 and IL-10 (11.24 [IQR 7.21-17.85]pg/ml and 0.21 [IQR 0.14-0.42]ng/ml vs 4.99 [IQR 2.59-26.09]pg/ml and 0.15 [IQR 0.13-0.21]ng/ml, p=0.203 and 0.261 respectively) in *Ascaris lumbricoides*–infectedpre-school aged children compared with post vaccination levels in helminth uninfected pre- school aged children were not statistically significant.

Table 4.7c shows theserum cytokine levels in *Ascaris lumbricoides*-infected pre-school aged children before and after oral rotavirus vaccination. Pre-vaccination serum levels of IFN–γ (170.15 [IQR 74.45-246.05] vs 96.52 [IQR 46.03-151.06]pg/ml, p=0.023), IL-4 (365.1 [IQR 156.8-732.1] vs 169.2 [IQR 73.3-378.2]pg/ml, p=0.023)and IL-8 (1122.3 [IQR 262.6-1447.6] vs 539.2 [IQR 266.1-808.0]pg/ml, p=0.002) were significantly higher compared with post-vaccination levels. Pre-vaccination serum level of IL-10 (0.11 [IQR 0.07-0.21] vs 0.21 [IQR 0.14-0.42]ng/ml, p=0.012) was significantly lower compared with post-vaccination level. The higher pre-vaccination serum levels of TNF–α and IL-6 (41.43 [IQR 35.56-57.71]pg/ml and 14.60 [IQR 8.18-38.34] pg/ml vs 39.69 [IQR 31.63-45.18.]pg/ml and 11.24 [IQR 7.21-17.85]pg/ml, p=0.209 and 0.116 respectively) compared with post vaccination levels were not statistically significant.

Table 4.7d shows the serum cytokine levels in helminth uninfected pre-school aged children before and after oral rotavirus vaccination. Pre-vaccination serum level of IL-10 was significantly lower compared with post-vaccination levels (0.09 [IQR 0.07-0.13] vs 0.15 [IQR 0.13-0.21]ng/ml, p=0.024). The higher pre-vaccination serum levels of TNF–α(48.08 [IQR 39.26-57.39] vs 44.21 [IQR 32.78-49.40]pg/ml, p=0.203), IL-4 (217.4 [IQR 128.7-269.8] vs 184.1 [IQR 129.6-321.9]pg/ml, p=0.959), IL-8 (501.3 [IQR 250.6-0705.5] vs 321.5 [IQR 171.5-451.7]pg/ml, p=0.074) and IL-6 (9.57 [IQR 5.76-11.22] vs 4.99 [IQR 2.59-26.09]pg/ml, p=0.958) and the lower pre-vaccination level of IFN –γ(95.92 [IQR 74.01-156.25] vs 99.61 [IQR 67.90-130.42]pg/ml, p=0.721) compared with post vaccination levels were not statistically significant.

Table 4.7a: Serum cytokine levels in *Ascaris lumbricoides* – infected and helminth uninfected pre-school aged children before oral rotavirus vaccination.

	***Al*-infected (n=19)**	**Helminth -uninfected (n=19)**	**U**	**p-value**
IFN–γ (pg/ml)	161.65(69.05-239.67)	90.01(70.98-141.32)	41.000	0.210
TNF–α(pg/ml)	40.67(36.87-58.92)	49.32(35.57-52.86)	47.000	0.391
IL–4 (pg/ml)	354.90(116.6-701.8)	222.3(121.9-250.7)	32.000	0.065
IL–10 (ng/ml)	0.12(0.07-0.26)	0.08(0.05-0.12)	46.500	0.372
IL-8 (pg/ml)	1069.0(257.8-1489.5)	462.2(227.4-692.8)	25.000	0.021*
IL-6 (pg/ml)	15.31(8.29-41.44)	9.03(5.00-10.19)	29.000	0.041*

*Significant at $p<0.05$
U - Mann Whitney U Test
Al – Ascaris lumbricoides

Table 4.7b: Serum cytokine levels in *Ascaris lumbricoides* – infected and helminth uninfected pre-school aged childrenafteroral rotavirus vaccination.

	***Al*-infected (n=19)**	**Helminth -uninfected (n=19)**	**U**	**p-value**
IFN–γ (pg/ml)	96.52 (46.03-151.06)	99.61 (67.90-130.42)	54.00	0.692
TNF–α (pg/ml)	39.69 (31.63-45.18)	44.21 (32.78-49.40)	53.00	0.644
IL–4 (pg/ml)	169.2 (73.30-378.2)	184.1 (129.6-321.9)	56.50	0.817
IL–10 (ng/ml)	0.21 (0.14-0.42)	0.15 (0.13-0.21)	43.00	0.261
IL-8 (pg/ml)	539.2 (266.1-808.0)	321.5 (171.5-451.7)	30.00	0.049*
IL-6 (pg/ml)	11.24 (7.21-17.85)	4.99 (2.59-26.09)	40.00	0.203

*Significant at $p<0.05$
U - Mann Whitney U Test
Al – Ascaris lumbricoides

Table 4.7c: Serum cytokine levels in *Ascaris lumbricoides* - infected pre-school aged children before and after oral rotavirus vaccination.

	Pre-vaccination (n=19)	Post-vaccination (n=19)	Z	p-value
IFN–γ (pg/ml)	161.65 (69.05-239.67)	96.52 (46.03-151.06)	2.275	0.023*
TNF–α (pg/ml)	40.67 (36.87-58.92)	39.69 (31.63-45.18)	1.255	0.209
IL–4 (pg/ml)	354.90 (116.60-701.80)	169.2 (73.30-378.2)	-2.275	0.023*
IL–10 (ng/ml)	0.12 (0.07-0.26)	0.21 (0.14-0.42)	2.499	0.012*
IL-8 (pg/ml)	1069.0 (257.8-1489.5)	539.2 (266.1-808.0)	3.059	0.002*
IL-6 (pg/ml)	15.31 (8.29-41.44)	11.24 (7.21-17.85)	1.571	0.116

*Significant at $p<0.05$
Z - Wilcoxon Signed Ranks Test
Al – Ascaris lumbricoides

Table 4.7d: Serum cytokine levels in helminth uninfected pre-school aged children before and after oral rotavirus vaccination.

	Pre-vaccination (n=19)	Post-vaccination (n=19)	Z	p-value
IFN–γ (pg/ml)	90.01(70.98-141.32)	99.61(67.90-130.42)	0.357	0.721
TNF–α (pg/ml)	49.32(35.57-52.86)	44.21(32.78-49.40)	1.274	0.203
IL–4 (pg/ml)	222.3(121.9-250.7)	184.1(129.6-321.9)	0.051	0.959
IL–10 (ng/ml)	0.08(0.05-0.12)	0.15(0.13-0.21)	2.550	0.024*
IL-8 (pg/ml)	462.2(227.4-692.8)	321.5(171.5-451.7)	1.784	0.074
IL-6 (pg/ml)	9.03(5.00-10.19)	4.99(2.59-26.09)	0.051	0.959

*Significant at $p<0.05$
Z - Wilcoxon Signed Ranks Test
Al – Ascaris lumbricoides

Table 4.8a shows the serum cytokine levels in *Ascaris lumbricoides*–infected and uninfected school aged children before oral polio vaccination. Pre-vaccination serum levels of IFN–γ (108.21 [IQR 75.17-137.5] vs 64.14 [IQR 25.68-88.07]pg/ml, p=0.014), IL-4 (204.4 [IQR 139.2-299.3] vs 89.3 [IQR 65.9-134.1]pg/ml, p=0.001), IL-8 (1193.9 [IQR 659.6-1321.9] vs 765.3 [IQR 218.7-802.8]pg/ml, p=0.014) and IL-6 (12.6 [8.02-20.39] vs 4.92 [IQR 2.69-6.52]pg/ml, p=0.000) were significantly higher in *Ascaris lumbricoides*–infected school aged children compared with pre-vaccination levels in helminth uninfected school aged children. The higher pre-vaccination serum level of TNF–α(51.30 [IQR 40.89-61.45] vs 43.36 [IQR 36.72-52.59]pg/ml, p=0.145) and the lower pre-vaccination serum level of IL-10 (0.12 [IQR 0.07-0.52] vs 0.14 [IQR 0.13-0.36]ng/ml, p=0.720) in *Ascaris lumbricoides*–infected school aged children compared with pre-vaccination levels in helminth uninfected school aged children were not statistically significant.

Table 4.8b shows the serum cytokine levels in *Ascaris lumbricoides*–infected and uninfected school aged children after oral polio vaccination. Post vaccination serum levels of IFN –γ (96.23 [IQR 74.83-123.29] vs 25.86 [IQR 17.01-30.57]pg/ml, p=0.000), IL-4 (170.8 [IQR 133.0-199.3] vs 41.1 [IQR 31.2-64.4]pg/ml, p=0.000) and IL-8 (805.6 [IQR 603.2-821.4] vs 233.4 [IQR 205.0-251.7]pg/ml, p=0.000) were significantly higher in *Ascaris lumbricoides*-infected school aged children compared with post-vaccination levels in helminth uninfected school aged children. The higher post-vaccination serum levels of TNF–α(44.19 [IQR 35.41-54.59] vs 33.53 [IQR 29.12-51.56]pg/ml, p=0.063), IL-6 (7.58 [6.01-10.55] vs 6.33 [IQR 3.87-8.17]pg/ml, p=0.174) and the lower post–vaccination serum levels of IL-10 (0.08 [IQR 0.06-0.21] vs 0.09 [IQR 0.08-0.19]ng/ml, p=0.800) in *Ascaris lumbricoides*–infectedschool aged children compared with post-vaccination levels in helminth uninfected school aged children were not statistically significant.

Table 4.8c shows theserum cytokine levels in *Ascaris lumbricoides*-infected school aged children before and after oral polio vaccination. Pre-vaccination serum levels of IL-8 and IL-6 were significantly higher compared with post-vaccination levels (1193.9 [IQR 659.6-1321.9]pg/ml and 12.67 [IQR 8.02-20.39]pg/ml vs 805.6 [IQR 603.2-821.4]pg/ml and 7.58 [IQR 6.01-10.55]pg/ml, p=0.027 and 0.000 respectively). The higher pre-vaccination serum levels of IFN –γ (108.21 [IQR 75.17-137.58] vs 96.23 [IQR 74.83-123.29]pg/ml, p=0.795), TNF–α(51.30 [IQR 4.89-61.45] vs 44.19 [IQR 35.41-54.59]pg/ml, p=0.381), IL-4 (204.4 [IQR 139.2-299.3] vs 170.8 [IQR 133.0-199.3]pg/ml, p=0.149) and IL-10 (0.12 [IQR 0.07-0.52] vs 0.08 [IQR 0.06-0.21]ng/ml, p=0.101) compared with post vaccination levels were not statistically significant.

Table 4.8d shows the serum cytokine levels in helminth uninfected school aged children before and after oral polio vaccination. Pre-vaccination serum level of IFN –γ (64.14 [IQR 25.68-88.07] vs 25.86 [IQR 17.01-30.57]pg/ml, p=0.037), IL-4 (89.3 [IQR 65.9-134.1] vs 41.1 [IQR 31.2-64.4]pg/ml, p=0.013) and IL-8 (765.3 [IQR 218.7-802.8] vs 233.4 [IQR 205.0-251.7]pg/ml, p=0.012) were significantly higher compared with post-vaccination levels. The higher pre-vaccination serum levels of TNF–α(43.36 [IQR 36.72-52.59] vs 33.53 [IQR 29.12-51.56]pg/ml, p=0.114), IL-10 (0.14 [IQR 0.13-0.36] vs 0.09 [IQR 0.08-0.19]ng/ml, p=0.201) and the lower pre-vaccination level of IL-6(4.92 [IQR 2.69-6.52] vs 6.33 [IQR 3.87-8.17]pg/ml, p=0.241) compared with post vaccination levels were not statistically significant.

Table 4.8a: Serum cytokine levels in *Ascaris lumbricoides* – infected and helminth uninfected school aged children before oral poliovaccination.

	***Al*-infected (n=19)**	**Helminth -uninfected (n=19)**	**U**	**p-value**
IFN–γ (pg/ml)	113.81 (68.41-146,52)	67.21 (23.46-93.29)	36.000	0.014*
TNF–α (pg/ml)	50.90 (40.41-69.34)	40.09 (32.97-58.30)	56.000	0.145
IL–4 (pg/ml)	191.2 (127.9-320.6)	92.7 (66.8-151.1)	21.000	0.001*
IL–10 (ng/ml)	0.11 (0.05-0.61)	0.12 (0.06-0.43)	78.000	0.720
IL-8 (pg/ml)	1211.1 (696.4-1226.7)	778.4 (232.9-899.9)	36.000	0.014*
IL-6 (pg/ml)	13.12 (9.37-22.15)	5.54 (3.02-7.29)	12.000	0.000*

*Significant at $p<0.05$
U - Mann Whitney U Test
Al – Ascaris lumbricoides

Table 4.8b: Serum cytokine levels in *Ascaris lumbricoides* – infected and helminth uninfected school aged childrenafteroral poliovaccination.

	***Al*-infected (n=19)**	**Helminth -uninfected (n=19)**	**U**	**p-value**
IFN–γ (pg/ml)	96.23 (74.83-123.29)	25.86 (17.01-30.57)	0.000	0.000*
TNF–α (pg/ml)	44.19 (35.41-54.59)	33.53 (29.12-51.56)	48.000	0.063
IL–4 (pg/ml)	170.8 (133.0-199.3)	41.1 (31.2 - 64.4)	4.000	0.000*
IL–10 (ng/ml)	0.08 (0.06-0.21)	0.09 (0.08-0.19)	80.000	0.800
IL-8 (pg/ml)	805.6 (603.2-821.4)	233.4 (205.0-251.7)	6.000	0.000*
IL-6 (pg/ml)	7.58 (6.01-10.55)	6.33 (3.87-8.17)	58.000	0.174

*Significant at $p<0.05$
U - Mann Whitney U Test
Al – Ascaris lumbricoides

Table 4.8c: Serum cytokine levels in *Ascaris lumbricoides* - infected school - aged children before and after oral poliovaccination.

	Pre-vaccination (n=23)	**Post-vaccination (n=23)**	**Z**	**p-value**
IFN–γ (pg/ml)	113.81(68.41-146,52)	96.23(74.83-123.29)	0.260	0.795
TNF–α (pg/ml)	50.90(40.41-69.34)	44.19(35.41-54.59)	0.876	0.381
IL–4 (pg/ml)	191.2(127.9-320.6)	170.8(133.0-199.3)	1.444	0.149
IL–10 (ng/ml)	0.11(0.05-0.61)	0.08(0.06-0.21)	1.642	0.101
IL-8 (pg/ml)	1211.1(696.4-1226.7)	805.6(603.2-821.4)	2.208	0.027*
IL-6 (pg/ml)	13.12(9.37-22.15)	7.58(6.01-10.55)	3.011	0.003*

*Significant at p<0.05
Z - Wilcoxon Signed Ranks Test
Al – Ascaris lumbricoides

Table 4.8d: Serum cytokine levels in helminth uninfected school aged children before and after oral poliovaccination.

