HAEMOPARASITOSIS, HELMINTHIASIS AND THEIR PLASMA CYTOKINES RESPONSES IN HUMANS IN BAYELSA STATE, NIGERIA

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BENIN CITY, NIGERIA

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HAEMOPARASITOSIS, HELMINTHIASIS AND THEIR PLASMA CYTOKINES RESPONSES IN HUMANS IN BAYELSA STATE, NIGERIA

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A THESIS WRITTEN IN THE DEPARTMENT OF ANIMAL AND ENVIRONMENTAL BIOLOGY, AND SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (Ph.D) IN PARASITOLOGY, UNIVERSITY OF BENIN, BENIN CITY, NIGERIA.

MARCH, 2021

CERTIFICATION

We certify that this work was carried out by **Dr. Ebube Manfred ODOYA** (DVM) in the Department of Animal and Environmental Biology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria.

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CERTIFICATION OF THESIS

We the undersigned attest and declare that the thesis of **Dr. Ebube Manfred ODOYA** (DVM) titled: Haemoparasitosis, Helminthiasis and their Plasma Cytokines Responses in Humans in Bayelsa State, Nigeria, have successfully passed the anti-plagiarism test and do not violate any copyright regulations.

Prof. Evelyn. U. Edosomwan **Date**
Supervisor

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Prof. Abigail A. Imasuen **Date**

Head of Department

DEDICATION

This thesis is dedicated to the blessed memory of my father Reverend Manfred E. Odoya; my younger brother, Pastor Dianikume Manfred Odoya; both were of the Nigerian Baptist Mission; and to my other siblings who are of blessed memory: Mrs Iziadu Brantley Gival Akuru (Nee Iziadu Manfred Odoya) and Miss Oisoma Manfred Odoya.

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ABSTRACT

The co-infection of malaria parasites and helminths is common in the tropics. Their pathogenicity lies in the infectivity of parasites and their modulation of the host immune system. This study aimed to generate epidemiological data of malaria and helminths in apparently healthy humans in a rural population in Bayelsa State; determine the serum concentration of Interleukin-4, Interferon-gamma -IFN-γ, Macrophage Inflammatory protein-MIP-β/CCL-4 and Eotaxin-3 /CCL 26 in healthy volunteers infected with malaria and helminths and their values after treatment.

Two sets of data; malaria and helminths were generated from school and community based study, carried out between May 2016 and July 2018 in four rural communities- Otuegela, Immiringi, Otuesega and Ibelebiri in which there was ongoing mass deworming and anti-malaria administration. Ethical approval was obtained from the Ethical Committee, College of Medicine, University of Benin, Nigeria (CMS/REC/2017/016). From every participant, blood and stool samples were collected; from 1441 volunteers, age-range 4 – 80 years. Diagnosis of helminths, malaria parasites were by standard procedures. The body mass index (BMI) of children was determined. Measurement of haemoglobin concentration and blood cells was automated (ABX Micros 60). Infected volunteers were treated specifically and after 18 days the second round of blood and stool samples were collected from treated participants only and analyzed. Immune molecules were measured by ELISA (PeproTech, USA) protocols. Data were analysed with the "R" Programme (version 2016) and a P-value of < 0.001 was considered significant.

- The prevalence of helminths was: community-based, 26.0% and school children, 30.3%. Helminths identified in communities were *Schistosoma intercalatum* (10.4%), *Schistosoma mansoni* (4.2%), and a variant of *Schistosoma intercalatum* (0.2%); *Ascaris lumbricodes* (6.5%),), *Trichuris trichiura* (2.5%), hookworm (2.0%) and *Taenia* spp (0.2%). In school-based, *Ascaris lumbricoides* had 10.5%, *Schistosoma mansoni* 8.0 %, *Schistosoma intercalatum* 5.0% and *Strongyloides* 1.0% ; *Trichuris trichiuria* 1.8%, hookworm 1.6%, *Taenia* species 1.3%. In co-infection, 18.0% prevalence was obtained in the community and 10.5% in schools. The prevalence of malaria parasites in community study was

42.0%. In a School-based study, the prevalence of malaria disease was 53.0% and 32.1% for first and second school-based study, respectively. Using Polymerase chain reaction (PCR) *Plasmodium falciparum* was identified at 205 bp and *Plasmodium ovale* at 787 bp. The mean values, before and after treatment for Eotaxin (5718pg/ml/ 5725pg/ml) and MIP-β (344.1pg/ml/642.6pg/ml) were close and had numerous outlines. The concentration of IFN-γ and IL-4 were higher in all categories of infection than after treatment but with no significant difference. IFN-γ had the highest mean expression (135.6pg/ml) in the co-infection group and least (59.8pg/ml) in the population infected by intestinal helminths only. The value for *Plasmodium falciparum* was 84.0pg/ml. Similarly, the expression of IL-4 was highest (68.8pg/ml) in co-infection and lowest (40.3 pg/ml) in helminths infected group. The value of IL-4 for those infected by *Plasmodium falciparum* only was 61.0pg/ml. There In all study groups, IFN-γ and IL-4 were positively correlated before and after treatment; which was significant (r = 0.60) in those infected by *P. falciparum* only. After treatment, the correlation between IFN-γ and IL-4 was significant in those who were treated for malaria infection (r = 0.7) and those who were treated for co-infection of *P. falciparum* and helminths (r = 0.6). There was a decrease in values of platelets, White Blood Cells and granulocytes during infection but platelet count was reduced after anthelminthic treatment and increased after anti-malaria administration**.** The ova of 7 species of helminths were diagnosed in this study. Treatment lowered the concentration of IFN-γ and IL-4 immune molecules in serum, which is of clinical relevance. This study proves that sub-clinical infection brought about a low concentration of IL-4/IFN-γ, altering their counter-inflammatory properties. They rather depended on each other positively. The clinical consequence of IL-4 suppression is the disability in class switch: antibody production is suppressed, resulting in susceptibility to infectious diseases. The presence of *P.ovale* in co-infection with *P. falciparum* is significant for the epidemiology and control of malaria disease in the Niger Delta.

CHAPTER ONE

INTRODUCTION

1.1. Background of Study

It is common in humans to have a concurrent simultaneous infection of malaria parasites and intestinal helminths (Ojurongbe *et al.,* 2011). More so, many infectious diseases in the sub-tropics share similar clinical features (Schwan *et al.,* 2012). In sub-Saharan Africa, malaria and typhoid fever are treated together because both diseases display similar signs and symptoms (Nsutebu *et al.,* 2003). Similarly, non-infectious disease conditions such as snake bites, burns, trauma, and haemorrhagic shock have related molecular pathogenesis by eliciting pro-inflammatory responses in the body (Clark *et al.,* 2004). As stated, in most tropical regions, malaria and intestinal helminth infections are common (Hartgers *et al.*, 2009). Malaria is a blood-borne parasitic disease, with over two billion people at risk, and 100 million developing clinical disease (Stoppacher and Adams, 2003). The female anopheles mosquitoes are vectors of malaria parasites transmission in humans. There are four species of malaria parasites which infect humans; namely *Plasmodium falciparum, P. ovalae, P. malariae* and *P. vivax*. An additional simian species, *P. knowelsi* is also known to infect humans (Monday, 2014). Among the species, only *P falciparum* causes fatal malaria disease in humans.

Helminths, on the other hand, are highly infectious (Lustigman *et al.*, 2012) infecting over a billion people worldwide (Wang *et al.,* 2008). There are different species of intestinal helminths: *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworms (*Necator americana* and *Ancylostoma duodenale*), *Schistosoma mansoni, Hymenoelepis nana*, *Strongyloides* and *Enterobius vermicularis* (Harhay *et al.,* 2010).

Human physiological response against malaria infection is by the production of pro-inflammatory cytokines such as interferon-gamma (IFN-γ) (Hartgers *et al.,* 2009). Also, the body responds to helminths infection is by expression of anti-inflammatory cytokines such as interleukin 4 (IL-4). The outcome of malaria and intestinal helminths infections depends on the ability of the parasites to modulate the immune system of the host (Hartgers *et al.,* 2009). To check the virulence of infective parasites, human elicits rapid inflammatory responses to infectious agents like *Plasmodium falciparum* and these inflammatory responses are deleterious and must be regulated to prevent severe disease condition. For instance, in cerebral malaria infection, the body responds with the uncontrolled production of pro-inflammatory cytokines but in simultaneous infection with intestinal helminths, counter-inflammatory cytokines are secreted in high concentration to check the activities of pro-inflammatory cytokines thereby decreasing the pathology of cerebral malaria in children (Besnard *et al.,* 2015). Although host innate immunity could clear malaria parasites through the spleen and liver (White, 2017) a thin balance is therefore required in the expression of counter-inflammatory cytokines response in determining the outcome of malaria in patients (Riley *et al.,* 2006).

A network of cytokines is involved in the pathogenesis of malaria (Wroczyńska *et al*., 2005) and intestinal helminthiasis (Moreau and Chauvin, 2010). For effective body defence, both cells mediated immunity and humoral antibody is required and they depend on the alpha and beta arms of clusters of differentiation-4 (CD4+ lymphocytes). There are two functional subsets of CD4+ lymphocytes which can be identified through the secretion of thymus helper 1(Th1) and thymus helper 2 (Th2). Th 1 secretes interferon-gamma while Th2 produces interleukin-4 (Prakash *et al.*, 2006). Early response of Th 1 cytokines such allows for quick clearance of blood parasites. Thus, Th1 cytokines are important in controlling early parasitaemia and later

counterbalanced by the Th2 response which leads to antibody production (Torre *et al.*, 2002). This explains that a switch from Th1 to Th2 is important and is a function of anti-inflammatory molecules. Early response of Th2 cytokines in *Plasmodium* infections has an antagonizing effect on Th1responses, resulting in susceptibility to infection. Besides, the concentration and time of response of Th1 and Th2 are essential in the control of malaria infections (Kobayashi *et al.*, 1996).

Besides *Plasmodium* infections, pro-inflammatory cytokines responses are observed in intestinal parasitic infections. (Hamm *et al.,* 2009) reported that co-infections of *Schistosoma haematobium/ Schistosoma mansoni* with protozoa parasites and *Necator americana* generated pro-inflammatory cytokines and chemokines responses, but after antiparasitic treatment, the pro-inflammatory response was reduced while Th2 responses appreciated. Th2 immunity is protective against helminth infections and it requires innate cells to induce (initiate) and to play effector (conclude) roles at different stages of helminth infections (Maizels *et al.,* 2012). Th2 immune responses reduce the virulence of helminths infections in the intestinal tract and repairs damage in the tissues caused by the parasites (Pulendran & Artis, 2012). The cytokines associated with Th2 responses are IL-4, IL-5, IL-9, and IL-13 (Berger 2000). IL-4 is associated with the proliferation of goblet cells in the intestine, which is a pathological response to the expulsion of worms in the intestine (Marillier *et al.,* 2008). IL-4 is a pleiotropic cytokine that regulates the growth and functions of B and T cells. Among other functions, interleukin - 4 is anti-inflammatory and suppresses inflammation associated with Th 1 responses but in colitis induced by the hapten, Kampten et al, (2005) observed an earlier response Th2, which was attributed to high expression of IL-4. This explains that high expression of IL-4 in the mucosa of the intestine of healthy mice elicited pro-inflammatory responses, which (Van Kampen *et al.,* 2005) reported

that the magnitude and duration of inflammatory activities were lower compared to colitis induced by classical pro-inflammatory cytokines such as IL12.

Malaria parasites are intracellular and it's an infection in humans' features prominently through Th1 responses by the production of pro-inflammatory immune molecules (Noone *et al.,* 2013). Interferon-gamma (IFN-γ) is a pro-inflammatory immune molecule induced primarily by interleukin 12 (IL-12) and it is the key cytokines in both blood and liver stages of *Plasmodium* infections (King and Lamb 2015). During an innate immune response, natural killer cells secrete IFN-γ but in adaptive responses, CD4+ and CD8+ cells take over its production (Dohi *et al.,* 2000). For IFN-γ to be protective it must be expressed early in infection (D'Ombrain *et al.,* 2008). Simultaneous infection of malaria parasites and other pathogens could account for the conflicting data of the time of expression of immune molecules and their level of productivity (King and Lamb, 2015). Nonetheless, the early expression of IFN-γ in *Plasmodium* infections confers protectivity (D'Ombrain *et al.,* 2008). It is documented that mice deficient in IFN-γ have more prolonged blood-stage parasitaemia (van der Heyde *et al.,* 1997). In Mali, it was reported that Fulani tribe have the natural resistance to *Plasmodium* infections and this was positively correlated with elevated IFN-γ reported (Mccall *et al*., 2010).

Another pro-inflammatory immune molecule is Macrophage inflammatory protein (MIP). Serum value for MIP-β also known as CCL-4 was reported to be elevated during acute inflammation (O'Grady *et al.,* 1999) but after antiparasitic treatment, the concentration was reduced. (Hamm *et al.,* 2009) reported a reduction in the seral value of MIP-1β and cellular production of interferon-gamma (IFN-γ) after Praziquantel treatment for *Schistosoma mansoni* infection.

Eotaxin is an immune molecule with chemotactic properties. It is expressed continuously in the intestine as well as other organs in the body and has been identified as chemo-attractants for eosinophils (Matthews *et al.,* 1998). Interleukin-4 is reported to induce the expression of eotaxins during an inflammatory response (Mochizuki *et al.,* 1998).

Generally, immune protectivity requires an organized network of multiple pathways of molecular mechanisms, cells phenotypes and haematological changes which are the common complication of infectious diseases (Kotepui *et al.,* 2015). In helminths infections, there is an increase in eosinophil and anti-inflammatory cytokines as well as expression of antibody isotopes immunoglobins (IgG1, IgG4 and IgE (Makepeace *et al.,* 2012). Malaria infection alters the values of blood cells such as red blood cells (RBC), leukocytes and Platelets (Kotepui *et al.,* 2015). These changes are due to factors that include a level of endemicity of malaria disease, malaria immunity, nutritional status and background haemoglobinopathy (Erhart *et al.,* 2004).

In heavy malaria parasitaemia, neutrophils count is usually high white blood cells count on lymphocytes and monocytes are significantly reduced. RBCs and platelet counts, as well as haemoglobin concentration, are reduced in a patient with Plasmodium parasitaemia (Kotepui *et al.,* 2015). Thrombocytopenia was reported as the most consistent parameter in malaria infection (Gupta *et al.,* 2013). Taking into account that co-infection of helminths and *Plasmodium* parasites are frequently observed in the tropics, and infection with *Plasmodium* elicit a different degree of inflammatory responses in the host and such responses needs to be regulated to reduce tissue damage, and these regulatory cytokines are evoked by helminths infections to prevent severe clinical complication.

Considering, therefore, the pathophysiological relationship between malaria and intestinal helminths infection, the following questions are needful about this study.

In humans, could the population infect by intestinal helminths higher than *Plasmodium* parasites? What could be the predominant species of intestinal helminths in single and multiple infections? What could be the predominant species of *Plasmodium* in the study area? Could treatment of infections reduce the concentration of immune molecules?

What could be the association between pro-inflammatory and anti-inflammatory cytokines in infections and after treatment? Could pro-inflammatory cytokines express more in patients with Plasmodium infections? Are anti-inflammatory cytokines expressed more in patients with intestinal helminth infections? Could the expression of cytokines influence by the sex and age of the individual volunteer? Which types of blood cells are associated with *Plasmodium*/ intestinal helminth infections? Which blood cells are most affected after antiparasitic treatment of malaria or helminths?

1.2. Justification of the Study

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Both malaria and intestinal helminths are endemic in Niger Delta, Nigeria. Malaria is a life-threatening disease that affects anyone bitten by infected female anopheles mosquitoes. The initial signs of malaria disease are fever, headache and chills which if untreated progress to a severe disease condition. In children, common symptoms are anaemia, respiratory distress and much worse, parasites could invade the brain tissues to cause cerebral malaria. Similarly, in adults, many organs are target resulting in their reduced functionalities. The intensity of transmission depends on the presence of the parasites, the human host and a suitable environment for the sustenance of the vector.

Intestinal helminths are nematodes, trematodes and cestodes which are highly infectious, with high morbidity and some mortality. The symptoms of intestinal helminths infection are confined to the intestinal tract with numerous systemic involvements. Transmission to humans is through exposure to contaminated soil, water, or food. Signs and symptoms are often mild, but severe complications may develop in some cases.

The physiological responses of humans' body to infection of malaria and intestinal helminths are counter-inflammatory, such that a malaria infection response is pro-inflammatory while helminths infections are anti-inflammatory responses.

The study was conducted in rural communities where there were several built (man-made) and natural factors in the environment that can sustain the transmission of malaria and intestinalparasites

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1.3 Aim and Objectives

This study aimed to generate epidemiological data for malaria parasites and intestinal helminths in humans in Bayelsa State, Nigeria and evaluate their responses to serum cytokines (IFN-γ and IL-4) and chemokines (MIP-β and Eotaxin-3) in malaria and helminths parasitic infections.

The specific objectives of the study were to:

1. determine the prevalence of malaria parasites, intestinal helminths and the co-infection of malaria parasites and intestinal helminths infections in Bayelsa States.
2. determine the sex and age, weight and height of children infected with *Plasmodium* parasites in Bayelsa State.
3. determine the species of malaria parasites by Polymerase Chain Reaction(PCR).
4. determine the relationship between the concentration of cytokines (IL-4, IFN-y, MIP-β/CCL-4 and eotaxin-3 /CCL 24) in co-infection of intestinal helminths/ malaria parasites; in.
5. determine the relationship between the concentration of cytokines (IL-4, IFN-y, MIP-β/CCL-4 and eotaxin-3 /CCL 24) in co-infection with intestinal helminths/ malaria parasites after treatment.
6. determine the relationship of the different blood cells- WBCs, lymphocytes, Platelets and haemoglobin concentration during infections.
7. determine the relationship of the different types of blood cells after treatment.

CHAPTER TWO

LITERATURE REVIEW

2.1 Epidemiology of *Plasmodium*

Plasmodium, a genus of parasitic protozoan belongs to the class Haematozoa (Babesia inclusive), which infects the blood of the vertebrate hosts and is the causative agent of malaria (Arora and Arora, 2010). *Plasmodium* could be described as eukaryotic microbe (compartmentalized cell) - it has a distinct nucleus and cytoplasm and possesses features capable of penetrating host cells and modifies the cellular function of the host (Wiser, 2018). There are about 150 named species of *Plasmodium* which infect various species of vertebrate (Arora and Arora, 2010). The five species of *Plasmodium* known to cause malaria in human: *Plasmodium vivas*; is the most widely distributed form; *Plasmodium falciparum* is common in tropical region and it produces the most severe disease, *P. ovale* is relatively uncommon but confined to central west Africa and South Pacific island; and *P.malariae* and *P.knowlesi* (Rogers, 2019) *P. knowlesi* is also known as simian malaria and it is considered zoonotic (CDC, 2019). In the rate of morbidity and mortality, malaria, caused by *Plasmodium* is the most virulent parasitic disease, with over two billion people in the world at risk, 100 million developing clinical disease and 1.5- 2.7 million people die every year of the disease (Stoppacher and Adams, 2003).

There are three stages in the life cycle of malaria parasites; the gametocytes stage, the sporozoites and merozoites stages. The gametocytes stage develops within mosquitoes into sporozoites. The sporozoites migrate to the mouthparts of mosquitoes where they are transmitted when they bite humans. In humans, sporozoites invade liver

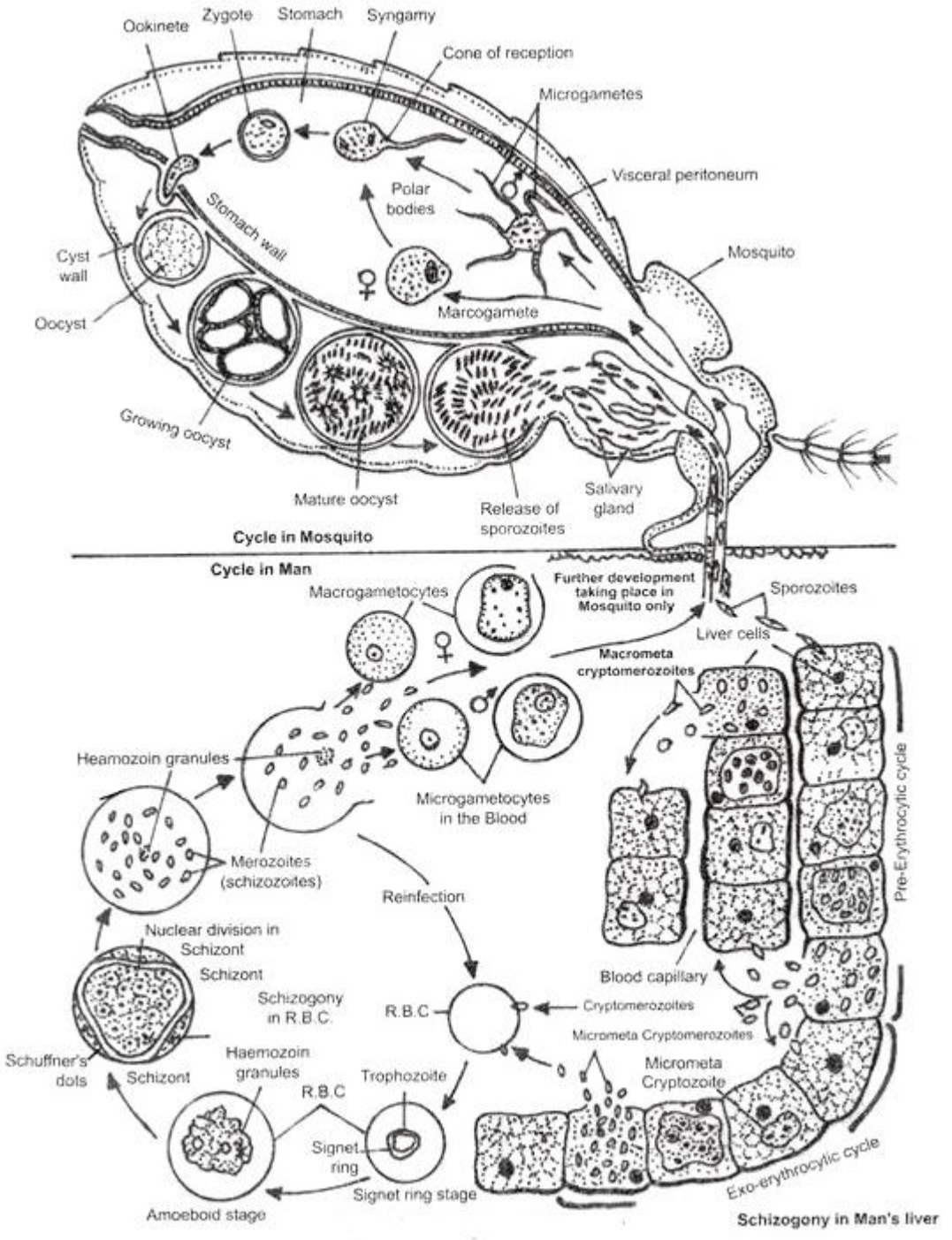

Figure 2.1: Life cycle of *Plasmodium* parasites.

(Rogers, 2019)

parenchyma, feed on liver cytoplasm to grow into large round cryptozoites. By nuclear fission in a process called schizogony crptozoites develop into round multinucleated cryptomerozoites which are liberated into the bloodstream where they invade red blood cells and some re-invade hepatocytes. Merozoites inside red blood cells (RBC) divide rapidly, destroying the RBC and invade uninfected red blood cells. Eventually, some of the merozoites develop into gametocytes which are taken up by mosquitoes while feeding on humans.