	Pre-vaccination (n=23)	**Post-vaccination (n=23)**	**Z**	**p-value**
IFN–γ (pg/ml)	67.21(23.46-93.29)	25.86(17.01-30.57)	2.090	0.037*
TNF–α (pg/ml)	40.09(32.97-58.30)	33.53(29.12-51.56)	1.580	0.114
IL–4 (pg/ml)	92.7(66.8-151.1)	41.1(31.2 - 64.4)	2.497	0.013*
IL–10 (ng/ml)	0.12(0.06-0.43)	0.09(0.08-0.19)	1.279	0.201
IL-8 (pg/ml)	778.4(232.9-899.9)	233.4(205.0-251.7)	2.505	0.012*
IL-6 (pg/ml)	5.54(3.02-7.29)	6.33(3.87-8.17)	1.174	0.241

*Significant at p<0.05
Z - Wilcoxon Signed Ranks Test
Al – Ascaris lumbricoides

Table 4.9 shows the serum levels of rotavirus-specific IgA antibody (RV-IgA) in pre-school aged children with and without *Ascaris lumbricoides* infection before and after rotavirus vaccination. There was significantly higher post-vaccination median serum level of RV-IgA compared with its level before vaccination in helminth-free pre-school aged children (6.477 [IQR 5.847-7.419] vs 7.080 [IQR 6.636-9.067]mg/dl, p=0.028). There was also significantly lower post vaccination median serum level of RV-IgA in *Ascaris lumbricoides*-infected pre-school aged children compared with post vaccination level in helminth-free pre-school aged children. (6.087 [5.381 – 6.563] vs 7.080 [6.636 – 9.067]mg/dl, p=0.004). However, there was insignificantly reduced post-vaccination serum level of RV-IgA compared with its level before vaccination in *Ascaris lumbricoides*-infected pre-school aged children (6.087 [5.381 – 6.563] vs 6.449 [5.831 – 6.679]mg/dl). There was also no significant difference in the pre-vaccination level of RV-IgA in *Ascaris lumbricoides*-infected pre-school aged children compared with helminth-free pre-school aged children (6.449 [5.831 – 6.67] vs 6.477 [5.847 – 7.419]mg/dl).

Table 4.9: Serum levels of Rotavirus-Specific IgA in pre-school aged children with and without *Ascaris lumbricoides* infection before and after rotavirus vaccination

		Rotavirus specific –IgA (mg/dl)
Pre-vaccination	*A. lumbricoides*-positive(n=19)	6.449 (5.831 – 6.679)
	Helminth-negative (n=19)	6.477 (5.847 – 7.419)
Post-vaccination	*A. lumbricoides*-positive(n=19)	6.087 (5.381 – 6.563)
	Helminth-negative (n=19)	7.080 (6.636 – 9.067)
Z, p-value[a]		1.400, 0.161
Z, p-value[b]		2.191, 0.028*
U, p-value[c]		0.556, 0.608
U, p-value[d]		2.776, 0.004*

*Significant at p<0.05

[a]*A. lumbricoides*-positive pre-vaccination vs *A. lumbricoides*-positive post-vaccination

[b]Helminth-negative pre-vaccination vs Helminth-negative post–vaccination

[c] *A. lumbricoides*-positive pre-vaccination vs Helminth-negative pre-vaccination

[d]*A. lumbricoides*-positive post-vaccination vs Helminth-negative post–vaccination

U = Mann Whitney U Test

Z = Wilcoxon Signed Ranks Test

Table 4.10 shows the serum levels of poliovirus-specific IgA (PV-IgA) in school aged children with and without *Ascaris lumbricoides* infection before and after oral poliovirus vaccination. There was no statistically significant difference in median serum levels of PV-IgA in *Ascaris lumbricoides*-infected school aged children compared with helminth-free school aged children, before or after oral poliovirus vaccination. However, there was insignificantly lower post-vaccination median serum level of PV-IgA compared with its level before vaccinationin *Ascaris lumbricoides*-infected school aged children (1.782 [1.381 – 2.979] vs 1.831 [1.609 – 2.575]U/ml, p=0.831). However, there was insignificantly higher post-vaccination median serum level of PV-IgA compared with its level before vaccinationin helminth-free school aged children (2.488 [1.597 – 3.641] vs 1.983 [1.368 – 4.234]U/ml, p=0.878). Also, there was insignificantly lower post-vaccination median serum level of PV-IgA in *Ascaris lumbricoides*-infected school aged children compared with post vaccination level in helminth-free school aged children (1.782 [1.381 – 2.979] vs 2.488 [1.597 – 3.641]U/ml, p=0.980). There was also insignificantly higher level of PV-IgA in helminth-free school aged children compared with its level in *Ascaris lumbricoides*-infected school aged children (1.983 [1.368 – 4.234] vs 1.831 [1.609 – 2.575]U/ml, p=0.223).

Table 4.10: Serum levels of Poliovirus-Specific IgA in school aged children with and without helminth *Ascaris lumbricoides* before and after oral polio vaccination

		Poliovirus specific –IgA (U/ml)
Pre-vaccination	*A. lumbricoides*-positive(n=23)	1.831 (1.609 – 2.575)
	Helminth-negative (n=23)	1.983 (1.368 – 4.234)
Post-vaccination	*A. lumbricoides*-positive(n=23)	1.782 (1.381 – 2.979)
	Helminth-negative (n=23)	2.488 (1.597 – 3.641)
Z, p-value[a]		0.213, 0.831
Z, p-value[b]		0.153, 0.878
U, p-value[c]		0.025, 0.980
U, p-value[d]		0.209, 0.223

*Significant at $p<0.05$

[a]*A. lumbricoides*-positive pre-vaccination vs *A. lumbricoides*-positive post-vaccination

[b]Helminth-negative pre-vaccination vs Helminth-negative post–vaccination

[c] *A. lumbricoides*-positive pre-vaccination vs Helminth-negative pre-vaccination

[d]*A. lumbricoides*-positive post-vaccination vs Helminth-negative post–vaccination

U = Mann Whitney U Test

Z = Wilcoxon Signed Ranks Test

CHAPTER FIVE

DISCUSSION

5.1 Prevalence and Demographic Characteristics of *A. lumbricoides* Infection

Intestinal helminth infection is one of the commonest chronic infections that affect children in developing countries where basic amenities are absent (Crompton, 1999). It continues to be a major burden in Nigeria with children of school age living in rural areas and urban slums being most affected (Arinola and Fawole, 1995; Ekundayo *et al.,* 2007). The observed prevalence (29%) of helminth infection in this studyis higher than that (20.8%) reported by Edem and Arinola (2015) among children in Ibadan. It is however, similar to that of Ogunkambi and Sowemimo (2014) who reported a prevalence of 28.1% among pre-school aged children of Ile Ife, but lower than the prevalence rates of 94.3%, 52% and 74% reported by Nmorsi *et al* (2009), Adefioye *et al* (2011), and Arinola *et al* (2012), among children of Ibilo, Edo State, Ilie, Osun State and Ibadan suburbs respectively. The relatively reduced prevalence of helminth infection as observed in this present study could be related to the effectiveness of deworming exercises and environmental sanitation among the populace. The present study however shows that helminth infection in still present in Nigerian children despite all control measures to eradicate it.

Ascaris lumbricoides is the commonest helminth that infects the children as described inprevious studies. (Ekundayo *et al.,* 2007; Arinola *et al,* 2012). The observation that the infection was more common among male children and children between ages 5 – 9 years old is in line with the findings of Ogunkambi and Sowemimo (2014). Children within this age group have direct contact with soil when playing, sleeping or eating. The children may become infected when the eggs of *Ascaris lumbricoides* from contaminated soil are ingested while playing with the soil. Eggs of *Ascaris lumbricoides* contaminates soil when an infected person defecates openly on the soil,releasing several hundreds of thousands of unfertilized

eggs which become fertilized and infective after 18 days to several weeks after contaminating the soil and may remain viable for years.

This study revealed a strong relationship between defecation method and period of living in the community with intestinal helminth infection. Defecation on open soil and bushes observed in this study has strongly been linked with prevalence of helminth infection. Rural and semi-urban communities in Nigeria lack portable tap water, adequate sanitation, planned housing structure and access to standard healthcare, which are factors that reduces infection in a community (Crompton, 1999). The findings in this study provided additional information that length of stay in the community contributes to prevalence of helminth infection.

This study revealed that stunting is more prevalent in helminth infected children compared with underweight and thinness. Kirwan *et al* (2009) and Francis *et al*, (2012) reported lower prevalence of stunting and higher prevalence of underweight and thinness among children in Ile-Ife, Osun State, Nigeria and Uganda respectively. On the contrary, Amare et al (2012) revealed a higher prevalence of stunting and lower prevalence of underweight and thinness among school children in Ethiopia. Stunting is a consequence of chronic malnutrition which begins in childhood at age 6 – 36 months (Amare et al, 2012) which is resolved by adequate nutrition (Thompson *et al*, 2011). High prevalence of stunting in this study population, especially females indicate lack of adequate adequate nutrition for the children after weaning which may be associated with low education or socio-economic status.

5.2 Effect of *A. lumbricoides* Infection on Serum Micronutrient Levels

Zinc is an integral part of many enzymes and it plays major role in nucleic acid metabolism, tissue repair, cell replication and growth. It supports antioxidant functions of selenium in glutathione peroxidase (McKenzie *et al*., 1998; Nicola *et al.,* 2002). It is critical for normal

functioning and development of both the innate and adaptive immune cells (Prasad, 1998). Deficiency of zinc leads to impairment of many processes including the complement system, natural killer cells'cytotoxicity, of neutrophils' and macrophages' phagocytic activity as well as theimmune cell's ability to generate oxidants (Kruse-Jarres, 1989; Ibs and Rink, 2004) and reduced lymphocyte number and function (Shankar and Prasad, 1998). Poor intake of zinc therefore predisposes to helminth infections, prolong helminth survival and suppress immune responses which may conversely affect vaccine efficacy in children (Koski and Scott, 2001). Some studies found no association between zinc and helminth infection (Furnée *et al.,* 1997; Osei *et al.,* 2010) while others have reported low serum zinc in helminth infected children (Dehghani *et al.*, 2011; Amare *et al.*, 2012). The lower levels of zinc in pre-school aged and school-aged children with *A. Lumbricoides*infection compared with those without helminth infection as observed in this study may therefore be associated with poor micronutrients intake or poor zinc absorption due to the *A. lumbricoides* infestation, which survives through continuous supply of nutrients digested by the host intestine (Chandra, 2007).

Selenium is an integral part of glutathione peroxidase enzyme, which is a major cellular antioxidant (McKenzie *et al,* 1998).It also plays major role in regulating the expression of cytokines (Baum *et al,* 2000). The observed higher selenium level in helminth-infected pre-school aged and school-aged children compared with helminth-free children as observed in this study disagrees with earlier studies which reported low selenium levels in school children in Zaire (Thorpe *et al.,* 1990)and Ethiopia (Amare *et al.,* 2012). The elevated selenium level in the helminth infected children is suggested to be a compensatory mechanism for low zinc level (House and Welch, 1989) as selenium provokes release of zinc by metallothioneins therough reduction of glutathione peroxidase production (Mocchegiani*et al.*, 2008).

Iron functions as a constituent of haemoglobin, and also is important in energy generation as well as proper functioning of the immune system (Gebre-Medhin and Birgegård, 1981). It aids in the proliferating and differentiating T lymphocytesas well as generation of reactive oxygen species (Beard, 2001). Iron depletion could therefore lead to decreased myeloperoxidase activity of neutrophils and impaired cell mediated immunity (Ghio *et al*, 1997). The reduced level of iron in pre-school aged children observed in this study supports earlier findings (Ngui *et al,* 2012). Previous studies have not established significant association between serum iron and *Ascaris lumbricoides,* however, hookworms and *Trichuris trichuia* have been strongly linked with iron-deficiency anaemia (Rajacopal *et al,* 2014). The observations from this study could therefore be due to *A. lumbricoides* – induced intestinal malabsorption of iron, or insufficient intake of iron-rich foods by the children.

This study also shows a significantly lower serum level of transferrin in *A. lumbricoides* – infected pre-school aged children compared with helminth – freepre-school aged children but significantly higher level in *A. lumbricoides*–infected school aged children compared with helminth-free school aged children. Also, serum ferritin level was significantly lowerin *A. lumbricoides* – infected pre-school aged children compared with helminth – free pre-school aged children. Ferritin is the major iron store while transferrin is a transport protein for iron (Jason *et al,* 2001). The low ferritin and transferrin levels in the *A. lumbricoides*- infected pre-school aged children may therefore indicate low iron store, which might also be associated with reduced iron intake or absorption. The raised transferrin level in *A. lumbricoides*- infected school aged children might be in response to increased level of serum iron. Iron bound to transferrin are not available to blood microbes for replication thus raised transferring level might be an innate mechanism to reduce secondary bacterial infection in *A. lumbricoides* – infected children.

The study showed that serum levels of iron and iron – binding proteins (ferritin, transferrin and haptoglobin) are generally significantly lower in *A. lumbricoides* – infected pre-school aged children compared uninfected pre-school aged children but that of iron, ferritin and transferrin was insigniflcantly higher and that of haptoglobin significantly higher in *A. lumbricoides* – infected school aged children compared with uninfected ones. Alhough this should be subjected to further studies, it could however be suggested to be due to the effect of the duration of the infection in the children (as the infection in the pre-school aged children may be shorter that that in the school aged children). This however may distinguish the effect ofacute and chronic *A. lumbricoides* infection on the micronutrients status.

The reduced serum vitamin A level in *A. lumbricoides*- infected pre-school aged and school-aged childrenas observed in this study is in consonance with previous finding (Ahmed *et al.,* 1993).Vitamin A functions in the regulation of growth and differentiation of virtually all the cells of the human body. It plays major roles in immune functions as well as the development of the eyes and vision (Higdon, 2000). It maintains the epithelial integrity in the respiratory and gastrointestinal tracts. Studies have shown that helminth – infected children absorb less vitamin A due to mucosal changes in the gastro-intestinal tract following *Ascaris lumbricoides* infection (Tripathy *et al.,* 1972). Severe complication due to vitamin A deficiency that result in blindness in some cases, is more common among children with *Ascaris lumbricoides* infection and deficiency can lead to increased susceptibility to illnesses such as lower respiratory infections and measles (Imdad, 2010). Animal studies have provided evidence that vitamin A deficiency reduced intestinal T-helper 2 immune responses (Hurst and Else, 2012) and antibody responses to different vaccines (Ross, 2000). The reduced vitamin A in *A. lumbricoides*- infected pre-school aged and school-aged children as observed in this study may be associated with *A. lumbricoides* – induced malabsorption of

vitamin A. It may also be as a result of reduced zinc level because zinc is important for vitamin A synthesis and release from the liver.