In humans toxins (haemozoin waste) are liberated by merozoites-destroyed RBC to cause a cycle of chills-and-fever.Tertian malaria: this runs a cycle of 48 hours and is expressed in infections of

P. vivas. *P. ovale* and *P. falciparum.*

Quartan malaria occurs every 72 hours and it's typical of *P.malariae*.

P. knowelsi runs the daily cycle of chill-and-fever.

Symptoms of chills-and-fever develop after 5 erythrocytic cycles. Moreso, malaria parasites have a latent period of 10-15 days, from infection to manifestation of symptoms (Rogers, 2019).

2.2 Distribution of Malaria

In the past, malaria was endemic in much of North America, Europe and part of Nothern Asia. Today, malaria is restricted to tropical and subtropical areas, in Korean Penninsula and in areas with an altitude below 1,500m (Herrera *et al*., 2012). By the early 1970s, malaria disease had been eradicated from the whole of Europe, most of

North America, most of the Caribbean and large parts of South America; from Singapore, Japan, Korea, Taiwan and Australia (Bryan, 1996). The position of eradication, however, has deteriorated in some countries where control efforts were not sustained, such that the disease has returned to areas previously considered free of malaria. In Africa south of the Sahara, not much eradication effort has been attempted; the distribution of malaria has remained unchanged (Russel, 2018). The reasons malaria is endemic could be attributed to fast deforestation, poor agricultural practices, climate change, unstable political structure and surge in parasite drug resistance and the resistance of vectors to insecticides (Herrera *et al*., 2012). The reasons why malaria disease is a great burden in Africa is an efficient mosquito that transmits the infection, a high prevalence of the most deadly species of the parasite, favourable climate, weak infrastructure to address the disease, and high intervention costs that are difficult to bear in poor countries (CDC, 2019).

2.2.1 Malaria: North America Perspective

Faust (1951) described the prevalence of infectious disease to depend on the co-ordination of two essential factors: the presence of the vector and the parasitic agent. There is evidence from Medical Historian that malaria parasites existed in the United States prior to the advent of the European Conquerors. Nonetheless, malaria became fully established when a virulent strain of parasites was imported by the immigration of Negro slaves from the west coast of Africa, and for three centuries constituted one of the very important diseases in the United States. By 1850, malaria was well established in the US except in Northernmost New England, the Alleghany Islands, the Rocky Mountains, the Great Island Desert and the western Sierras. In the warmer areas, malaria

was hyperendemic, mildly endemic in the cooler region but frequently develop to epidemic proportion during the warm summer months (Faust, 1951).

The elimination of malaria in the US was through the establishment of National Malaria Eradication Programme, which was a cooperative undertaking by States and Local Health Agencies and the Communicable Disease Center (CDC) of the US public health services. The procedure was by the application of DDT to the interior surfaces of rural homes or entire premises in counties where malaria was reported to have been prevalent in recent years. Several house spray applications had been made by the end of 1949. Drainages and other breeding sites for mosquitoes were sprayed (occasionally from aircraft) with insecticides. Total elimination of transmission was slowly achieved. In 1949, malaria was no longer a public health problem in the country. By 1951, CDC gradually withdrew from active participation in the operational phases of the program and shifted its interest to surveillance, and in 1952, CDC participation in operations ceased altogether (CDC, 2019). That means that by 1951, malaria was considered eliminated from the United States. However, to the present day, malaria remains a major field of activities at CDC (CDC, 2019). Presently, the majority of malaria infections in the United States occur among persons who have travelled to regions with ongoing malaria transmission.

2.2.2 Malaria in Australia

According to (Walker, 1998) the three elements necessary for the establishment of endemic malaria (infected humans, susceptible mosquitoes and a suitable climate) occur in parts of Australia. Most outbreaks of Malaria in Australia have occurred in small, isolated populations of the Northern Territory. The last epidemic was at the Roper River Mission in the Northern Territory in 1962 but untill 1983, Australia was

declared free of endemic malaria, although sporadic cases of local transmission do occur. Between 2010- 2011 there were 414 notification of malaria and the infecting species were *P.falciparum* and *P.vivas* (Wright *et al*., 2012). However, laboratory investigations have revealed that a number of local *Anopheles* species are susceptible to infection, and *Anopheles annulipes*, *An. bancroftii*, *An. farauti* and *An. hilli* have been possibly involved in field transmission (Rusell, 2018).

2.2.3 Malaria in Africa

The African Region accounts for 85% of malaria cases and 90% of malaria deaths worldwide (WHO, 2017). This is because malaria infection in Africa is caused by the most virulent strain, *Plasmodium falciparum*. Another reason is, *Anopheles gambiae* is the most effective vector of malaria transmission and it is most widespread and most difficult to control. Between 2000 and 2013 there was a decline in estimated cases of malaria death by 54% in the African Region. However, an estimated 163 million cases of malaria were reported in 2013, with 528000 deaths (WHO, 2017).

In the five northernmost countries of Africa- Algeria, Egypt, Libya, Morocco and Tunisia, malaria has been well controlled possibly because *Plasmodium vivas* is the predominant parasite and transmitted by the less tractable breed of mosquitoes. However, surveillance effort is maintained in these countries to prevent the reintroduction of malaria parasites to the local population, and the introduction of other species of mosquitoes capable of transmitting malaria more effectively

(World Health Report, 2002). However, malaria is endemic in most of the offshore islands Africa- Sao Tome and Principe and São Tiago Island of Cape Verde. Malaria transmission was controlled in the 1950s in Mauritius although, after the cyclone in

1982, sporadic outbreaks of *vivax* malaria occur. Afterwards, there has been a steady decrease in cases and risk is now extremely low. Seychelles has been free of malaria since 1930, and malaria vectors are believed to no longer exist there (World Health Report, 2002).

Ayele (2012) reported that more than 75% of the total area of Ethiopia is endemic in malaria transmission. Malaria presents a serious health problem in Ghana; it is hyperendemic with a crude parasite rate ranging from 10 – 70% and *Plasmodium falciparum* the major malaria parasite, dominating. However, Gakpey *et al*. (2016) reported that in-patient death cases from malaria in Ghana dropped from 3,259 in 2011 to 2,815 in 2012. Also, malaria deaths among children under five years for the same periods were 1,539 for 2011 and 1,129 for 2012, whereas death among pregnant women due to malaria was 918 for 2011 and 476 for 2012, the decline was attributed largely to the successful distribution of long-lasting insecticide-treated mosquito nets. In 2012, Ghana carried out national coverage of two persons per long-lasting insecticide-treated mosquito net campaign during which 12 million people were covered, which represents the coverage of 24 million of the population.

The following reports were cases of malaria transmission in South African National Department of Health Malaria Control Programme; the number of cases in January 2012 as 2267 with 16 deaths, and in February 2012 as 968 cases with 10 deaths. A total of 67% of February cases came from the endemic regions of KwaZulu-Natal, Mpumalanga and Limpopo, while 31% came from Gauteng, mainly in travelers (Peter, 2012).

2.2.4 Malaria in Nigeria

Malaria disease has public health significance in Nigeria. In Nigeria, malaria accounts for more cases and deaths than any other country in the world. About 97% of Nigeria's population is at risk of malaria. Just about 3% of the population lives in the malaria-free highlands. The hyper-endemicity of malaria in Nigeria with over 300,000 deaths per year could be compared with 215,000 deaths per year in Nigeria from HIV/AIDS (Nigeria Malaria Fact sheeth, 2011).

In urban cities and rural settlements in Nigeria, a number of common factors may contribute to the maintenance of malaria disease, vector and the high level of transmission: both identifiable among these are ditches, gutters and other man-made temporary pools of water, some of which results from broken pipes and improper or blocked drainage systems (Aderounmu, 2012). Poverty along with ignorance, urban farming, deteriorating infrastructures and overcrowding in the urban cities form a vicious cycle that makes it extremely difficult to control malaria and perhaps other diseases. Other key factors contributing to this unfortunate situation are social attitude including, poor water delivery systems, lack of sanitation service development, inadequate knowledge of mosquito-human interaction and haphazard urbanization (Aderounmu, 2012).

In Abeokuta, south-west Nigeria, work done by Okonko *et al.* (2009) between January 2002 and December 2004, using thick film techniques showed high malaria parasites prevalence of 81.5%. The total prevalence of 80.4% was observed in a study carried out by Kalu *et al*. (2012) in Abia State, Eastern Nigeria. *Plasmodium falciparum*, *Plasmodium vivax* and *Plasmodium malariae* occurred in urban areas with higher infection of *Plasmodium falciparum* in Aba and Umuahia. Wariso and Oboro (2015)

observe a prevalent rate of *Plasmodium* parasites of 67.5% among blood donors at the University of Port Harcourt, Nigeria. A hospital-based study among pregnant women in a semi-urban city in northern Nigeria, the incidence of malaria parasitaemia was found to be 308 (42.4%); *Plasmodium falciparum* and *Plasmodium malariae* accounted for 302 (98%) and 6 (2%) of the isolates, respectively (Jombo *et al*., 2011). In an investigation (Zama *et al.,* 2013) in Sokoto, Nothern Nigeria, 228 blood donors were screened for malaria parasites, 74 of the subjects (32.5%) were positive for Malaria and 154 (67.5%) tested negative.

The prevalence rate of malaria infection in southern Nigeria is higher than is found in the northern region due difference s in predisposing factors. Constant minimum temperatures of 16–18.8°C (optimum: 20–30°C) and high humidity for several weeks are pre-conditions for vectorial transmission of malaria [Fritz, 2005]. In a very recent study, a high malaria prevalence of 68.0% was reported by Edosomwan *et al*. (2020) in children in an Internally Displace Persons (IDPs) camp in Benin City.

2.3 Epidemiology of Intestinal Helminthes

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Normal Stoll in 1947 in his monograph titled "This Wormy World" reckoned that over 25% of the human population harboured one or more helminths infection. The leading helminths were *Ascaris*, hookworm and *Trichuris* (Arora and Arora, 2010). However, the recent improvement in social and economic conditions, as well as the implementation of control strategies in some regions of the world, has altered the global picture of intestinal helminths infections (De Silva *et al.,* 2003). An estimate was presented in 1994 (Chan *et al.,* 1994) building upon application of geographical information system to derive updated atlases of helminths infections.

In the United States, there are few diseases caused by parasitic, bacterial and congenital infections, (known as the neglected infections of poverty and this refers to infections like helminthiasis). Helmiths infections promote poverty, because of their impact on child development, pregnancy outcomes, and worker's productivity (Hotez and Ferris, 2006). Poverty in the US is focally concentrated into several defined geographic regions, each with unique socioeconomic characteristics. Six major distresses regions of poverty in the US have been identified: Mississippi Delta, Native American tribal lands, Appalachia, the borderland between the United States and Mexico, black metro adjacent to the Great Lakes and in the Northeast (Glasmeier, 2006). In 2000, it was estimated that 169,000 housing units in Appalachia had no indoor plumbing for toilet facilities (Glasmeier, 2006). The high rate of ascariasis in the 1930s in Appalachia was associated with poverty and poor sanitation (Kitchen *et al.,* 2000). A recent report shows that 14% of school children in Eastern Kentucky were infected with *A. lumbricoides* and 13% with *T.trichiuria*. *Strongyloides* is highly under-reported in the US partly because of the difficulty of diagnosing the infection by faecal examination (Kitchen *et al.*, 2000). The overall prevalence of *Strongyloides* was approximately 1% (Douce *et al*., 1987). A high percentage of patients infected with *Strongyloides* were older males, most of whom had underlying chronic illnesses (Douce *et al*., 1987). The Mississippi Delta is composed of the delta region Louisiana, Arkansas and Tennessee is one of the poorest regions in the United States characterized by inadequate housing and high poverty rate (Glasmeier, 2006). Hookworm infection and malaria combined to produce a generation of anaemic, weak and unproductive children (Humpherys, 2001).

Beginning in 2000, there was increase in the migration of refugees from sub-Saharan Africa with an estimation of 70,000 refugees annually (Barnett, 2004). Almost all the population of immigrants tested was seropositive for both schistosomiasis and

strongyloidiasis (Posey *et al.*, 2007). Seropositivity was a result of prolonged untreated infections (Franco-Paredes *et al.,* 2007). In a sero-prevalence study of rural Ventura County, California, it was found that 1.8% of the population had cysticercosis (Degiorgio *et al*., 2005). Owing to the endemicity of intestinal helminths, in 1999, CDC recommended that all refugees older than two years of age departing to the United States from sub-Saharan Africa or Southeast Asia receive a single dose of 600mg of albendazole (CDC, 1999).

Neglected tropical diseases are not exclusive to low-income countries (Hotez and Kamath, 2009). In the United State, such infections account for a sizeable but largely hidden disease burden among minority populations living in poverty and among people of African descent in particular. Similar infection occurs in Europe. Thus as an effort to control neglected tropical disease expands through Africa, parallel effort should also target poor and forgotten people in wealthy nations (Hotez and Kamath 2009).

Hotez and Gurwith (2011) reported the endemicity of soil-transmitted helminth infection in Eastern Europe. Selected foodborne helminths such as trichinellosis, taeniasis and echinococosis had high incidence rate. Turmoil and economic recession following the war in the Balkans, the fall of communism helped to promote their high incidence and prevalence rate. The vulnerable groups noted were the Roma, orphans destined for international adoption and some immigrant groups.

Current global estimates of helminths infection show a remarkable decline in prevalence in both the Americas and Asia. The decline is a reflection of the improvement in socioeconomic status and good implementation of national control activities. For instance, in regions of rapid economic development such as a shift from an agrarian to a suburban economy in Jiangsu Province, China, a reduction in helminths infection was

observed. Japan and Korea are common examples with improvement in living standards and specific control measures (de Silva *et al*., 2003). However little change in prevalence rates have been observed in sub- Saharan Africa, rather with population increase, the absolute numbers of infections has dramatically increased. Thus the poorest people in the world suffer the greatest burden of infectious diseases, including helminths infections. In the developing world, inadequate water and sanitation and crowded living conditions, combined with lack of access to health care and low level of education make the poor particularly susceptible to helminths infections.

In the Caribbean communities, *Trichuris trichiura* had equal prevalence with *Ascaris lumbricoides* (Bundy, 1986). South Saint Lucia showed a prevalence of 45% helminths infections. Helminths identified included *A.lumbricoides*, hookworm, *Strongyloides stercoralis*, *Trichuris trichiura, Enterobius vermicularis*, *Taenia solium,* and *Schistosoma mansoni*. The study recorded more helminths infection in females (51.9%) than males (39.2%). Population examined with single infection (50.9%), with double infection (10.1%) and triple infection (0.5%). Egg intensity of helminths showed most cases with light infections. In the report, *Ascaris* had six cases of heavy infections and twelve cases of moderate infections. Hookworm had six cases of heavy and six moderate infections. *Trichuris* had only one heavy and five moderate infections; *Schistosoma mansoni* recorded only one moderate infection. A similar report showed a high prevalence of the three major intestinal helminths infections (ascariasis, trichuriasis and hookworm) in the poorest areas of the Caribbean- Dominican Republic, Haiti, and Jamaica, Barbados and Trinidad and Tobago as well as other parts of the world ((De Silva *et al*., 2003). Although the study by Kurup and Hunjan (2010) showed a low rate of infection of *Schistosoma mansoni* in children with only one moderate case, the

authors suggested prompt treatment of infected case because such could act as a potential infective pool for the rest of the community.

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high poverty rate (Glasmeier, 2006). Hookworm infection and malaria combined to produce a generation of anaemic, weak and unproductive children (Humpherys, 2001). Beginning in 2000, migration of refugees from sub-Saharan Africans marked increased with an estimation of 70,000 refugees annually (Barnett 2004).

In a South Indian fishing village (Naish *et al*., 2004) studied the prevalence, intensity and risk factors for soil-transmitted helminths infection among school children aged 5-9 years and showed 92.6% prevalence with *Ascaris lumbricoides* as the predominant parasites (prevalence of 91%), followed by *Trichuris trichiura* (72%) and hookworm (54%). The intensity of infection was highest in *A.lumbricoides* among younger children than older children. While aggregation of helminth infection was observed, hookworm infection was more highly aggregated than either *A. lumbricoides* or *T. trichiura,* suggesting vulnerability of affected population to anaemia.

The epidemiology of soil-transmitted helminths in Malaysia was investigated by (Ahmed *et al.,* 2011) and observed high prevalence among the rural Aborigines, estate workers and in urban slums and squatter areas. *Trichuris trichiura* was the most prevalent helminth in Malaysia ranging from 2.1% to 98.2%, followed by *A. lumbricoides* with a prevalence of 4.6 - 86.7%. Hookworm was the least prevalent (37.0%).

In western Tajikistan, the overall prevalence of infection with helminths was 32.0%. The most common helminth was *Hymenolepis nana*, with 25% prevalence. The prevalence rate of *Ascaris lumbricoides*, hookworm and *Enterobius vermicularis* were below 5% (Matthys *et al.,* 2011).

In the Middle East and Asia an estimated 700 million people are at risk of infection and more than 200 million are infected annually (Aryeetey *et al.,* 2000).

In sub-Saharan Africa, helminth infection is common (De Silva *et al.,* 2003). There are several million school-aged children in sub-Saharan Africa and about half are infected with one or more of these intestinal helminths (Brooker *et al.,* 2006). Children are more affected than adults fundamentally due to behavioural, biological and environmental bases. By behaviour, children are known to rarely employ good sanitary habits. Children who live in a crowded area are highly susceptible to helminths infection. Immunologically, however, helminths infections elicit an immune response from the host to expel the helminths (Harhay *et al.,* 2010).

Commonly, the prevalence curves of *Ascaris lumbricoides* and *T trichiura* go with a steady rise from infancy to mid-teens and then declining into the adult age classes. In hookworm, the curves begin from early childhood and adolescence but rise through adult life and may decline at some point in life. *Strongyloides* lack data on the age-associated prevalence (Olsen *et al.,* 2009).

A connection exists between prevalence and intensity of infection in helminthiasis. Prevalence represents the population affected but intensity of infection is reflected in morbidity, thus while with a prevalence of 80%, measurable morbidity may perhaps be 15% (Harhay *et al.,* 2010).

Global epidemiology of cestodes proves that cestodes infections are not frequent in children living in tropical poverty as they have limited access to meat that serves as a source of infection (Hall *et al.,* 2008). Adolescent who earn money through menial jobs however, can purchase “Suya” meat (from beef) and improperly-boiled pork which are

sources of cestode infection. Low rate of tapeworm infection is observed in large Muslim communities in Africa and in parts of Asia (Ito *et al.,* 2003). Detection of *Taenia* in stool samples is not common. However, the incidence of neurocysticercosis (larvae of *T. solium* infection) can be an indicator of the presence of this tapeworm and is a leading cause of epilepsy in the developing world (Zoli *et al.,* 2003). Human infection of intestinal flukes is by consuming food or water that is contaminated with intermediate hosts such as infected fish and aquatic animals. *Schistosoma mansoni* causes intestinal schistosomiasis and is found in sub-Saharan Africa, Eastern Mediterranean and Latin America (Steinmann *et al.,* 2006). A recent review suggests that fascioliasis and clonorchiasis (live in the biliary system) are increasing in global prevalence with approximately 750 million people at risk of infection (Keiser *et al.,* 2011).

2.5 Africa Perspective

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In Zanzibar study on prevalence of intestinal helminth infection was carried out among school children (Stephanie *et al.,* 2009; Knopp *et al.,* 2010), reported *Trichuris trichiura* (46.6%), Hookworm (21.6%), *Ascaris lumbricoides* (16.9%) and *Strongyloides stercoralis* (10.2%) prevalence. Similar work conducted in school children in Cape Town, South Africa showed an overall prevalence of STH infection of 55.8%, with *Trichuris trichiura*, 50.6%; *A. lumbricoides*, 24.8%; *Hymenolipis nana*, (2.2%); *Trichostrongylus*, 0.1% and hookworm, 0.08%. The authors attributed sewage sludge and effluent to be a source of infection, besides promiscuous defecations and poor urban sewage disposal facilities (Adams *et al.,* 2006). Similarly, the relationship between intestinal parasitism and excreta disposal technologies was investigated in three countries in Africa- Botswana (Gaborone), Zambia (Ndola) and in Ghana (Kumasi).

Despite the difference in toilet types in the study design, no evidence was obtained of any differences in intestinal parasitism among the studied groups. The study suggested that provisions of superior water and sanitation facilities to houses may not be protected from infection if the overall level of faecal contamination of the environment is high (Bain *et al.,* 2014).

A study by Glickman *et al.* (1999) on 286 school children aged 1-18 years in rural Guinea in West Africa showed 53% prevalence of one or more intestinal helminths. The epidemiology of hookworm infection and its contribution to anaemia among preschool children on the Kenyan coast was investigated by Brooker *et al.* (2006). The authors observed that infection with each species of helminths increased with age and the prevalence of heavy infection with hookworm and mean intensity of hookworm were markedly age-dependent.In Nigeria, there are over 15 million individuals suffering from ascariasis alone while there are many more others with strongyloidiasis, trichuriasis, enterobiasis, hookworm, tapeworm infections among others (Karshima, 2018). Several reports exist on the epidemiology of intestinal helminthes, including reports on helminth morbidity and mortality in Nigeria (Anosike *et al.,* 2006). Helminthiasis in selected children seen at the University of Benin Teaching Hospital had an overall prevalence of 22.2% with *A. lumbricoides* having the highest prevalence of 11.1%, followed by hookworm, 5.8%; *Trichuris trichiura*, 3.8%; *Schistosoma mansoni*, 1.0%; *Strongyloides stercoralis*, 0.5% while multiple infections was 5.3%. More females (13.5%) had infection than males (8.7%) but not statistically significant. Mother's level of education more than father's level seemed to influence the pattern of intestinal helminthiasis among the children but the association was not statistically significant (Wagbatsoma and Aisien 2005). In a study by Ekpo *et al*. (2013) and Mogaji et al. (2017) the prevalence of helminthes infection was 54.9% in the urban government school, 63.5%

in rural government school and 28.4% in the urban private school. Prevalence of infection in the government owned-schools was significantly higher than in the private school. Lack of portable water, poor sanitation of latrines, unavailability of hand washing soap and presence of garbage around the school compound was believed to be responsible for the burden of parasitic infection. Similarly**,** the burden of parasitic infection was observed to be greater in government schools than in private schools in Ile Ife, Osun State. Sowemimo and Asaolu (2011) reported an overall prevalence of 34.4%, with *A. lumbricoides, T. trichiura* and hookworm were 33.2%, 3.7% and 0.7%, respectively. The authors reported a prevalence of soil transmitted helminthes of 47.8% in government schools and was significantly higher than in private schools (16.1%). A co-infection of *Ascaris lumbricoides* and *Trichuris trichiura* (6.8%) was most common. The nutritional status of children infected with intestinal helminths was studied in Ibadan, Oyo State by Adekunle (2002). From the results, children with heavy worm burden were shorter in height and lower in weights than non-infected children. The author attributed the infection with *Ascaris lumbricoides* (39% prevalence) and hookworm (26.5% prevalence) as cause of remarkable effect on the weight gain, skin and mouth condition of the infected children compared to non infected children. The study to determine the parasitological contamination of fruits was carried out in Ibadan Oyo State (Alli *et al.,* 2011). Pineapple had the highest number of intestinal parasites which was 62.5% positive and the lowest was water melon (12.5%). Helminths identified were ova of *A. lumbricoides* (55.9%), ova of hookworm (32.3%) and larvae of *S. stercoralis* (11.8%). The consumption of fruits not properly washed could account for the risk of acquiring intestinal parasites. Vegetables too need proper washing before consumption. Edosomwan *et al.,* (2011) conducted similar study on the prevalence of geohelminths eggs contamination of vegetables in Edo State, Nigeria. Prevalence of

geohelminth was higher in the rainy season (61.5%) than in the dry season (38.5%). The researchers observed 7 species of helminths eggs, namely, *A. lumbricoides* (35.5%); hookworm (26.0%); *T. trichiura* (15.0%); *Toxocara canis* (14.1%); *S. stercoralis* (7.3%); *Taenia* spp. (2.9%) and *Enterobius vermicularis* (1.28%).