This study observed a significantly increased serum level of vitamin A at one month after anthelminthic drug treatment and further increase at two months after anthelminthic drug treatment compared with the baseline level before anthelminthic drug treatment. This indicates that recovery and subsequent normal absorption of vitamin A occur within a month post-treatment of helminth infection. This supports earlier studies which reported significant rise in serum vitamin A levels following anthelminthic treatment of infected children compared with its levels in untreated children (Jalal *et al*., 1998; Jinabhai *et al.*, 2001; Mwaniki *et al.,* 2002) but these previous studies did not specify the actual period of recovery after treatment. This study therefore suggests that anthelminthic treatment leads to improved intestinal absorption of vitamin A in *A. lumbricoides*- infected Nigerian children within one month of anthelminthic treatment.

5.3 Effect of *A. lumbricoides* Infection on Serum Cytokine Levels

IL-8 is a pro-inflammatory cytokine, secreted by different cells including monocytes, neutrophils, T-lymphocytes, dermal fibroblasts, vascular endothelial cells, hepatocytes and keratinocytes (Thornton *et al.*, 1990). Its major function is neutrophil activation and recruitment (Harada *et al.*, 1994). Th-2 cells contribute to eosinophil differentiation and recruitment which in turn secrete IL-8 (Hannsel *et al.,* 1993). Increased eosinophils are observed in acute helminth infection through the helminth-induced IL-5 secretion which induces eosinophil proliferation and differentiation (Maizels and Yazdanbaksh, 2003). The elevated IL-8 levels in *A. lumbricoides*- infected pre-school and school aged children compared with helminth-free children as observed in this study is consistent with the report of Wang *et al,* (2008). This may be due to eosinophilia usually associated with helminth

infection. This study also suggests that IL-8 - mediated neutrophil proliferation and activation may occur due to *A. lumbricoides* – induced hypersensitivity reaction during re-infection (Loffler's syndreomme) (Lora, 2004), which reverses following anthelminthic treatment. The significantly reduced IL-8 level at one month after anthelminthic drug treatment suggests reduced proliferation of neutrophils and eosinophils within one month after anthelminthic drug treatment.

IL-6 is a highly pleiotropic molecule, with diverse pro-inflammatory and anti-inflammatory properties depending on the prevailing circumstance (Scheller *et al.,* 2011). IL-6 is involved in the induction of switch from neutrophil to monocytes recruitment by suppressing neutrophil-attracting chemokines and enhancing neutrophil apoptosis thereby contributing to the resolution of acute neutrophil infiltration (Kaplanski *etal.,* 2003). IL-6 has been reported to limit Th2 responses, modifies the Treg-cell phenotype, and promotes the host's susceptibility after helminth infection (Smith and Maizels, 2014). The elevated IL-6 levels in *A. lumbricoides*- infected pre-school and school aged children compared with helminth-free children as observed in this study support the report of Nagy *et al.* (2012) who reported elevated IL-6 level in children with *Toxocara canis* infection. This may be attributed to the role of IL-6 as an enhancer of Th2 cells differentiation, involved in helminth infection control. IL-6 increase in this study may also be attributed to its role in suppressing IL-8-induced neutrophil proliferation, which may occur due to *A. lumbricoides* – induced hypersensitivity reaction during re-infection.

This study also reported a significantly increased serum level of IFN–γ and IL-4 in *A. lumbricoides*-infected school aged children compared with helminth-free school aged children. IFN-γ is a critical cytokine for adaptive and innate immunity against some bacterial, viral and protozoal infections. It is produced majorly as part of the innate immune response by natural killer cells and by CD4 Th1 and CD8 cytotoxicT-lymphocyte effector T cells in

cases of specific immunity (Schoenborn and Wilson, 2007). Production of IL-4 by leukocytes is a key regulatory factor that occurs early in the Th-2 response, which induces allergic reactions and mediates parasites expulsion. Studies have demonstrated the reciprocal roles that IFN-γ and IL-4 play in worm expulsion. Depletion of IFN-γ and increased expression of IL-4 with significant IgE secretion is required for worm expulsion. The increased IFN-γ in this study disagrees with these earlier findings (King *et al.*, 1993). However, the increased expression of IL-4 in *A. lumbricoides*-infected children in this study may be associated with increased expression of IL-4 in *A. lumbricoides* infections as observed in previous studies (Nmorsi *et al.*, 2010). Also, the increased IFN-γ in *A. lumbricoides*- infected children as observed in this study, in addition to increased expression of IL-8, indicated systemic inflammation in the *A. lumbricoides* -infected children.

5.4 Effect of *A. lumbricoides* Infection on Vaccination and Associated Immune Responses

The observed reduced post vaccination serum level of IL-8 in pre-school aged children with *A. lumbricoides* infection that received rotavirus vaccine compared with the pre-vaccination level, and also compared with helminth negative children in this study implies that IL-8 was up-regulated due to *A. lumbricoides* infection but this up-regulation was counteracted by the effect of the vaccine. Th2 cell-derived cytokines can stimulate eosinophils to secrete IL-8 (Harada*et al.*, 1994). Increased expression of IL-8 occurs in acute helminth infection through eosinophil proliferation and differentiation (Maizels and Yazdanbaksh, 2003). Also, Induction of IL-8 occurs in acute rotavirus infection where they serve as potent chemoattractants for intestinal intraepithelial lymphocytes (Ebert, 1995) thereby promoting cell proliferation and epithelial repair (Wang et al, 2005). This occurs in active and inactivated rotavirus infection through NF-κB mediated epithelial expression (Clemente *et al.,* 2008). Although the mechanism of the antagonistic effect of *A. lumbricoides* infection on

IL-8 expression in rotavirus infection requires further study, it might be suggested from this study that *A. lumbricoides* infection contributed to down-regulation ofNF-κB mediated expression of IL-8, while it up-regulates its secretion through induction of eosinophils.

The reduced post vaccination serum levels of IL-8 in poliovirus-vaccinated school aged children with and without *A. lumbricoides* infection that received oral poliovirus vaccine compared with the pre-vaccination levels may suggest interplay between the effect of previous vaccination and *A. lumbricoides* infection, in counteracting the vaccine recently administered. Oral polio vaccine induces strong Th1 response (Mahon *et al.,* 1995) and may attenuate immune response to other vaccines co-administered (Jensen *et al.,* 2014). It is however suggested from the findings of this study that immune responses to poliovirus vaccination in this study might be through specific adaptive immune processes because of previous immunization.

The observed reduced post vaccination serum level of IL-6 in oral poliovirus- vaccinated school aged children with helminth infection compared with the pre-vaccination level might suggest inhibitory effect of *A. lumbricoides* – induced Th2 immunity on IL-6. This may also support the function of IL-6 as promoting host susceptibility to helminth infection (Smith and Maizels, 2014).

IL-10 is produced by macrophages, dendritic cells (DC), B cells, and different subsets of $CD4^+$ and $CD8^+$ T cells (Moore *et al.*, 2001). It is a key immune-regulator during infection with viruses, helminths, protozoa, bacteria, and fungi, and functions to ameliorate the excessive Th1, Th2 and $CD8^+$Tcell responses (Hoffman *et al.,* 2000; Wilson *et al.,* 2005). IL-10 plays important role in regulating immune responses to parasitic infections through upregulation of regulatory T-cells (Tregs) induced by helminths (Takahashi *et al.*, 2000). High levels of IL-10 have been observed in individuals heavily infected with chronic

intestinal helminth infections (Figueiredo, 2010). Increased level of IL-10 has been reported in helminth infected children compared with helminth-free children (Arinola *et al.*, 2015). The increased post vaccination level of IL-10 in pre-school aged children with and without helminth infection compared with their pre-vaccinatin levels may therefore indicate up-regulation of Tregs due to vaccination irrespective of the helminth status. This might be to regulate excessive immune response to vaccines. The observations from this study is also in consonance with reports of Azevedo *et al* (2006), who demonstrated increased level of IL-10 after rotavirus vaccination in pigs.

Vaccines induce immune effectors which are majorly antibodies produced by B-lymphocytesand cytotoxic $CD8^+$ T lymphocytes that may recognise and kill invaded cells, or secrete specific antiviral cytokines (Casadevall, 2004). They are supported by factors and signals made available by $CD4^+$ T helper cells, which are of T helper 1 (Th1) and T helper 2 (Th2) subtypes (Igietseme *et al.,* 2004). Rotavirus –specific IgA antibody (RV-IgA) as measured in this study indicated a significant increase in the level of the antibody in helminth free pre-school aged children after vaccination compared with its level before vaccination, but insignificant decrease in its level in *A. lumbricoides*-infected pre-school aged children after vaccination compared with its level before vaccination. This implies that *A. lumbricoides* infection affects production of rotavirus specific IgA (mucosal) antibodies after rotavirus vaccination of infected hosts, thereby compromising the vaccine efficacy. This might have occured through alteration of cytokines expression during immune responses to the vaccine, by *A. lumbricoides*. The reduced post vaccination level of RV-IgA in *A. lumbricoides*-infected children compared with post vaccination level in helminth-free children further corroborates the effect of *A. lumbricoides* infection on efficacy of oral rotavirus vaccination.

Although not statistically significant, this study also showed a reduced serum level of Poliovirus-specific IgA antibody (PV-IgA) in *A. lumbricoides*-infected school aged children after vaccination with oral poliovirus compared with that of helminth-free school aged children which showed increase level of the PV-IgA antibody. This confirms the fact that children with *A. lumbricoides*infection reduced poliovirus vaccine efficacy. A similar non significantly raised post vaccination level of PV-IgA in helminth-free school aged children compared with post-vaccination level in *A. lumbricoides*-infected children further corroborates a case of compromised vaccine efficacy in the infected children.

CHAPTER 6

6.0 CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

It can be concluded from this study that:

The prevalence of Intestinal helminth infection is still high in children of sub-urban communities in Ibadan, which confirms the report of Ekundayo et al (2007). Although the regular dewormimg exercise might have relatively reduced the prevalence rate, the relatively higher prevalence rate this time round could be attributed to constant re-infection due to lack of knowledge of environmental infection control. Futher studies to assess prevalence of helminth infection after proper education of sub-urban dwellers on infection control is also required.

Ascaris lumbricoides infection causes micronutrient deficiency (essentially Zinc and Vitamin A) in infected children, which may contribute to reduced vaccine efficacy and immune responses in the children. It is therefore essential to carry out deworming exercise before vaccination, to achieve optimal outcome of the vaccination exercise.

Anthelminthic drug treatment reverses the negative effect of *A. lumbricoides* infection in children through restoration of adequate basic micronutrients. Longitudinal studies would be required to determine the actual period of restoration of other essential micronutrients beyond those carried out in this study. This however suggests that a particular time frame should be allowed after deworming exercise for restoration of basic micronutrients in children before vaccination, to achieve optimal result.

Intestinal helminth infection caused systemic inflammation in *A. lumbricoides*-infected children through expression of IL-6 and IL-8, which ultimately affected vaccine responses. Further studies are however required to determine other indicators of inflammation and their direct or indirect effects on vaccine response. The mechanism of the antagonistic effect of *A. lumbricoides* infection on IL-8 expression in rotavirus infection also requires further study.

The main addition to knowledge in this study is the demonstration that vaccinated children with *Ascaris lumbricoides* may not derive full benefit of the effect of the vaccination. This is demonstrated through reduced rotavirus-specific and poliovirus-specific IgA antibody production in *Ascaris lumbricoides* – infected children compared with uninfected children. This study however need more verification through a lagre-scale study with higher number of study subjects.

6.2 RECOMMENDATION

It is therefore recommended that periodic anthelminthic drug treatment coupled with micronutrient supplementation in pre-school and school age children should periodically be carried out. This is essential before any oral vaccination to achieve maximum advantage of the vaccination.

REFERENCES

Ada, G. L. 1990. The Ideal Vaccine. *World Journal of Microbiology and Biotechnology*7:105-109.

Adekunle, L. V., Bammeke, A. O., Lucas, A. O. 1986. Family influence on incidence of intestinal parasites among Nigerian children. *Journal of Royal Society of Health* 106:66-68.

Adefioye, O., Efunshile, A., Ojurongbe, O. 2011: Intestinal Helminthiasis among School Children in Ilie, Osun State, Southwest, Nigeria .*Sierra Leone Journal of Biomedical Research*. 3(1): 36-42.

Adeyeba, O. A. and Dipeolu, O. O. 1984. A survey of gastrointestinal parasites in a local government area of south-west Nigeria. *International Journal of Zoonoses* 11:105-110.

Agrawal, S., Agrawal, A., Doughty, B., Gerwitz, A., Blenis, J., Van Dyke, T., Pulendran, B. 2003. Cutting edge: different Toll-like receptor agonists instruct dendritic cells to induce distinct Th responses via differential modulation of extracellular signal-regulated kinase-mitogen-activated protein kinase and c-Fos. *Journal of Immunology*; 171: 4984-4989.

Ahmed, F., Mohiduzzaman, M., Jackson, A.1993. Vitamin A absorption in children with ascariasis. *British Journal of Nutrition*; 69:817e25.

Airauhi, L. U., Idogun, E. S. 2008. Demographic and biochemical features associated to children infected with intestinal helminths.*Pakistan Journal of Medical Sciences*. 1(2):269-273.

Akogun, O. B**.** 1989. Some social aspects of helminthiasis among the people of Gumau District, Bauchi State, Nigeria. *Journal of Tropical Medicine and Hygiene;* 92:193-196

Alberta, A., Di Pasquale A., Preiss, S., Da Silva, F., Garçon, N. 2015. Vaccine Adjuvants: from 1920 to 2015 and Beyond. *Vaccines,* 3(2): 320-343

Allen, J. E, Lawrence, R. A., Mizels, R. M. 1996. Antigen Presenting Cells from mice harbouring the filarial nematode, Brugia may, prevent cellular proliferation but not of cytokine production. *International Immunology* 8: 143-151.

Allen, J. E. and Maizels, R. M.2011. Diversity and dialogue in immunity to helminths. *Nature Reviews Immunolology*11:375–388.

Allen, J. E and McDonald, A. S.1998. Profound suppression of cellular proliferation mediated by the secretions of nematodes. *Parasite Immunolology* 20: 242-247.

Amare, B., Moges, B., Fantahun, B.,Tafess, K., Woldeyohannes, D., Yismaw, G., Ayane, T., Yabutani, T., Mulu,A., Ota F., Kassu, A. 2012. Micronutrient levels and nutritional status of school children living in Northwest Ethiopia.*Nutrition Journal*. 11:108.

Anderson, R., Oosthuizen, R., Maritz, R., Theron, A., van Rensburg A. 1980. The effects of increasing weekly doses of ascorbate on certain cellular and humoral immune functions in normal volunteers. *American Journal of Clinical Nutrition*; 33(1): 71-76.

Anthony, R. M., Urban, J. F. Jr., Alem, F. A., Hamed, H. A., Rozo, C. T., Boucher, J.L., van Rooijen, N., Gause, W. C. 2006. Memory Th2 cells induce alternatively activated macrophages to mediate protection against nematode parasites. *Nature Medicine*12: 955-960.