In Konduga, Borno State, north east Nigeria, intestinal helminthiasis was high (85.7% - 77.7%) among Almajiri pupils 13-16 age. *Ascaris lumbricoides* had the highest prevalence of 19.1% while the least prevalence was 3.5% for *T. trichiura* (Damen *et al.,* 2011). Bala and Yakubu (2010) in a survey among school-aged pupils in Jos North, Nothern Nigeria reported that out 647 pupils examined for helminth infections, 23.11% were infected by helminth parasites including *Ascaris lumbricoides* (8.46%), *Entamoeba histolytica* (5.64%), Hookworm spp. (5.14%), *Giardia* (2.61%) and *Trichuris trichiura* (1.29%). The result showed that 144 of the pupils had hookworm ova in their stool samples.

In Ebenebe town, Anambra State, eastern Nigeria, a study of geohelminth infection of pupils showed a prevalence of 53.6% in soil and 87.7% in stool samples. Prevalence in soil samples showed 24.0% of *Ascaris lumbricoides* eggs, 25.9% of hookworm eggs and 9.5% larvae of *S. stercoralis*. The ova recovered from stool samples were eggs of *Ascaris lumbricoides* (54.1%); hookworm (45.5%), *Trichuris* (18%) and larvae of *Strongyloides* (5.9%). Male pupils had higher infection with *A. lumbricoides* and *T. trichiura* than females. However, female pupils had higher prevalence in infection with hookworm and *Strongyloides*. Mixed infection of *Ascaris lumbricoides* and hookworm was observed (Chukwuma *et al.,* 2009). Similarly, study of geohelminths in contaminated foci in Abua, Niger Delta, helminthes eggs identified were: *A. lumbricoides* (44%), hookworm, 29%, *T.trichiura*, 17% and larvae of hookworm, 54%. The occurrences of geohelminthes were a result of feacal contamination; human feaces

was found at common spots and was attributed to be responsible for the high worm egg recovery (Amadi *et al.,* 2010). A study of hookworm infection was conducted in Amassoma community in the Niger Delta, Nigeria. Agi and Awi-Waduu (2008) reported a prevalence of 34.9% of hook worm infection, with 33.6% infection in males and 36.6% in females. Highest incidence (12.2%) of hookworm infection occurred in September and lowest (1.8%) was in February. Infectivity was noted to be all round the year and was attributed to the high moisture content of the soil and temperature of the study area. However, the incidence rate was lowest in February, at which time moisture content of soil was significantly low, suggesting a suitable time for intervention. Apart from human factors involved in hookworm transmission, rainfall patterns and high tropical temperatures constitute independent variables which enhanced or sustained infection.

The pathology associated with hookworm infections declines with increase in age. Agi and Awi-Waduu (2008) reported increasing prevalence of hookworm infection with age in females and was indicative of a strong female association with soil through farming. The influence of community ecology and behavior on the bionomics of intestinal helminthiasis was investigated by Amadi *et al*. (2010) in four communities in Asari-toru, Niger Delta Nigeria. A total prevalence of 55.3% was reported and helminth species involved were *Ascaris*, hookworm, *Trichuris, Strongyloides and Enterobius*.

Life cycle of some intestinal helminths

Ascaris lumbricoides

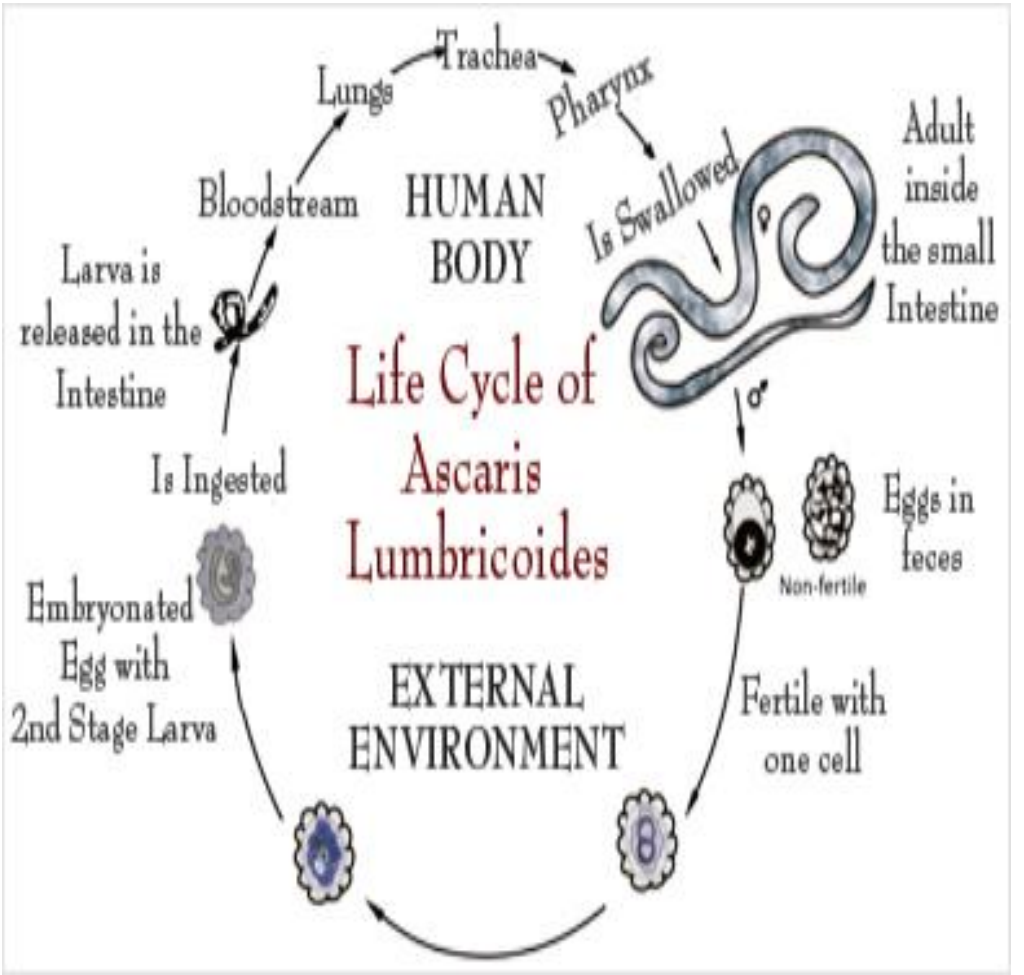

Figure 2.2: Life cycle of *A.lumbricoides.* Source: Dreamstime.com. 20/09/19

Trichuris

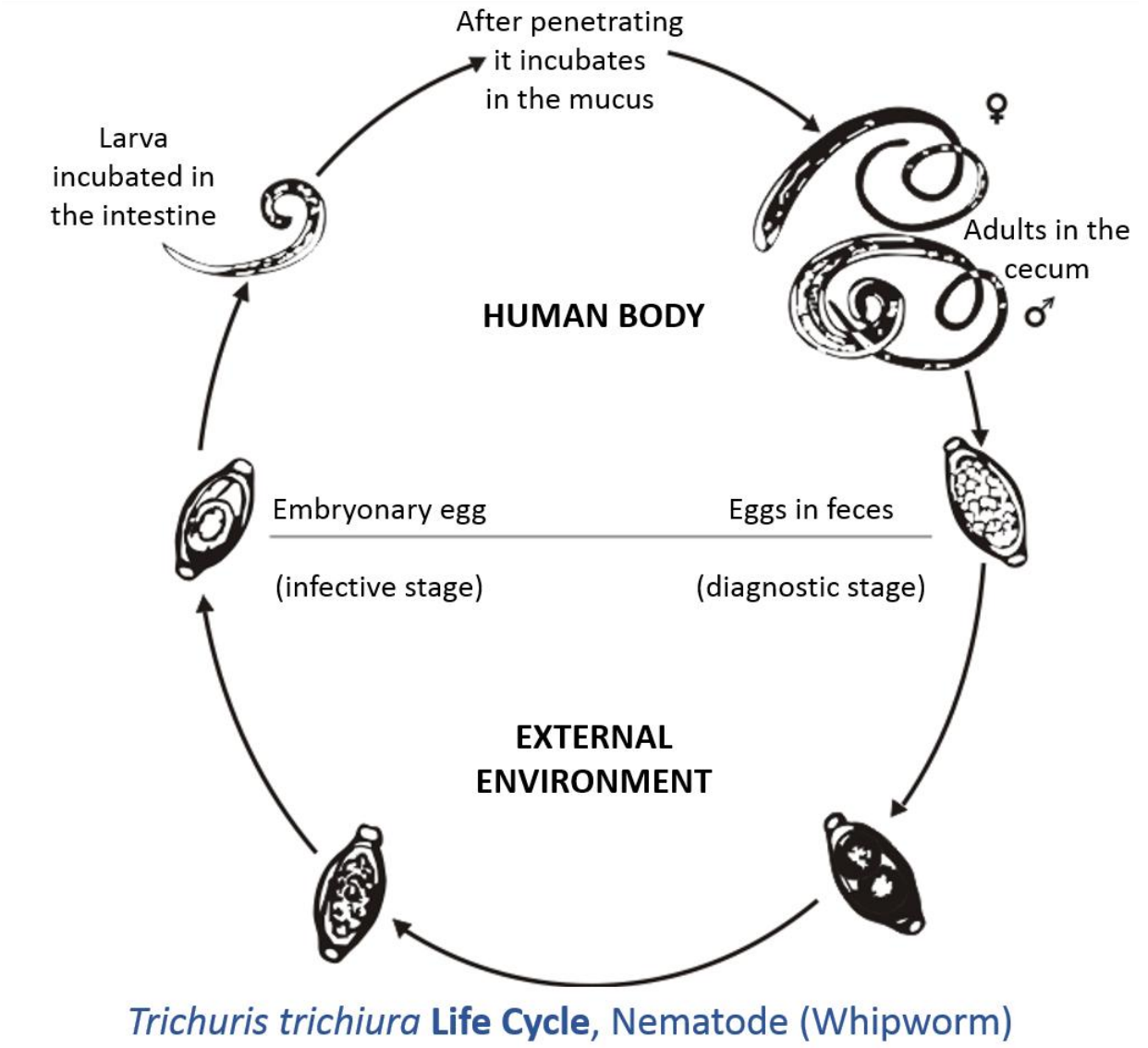

Figure 2.3. Lifecycle of *Trichuris trichiura*. Source: Dreamstime.com. 20/09/19

Hookworm

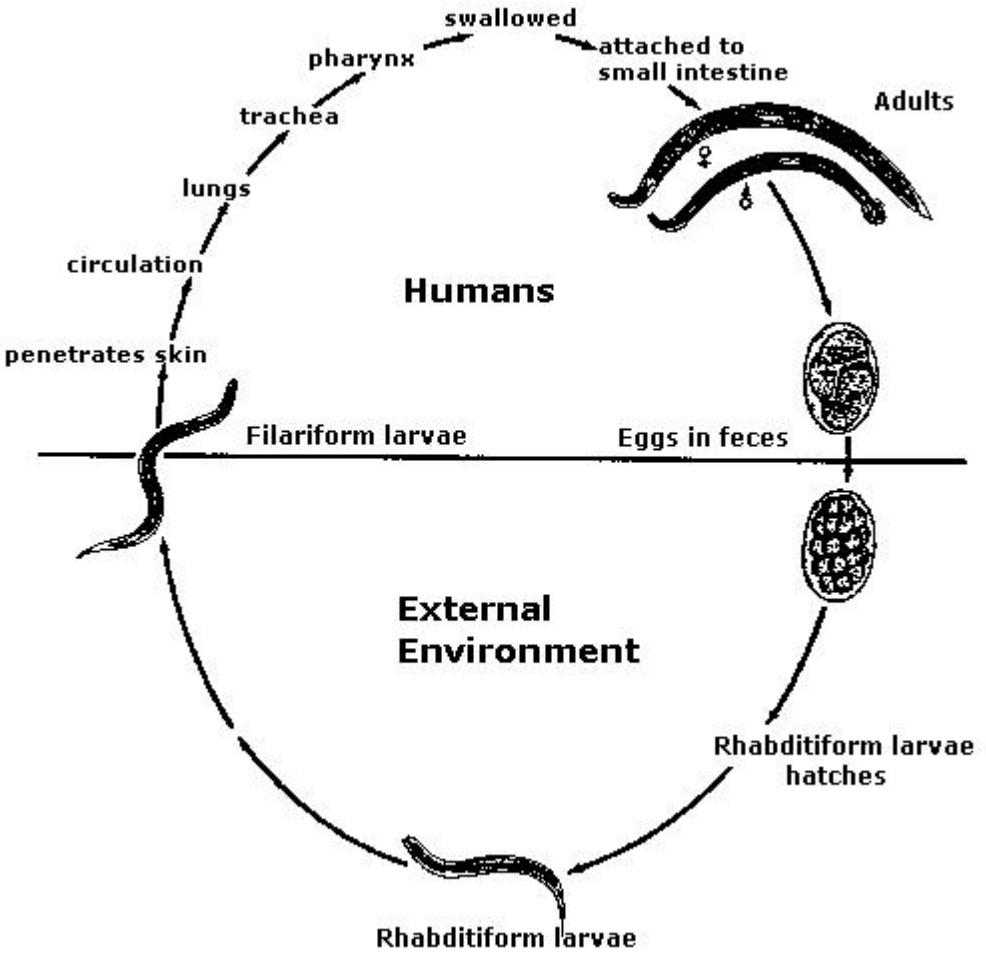

Figure 2.4 : Life cycle of Hookworm. Source: Dreamstime.com. 20/09/19

Tapeworm

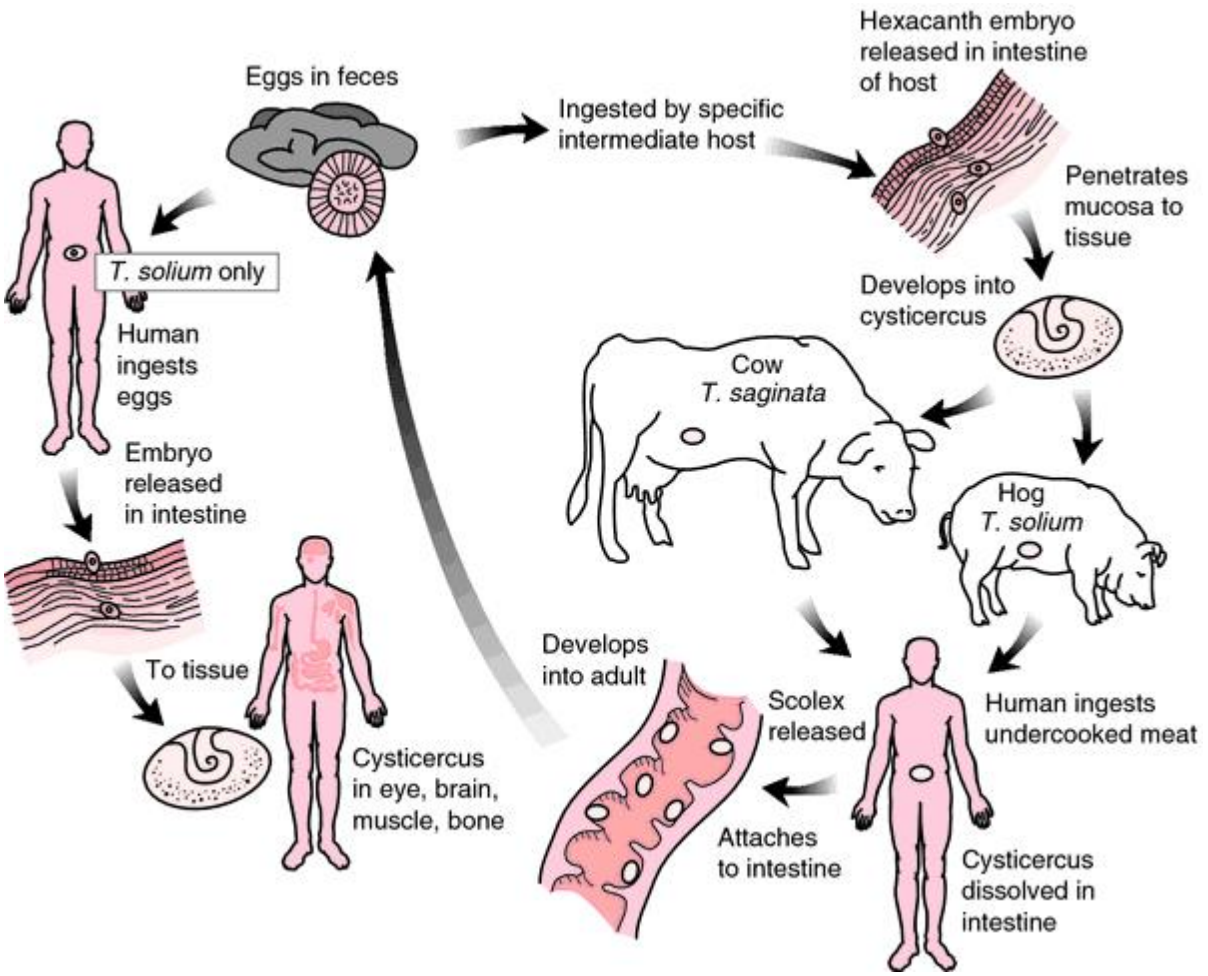

Figure 2.5: Life cycle of Tapeworm

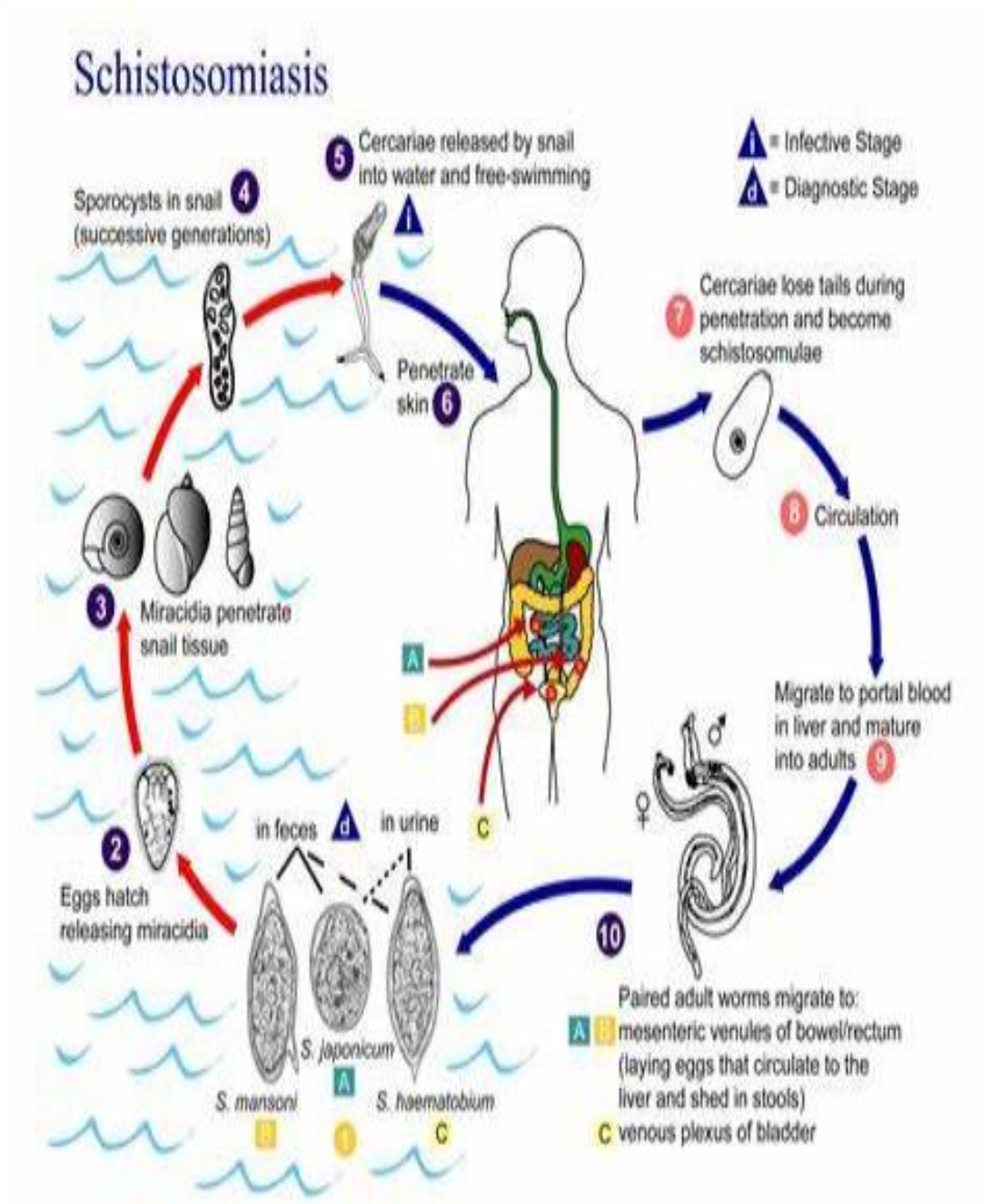

Figure 2.6: Life cycle of schistsosome. Source: Dreamstime.com. 20/09/19

2.6 Immunology of Intestinal Epithelial Cell (IEC)

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Intestinal helminths are common pathogens in the intestine. The intestinal epithelial cell (IEC) establishes a barrier which determines the persistency and susceptibility of helminths (Atis and Grencis, 2008). Besides, IECs expresses a wide range of cytokines and chemokines including tumour-necrosis factor (TNF), transforming growth factor-β (TGF-β), IL-1, IL-6, IL7, IL8, IL10, monokine induced by interferon-gamma (MIG), CXCL9, CXCL10, CXCL 11, MIP-3α and fractalkine among others that can promote the recruitment or activation of immune cells (Hershberg and Mayer, 2000). When IECs are exposed to intestinal helminths, they are activated to secrete a wide range of physiological molecules, including regulatory cytokines. The effectiveness of these effectors varies depending on the species of helminths. Effective responses result in the expulsion of helminths via an adaptive mediated immunity. The host establishes immunological memory and is able to expel future challenge infections. If for whatever reasons, protective immunity is not established, chronic infection then ensues. To prevent immunopathological damage in the host, regulation is required. It is undoubtedly clear that intestinal helminths are not commensals, but pathogens, and as such the host body would recognize it as antigenic and potentially dangerous (Hershberg and Mayer, 2000).