Araujo, M. I. and de Carvalho, E. M**.** 2006. Human schistosomiasis decreases immune responses to allergens and clinical manifestations of asthma. *Chemical Immunology and Allergy*90:29–44.

Arinola, O. G. and Fawole, O. O. 1995. Prevalence of Protozoan and Helminthic infections among different occupational and age groups in Iroko village, Oyo state*Nigerian Journal of Science. Engineering and Technology*2(1):51-57

Arinola, G. O., Morenikeji, O. A., Akinwande, K. S., Alade, A. O., Olateru-Olagbegi, O., Alabi, P. E., Rahamon, S. K. 2015. SerumLevels of Cytokines and IgE in Helminth-InfectedNigerianPregnantWomen and Children.*Annals of Global Health* 81(5):689-93.

Arinola, O. G., Morenikeji, O. A., Akinwande, K. S., Alade, A. O., Olateru-Olagbegi, O., Alabi, P. E., Rahamon SK. 2015b. Serum Micronutrients in Helminth-infected Pregnant Women and Children: Suggestions for Differential Supplementation During Anti-helminthic Treatment. *Annals of Global Health.* 81(5): 1-6.

Arinola, O. G., Oluwole, O., Oladokun, R., Adedokun, B. O., Olopade, O. I., Olopade, C. O. 2014. Intestinal Helminthic Infection Increases Serum Levels of IL-2 and Decreases Serum TGF-Beta Levels in Nigerian Asthmatic Patients. *Open Journal of Immunology* 4:1-8.

Arinola, O. Ganiyu., Yaqub, A. Suraj, Rahamon, K. Sheu.2012. Reduced serum IgE level in Nigerian children with helminthiasis comparedwith protozoan infection: Implication on hygiene hypothesis. *Annals of Biological Research* 3(12):5754-5757.

Atkinson, W., Hamborsky, J., McIntyre, L., Wolfe, S. (Eds.). 2009. "Poliomyelitis". Epidemiology and Prevention of Vaccine-Preventable Diseases (11th ed.). Washington DC: Public Health Foundation. pp. 231–44.

Armah, G. E., Sow, S. O., Breiman, R. F., Dallas, M. J., Tapia, M. D., Feikin, D. R., Binka, F. N., Steele, A. D., Laserson, K. F., Ansah, N. A., Levine, M. M., Lewis, K., Coia, M. L., Attah-Poku, M., Ojwando, J., Rivers, S. B., Victor, J. C., Nyambane, G., Hodgson, A., Schödel, F., Ciarlet, M., Neuzil, K. M. 2010. Efficacy of pentavalent

rotavirus vaccine against severe rotavirus gastroenteritis in infants in developing countries in sub-Saharan Africa: a randomised, double-blind, placebo-controlled trial. *Lancet*376(9741):606–614.

Arthur, J. R., McKenzie, R.C., Beckett, G. J. 2003. Selenium in the immune system. *Journal of Nutrition*; 133.5 Suppl 1: 1457S-9S

Ayanwale, F. O., Esuruoso, G.O., Dipeolu, O. O. 1982. The epidemiology of human intestinal helminthiasis in Ibadan, South Western Nigeria. *International Journal of Zoonoses* 9:69-72.

Azevedo, M. S. P., Yuan, L., Pouly, S., Gonzales, A. M., Jeong, K. I., Nguyen, T. V., Saif, L. J. 2006. Cytokine responses in gnotobiotic pigs after infection with virulent or attenuated human rotavirus. *Journal of Virology* 80(1): 372–382.

Bacchetta, R., Gregori, S., Roncarolo, M. G. 2005. CD4+ regulatory T cells: mechanisms of induction and effector function. *Autoimmune Reviews* 4:491–496.

Bárány, E., Bergdahl, I. A., Bratteby, L. E., Lundh, T., Samuelson, G., Schütz, A., Skerfving, S., Oskarsson A. 2002. Trace element levels in whole blood and serum from Swedish adolescents.*Science of the Total Environment*286**:**129–141.

Barton, G. M. and Medzhitov, R. 2002. Control of adaptive immune responses by Toll-like receptors. *Current Opinionin Immunology*14: 380-383

Bates, C. J.1997. Vitamin Analysis.*Annals of Clinical Biochemistry*. 54. 599 - 626

Baum, M. K., Miguez-Burbano, M. J., Campa, A., Shor-Posner, G. 2000. Selenium and interleukins in persons infected with human immunodeficiency virus type 1. *Journal of Infectious Diseases*182(1): S69-73.

Beard, J. L. 2001. Iron biology in immune function, muscle metabolism and neuronal functioning*Journal of Nutrition* 131(2S-2): 568S-579S; discussion 580S.

Bechir, M., Hamit, M. A., Schelling E., Tanner M., Zinsstag J. 2012. Parasitic Infections, Anemia and Malnutrition Among Rural Settled and Mobile Pastoralist Mothers and Their Children in Chad. *Ecohealth* 9(2): 122–131.

Belkaid, Y., and Rouse B. T. 2005. Natural Regulatory T-cells in infectious disease. *Nature Immunology;* 6: 353-360

Benhamou, E., Courouce, A. M., Jungers, M. P. 1984. Hepatitis B vaccine: randomized trial of immunogenicity in hemodialysis patients,*Clinical Nephrol*ogy 21: 143–147.

Bethony, J., Brooker, S., Albonico, M., Geiger, S. M., Loukas, A., Diemert, D., Hotez, P. J. 2006. Soil transmitted helminth infections: ascariasis, trichuriasis, and hookworm. *Lancet.*; 67: 1521-1532.

Bleys, J., Navas-Acien, A., Guallar, E. 2007. Serum selenium and diabetes in U.S. adults. *Diabetes Care*30(4):829-834.

Bluestone, J. A. and Abbas, A. K. .2003. Natural versus adaptive regulatory T cells. *Nature Review Immunology*3: 253-257

Borkow, G. and Bentwich, Z. 2006. HIV and helminth co-infection. *Parasite Immunology*28:605–612.

Borkow, G., Weisman, Z., Leng, Q., Stein, M., Kalinkovich,A., Wolday D. 2001. Helminths, human immunodeficiency virus and tuberculosis. *Scandinavian Journal of Infectious Diseases* 33(8):568–571.

Bresee, J., Fang, Z. Y., Wang, B., Nelson, E. A., Tam, J., Soenarto, Y., Wilopo, S. A., Kilgore, P., Kim, J. S., Kang, J. O., Lan, W. S., Gaik, C. L., Moe, K., Chen, K. T., Jiraphongsa, C., Ponguswanna. Y., Nguyen, V. M., Phan, V. T., Le, T. L., Hummelman, E., Gentsch, J. R., Glass, R. Asian Rotavirus Surveillance Network. 2004.First report from the Asian Rotavirus Surveillance Network.*Emerging Infectious Diseases*.10(6):988-95.

Brooker, S., Akhwale, W., Pullan, R., Estambale, B., Clarke, S. E., Snow, R. W., Hotez, P. J. 2007. Epidemiology of *Plasmodium-helminth* co-infection in Africa: population at risk, potential impact on anemia, and prospects for combining control.*American Journal of Tropical Medicine and Hygiene* 77(6 Suppl):88-98.

Brussow, H., Sidoti, J., Dirren, H., Freire, W. B. 1995. Effect of malnutrition in Ecuadorian children on titers of serum antibodies to various microbial antigens.*Clinical and Diagnostic Immunology*2**:**62–68.

Bundy, D. A. P and Cooper, E. S. 1989. Trichuris and trichuriasis in humans. *Advances in Parasitology*28:107–73.

Carr, A. C. and Frei, B. 1999. Toward a new recommended dietary allowance for vitamin C based on antioxidant and health effects in humans. *American Journal of Clinical Nutrition.*69(6):1086-1107.

Casadevall, A. 2004. The methodology for determining the efficacy of antibody-mediated immunity. *Journal of Immunology Methods* 291:1–10.

Cassidy, W.M., 2001. Adolescent hepatitis B vaccination. A review. *Minerva Pediatrics*, 53: 559-566.

Castro, G. A. 1996. Helminths: Structure, Classification, Growth, and Development. In: Baron S, (Ed) Medical Microbiology. 4th edition.University of Texas Medical Branch, Galveston (TX). USA**.**pp 110-114.

Centres for Disease Control and Prevention 2014. Immunisation: The Basics. Vaccines and Immunizations Assessed at *http://www.cdc.gov/vaccines/vac-gen/imz-basics.htm* 2016

Centers for Disease Control and Prevention. 2015. Laboratory Identification of Parasitic Diseases of Public Health Concern. Hookworm. Assessed: *http://www.cdc.gov/dpdx/hookworm/index.html*.

Centres for Disease Control and Prevention. 2015b.Micronutrient Facts. Assessed:http://www.cdc.gov/immpact/micronutrients/.

Centers for Disease Control and Prevention. 2016. DPDx - Laboratory Identification of Parasites of Public Health Concern. Assessed at https://www.cdc.gov/dpdx/diagnostic procedures/stoolspecimenproc.html.

Centersfor Disease Control and Prevention. 2018. Micronutrient Facts. Assessed. https://www.cdc.gov/nutrition/micronutrient-malnutrition/micronutrients/index.html

Chandra, R. K.1997. Nutrition and the immune system: an introduction.*American Journal of Clinical Nutrition*66:460S-463S.

Chen, W. and Gluud, C. 2005. Vaccines for preventing hepatitis B in health-care workers.*Cochrane Database System Reviews*.19.4:CD000100.

Chiba, S., Yokoyama, T., Nakata, S., Morita, Y., Urasawa, T., Taniguchi, K., Urasawa, S., Nakao, T. 1986. Protective effect of naturally acquired homotypic and heterotypic rotavirus antibodies *Lancet* 2(8504):417-21.

Clément, J.-F., Meloche, S., Servant, M. J. 2008. The IKK-related kinases: from innate mmunity to oncogenesis. *Cell Research*.18(9):889–899.

Cohen, J. I.. 2004. "Chapter 175: Enteroviruses and Reoviruses". In Kasper DL; Braunwald E; Fauci AS; et al. Harrison's Principles of Internal Medicine (16th Ed.). McGraw-Hill Professional. p. 1144.

Combs, J. and Gerald, F. 2012. The Vitamins. 4 ed. Burlington, USA.Elsevier Science, pp: 221-227

Cools, N., Ponsaerts, P., Van Tendeloo, V. F., Berneman, Z. N. 2007. Balancing between immunity and tolerance: an interplay between dendritic cells, regulatory T cells, and effector T cells. *Journal of Leukocyte Biology*82: 1365-1374

Cooper, N. R., Nemerow, G. R. 1984. The role of antibody and complement in the control of viral infections. *Journal of Investigative Dermatology* 83: 121s–127s.

Cooper, P. J.2009. Interactions between helminth parasites and allergy. *Current Opinion in Allergy and Clinical Immunology*9:29 –37.

Cooper, P. J., Chico, M. E., SandovaL, C., Espinel, I., Guevara, A., Kennedy, M. W., Urban, J. F. Jr., Griffin, G. E., Nutman, T. B. .2000. Human infection with *Ascaris lumbricoides* is associated with a polarized cytokine response. *Journal of Infectious Diseases* 182: 1207–1213.

Correale, J. and Farez, M. 2007. Association between parasite infection and immune responses in multiple sclerosis. *Annals of. Neurology*. 61:97–108

Coulson, B., Grimwood, K., Hudson, I., Barnes, G., Bishop, R. 1992. Role of coproantibody in clinical protection of children during reinfection with rotavirus. *Journal of Clinical Microbiology* 30:1678-84.

Cousins, R. J. 2006. Zinc. In: Bowman, B. A., Russell, R. M, eds. Present Knowledge in Nutrition. 9th ed, Vol. 1. Washington, D.C.: ILSI Press; pp:445-457.

Cox, F.E.G. 2002. History of human parasitology. *Clinical MicrobiologyReviews*15:595–612.

Crompton, D. W. 1999. How much human helminthiasis is there in the world? *Journal of Parasitology*85:397-403.

Crompton, D.W., Nesheim, M. C., Pawlowski, Z. S. 1989. Ascariasis and human nutrition. In: Ascariasis and Its Prevention and Control. London: Taylor & Francis. pp. 93–94.

Crompton, D. W. and Nesheim, M. C. 2002. Nutritional impact of intestinal helminthiasis during the human life cycle. *Annual Review of Nutrition* 22:35–59.

Culley, F. J., Brown, A., Conroy, D. M., Sabroe, I., Pritchard, D. I. & Williams, T. J. 2000. Eotaxin is specifically cleaved by hookworm metalloproteases preventing its action in vitro and in vivo. *Journal of Immunology*165: 6447–53.

Deehan, M. R., Goodridge, H. S., Blair D. 2002. Immunomodulatory properties of *Ascaris suum* glycosphingolipids: phosphorylcholine and non-phosphorylcholine-dependent eff ects. *Parasite Immunology* 24: 463–69.

Deenick, E. K., Chan, A., Ma, C.S., Gatto, D., Schwartzberg, P.L., Brink, R., Tangye, S.G. 2010.Follicular helper T cell differentiation requires continuous antigen presentation that is independent of unique B cell signaling. *Immunity*. 33:241–253.

de Gier, B., Mpabanzi, L., Vereecken, K. 2015. Height, zinc and soil transmitted helminth infections in schoolchildren: a study in Cuba and Cambodia. Nutrients 7:3000e10.

Dehghani, S. M., Katibeh, P., Haghighat, M., Moravej, H., Asadi, S., 2011. Prevalence of Zinc Deficiency in 3–18 Years Old Children in Shiraz-Iran. *Iran Red Crescent Medical Journal* 13(1):4–8.

de Lalla, F., Rinaldi, E., Santoro, D. , Pravettoni, G.1988. Immune response to hepatitis B vaccine given at different injection sites and by different routes: A controlled randomized study.*European Journal of Epidemiology*4(2): 256–258.

Deming, M. S., Linkins, R.W., Jaiteh, K., Hull, O. H. 1997. The Clinical Efficacy of Trivalent Oral Polio Vaccine in the Gambia by Season of Vaccine Administration *Journal of Infectious Diseases.*175(8):8254-8257.

de Jong, E. C., Vieira, P. L., Kalinski, P., Schuitemaker, J. H., Tanaka, Y., Wierenga, E. A., Yazdanbakhsh, M., Kapsenberg, M. L. 2002. Microbial compounds selectively induce Th1 cell-promoting or Th2 cell-promoting dendritic cells in vitro with diverse Th cell-polarizing signals. *Journal of Immunology*168: 1704-1709.

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.

Del Valle, A., Jones, B. F., Harrison, L. M., Chadderdon, R. C., Cappello, M. 2003. Isolation and molecular cloning of a secreted hookworm platelet inhibitor from adult *Ancylostoma caninum*. *Molecular and Biochemical Parasitology*129: 167–77.

Doetze, 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-b but not by a Th1 to Th2 shift. *International Immunology*12:623– 630.

Doherty, C. P. 2007. Host-pathogen interactions: the role of iron. *Journal of Nutrition*. 137(5): 1341-4.

Dold, C. and Holland, C. V. 2011. *Ascaris* and ascariasis. *Microbes and Infection*. 13(7): 632–637.

Ebert, E. 1995. Human intestinal intraepithelial lymphocyres have potent chemotactic activity. *Gastroenterology* 109:1154-1159.

Edem, V. F. and Arinola, O.G. 2015. Differential effects of age and intestinal helminthiasison positivity rate of skin prick reaction toenvironmental allergens among Nigerians. *Journal of Medicine in the Tropics* 17(2):76-80.

Ejezie, F. and Nwagha, U. 2011. Zinc concentration during pregnancy and lactation in Enugu, South-East Nigeria. *Annals of Medical and Health Science Research*1: 69-76.

Ekundayo, O. J., Aliyu, M. H. and Jolly, P. E. 2007. A review of intestinal helminthiasis in Nigeria and the need for school-based intervention *Journal of Rural and Tropical Public Health*6: 33-39.

Elias, D., Akuffo, H. & Britton, S. 2006. Helminthes could influence the outcome of vaccines against TB in the tropics. *Parasite Immunology* 28(10):507–513.

Elias, D., Wolday, D., Akuffo, H., Petros, B., Bronner, U., Britton S. 2001. Effect of deworming on human T cell responses to mycobacterial antigens in helminth-exposed

individuals before and after bacilli Calmette-Guerin (BCG) vaccination. *Clinical and Experimantal Immunology* 123(2):219–225.

Elliott, A. M. 2011. Treatment with anthelminthics during pregnancy: what gains and what risks for the mother and child? *Parasitology* 138:1499 –1507.

Else, K. J., Finkelman, F. D., Maliszewski, C. R., Grencisn, R. K. 1994.Cytokine-mediated regulation of chronic intestinal helminth infection. *Journal of Experimental Medicine*179(1): 347 -355

Estes, M. K. and Kapikian, A. Z. 2007. Fields Virology. 5th Ed. Williams and Wilkins, Lippincott, Philadelphia. pp 1917–1974.

Evans, R. M., Currie, L., Campbell, A. 1982. The distribution of ascorbic acid between various cellular components of blood, in normal individuals, and its relation to the plasma concentration. *British Journal of Nutrition* 47(3): 473-82.

Failla, M. L. 2003. Trace elements and host defense: recent advances and continuing challenges. *Journal of Nutrition* 133: 1443S-1447S.

Feary, J., Britton J., Leonardi-Bee, J. 2011. Atopy and current intestinal parasite infection: a systematic review and meta-analysis. *Allergy* 66: 569–578.

Feigen, G. A., Smith, B. H., Dix, C. E.1982. Enhancement of antibody production and protection against systemic anaphylaxis by large doses of vitamin C. *Research Communication in Chemical Pathology and Pharmacology* 38(2): 313-33.

Figueiredo, C. A., Barreto, M. L., Rodrigues, L. C., Cooper, P. J., Silva, N. B., Amorim, L. D., Alcantara-Neves, N. M. 2010. Chronic intestinal helminth infections are associated with immune hyporesponsiveness and induction of a regulatory network. *Infection and Immunity*78:3160 –3167.

Finkelman, F.D., Shea-Donohue, T., Goldhill, J. 1997.Cytokine regulation of host defense against parasitic gastrointestinal nematodes: lessons from studies with rodent models. *Annual Review of Immunology* 15: 505-513.

Fleming, J. O. and Cook, T. D. 2006. Multiple sclerosis and the hygiene hypothesis. *Neurology*67:2085–2086.

Flohr, C., Quinnell, R. J. & Britton J. 2009. Do helminth parasites protect against atopy and allergic disease? *Clinical and Experimental. Allergy*39:20 –32.

Fort, M. M., Cheung, J., Yen, D., Li, J., Zurawski, S. M., Lo, S., Menon, S., Clifford, T., Hunte, B., Lesley, R., Muchamuel, T., Hurst, S. D., Zurawski, G., Leach, M. W., Gorman, D. M., Rennick, D. M. 2001. IL-25 induces IL-4, IL-5, and IL-13 and Th2-associated pathologies in vivo. *Immunity*15: 985-995.

Francis, L., Kirunda, B. E., Orach, C. G. 2012. Intestinal Helminth Infections and Nutritional Status of Children Attending Primary Schools in Wakiso District, Central Uganda *International Journal of Environmental Research and Public Health* 9: 2910- 2921.

Fried, B., Reddy, A., Mayer, D. 2011. Helminths in human carcinogenesis. *Cancer Letters.*305:239 –249.

Furnée, C.. A., West, C.E, van der Haar, F., Hautvast, J. G. 1997. Effect of intestinal parasite treatment on the efficacy of oral iodized oil for correcting iodine deficiency in schoolchildren. *American Journal of Clinical Nutrition*: 66(6):1422–1427.

Gallagher, M., Malhotra, I., Mungai, P. L., Wamachi, A. N., Kioko, J. M., Ouma, J. H., Muchiri, E., King, C. L. 2005. The effects of maternal helminth and malaria infections on mother-to-child HIV transmission. *AIDS*19:1849–1855.

Ghio, A. J., Piantadosi, C. A., Crumbliss, A. L. 1997. Hypothesis: Iron chelation plays a vital role in neutrophilic inflammation. *BioMetals* 10: 135–142.

Gladyshev, V. N. 2006. Selenoproteins and selenoproteomes. In: Hatfield, D. L., Berry, M. J., Gladyshev, V. N., eds. Selenium: Its molecular biology and role in human health, 2nd ed. New York: Springer, pp 99-114.

Global Report, 2009. Investing in the future: A united call to action on vitamin and mineral deficiencies. Available at http://www.unitedcalltoaction.org/flashreport.asp

Gordon, S. 2003. Alternative activation of macrophages.*Nature Reviews Immunology* 3(1):23-35.

Granoff, D. M., Feavers, I. M., Borrow, R. 2004. Meningococcal vaccines. In: Plotkin SAO, Walter A, editors. Vaccines. Saunders; Philadelphia, PA. pp. 959–988.

Greene, B. M., Fanning, M. M., Eliner, J. J. 1983. Non specific suppression of antigen-induced lymphocyte blastogenesis in *Onchocerca volvolus* infection in man. *Clinical and Exerimental. Immunology* 52: 259-265.

Grimble, R. F. 1994. Malnutrition and the immune response 2.Impact of nutrients on cytokine biology in infection. *Transactiions of the Royal Society of Tropical Medicine and Hygiene88:*615–619.

Grimble, R. F. 1996. Interaction between nutrients, pro-inflammatory cytokines and inflammation.*Clinical Science.* 91; 121-130.

Groff, J. L. 1995. Advanced Nutrition and Human Metabolism. 2nd ed. St. Paul: West Publishing pp 39-45.

Groothuis, T.A., Griekspoor, A.C.,Neijssen, J.J.,Herberts, C.A.,Neefjes, J. J. 2005. MHC class I alleles and their exploration of the antigen-processing machinery. *Immunology Reviews* 207:60–76.

Gross, C.P. and Sepkowitz, K.A. 1998.The myth of the medical breakthrough: Smallpox, vaccination, and Jenner reconsidered. *International. Journal of InfectiousDiseases*3: 54–60.

Hall, A., Hewitt, G., Tuffrey, V., de Silva, N. 2008. A review and meta-analysis of the impact of intestinal worms on child growth and nutrition.*Maternal and Child Nutrition.* 4(1):118-236.

Hanekom, W. A. 2005. The immune response to BCG vaccination of newborns. *Annals of the New York Academy of Sciences* 1062: 69–78.

Hansel, T. T., Braun, R. K., De Vries, I. J., Boer, C., Boer, L., Rihs, S., Walker, C. 1993. Eosinophils and cytokines. *Agents and Actions Supplements*43:197-208.

Harada, A., Sekido. N., Akahoshi, T., Wada, T., Mukaida, N., Matsushima, K. 1994. Essential involvement of interleukin-8 (IL-8) in acute inflammation. *Journal of Leukocyte Biology* 56(5):559-64.

Harnett, W., Harnett, M. M. 2006. Molecular basis of worm-induced immunomodulation. *Parasite Immunology* 28: 535-543.

Haskell, B. E.and Johnston, C. S. 1991. Complement component C1q activity and ascorbic acid nutriture in guinea pigs. *Americal Journal of Clinical Nutrition* 54(6): 1228S-1230S.

Haswell-Elkins, M. R., Mairiang, E., Miriang, P. 1994. Cross-sectional study of *Opisthorchis viverrini*infection and cholangiocarcinoma in communities within ahigh-risk area in northeast Thailand. *International Journal of Cancer*59:505–509.

Higdon, J. 2000. Vitamin A. Linus Pauling Institute Micronutrient Information Center. Oregon State University. Assessed at: https://lpi.oregonstate.edu/mic/vitamins/ vitamin-A

Hoebe, K., Janssen, E., Beutler, B.2004. The interface between innate and adaptive immunity. *Nature Immunology* 5(10): 971-974.

Hoffmann, K. F., Cheever, A. W., Wynn. T. A. 2000. IL-10 and the dangers of immune polarization: excessive type 1 and type 2 cytokine responses induce distinct forms of

lethal immunopathology in murine schistosomiasis. *Journal of Immunology* 164: 6406-6416.

Höhler, T., Reuss E., Evers, N., Dietrich, E., Rittner, C., Freitag, C. M., Vollmar, J., Schneider P. M., Fimmers R. 2002. Differential genetic determination of immune responsiveness to hepatitis B surface antigen and to hepatitis A virus: a vaccination study in twins. *Lancet* 360:991-995.

Hori, S., Nomura, T., Sakaguchi, S. 2003. Control of regulatory T cell development by the transcription factor Foxp3. *Science*299: 1057-1061

Hotez, P. J., Brooker, S., Bethony, J. M., Bottazzi, M. E., Loukas, A., Xiao S 2004. Hookworm infection. *NewEngland Journal of Medicine*351: 799–807.

Hotez, P. J., Molyneux, D. H., Fenwick, A., Ottesen, E., Ehrlich, S. 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

Hotez, P. J., Molyneux, D. H., Fenwick, A., Kumaresan, J., Sachs, S. E., Sachs, J. D., Savioli L. 2007. Control of neglected tropical diseases. *NewEngland Journal of Medicine*357(10):1018-1027.

Hotez, P. J., Brindley, P. J., Bethony, J. M., King, C. H., Pearce, E. J., Jacobson, J.2008. Helminth infections: the great neglected tropical diseases. *Journal of Clinical. Investigation*. 118:1311–1321.

House, W. A. and Welch, R. M., 1989. Bioavailability of and Interactions between Zinc and Selenium in Rats Fed Wheat Grain Intrinsically Labeled with 65Zn and 75Se *Journal of Nutrition* 119: 916-921.

Hsieh, G. C., Loukas, A., Wahl, A. M., Bhatia, M., Wang, Y., Williamson, A. L., Kehn K. W., Maruyama, H., Hotez, P. J., Leitenberg, D., Bethony, J., Constant, S. L. 2004. A secreted protein from the human hookworm necatoramericanus binds selectively to

NK cells and induces IFN-gamma production.*Journal of Immunology*173(4):2699-704.

Hurst, R. J., Else, K.J. 2012. Retinoic acid signalling in gastrointestinal parasite infections: lessons from mouse models. *Parasite Immunology* 34(7):351–359.

Ibs, K. H. and Rink, L. 2003. Zinc-altered immune function. *Journal of Nutrition* 133.(5): 1452S-1456S.

Ibs, K.H. and Rink, L. 2004. Zinc. In: Hughes DA, Darlington LG, Bendich A, eds. Diet and human immune function. Totowa, New Jersey: Human Press Inc.pp 241-259.

Igietseme, J. U., Eko, F. O., He, Q., Black, C. M.2004. Antibody regulation of T-cell immunity: implications for vaccine strategies against intracellular pathogens. *Expert Review of Vaccine*s3:23–34.

Ilic, N., Colic, M., Gruden-Movsesijan, A., Majstorovic, I., Vasilev, S., Sofronic-Milosavljevic L. 2008. Characterization of rat bone marrow Dendritic cells initially primed by Trichinella spiralis antigens. *Parasite Immunology*30: 491-495.

Imdad, A., Herzer, K., Mayo-Wilson, E., Yakoob, M. Y., Bhutta, Z. A. 2010. Vitamin Asupplementation for preventing morbidity and mortality in children from 6 months to 5 years of age. *Cochrane Database System Reviews* 12: 8524–8524.

Iwasaki, A. and Medzhitov, R. 2004. Toll-like receptor control of the adaptive immune responses. *Nature Immunology*5(10):987-95.

Jackson, J. A., Turner, J. D., Rentoul, L., 2004. Cytokine response profiles predict speciesspecific infection patterns in human GI nematodes. *International Journal of Parasitology*. 34.11: 1237–1244.

Jaimes, M. C., Rojas, O. L., Kunkel, E. J., Lazarus, N. H., Soler, D., Butcher, E. C., Bass D., Angel, J., Franco, M. A., Greenberg, H. B. 2004. Maturation and Trafficking Markers on Rotavirus-Specific B Cells during Acute Infection and Convalescence in Children. *Journal of Virology*, 78(20):10967–10976.

Jalal, F., Metheim, M. C., Agns, Z., Sanjur, D. Habicht, J.P. 1998. Serum retinol concentrations are affected by food sources of b-carotene, fat intake, and anthehelmintic drug treatment. *American Journal of Clinical Nutrition* 68: 623–629.

Jason, J., Archibald, L. K., Nwanyanwu, O. C. 2001. The effects of iron deficiency on lymphocyte cytokine production and activation: preservation of hepatic iron but not at all cost. *Clinical and Experimental Immunology*; 126(3): 466–473.

Jensen, K.J., Karkov, H.S., Lund, N., Andersen, A., Eriksen, H.B., Barbosa A.G., Kantso, B., Aaby, P., Benn C.S. 2014.The immunological effects of oral polio vaccine provided with BCG vaccine at birth: A randomised trial. *Vaccine*32(45): 5949-5956.

Jinabhai, C.C., Taylor, M., Coutsoudis, A., Coovadia, H.M., Tomkins, A.M., Sullivan, K.R. 2001. Epidemiology of helminth infections: implications for parasite control programmes, a South African perspective. *Public Health Nutrition* 4: 1211-1219.

Josefowicz, S. Z., Lu, L. F., Rudensky, A. Y. 2012. Regulatory T cells: mechanisms of differentiation and function. *Annual Review of Immunology*30:531–564.

Imdad, A., Herzer, K., Mayo-Wilson, E., Yakoob, M. Y., Bhutta, Z. A. 2010. Vitamin A supplementation for preventing morbidity and mortality in children from 6 months to 5 years of age. *Cochrane Database Systemic Reviews* 12: 8524–8524.