2.7 Clinical Features of Human Intestinal Helminthiasis

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The disease condition, *ascariasis* is caused by both adult worm and migratory larvae (Arora and Arora, 2010). Majority of infection are asymptomatic. Pathophysiological condition is; direct tissue damage, the immunological response of the host to infection with larvae, eggs or adult worm; psychological effect (Nmorsi, 2010) and nutritional sequel of infections. According to Nmorsi (2010), the mechanical effect is due to worm

clog causing blockage of the intestinal lumen. Worm clog can occur in response to treatment of hookworm infection with tetrachloroethylene. Tissue damage can occur when worm invades the intestinal lumen into the peritoneal where they migrate to the common bile duct, they obstruct the bile flow resulting in obstructive jaundice and recurrent cholangitis. Other organs invaded could cause tissue reaction and non specific symptom. Brief respiratory symptoms can occur in the previously infected host during the stage of larval migration through the lungs causing pneumonitis known as Loffler's syndrome (Ledders and Weller, 2009) or Loffler's pneumonia (Nmorsi, 2010). This is a result of localized accumulation of fluid and blockage of air space in association with the massive deposit of white blood cells. Such an allergic reaction is a result of the host response to metabolites produced by the worm (Nmorsi, 2010). The nutritional effect is due to the fact that *Ascaris lumbricoides* feed on the content of the intestinal lumen; therefore, it is associated with malnutrition and underdevelopment in children (Nmorsi, 2010). Children go through psychological trauma when they vomit, as migratory larvae pass through the bronchial tree. Occasionally adult worm may be passed out from the rectum, from the nose and from the mouth (Nmorsi, 2010).

Hookworm infection is "silent and insidious", extremely dangerous. Major morbidity is caused by adult ingestion of blood resulting in intestinal blood loss, iron deficiency anaemia and protein malnutrition (Bethony *et al.,* 2006). Physically, this manifests as facial and peripheral oedema, eosinophilia, and pica. There is the paleness of tongue, conjunctiva, and skin and associated oedema of feet and ankles (Arora and Arora, 2010). General pathology includes ground itch allergic reaction at the site of skin penetration cough and pneumonitis as a result of larval migration through the alveoli and travels up the trachea. When the larvae arrive at the gut, it provokes diarrhoea and another abdominal discomfort. Chronic hookworm infection in children can result in growth

retardation as well as intellectual and cognitive impairment. Hookworms ingest blood; one *A. deudenale* can ingest 0.2ml blood daily with smaller amounts (0.33ml) by *A. americana*. The worm migrates in the body and its secretion contain anticoagulant, resulting in bleeding from abdominal sites (Arora and Arora, 2010).

Hookworm infections tend to be occupational, affecting plantation workers, coal miners etc. through a contaminated environment. Anaemia from hookworm infection affects adult women most because of physiological needs for iron (menstruation and pregnancy). *Ancylostoma deudonale* infection is trans-lactational. The skin invasive larvae do not all pass through the lungs and into the gut but spread around the body via circulation, to become lodged inside muscle fibres. During pregnancy, due to hormonal changes, some of the larvae in human gut are stimulated to invade the vascular system, and then pass into the mammary glands to infect the newborn baby.

Strongyloidiasis affects indivuals with depressed immunity. However, hyperinfection syndrome called 'disseminated *Strongyloides'* can ensue from an increase in reproductivity of the helminths in the host. The mortality rate in hyperinfection syndrome can be close to 90 % if disseminated (Igrasiegman *et al.,* 1981). Lesions from strongyloidiasis can be found in the skin, lungs, intestine as a result of hyperinfection (Arora and Arora, 2010). In the skin pruritis and urticaria involving around the anus and buttocks are common in chronic disease case. A serpiginous, pruritic eruption usually found on the trunk or buttocks, is a skin scarification caused by autoinfection filariform larvae and the condition is known as *larva currens*.

In the lungs, eosinophilia is common. Larvae may lodge in the epithelium of the bronchia and develop to adult. This may lead to chronic bronchitis or asthmatic symptoms. Expectedly, eggs and rabditiform larvae of *Strongyloides stercoralis* may be

found in the sputum of patients. In the intestine, patients develop intermittent abdominal pain, distention, bloating, and diarrhoea alternating with constipation. Abdominal pain associated with paralytic ileus, gut bleeding and perforation is a consequence of hyperinfection (Nmorsi, 2010).

Infection with *S. mansoni* has two pathological courses; acute and chronic schistosomiasis (CDC, 2019). *Acute schistosomiasis* (Katayama's fever): this a consequence of the growth of fibrous tissue to wall off the antigenic effect of the trapped eggs in the liver and intestinal wall, associated with hepatosplenomegally, and high leucocytes and eosinophilic counts. This phase of infection is asymptomatic but when symptoms do occur, they include fever, nausea, headache, irritating cough and extreme diarrhoea mixed with blood and necrotic tissues. Symptoms last a few weeks to several months (Arora and Arora, 2010). In chronic intestinal schistosomiasis, infection is prolonged with granulomatous inflammation around the trapped eggs. The entire intestine may be involved with the large intestine most affected.

Chronic hepatosplenic schistosomiasis*:* This also runs a chronic course in the liver-spleen, with helminth eggs trapped in granulomatous inflammation tissues.

2.8 Immunology in Helminthiasis

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In helminths endemic areas, immunity to infection in previously exposed individuals is associated with expression of TH2 cytokines. Persistent heavy infection can result in overproduction of proinflammatory cytokines and the development of severe intestinal inflammation (Macdonald, 2004). Murine nematode infection models proved that CD4 TH1 expresses IFN-γ, promotes parasite persistence, resulting in host susceptibility; CD 4 TH2 expresses TH2 associated cytokines-IL4, IL9, IL13, IL25 and IL 35, resulting in

host resistance and consequent expulsion of worms (Else *et al.,* 1994). However, IL-4 is a potent factor in stimulating progenitor cells in the bone marrow to differentiate into eosinophils which can produce Th2 cytokine (Lugiu *et al*., 2004).

2.9 Granulocytes and Helminthes Immunology

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Granulocytes are a class of leukocytes whose cytoplasm is granulated and their nuclei, lobulated. They originate from hematopoietic stem cells, which differentiate into common lymphoid progenitor cells or common myeloid progenitor cells and acquire specialized features of cell types (Kondo, 2010). The lineage for lymphoid cells are Thymus dependent, Bursar and natural killer cells; while myeloid progenitor cells differentiate into mast cells, erythrocytes, dendritic cells (DC), megakaryocytes, macrophages and granulocytes (Doulatov *et al.,* 2010). In response to an infection, IFNγ is produced by Th 1; IFNγ acts on macrophages resulting in the clearance intracellular parasites. Extracellular pathogens are cleared from the body system through Th 2 immunity which features- elevation of peripheral blood eosinophilia and increased expression of cytokine (IL-4), granulocyte-macrophage colony-stimulating factors (GM-CSF) and the consequent production of antibody isotypes immunoglobin (Ig) G1, IgG4 and IgE (Makepeace *et al*., 2012).

2.9.1 Eosinophils

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The cell membrane of eosinophils possesses receptors for chemokines, cytokines, immunoglobins, and complement and serine proteases. Through these receptors eosinophils is recruited into affected tissue sites where they release granular content (Shamri *et al*., 2011). The chemokines, CCL 11 and CCL 26 mediate recruitment of eosinophils. Upon activation, eosinophils fuse with extracellular membrane and the

granular content are released by exocytosis (Shamri *et al*., 2011). Eosinophils are potent pro-inflammatory cells and are active in host defense against parasitic diseases. In other instances, the accumulation of eosinophils cells in host tissues can cause severe damage if it is not controlled. Eosinophils kill helminths, especially the larval stages (Mathew *et al*., 1998).

2.9.2 Neutrophils

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Neutrophils are the first cell type recruited to the site of an acute inflammatory response and are characterized by their ability to act as phagocytic cells, to release lytic enzymes and to produce reactive oxygen species with antimicrobial potentials (Mollinedo *et al*., 1999). The released products act in conjunction with cells resident in the affected tissue, such as macrophages and mast cells, to amplify the initial inflammatory response and induce the recruitment of additional neutrophils, monocytes and lymphocytes; and in TH2 scenarios, eosinophils and basophils (Makepeace *et al*., 2012).

2.9.3 Basophils

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Basophils are characterized by the presence of granules and surface expression of high-affinity FcεR1 for binding of IgE in addition to cytokine receptors, chemokine receptors and complement receptors (Stone *et al*., 2010). Upon ligation, they release chemical mediators, such as leukotriene C4 and histamine, and particularly the TH2 cytokines IL-4 and IL-13, which implicates basophils in immune response elicited by helminthiasis (Sullivian *et al*., 2009). In response to helminth infections, basophils releases histamine and a large volume of preformed IL 4 which interacts with polyclonal IgE (Capron *et al*., 2006).

2.10 Immunology of *Plasmodium*

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Critical analysis of the mechanism of falciparum malarial disease was made possible through the seminal work of Peter Mitchell who identified mitochondria as the ATP-energy generating source for aerobic and aerobic organisms. The understanding of severe infectious diseases was seen through the perspective of these organelles (Reid *et al*., 1967). For over twenty years there have been two opposing debates: first is the mechanical hypothesis, based on the concept of poor oxygenation of vital organs; the second is the cytokine hypothesis, in which there is uncontrolled release of pro-inflammatory cytokines. The mechanical hypothesis stresses the uniqueness of the pathophysiology of falciparum malaria compared to that of other severe systemic infectious diseases, whereas the latter sees malaria as having fundamentally the same basis as these other conditions, with the adhesive property of parasitized erythrocytes giving it no more than a distinctive flavour (Clark *et al.,* 2004).

Tumour necrosis factor, TNF, IL-1 beta and LT generate the inducible form of nitric oxide synthase iNOS. Anti-inflammatory cytokines such as IL-10, IL-4, and transforming growth factor-beta (TGF-beta), also play active roles, and an imbalance between these and their pro-inflammatory counterparts often determines outcome in disease. The pro-inflammatory cytokines most closely investigated in malaria, such as TNF, usually act as homeostatic agents but can cause pathology if produced excessively (Clark *et al*., 2004).

The balance between pro- and anti-inflammatory cytokines plays a pivotal role in the regulation of immune responses and pathogenesis in *P. falciparum* malaria, although, to date, their roles in disease pathogenesis and relationship to host protection have remained unclear. In a work by Prakas *et al* (2006) the pro-inflammatory cytokines

composed of IFN-γ, IL-2, IL-5, IL-6, and IL-12, the levels of which were significantly elevated during infection but were predominant in patients with Mild Malaria allowed their distinction from patients with Severe Malaria or Cerebral Malaria (CM). A second study group was composed of anti-inflammatory cytokines such as TGF-b, TNF-a, IL-10, and IL-1b, the levels of which were highly correlated with each other in the different clinical groups of patients and significantly increased with disease severity, particularly in CM. Through discriminant analyses cytokines such as IL-5, IL-1b, IL-10, and IL-2 increase with infection. Levels of IL-12, IL-5, and IL-6 discriminate severe forms of malaria from mild Malaria. Finally, levels of IL-1b, IL-12, and IFN-γ are relevant for the discrimination of Cerebral Malaria from Severe Malaria: high IL-1b levels are implicated in cerebral malaria, and high IL-12 and IFN-γ levels are associated with severe malaria.

Malaria tends to be more severe in children than in adults, presumably because partial immunity develops with age. Jason *et al*. (2001) in a study on cytokine and malaria parasitaemia, reported that a higher level of serum interleukin (IL)-10 (an anti-inflammatory, immunoregulatory, and type 2 cytokine) in malaria patients than in other patients (medians 502 pg/mL vs 16 pg/mL, $P = 0.002$), and IL-6 (a pro-inflammatory, type 2 cytokine regulating iron distribution) were lower in malaria patients than in other patients. For adult patients, IL-4 (a type 2 cytokine) was significantly lower in those with malaria than in those without malaria. IL-8 (a chemotactic, pro-inflammatory chemokine) was higher in parasitemic children than in parasitemia adults). Several cellular pro-inflammatory, type 1 parameters were significantly higher in all children (with or without malaria) than in all adults; these included the percentages of various lymphocyte populations making IL-6, both IL-6 and interferon-γ or IL-8. Nmorsi *et al*. (2010) in examining the array of some pro-and anti-inflammatory cytokines in the

serum of children, reported that anti-inflammatory cytokines status of IL-4 were 4.7 pg/mL versus 20.3 pg/mL, and IL-10 were 216 pg/mL versus 143.8 pg/mL in uncomplicated versus complicated. The mean pro-inflammatory cytokines in serum of children with uncomplicated and complicated malaria were IL-5 482.2 pg/ml versus 526.7 pg/ml, IL-6 98.8 pg/ml versus 82.6 pg/ml, IL-12 24.1 pg/ml versus 15.9 pg/ml, TNF-α 107 pg/mL versus 511.7 pg/ml and IFN-γ 2.1 pg/ml versus 2.5 pg/ml .