Ince, M.N., Elliott, D. E., Setiawan, T., Blum, A., Metwali, A., Wang, Y., Urban, J. F. Jr, Weinstock J. V. 2006. Heligmosomoides polygyrus induces TLR4 on murine mucosal T cells that produce TGFbeta after lipopolysaccharide stimulation. *Journal of Immunol*ogy176: 726-729.

Ince, M.N., Elliott, D. E., Setiawan, T., Metwali, A., Blum, A., Chen, H.L., Urban, J.F., Flavell, R.A., Weinstock J.V. 2009.Role of T cell TGF-β signaling in intestinal cytokine responses and helminthic immune modulation. *European Journal of Immunology*. 39:1870–1878.

Kapsenberg, M. L. 2003.Dendritic-cell control of pathogen-driven T-cell polarization.*Nature Reviews Immunology*. 3(12):984-93.

Kaplan, K. J., Goodman, Z. D. Ishak, K. G. 2001. Eosinophilic granuloma of the liver: a characteristic lesion with relationship to visceral larva migrans. *American Journal of Surgical Pathology*25: 1316–21.

Kaplanski, G., Marin, V., Montero-Julian, F., Mantovani, A., Farnarier C. 2003. IL-6: A regulator of the transition from neutrophil to monocyte recruitment during inflammation. *Trends in Immunology* 24: 25–29.

Karki, G. 2017. Ascaris lumbricoides: Morphology, life cycle, pathogenesis, lab diagnosis and Treatment. Online Biology Notes. Retrieved from https://www.onlinebiologynotes.com/ascaris-lumbricoides-morphology-life-cycle-pathogenesis-lab-diagnosis-treatment/

Katz, N., Chaves, A., Pellegrino J. 1972. A simple device for quantitative stool thick-smear technique in Schistosomiasis mansoni. *Revista do Instituto de Medicina Tropica de S'ao Paulo*. 14(6): 397 – 400.

Katz, S. L. Gershon, A. A., Krugman, S., Hotez, P. J. 2004. Krugman's infectious diseases of children. St. Louis: Mosby. pp. 81–97.

Kernan, F.A.J. 1959. The blood fluke that saved Formosa. Harper's Magazine. Harpers Publishers. New York. pp. 45–47.

Khuroo, M. S., Zargar, S. A., Mahajan R. 1990. Hepatobiliary and pancreatic ascariasis in India. *Lancet* 335: 1503–06.

King, C. L. 1996. Cytokine control of parasite-specific anergy in human urinary schistosomiasis. IL-10 modulates lymphocyte reactivity. *Journal of Immunology*156:4715– 4721.

King, C. L. , Low, C. C., Nutman, T. B. 1993. IgE production in human helminth infection. Reciprocal interrelationship between IL-4 and IFN-gamma. *Journal of Immunology*150(5):1873-80.

King, C. L., Medhat, A., Malhotra, I., Nafeh, M., Helmy, A., Khaudary, J., Ibrahim, S., El-Sherbiny, M., Zaky, S., Stupi, R. J., Brustoski, K., Shehata, M., Shata, M.T.1996. Cytokine control of parasite-specific anergy in human urinary schistosomiasis - IL-10 modulates lymphocyte reactivity.*Journal of Immunology.* 156(12):4715-21.

Kirwan, P., Asaolu, S.O., Abiona, T.C., Jackson, A.L., Smith, H.V., Holland, C.V.2009. Soil-transmitted helminth infectionsin Nigerian children aged 0–25 months. *Journal of Helminthology* 00: 1–6..

Kongsbak, K., Wahed, M. A., Friis, H., Thilsted, S. H. 2006. Acute phase protein levels, T. trichiura, and maternal education are predictors of serum zinc in a cross-sectional study in Bangladeshi children. *Journal of Nutrition*136:2262e8.

Koski, K. G. and Scott, M. E. 2001. Gastrointestinal nematodes, nutrition and immunity: breaking the negative spiral. *Annual Review of Nutrition.*21:297–321

Kreider, T., Anthony, R. M., Urban, J. F. Jr, Gause, W. C. 2000. Alternatively activated macrophages in helminth infections. C*urrent Opinion in Immunology* 19(4):448–453.

Kremer, M. and Miguel, E. 2004. Worms: Identifying impacts on education and health in the presence of treatment externalities. *Econometrica* 72(1): 159–217

Krogsgaard, M. and Davis, M. M. 2005. How T cells 'see' antigen. *Nature Immunology*6(3):239-45.

Kruse-Jarres, J. D. 1989. The significance of zinc for humoral and cellular immunity. *Journal of Trace Elements and Electrolytes in Health and Disease* 3(1): 1-8.

Lavelle, E. C., and D. T. O'Hagan. 2006. Delivery systems and adjuvants for oral vaccines. *Expert Opinion on Drug Delivery.* 3:747–762.

Ledizet, M., Harrison, L. M., Koskia, R. A., Cappello M. 2005. Discovery and pre-clinical development of antithrombotics from hematophagous invertebrates. *Cardiovascular and Hematological Agents in Medicinal Chemistry* 3:1–10.

Lee, C. J., Lee, L. H., Lu C. S.2001. Bacterial polysaccharides as vaccines–immunity and chemical characterization. *Advances in Experimental Medicine and Biology* 491:453–471.

Levine, M. M. 2010.Immunogenicity and efficacy of oral vaccines in developing countries: lessons from a live cholera vaccine. *BMC Biology* 8:129.

Levy, R., Shriker, O., Porath, A., Riesenberg, K., Schlaeffer, F. 1996. Vitamin C for the treatment of recurrent furunculosis in patients with imparied neutrophil functions. *Journal of Infectious Diseases* 173(6): 1502-1505.

Liu, A. H. 2007. Hygiene theory and allergy and asthma prevention*.* *Paediatric and Perinatal Epidemiology* 21(3):2–7.

Liu, Y.J., Soumelis, V., Watanabe, N.,Ito, T., Wang, Y. H., Malefyt, R. W., Omori, M., Zhou, B., Ziegler, S. F. 2007. TSLP: an epithelial cell cytokine that regulates T cell differentiation by conditioning dendritic cell maturation. *Annual Review ofImmunology*25:193–219.

Liu, Z., Liu, Q., Bleic,h D., Salgame, P., Gause, W. C. 2010. Regulation of type 1 diabetes, tuberculosis, and asthma by parasites*Journal of Molecular Medicine* 88(1): 27–38.

Lockhart, S. 2003. Conjugate vaccines. *Expert Review of Vaccines* 2:633–648.

Loke, P., Nair, M. G., Parkinson, J., Guiliano, D., Blaxter, M., Allen, J. E.2002. IL-4 dependent alternatively activated macrophages have a distinctive in vivo gene expression phentypa. *BMC Immunology*3:7

Lone, B. A., Ahmad, F., Tak, H. 2015. Impact of Soil-Transmitted Helminth Infections on Haematobiochemical Profile in Children. *Acta Parasitologica Globalis* 6(2): 79-82.

Lora, J. 2004. Ascariasis. Retrieved from https://web.stanford.edu/group/parasites/ ParaSites2005/ Ascaris/JLora_ParaSite.htm.

Lwambo, N. J., Bundy, D. A., Medley, G. F. 1992. A new approach to morbidity risk assessment in hookworm endemic communities. *Epidemiology and Infection*108: 469–481.

Macedo, M. F. and de Sousa, M. 2008. Transferrin and the transferrin receptor: of magic bullets and other concerns*Inflammation & Allergy Drug Targets*7(1): 41–52.

MacLennan, I.C., Toellner, K.-M., Cunningham, A.F., Serre, K., Sze, D.M., Zúñiga, E., Cook, M.C., Vinuesa, C.G. 2003. Extrafollicular antibody responses. *Immunology Reviews.* 194:8–18.

Madhi, S. A., Cunliffe, N. A., Steele, D., Witte, D., Kirsten, M., Louw, C., Ngwira, B., Victor, J. C., Gillard, P. H., Cheuvart, B. B., Han, H. H., Neuzil, K. M. 2010. Effect of human rotavirus vaccine on severe diarrhea in African infants. *The New England Journal of Medicine*. 362(4):289–298.

Madhi, S. A., Kirsten, M., Louw, C., Bos, P., Aspinall, S., Bouckenooghe, A., Neuzil, K. M., Steele, A. D. 2012. Efficacy and immunogenicity of two or three dose rotavirus-vaccine regimen in South African children over two consecutive rotavirus-seasons: a randomized, double-blind, placebo-controlled trial. *Vaccine* 30(Suppl 1):A44-51.

Mahanty, S., Mollis, S. N., Ravichandran, M., Abrams, J. S. Kumaraswami, V., Jayaraman, K., Ottesen, E. A., Nutman, T. B.1996. High levels of spontaneous and parasite antigendriven interleukin-10 production are associated with antigen-specific hyporesponsiveness in human lymphatic filariasis. *Journal of Infectious Diseases*. 173: 769–773

Mahon, B. P., Katrak, K., Nomoto, A., Macadam, A. J., Minor, P. D., Mills, K. H. 1995. Poliovirus-specific CD4+ Th1 clones with both cytotoxic and helper activity mediate

protective humoral immunity against a lethal poliovirus infection in transgenic mice expressing the human poliovirus receptor. *Journal of Experimantal Medicine*181(4):1285-92.

Maizels, R. M. 2005. Infections and allergy - helminths, hygiene and host immune regulation. *Current Opinion in Immunology*17:656–661.

Maizels, R. M., Balic, A., Gomez-Escobar, N., Nair, M., Taylor, M. D., Allen, J. E. 2004. Helminth parasites: masters of regulation. *Immunology Reviews*201: 89–116.

Maizels, R. M., Bundy, D. A., Selkirk, M. E., Smith, D. F., Anderson, R. M. 1993. Immunological modulation and evasion by helminth parasites in human populations. *Nature* 365: 797–805.

Maizels, R. M. and Yazdanbakhsh, M. 2003. Immune Regulation by Helminth Parasites: Cellular and Molecular mechanisms.*Nature Reviews Immunology* 3(9):733-44.

Malley, R., Trzcinski, K. & Srivastava, A. 2005. $CD4^{+}$T cells mediate antibody-independent acquired immunity to pneumococcal colonization. *Proceedings of the National Academy of Science, USA* 102:4848–4853.

Marrack, P., McKee, A. S., Munks, M. W. 2009. Towards an understanding of the adjuvant action of aluminium. *Nature Reviews Immunology* 9(4):287-93.

Maxwell, C., Hussain, R., Nutman, T. B.1987. The clinical and immunologic responses of normal human volunteers to low dose hookworm (*Necator americanus*) infection. *American Journal of Tropical Medicine and Hygiene* 37: 126–34.

McCall, K. A., Huang, C., Fierke, C. A. 2000. Function and mechanism of zinc metalloenzymes. *Journal of Nutrition*.130(5):1437S-1446S.

McKenzie, R.C., Rafferty, T. S., Beckett G. J. 1998. Selenium: an essential element for immune function *Immunology Today*19(8): 342-345.

McSharry, C., Xia, Y., Holland, C. V., Kennedy, M. W. 1999. Natural Immunity to *Ascaris lumbricoides* Associated with Immunoglobulin E Antibody to ABA-1 Allergen and Inflammation Indicators in Children. *Infection and immunity*67(2): 484–489.

McSorley, H. J.and Maizels, R. M. 2012. Helminth Infections and Host Immune Regulation *Clinical Microbiology Reviews*25(4): 585–608.

Miguel, E.A. and Kremer, M. 2003. Worms: identifying impacts on education and health in the presence of treatment externalities. *Econometrica*.72:159–217.

Mocchegiani, E., Malavolta, M., Muti, E., Costarelli, L., Cipriano, C., Piacenza, F., Tesei, S., Giacconi, R., Lattanzio, F. 2008. Zinc, Metallothioneins and Longevity: Interrelationships with Niacin and Selenium. *Current Pharmaceutical Design* 1426: 2719-2732.

Mocchegiani, E., Santarelli, L., Muzzioli, M., Fabris, N. 1995. Reversibility of the thymic involution and age-related peripheral immune dysfunction by zinc supplementation in old mice. *International Journal of Immunopharmacology* 17: 703–718.

Moreau, E. and Chauvin, A. 2010.Immunity against Helminths: Interactions with the Host and theIntercurrent Infections. *Journal of Biomedicine and Biotechnology* Article ID 428593.

Moriguchi, S., Muraga, M. 2000. Vitamin E and immunity. *Vitamins and Hormones* 59: 305-36.

Moore, K. W., de Waal Malefyt, R., Coffman, R. L., O'Garra,A. 2001. Interleukin-10 and the interleukin-10 receptor. *Annual. Reviiew of Immunoogy* 19: 683-765.

Mosmann, T. R and Coffman, R. L. 1989. TH1 and TH2 Cells: Different Patterns of Lymphokine Secretion Lead to Different Functional Properties *Annual Review of Immunology* 7:145-173

Moyle, M., Foster, D. L., McGrath, D. E, 1994. A hookworm glycoprotein that inhibits neutrophil function is a ligand of the integrin CD11b/CD18. *Journal of Biological Chemistry*269: 10008–10015.

Murray, C. J.., Vos, T., Lozano, R., Naghavi, M., Flaxman, A. D., Michaud, C. &Ezzati, M. 2010. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990—2010: a systematic analysis for the global burden of disease study 2010. *Lancet*. 380: 2197-2223.

Mutapi, F,Imai,I. Nausch, N., Bourke, G.D.,Rujeni, N., Mitchell, K.M., Midzi,N., Woolhouse, M. E. J. Maizels,R. M., Mduluza M.2011. Schistosome infection intensity is inversely related to auto-reactive antibody levels. PLoS One 6:e19149.

Mwaniki, D., Omwega, A., Muniu, E., Mutunga, J., Akelola, R., Shako, B., Pertet, A. 2002. Anaemia and Status of Iron, Vitamin A and Zinc in Kenya. Nairobi, Kenya: The 1999 National Survey. Ministry of Health.

Nair, M. G., Cochrane, D. W., Allen, J. E. 2003. Macrophages in chronic type 2 inflammation have a novel phenotype characterized by the abundant expression of YM1 and FIZZ1 that can be partly replicated n vitro. *ImmunologyLetters* 85: 173-180.

Newport, M. J., Goetghebuer, T., Weiss, H. A., Whittle, H., Siegrist, C. A., Marchant, A. 2004. Genetic regulation of immune responses to vaccines in early life. *Genes Immunology.* 5, 122–129.

Nagy, D., Bede, O., Danka, J., SzÉnási, Z. Sipka, S. 2012. Analysis of serum cytokine levels in children with chronic cough associated with *Toxocara canis* infection *Parasite Immunology34*(12): 581-588.

Ngui, R., Lim, Y. A., Chong, K. L, Sek, C. C., Jaffar S. 2012. Association between anaemia, iron deficiency anaemia, neglected parasitic infections and socioeconomic factors in rural children of West Malaysia. *PLoS Neglcted Tropical Diseases*6:e1550.

Nicola, M. L., William, D. F., Malcolm, J. J. 2002. Is there a potential therapeutic value of copper and zinc for osteoporosis? *Proceedings of the Nutrition Society* 61:181–485.

Nmorsi, O. P, G, Irior, D. O., Abu, M. 2010. Serum cytokines profiles in Nigerian children with *Ascaris lumbricoides* infection. *Asian Pacific Journal of Tropical Medicine*3(4) 288-291.

Nmorsi, O. P. G., Isaac, C., Aashikpelokhai, J. S., Ukwandu, N. C. D., 2009. Geohelminthiasis among Nigeria preschool age children. *International Journal of Medical Sciences* 1(10):407 – 411.

Northrop-Clewes, C. A., Rousham, E. K., Muscie-Taylor, C. G., Lunn, P. G. 2001. Anthelminthic treatment of rural Bangladeshi children: effect on host physiology, growth, and biochemical status. *Americal Journal of Clinical Nutrition* 73: 53-60.

Nwaneri, D. U. and Omuemu V. O. 2013. Intestinal helminthiasis and nutritional status of children living in orphanages in Benin City, Nigeria. *Nigerian Journal of Clinical Practice* 16(2): 243 – 248.

O'Dell, B. L.2000. Role of zinc in plasma membrane function. *Journal of Nutrition*130.5:1432S-1436S

O'Garra, A. and Robinson, D. 2004. Development and function of T helper 1 cells.*Advances in Immunology* 83:133-62.

Ogunkambi, A. E. and Sowemimo OA. 2014.Factors associated with ascariasis among pre-school aged children in Ile Ife, Osun State, Nigeria. *Journal of Parasitology and Vector Biology*6(8):117 – 123.

Oluwole, A.S., Ekpo, U. F., Karagiannis-Voules, D. A., Abe, E. M., Olamiju, F. O., Isiyaku, S., Okoronkwo, C., Saka, Y., Nebe, O. J., Braide, E. I., Mafiana, C. F., Utzinger, J., Vounatsou, P. 2015. Bayesian geostatistical model-based estimates of soil-transmitted

helminth infection in Nigeria, including annual deworming requirements. *PLoS Neglcted Tropical Diseases*9.4:e0003740.

Ohri, L.K. and Jonathan G. M. 1999."Polio: Will We Soon Vanquish an Old Enemy?”*Drug Benefit Trends* 11(6): 41–54.

Offit, P. A., Cunningham, S. L., Dudzik, K. I. 1991. Memory and distribution of virus-specific cytotoxic T lymphocytes (CTLs) and CTL precursors after rotavirus infection. *Journal of Virology.* 65: 1318–1324.

Panush, R. S., Delafuente, J. C., Katz, P. Johnson, J. 1982. Modulation of certain immunologic responses by vitamin C. III. Potentiation of in vitro and in vivo lymphocyte responses. *International Journal of Vitamin andd Nutrition Research* 23: 35-47.

Parashar, U. D., Alexander, J. P., Glass, R. I., Advisory Committee on Immunization Practices (ACIP), Centers for Disease Control and Prevention (CDC). 2006. Prevention of rotavirus gastroenteritis among infants and children - Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recommendations and Reports*, 55(RR-12):1-13.

Pashine, A., Valiante, N. M., Ulmer, J. B. 2005. Targeting the innate immune response with improved vaccine adjuvants. *Nature Medicine* 11:S63–68.

Patel, M., Shane, A. L., Parashar, U. D., Jiang, B., Gentsh, J. R., Glass, R. I. 2009. Oral rotavirus vaccines: How will they work where they are needed most?*Journal ofInfectious Diseases* 200: S39-48.

Payne, D. C., Parashar, U. D., 2018. Rotavirus. In: Manual for the Surveillance of Vaccine-Preventable Diseases. Centers for Disease Control and Prevention. Assessed at: https://www.cdc.gov/vaccines/pubs/surv-manual/chpt13-rotavirus.html

Prasad, A. S. 1998. Zinc deficiency in humans: a neglected problem. *Journal of the American College of Nutrition* 17(6):542-543.

Prasad, A. S. 2008. Zinc in human health: effect of zinc on immune cells. *Molecular Medicine* 14(5-6): 353-7.

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. *Parasites and Vectors* 7:37.

Rajagopal, S., Hotez, P. J., Bundy, D. A. 2014. Micronutrient supplementation and deworming in children with geohelminth infections. *PLoS Neglcted Tropical Diseases*8:e2920.

Ramesh, G. N., Malla, N., Raju, G. S., Sehgal, R., Ganguly, N. K., Mahajan, R. C., Dilawari, J. B. 1991. Epidemiological study of parasitic infestations in lower socioeconomic group in Chandigarh (north India). *Indian Journal of Medical Reearch*93: 47- 50.

Randolph, G. J., Angeli, V., Swartz, M. A. 2005. Dendritic-cell trafficking to lymph nodes through lymphatic vessels. *Nature Reviews Immunolog*y 5:617–628.

Rayhan, I. and Khan, S. H. 2006.Factors causing malnutrition among under five children in Bangladesh, Asian Network for Scientific Information.*Pakistan Journal of Nutrition*5(6):558–562.

Rayman, M. P. 2000. The importance of selenium to human health. *Lancet* 356:233-241.

Raz, I., Eldor, R., Naparstek, Y. 2005. Immune modulation for prevention of type 1 diabetes mellitus. *Trends in Biotechnology*23(3):128–134.

Redmond, H. P., Gallagher, H. J., Shou J. 1995.Antigen presentation in protein energy malnutrition.*Cellular Immunology*163*:*80–87.

Reed, S. G., Orr, M. T., Fox, C. B. 2013. Key roles of adjuvants in modern vaccines. *Nature Medicine.* 19.12:1597-608.

Reif, K., Ekland, E.H., Ohl, H., Nakano, H. Lipp, M., Forster, R., Cyster, J. G. 2002. Balanced responsiveness to chemoattractants from adjacent zones determines B-cell position*Nature.* 416:94-99.

Richmond, P., Kaczmarski, E., Borrow, R., Findlow, J., Clark, S., McCann, R., Hill, J., Barker, M., Miller, E. 2000. Meningococcal C polysaccharide vaccine induces immunologic hyporesponsiveness in adults that is overcome by meningococcal C conjugate vaccine.*Journal of Infectious Diseases.*181(2):761-4.

Roberts, L. S. and Janovy, J. Jr. 2009. *Foundations of Parasitology*, 8th Ed.. McGraw-Hill, United States

Rosalind, S.G. 1993.Nutritional Assessment, A Laboratory Manual; Oxford University Press: New York, NY, USA.

Ross, A. C. 2000. Vitamin A status: relationship to immunity and then antibody response. *Proceedings of the Society for Experimental Biology and Medicine.* 119: 303-20.

Rujeni, N. 2012. Atopy is inversely related to schistosome infection intensity: a comparative study in Zimbabwean villages with distinct levels of *Schistosoma haematobium* infection. *International Archives of Allergy and Immunology*158: 288–298.

Ryan, K. J. and Ray, C. G. Eds. 2004. "Enteroviruses". Sherris Medical Microbiology (4th ed.). McGraw Hill. pp. 535–7.

Sabin, E. A., Araujo, M. I., Carvalho, E. M., Pearce, E. J. 1996. Impairment of Tetanus Toxoid – Specific Th1 – like Immune responses in humans infected with *Schistosoma mansoni*. *Journal of Infectious Diseases* 173:269-272.

Sack, D. A., Qadri, F., Svennerholm, A-M. 2008. Determinants of Responses to Oral Vaccines in Developing Countries. *Annales Nestlé England* 66:71–79.

SAGE. 2009. Meeting of the immunization Strategic Advisory Group of Experts – conclusions recommendations. *WHO Weekly Epidemiology Record*84:220–35.

Saleem A. F., Mach, O., Quadri, F., Khan, A., Bhatti,Z., Rehman, N., Zaidi, S., Weldon, W. C., Obestre, S. M., Salama, M., Sutter, R.W., Zaidi, A. K. M. 2015. Immunogenicity of poliovirus vaccines in chronically malnourished infants: A randomized controlled trial in Pakistan. *Vaccines*33(24):2757–2763.

Saunders, K. A., Raine, T., Cooke, A., Lawrence, C. E. 2007. Inhibition of autoimmune type 1 diabetes by gastrointestinal helminth infection. *Infection and Immunity* 75.1:397–407.

Scheller, J., Chalaris, A., Schmidt-Arras, D., Rose-John, S. 2011. The pro- and anti-inflammatory properties of the cytokine interleukin-6. *Biochimica et Biophysica Acta.* 1813(5):878-88.

Schoenborn, J. R. and Wilson, C. B. 2007. "Regulation of interferon-gamma during innate and adaptive immune responses". *Advances in Immunology* 96: 41–101.

Scott, M. E and Koski, K. G 2000. Zinc deficiency impairs immune responses against parasitic nematode infections at intestinal and systemic sites. Journal of Nutrition 130(5):1412S-20S.

Semba, R. D. 2004. Vitamin A. In: Hughes DA, Darlington LG, Bendich A, eds. Diet and human immune function. Totowa, New Jersey: Humana Press Inc. pp 105-131.

Shankar, A. H. and Prasad, A. S. 1998. Zinc and immune function: the biological basis of altered resistance to infection. American *Journal of Clinical Nutrition* 68(2):447S-463S.

Sher, A. and Coffman, R. L. 1992. Regulation of immunity to parasites by T cells and T cell-derived cytokines. *Annual Review of Immunology*10: 385-409.

Sher, A., Pearce, E., Kaye P. 2003: Shaping the immune response to parasites: role of dendritic cells. *Current Opininion in Immunology*; 15:421-429.

Shimizu, J., Yamazaki, S., Takahashi, T., Ishida, Y., Sakaguchi, S. 2002. Stimulation of CD25(+)CD4(+) regulatory T cells through GITR breaks immunological self-tolerance. *Nature Immunol*ogy3:135-142.

Shin, J. L., Gardiner, G. W., Deitel, W., Kandel, G. 2004. Does whipworm increase the pathogenicity of *Campylobacter jejuni*? A clinical correlate of an experimental observation. *Canadian Journal of Gastroenterology* 18:175–77.

Siegrist, C. A 2008. Vaccine Immunology. In: Plotkin SA, Oreinstein WA, Offit PA (Eds): Vaccines. 5th Ed. Saunders Elsevier. Philadelphia. USA.

Simon, J. A and Hudes, E. S. 2000. Serum ascorbic acid and gallbladder disease prevalence among US adults: the Third National Health and Nutrition Examination Survey (NHANES III). *Archives of Internal Medicine* 160(7):931-936.

Smith, K. A., Maizels, R. M. 2014. IL-6 controls susceptibility to helminth infection by impeding Th2 responsiveness and altering the Treg phenotype in vivo. *European Journal of Immunology* 44(1):150-61.

Smout, M. J, Laha, T., Mulvenna, J., Sripa, B., Suttiprapa, S., Jones, A., Brindley, P. J., Loukas, A. 2009. A granulin-like growth factor secreted by the carcinogenic liver fluke, *Opisthorchis viverrini*, promotes proliferation of host cells. *PLoS Pathology*. 5:e1000611.

Sommer, A. 2008.Vitamin a deficiency and clinical disease: an historical overview.*Journal of Nutrition*138:1835–1839,

Spallholz, J. E., Boylan, L. M. and Larsen, H. S. 1990. Advances in understanding selenium's role in the immune system. *Annals of the New York Academy of Science* 587: 123-39.

Stanberry, L. R. and Strugnell, R. 2011. Vaccines of the future: Understanding Modern Vaccines. *Perspectives in Vaccinology*. 1(1): 151-199.

Stetson, D.B., Voehringer, D., Grogan, J.L., Xu, M., Reinhardt, R.L., Scheu, S., Kelly, B. L., Locksley, R. M. 2004. Th2 Cells: Orchestrating Barrier Immunity.*Advances in Immunology* 83: 163– 189.

Stevens, G. A., Finucane, M. M., De-Regil, L. M., Paciorek, C. J., Flaxman, S. R., Branca, F., Peña-Rosas, P. J., Bhutta, Z. A., Ezzat, M. 2013.Global, regional, and national trends in haemoglobin concentration and prevalence of total and severe anaemia in children and pregnant and non-pregnant women for 1995—2011: a systematic analysis of population-representative data. *The Lancet Global Health* 1(1):e16 - e25.

Stevens, R. G., Graubard, B. I., Micozzi, M. S., Neriishi, K., Blumberg, B. S. 1994. Moderate elevation of body iron level and increased risk of cancer occurrence and death. *International Journal of Cancer* 56(3): 364-9.

Stephenson, L. S., Latham, M. C., Ottesen, E. A. 2000. Malnutrition and parasitic helminth infections. *Parasitology* 121:S23-38.

Suchdev, P.S., Davis,S.M., Bartoces, M.., Ruth,L. J., Worrell,C. M.., Kanyi, H., Odero, K., Wiegand,R.E., Njenga,S. M., Montgomery, J. M., Fox L.M. 2014. Soil-Transmitted Helminth Infection and Nutritional Status Among Urban Slum Children in Kenya. *American Journal of Tropical Medicine andHygiene*90(2): 299–305.

Takahashi, T., Tagami, T., Yamazaki, S., Uede, T., Shimizu, J., Sakaguchi, N., Mak, T. W., Sakaguchi, S. 2000. Immunologic selftolerance maintained by CD25(+)CD4(+) regulatory T cells constitutively expressing cytotoxic T lymphocyte-associated antigen 4. *Journal of Experimental Medicine* 192: 303-310

Taren, D. L., Nesheim, M. C., Crompton, D. W. 1987. Contributions of ascariasis to poor nutritional status in children from Chiriqui Province, Republic of Panama. *Parasitology*95: 603–13.

Taylor, B.C., Zaph C., Troy A.E. , Du, Y., Guild, K. J., Comeau, M. R., Artis, D. 2009. TSLP regulates intestinal immunity and inflammation in mouse models of helminth infection and colitis. *Journal of Experimental Medicine* 206:655–667.

Thomas, P. G,, Carter, M. R., Atochina, O., Da'Dara, A. A., Piskorska, D., McGuire, E., Harn, D. A. 2003. Maturation of Dendritic cell 2 phenotype by a helminth glycan uses a Toll-like receptor 4-dependent mechanism. *Journal of Immunol*ogy171: 5837-5841.

Thompson, B. and Amoroso, L. 2011. Combating Micronutrient Deficiencies: In Food-based Approaches. FAO &CABI:432. ISBN-13: 978 1 84593 714 0.

Thornton, A. J., Strieter, R. M., Lindley, I., Baggiolini, M., Kunkel, S. L. 1990. Cytokine-induced gene expression of a neutrophil chemotactic factor/IL-8 in human hepatocytes. *Journal of Immunology*144(7):2609–2613.

Thorpe, R., Ntambue, K., Dumont, J., Thilly, C. H., Diplock, A.T., 1990. Iodine and selenium deficiency associated with cretinism in Zaire. *American Journal of Clinical Nutrition* 52:1087–1093.

Traber, M. G. 2012. Vitamin E. In: Erdman J. W. J., Macdonald, I. A., Zeisel, S. H. Eds. Present Knowledge in Nutrition. 10th ed. Washington, D.C.: Wiley-Blackwell. pp 214-229.

Traber, M. G. and Atkinson, J. 2007. Vitamin E, antioxidant and nothing more. *Free Radical Biology and Medicine 43*(1): 4-15.

Tripathy, K., Duque, E., Bolaños, O., Lotero, H., Mayoral, L. G. 1972.Malabsorption syndrome in ascariasis. *American Journal of Clinical Nutrition*25: 1276–1281.

Truong-Tran, A. Q., Ho, L. H., Chai, F., Zalewski, P. D. 2000. Cellular zinc fluxes and the regulation of apoptosis/gene-directed cell death. *Journal of Nutrition* 130.5:1459S-1466S.

Ugbomoiko, U.S., Ariza, L., Heukelbach, J. 2008. Parasites of importance for human health in Nigerian dogs: high prevalence and limited knowledge of pet owner. *BMC Veterinary Research* 4:49.

United States Agency for International Development. 2014. USAID's NTD Program. Neglected Tropical Diseases. Soil Transmitted Helminthiasis. Retrieved: *http://www.neglecteddiseases.gov/target_diseases/soil_transmitted_helminthiasis/index.html*

van Der Wielen, M., Van Damme, P., Collard, F. 2000.A two dose schedule for combined hepatitis A and hepatitis B vaccination in children ages one to eleven years. *Paediatric Infectious Disease Journal.* 19(9): 848-53.

vanDuin, D., Medzhitov, R., Shaw, A. C. 2006. Triggering TLR signaling in vaccination. *Trends in Immunology* 27, 49–55.

van Panhuys, N.,Prout, M..,Forbes,E. , Min B,Paul, W. E.,Le Gros,G. 2011.Basophils are the Major Producers of IL-4 during Primary Helminth Infection. *Journal of Immunology* 186(5): 2719–2728.

Vekemans, J. Ota, M. O. C., Wang, E. C. Y., Kidd, M,, Borysiewicz, L. K., Whittle, H., Mcadam, K. P. W. J. , Morgan, G., Marchant, A. 2002. T cell responses to vaccines in infants: defective IFNγ production after oral polio vaccination *Clinical and Experimantal Immunology* 127(3): 495–498.

Velázquez, F. R., Matson, D. O., Calva, J. J., Guerrero, L., Morrow, A. L., Carter-Campbell, S., Glass, R. I., Estes, M. K., Pickering, L. K., Ruiz-Palacios, G. M. 1996. Rotavirus infection in infants as protection against subsequent infections.*New England Journal of Medicine*. 335(14):1022-8.

Vinuesa, C. G., Cook, M. C., Angelucci, C., Athanasopoulos, V., Rui, L., Hill, K. M., Yu, D., Domaschenz, H., Whittle, B., Lambe, T., Roberts, I. S., Copley, R. R., Bell, J.

I., Cornall, R. J., Goodnow, C. C.2005. A RING-type ubiquitin ligase family member required to repress follicular helper T cells and autoimmunity.*Nature.*435(7041):452-8.

Wang, L. J., Cao, Y. Shi, H. N. 2008. Helminth Infection and Intestinal Inflammation. *World Journal of Gastroenterology* 14(33): 5125- 5132.

Wang, Y., Huang, D. S., Watson, R. R. 1994. Dietary vitamin E modulation of cytokine production by splenocytes and thymocytes from alcohol-fed mice. *Alcoholism, Clinical and Experimental Research* 18(2): 355-62.

Wang, Y., Yang, J., Gao, Y., Du, Y., Bao, L., Niu, W., Yao, Z. 2005. Regulatory effect of e2, IL-6 and IL-8 on the growth of epithelial ovarian cancer cells.*Cellular and Molecular Immunology* 2(5):365-72.

Ward, R. L., McNeal, M. M., Sheridan, J. F. 1992. Evidence that active protection following oral immunization of mice with live rotavirus is not dependent on neutralizing antibody. *Virology*188:57–66.

Weng, M., Huntley, D., Huang, I. F., Foye-Jackson, O., Wang, L., Sarkissian, A, Zhou, Q., Walker, W. A., Cherayil, B. J., Shi, H. N. 2007. Alternatively activated macrophages in intestinal helminth infection: effects on concurrent bacterial colitis. *Journal of Immunology*179: 4721-4731.

Wilson, E. H., U. Wille-Reece, F. Dzierszinski, C. A. Hunter. 2005. A critical role for IL-10 in limiting inflammation during toxoplasmic encephalitis. *Journal of Neuroimmunology* 165: 63-74.

Wood, R. J. and Ronnenberg, A. G. 2006. Iron. In: Shils ME, Shike M, Ross, A. C., Eds. Modern nutrition in health and disease, 10th ed. Philadelphia: Lippincott Williams & Wilkins. pp 248-270.

World Bank, 1993. World Development Report: Investing in Health. Retrieved: https://openknowledge.worldbank.org/bitstream/handle/10986/5976/9780195208900_ch04.pdf..

World Health Organization. 1994. .Bench Aids for the Diagnosis of Intestinal Parasites; WHO: Geneva, Switzerland.

World Health Organization. 2007. WHO AnthroPlus Software for Assessing Growth and Development of the World's Children; WHO: Geneva, Switzerland,.

World Health Organization. 2008. Global Health Observatory (GHO) data: Nigeria. Retrieved: http://http://www.who.int/gho/countries/nga/country_profiles/en/

World Health Organization. 2011. Vitamin A supplementation for infants and children 6-59 months of age - Guideline. Retrieved: http://www.who.int/nutrition/publications/ micronutrients/guidelines/vas_6to59_months/en/

World Health Organization.2013. Global Vaccine Action Plan 2011–2020. Retrieved: http://www.who.int/immunization/global_vaccine_action_plan/GVAP_doc_2011_2020/en/

World Health Organization. 2016. Soil-transmitted helminth infections. Retrieved:http://www.who.int/mediacentre/factsheets/fs366/en/

World Health Organization. 2016b. Intestinal worms – Epidemiology and Geographical distribution. Retrieved:*http://www.who.int/intestinal_worms/epidemiology/en/*

World Health Organisation. 2016c. Nutrition: Micronutrients. Retrieved: http://www.who.int/nutrition/topics/micronutrients/en/

Yazdanbakhsh, M., van den Biggelaar, A., Maizels, R. M.. 2001.Th2 responses without atopy: immunoregulation in chronic helminth infections and reduced allergic disease. *Trends in Immunoogy*; 22: 372–77.

Zaccone, P., Fehervari, Z., Jones, F. M., Sidobre, S., Kronenberg, M., Dunne, D. W. 2003. Schistosoma mansoni antigens modulate the activity of the innate immune response and prevent onset of type 1 diabetes. *European journal of Immunology* 33(5):1439–1449.

Zaccone P, Fehervari Z, Phillips JM, Dunne DW, Cooke A. 2006 Parasitic worms and inflammatory diseases. *Parasite Immunology* 28(10):515–523.

Zepp, F. (2010): Principles of vaccine design-Lessons from nature. *Vaccine.* 28, C14–C24

PUBLICATIONS

Akinwande K.S. and Arinola O.G. 2016. Serum Cytokine Levels in Intestinal Helminth Infected Pre- School Aged Nigerian Children before and after Oral Rotavirus Vaccination *Archives of Basic and Applied. Medicine.* 4(1): 109 –114.

Akinwande K. S.,MorenikejiO.A., Arinola O. G. 2017. Anthropometric Indices and Serum Micronutrient Status of Helminth Infected School Children from Semi-Urban Communities in Southwestern Nigeria. *Nigerian Journal of Physiological Sciences*. 32(2):195-200.

O.G. Arinola, O.A. Morenikeji, **K.S. Akinwande**, A.O. Alade,O. Olateru-Olagbegi, P.E. Alabi, S.K. Rahamon. 2015. Serum Micronutrients in Helminth-infected Pregnant Women and Children: Suggestions for Differential Supplementation During Anti helminthic Treatment. *Annals of Global Health.*81(5): 705-710.

O.G. Arinola, O.A. Morenikeji, **K.S. Akinwande**, A.O. Alade,O. Olateru-Olagbegi, P.E. Alabi, S.K. Rahamon. 2015. Serum levels of cytokines and IgE in helminth infected Nigerian pregnant women and children. *Annals of Global Health*. 81(5):689-693.

APPENDIX I

MATERNAL AND CHILD HEALTH SURVEY IN GBADA-EFON COMMUNITIES, ONA ARA LOCAL GOVERNMENT, OYO STATE, NIGERIA

QUESTIONAIRE

1. Idetification number………… Name (optional)……………………………………………
2. Home Address / Location………………………………………..Phone no………………………
3. Access to drinkable water Well ☐ Rain ☐ Tap ☐ Others……….…
4. Cooking method Firewood ☐ Kerosene stove ☐ Gas stove ☐ How long?......
5. Latrine Type Bush ☐ Pit ☐ Water closet ☐
6. Animal ownership by parents Cat ☐ Dog ☐ Goat ☐ thers………….……..
7. Parental Education Primary ☐ Secondary ☐ Tertiary ☐ Postgraduate ☐
8. Occupation of Parents Farmiing ☐ Business ☐ Professional ☐
9. Sanitation habit Wash hands with soap: before breastfeeding? ☐ r toilet? ☐
10. Parent's duration of stay in community >1yr ☐ 1-2yrs ☐ 3-4yrs ☐ <5yrs ☐
11. Parent's access to healthcare PHC ☐ Govt Hospital ☐ Private Hospital ☐
12. Solid waste disposal Waste bins ☐ Refuse heaps ☐ Burning ☐ Others…………
13. Do you have children <2yrs of aage? Yes ☐ No ☐
14. Specify: Age of child……………..Sex …………………Name (optional)……….………………

Weight of baby: …………………………Height of baby……………..MUAC……………..…

Baby's common food; Breastfeeding Semi-solid foods ☐ Solid foods ☐ Vegetables ☐

Type of vaccine already given to baby……………………..… .... No of times………..…………..

Any food supplement in baby's food? Yes ☐ No ☐ Specify………..……………

Baby treated with diarrhoea or related illnessYes ☐ How lo☐go?.........……

Baby Dewormed recentlty? Yes NoH☐long ☐?..............................……

APPENDIX II

INSTITUTE FOR ADVANCED MEDICAL RESEARCH AND TRAINING (IAMRAT)

COLLEGE OF MEDICINE, UNIVERSITY OF IBADAN. IBADAN, NIGERIA.

Director: Prof. A. Ogunniyi, B.Sc(Hons), MBChB, FMCP, FWACP, FRCP (Edin), FRCP (Lond)
Tel: 08023038583, 08038094173
E-mail: aogunniyi@comui.edu.ng

UI/UCH EC Registration Number: **NHREC/05/01/2008a**

NOTICE OF FULL APPROVAL AFTER FULL COMMITTEE REVIEW

Re: Rotavirus Specific Serum IgA and Microbiomes in Pre-School Age Children after Rotavirus Vaccination

UI/UCH Ethics Committee assigned number: UI/EC/13/0331

Name of Principal Investigators: **Professor O. G. Arinola**

Address of Principal Investigators: Department of Chemical Pathology, College of Medicine, University of Ibadan, Ibadan

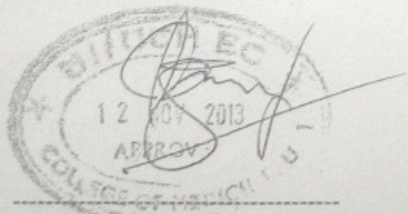
Date of receipt of valid application: 23/09/2013

Date of meeting when final determination on ethical approval was made: **N/A**

This is to inform you that the research described in the submitted protocol, the consent forms, and other participant information materials have been reviewed and *given full approval by the UI/UCH Ethics Committee.*

This approval dates from 12/11/2013 to 11/11/2014. If there is delay in starting the research, please inform the UI/UCH Ethics Committee so that the dates of approval can be adjusted accordingly. Note that no participant accrual or activity related to this research may be conducted outside of these dates. *All informed consent forms used in this study must carry the* UI/UCH EC *assigned number and duration of* UI/UCH EC *approval of the study.* It is expected that you submit your annual report as well as an annual request for the project renewal to the UI/UCH EC early in order to obtain renewal of your approval to avoid disruption of your research.

The National Code for Health Research Ethics requires you to comply with all institutional guidelines, rules and regulations and with the tenets of the Code including ensuring that all adverse events are reported promptly to the UI/UCH EC. *No changes are permitted in the research without prior approval by the* UI/UCH EC *except in circumstances outlined in the Code. The* UI/UCH EC *reserves the right to conduct compliance visit to your research site without previous notification.*



Professor A. Ogunniyi
Director, IAMRAT
Chairman, UI/UCH Ethics Committee
E-mail: uiuchirc@yahoo.com

▪ Drug and Cancer Research Unit Environmental Sciences & Toxicology ▪ Genetics & Cancer Research ▪ Molecular Entomology ▪ Malaria Research ▪ Pharmaceutical Research ▪ Environmental Health ▪ Bioethics ▪ Epidemiological Research Services ▪ Neurodegenerative Unit ▪ Palliative Care ▪ HIV/AIDS

APPENDIX III

TELEGRAMS................ TELEPHONE.................

MINISTRY OF HEALTH

DEPARTMENT OF PLANNING, RESEARCH & STATISTICS DIVISION

PRIVATE MAIL BAG NO. 5027, OYO STATE OF NIGERIA

Your Ref. No. ....

All communications should be addressed to the Honorable Commissioner quoting

Our Ref. No. AD 13/ 479/ 517

21st November, 2013

The Principal Investigator,
Department of Chemical Pathology,
Faculty of Basic Medical Sciences,
College of Medicine,
University of Ibadan,
Ibadan.

Attention: Arinola.O.Ganiyu

Ethical Approval for the Implementation of your Research Proposal in Oyo State

This acknowledges the receipt of the corrected version of your Research Proposal titled: "Rotavirus Specific Serum IgA and Microbiomes in Pre-School age Children after Rotavirus Vaccination."

2. The committee has noted your compliance with all the ethical concerns raised in the initial review of the proposal. In the light of this, I am pleased to convey, to you, the approval of committee for the implementation of the Research Proposal in Oyo State, Nigeria.

3. Please note that the committee will monitor, closely, and follow up the implementation of the research study. However, the Ministry of Health would like to have a copy of the results and conclusions of the findings as this will help in policy making in the health sector.

4. Wishing you all the best,

OYO STATE RESEARCH ETHICAL REVIEW COMMITTEE
Signature & Date
MINISTRY OF HEALTH, IBADAN

Sola Akande
Director, Planning, Research & Statistics
Secretary, Oyo State, Research Ethical Review Committee