In co-infection of *Plasmodium* and intestinal helminths, *Trichuris trichiura* infection was associated with increased malaria prevalence while increased worm burden of helminths as expressed by egg intensity was associated with increased malaria parasitaemia which could be a potential factor for development of severe malarial infection with the course of the disease (Mullu *et al*., 2013). The study observed that the rate of helminths co-infection among malaria patients was 67% (154/230).

However, Hatger *et al*. (2009) in a study indicated that the presence of helminth infection modulates the immune response to malaria parasites, making it more anti-inflammatory.

2.10.1 Cytokine

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Cytokines are small secreted proteins released by cells and have a specific effect on the interactions and communications between cells (Zhang and Jiaxoy, 2007). Cytokine is a general name; other names include lymphokine (cytokines made by lymphocytes), monokine (cytokines made by monocytes), chemokine (cytokines with chemotactic activities), and interleukin (cytokines made by one leukocyte and acting on other leukocytes) and cell signal molecules, such as tumour necrosis factor and the interferons, which trigger inflammation and respond to infections (Zhang and Jiaxoy, 2007).

2.10.2 Properties of Cytokines

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Cytokines controls the activities of cell, and hence tissue, growth, migration, development and differentiation. Cytokines have important roles in the repair of damaged tissue, in cancer development and progression, in the control of cell replication and apoptosis, and the modulation of immune reactions such as sensitization. Unlike hormones, cytokines are produced by cells which are not organized in special glands but act systemically to affect inflammation, wound healing, organogenesis and oncogenesis (Foster, 2001).

Cytokines are produced throughout the body by cells of diverse embryological origin. Cytokines may act on the cells that secrete them (autocrine action), on nearby cells (paracrine action), or in some instances on distant cells (endocrine action). Cytokines generally function as intercellular messenger molecules that evoke particular biological activities after binding to a receptor on a responsive target cell (Foster, 2001)..

The role of cytokines is sometimes similar to that of hormones, and this is why cytokines are also known as the hormones of the immune system. Cytokines are involved in the innate immune response because they induce macrophage and NK cell activation, generating inflammatory and chemotaxis processes (Foster, 2001)..

They also play a role in the adaptive immune response when they act on T and B lymphocytes, favouring communication among different cell populations. The main difference between cytokines and hormones is that cytokines have their effect on different cell populations and tissues, while hormones usually act on just one organ. Moreover, a single cell can produce different cytokines, and this does not happen with hormones (Foster, 2001).

Lastly, cytokines can act locally, both in the cell producing them (autocrine activity) and in the cells next to it (paracrine activity). More rarely they affect cells and tissues distant from the place where they are produced (endocrine activity). This is similar to hormones. However, some cytokines, especially those with inflammatory effects such as IL-1 and TNF have their effect after being transported through the blood to distant target cells (endocrine activity) (Sanchez-Vizcaino, 2001).

Summary of the properties of cytokines include:

(1) The family of cytokines consists mainly of smaller, water-soluble proteins.

(2) Generally (although not always) they act over short distances and short periods.

(3) They act by binding to specific membrane receptors and induce specific gene expression via a second messenger (often tyrosine kinases).

(4). Cytokines are often produced in a cascade, as one cytokine stimulates its target cells to secrete additional cytokines.

(5). Cytokines can act "synergistically" (two or more cytokines acting together) or "antagonistically" (causing opposing activities).

(6) Certain cytokines are "redundant" in their activity, (means similar functions can be stimulated by different cytokines)

(7) Cytokines express "pleiotropism" (literally means a single cytokine has many different functional effects on many different cell types, but sometimes even on the same cell).

(8) They act at very low concentrations (typically 10-10 -10-15 M) and possess a very short life span.

(9) They are produced de novo in response to an immune stimulus.

(10) Responses to cytokines include increasing or decreasing expression of membrane proteins (including cytokine receptors), proliferation, and secretion of effector molecules.

(11) Cytokines are released by many cell populations, but the predominant producers are helper T cells (TH) and macrophages.

2.10.3 Structural families of Cytokines

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Cytokines exist in broad families that are structurally related but may contain rather diverse cytokine functions. Families of cytokines share sequence similarity and exhibit homology and some promiscuity in their reciprocal receptor systems. They do not exhibit functional similarity. Cytokine families also contain important regulatory cell membrane receptor-ligand pairs, reflecting evolutionary pressures that use common structural motifs in diverse immune functions in higher mammals. A large family of cytokines produced by various cells of the body and the cytokine super-family includes interleukins, chemokines, colony-stimulating factors (CSF), interferon, and the transforming growth factors (TGF) and tumour necrosis factor (TNF) families (Taga and Kishimotor, 1997)

2.10.4 Cytokine receptor family

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For cytokine to exert their biological effects, it must first bind to specific receptors on the membrane of target cells. The cytokine receptors are glycoprotein-membrane bound.

They initiate signalling within cells. Besides membrane receptors are soluble receptors which act as antagonists of the membrane receptors (Sanchez-Vizcaino, 2001).

2.10.5 Divisions of Cytokines

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Functionally, cytokines can be divided into two groups: those that are pro-inflammatory and those that are essentially anti-inflammatory but promote allergic responses. The major source of cytokines is the T lymphocytes and they possess antigen-specific receptors on their cell surface to allow recognition of foreign pathogens. They can also recognise normal tissue during episodes of autoimmune diseases. T lymphocytes have two basic subsets, distinguished by the presence of cell surface molecules known as CD4 and CD8. T lymphocytes expressing CD4 are also known as helper T cells, and these are regarded as the most prolific cytokine producers. This subset, CD4 is further subdivided into Th1 and Th2, and the cytokines they produce are known as Th1-type cytokines and Th2-type cytokines. Th1-type cytokines produce proinflammatory responses responsible for killing intracellular parasites and for perpetuating autoimmune responses. Interferon-gamma is the main Th1 cytokine. The Th2-type cytokines include interleukins 4, 5, and 13, which are associated with the promotion of IgE and eosinophilic responses in atopy, and also interleukin-10, which has more of an anti-inflammatory response. Excessive pro-inflammatory responses can lead to uncontrolled tissue damage, so there needs to be a mechanism to counteract this. In likewise, excess of Th2 responses will counteract the Th1 mediated microbicidal action. The optimal scenario would, therefore, require the production of a well-balanced Th1 and Th2 response, suited to the immune challenge. Th1 cells produce pro-inflammatory cytokines like IFN-γ, TNF-β and IL-2, while Th2 cells produce the cytokines IL-4, IL-5, IL-6 and IL-13. The cytokines produced by Th1 cells stimulate innate phagocytic

responses and destruction of microbial pathogens while Th2 cytokines stimulate the production of antibodies against extracellular parasites. Thp, a non-antigen precursor cell type differentiates into Th 1 and Th 2. Exposure of Thp cells to antigen by antigen-presenting cells may result in their differentiation to Th0 cells, not yet committed to become either Th1 or Th2 cells, although the existence of Th0 cells is controversial. Cells committed as either Th1 or Th2 cells are called polarized, whether they are effector cells actively secreting cytokines or are memory cells. The stimulation of Thp cells by exposure to antigen-presenting cells induces the proliferation of undifferentiated cells and their expression of IL-2 and IL-2 receptor. The differentiation of Th1 cells and Th2 cells depends on the cytokines they are exposed to. IL-12 causes Th1 differentiation and blocks Th2 cell production while IL-4 causes Th2 differentiation and antagonizes Th1 development. IL-18 also induces Th1 differentiation. Polarized Th1 and Th2 cells also express distinct sets of chemokine receptors that further modify their homing and other cellular responses (Sverremark, 2010)..

Inflammation is mediated by a variety of soluble factors, including a group of secreted polypeptides known as cytokines. Inflammatory cytokines can be divided into two groups: those involved in acute inflammation and those responsible for chronic inflammation. Acute inflammation includes IL-1, TNF-alpha, IL-6, IL-11, IL-8 and other chemokines, G-CSF, and GM-CSF. Cytokines in chronic inflammation can be subdivided into cytokines mediating humoral responses such as IL-4, IL-5, IL-6, IL-7, and IL-13, and those mediating cellular responses such as IL-1, IL-2, IL-3, IL-4, IL-7, IL-9, IL-10, IL-12, interferons, transforming growth factor-beta, and tumour necrosis factor-alpha and beta. Some cytokines, such as IL-1, significantly contribute to both acute and chronic inflammation (Feghali and Wright, 1997). Acute inflammation is a short-lived response that is characterized by extravasation of leukocytes, erythrocytes,

and plasma components into the injured tissue. If left unchecked, the acute inflammatory process can lead to chronic inflammation. In chronic inflammation, tissues are infiltrated by lymphocytes and macrophages (Thermo-Fischer, 2015).

According to (Landskron *et al.,* 2014) acute inflammation is a response to an alteration induced by a pathogen or a physical or chemical insult, which functions to eliminate the source of the damage and restore homeostasis to the affected tissue. However, chronic inflammation triggers cellular events that can promote malignant transformation of cells and carcinogenesis. The benefit of inflammation is to restore injured tissues and elimination of pathogenic agents. However, if inflammation is not checked, it can become chronic, resulting in the transformation to malignant cells in the surrounding tissue.

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2.10.6 The Critical Balance between pro- and anti- inflammatory mediators

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The outcome of the inflammatory process is determined by the balance between pro- and anti-inflammatory mediators. Pro-inflammatory cytokines respond early in infection and amplify inflammatory reactions. Anti-inflammatory cytokines rather limit inflammatory responses (Kampen *et al*., 2005). Th1 cells develop in response to infections caused by intracellular bacteria and some viruses, whereas Th2 cells predominate in response to infestations by gastrointestinal nematodes (Romagnani, 1999).

2.10.7 Cytokines and Diseases

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Inadequately resolved chronic inflammatory process may progress to cancer. Several pathologies illustrate this link, such as endometriosis, chronic prostatitis, and chronic gastritis due to *Helicobacter pylori* (*H. pylori*), inflammatory bowel diseases (IBD), and primary sclerosing cholangitis (PSC). Inflammation increases the risk of cancer by providing bioactive molecules from cells infiltrating the tumour microenvironment, including cytokines; growth factors; chemokines that maintain a sustained proliferative rate; cell survival signals to avoid apoptosis; proangiogenic factors; and extracellular matrix-modifying enzymes such as metalloproteinases that promote epithelial-mesenchymal transition (EMT) and facilitate other carcinogenesis programs, such as genome instability, reprogramming of energy metabolism, and immune evasion (Hanahan and Weinberg, 2011).

2.10.8 Signalling Pathway of Cytokine

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Cytokines use multiple signalling pathways (Leonard and Lin, 2000). Important mediators for the main cytokine signal-transduction pathway are the Janus kinases (Jaks) and signal transducer and activator of transcription (STATs). Selective usage of members of the Jak and STAT families by a given cytokine receptor is partly responsible for the specificity of cytokine action. In addition to the Jak-STAT pathway, a cytokine receptor complex can simultaneously operate multiple signal-transduction pathways, which usually express contradictory properties. These contradictory signals from a single cytokine are orchestrated to evoke a unified biological response in the cell (Ishihara and Hirano, 2002). Cytokines regulate cellular behaviour by interacting with receptors on the plasma membrane of target cells and activating intracellular signal transduction cascades such as the JAK-STAT pathway. Suppressors of cytokine signalling (SOCS) proteins negatively regulate cytokine signaling. The SOCS family

consists of eight proteins: SOCS1-SOCS7 and CIS, each of which contains a central Src-homology 2 (SH2) domains and a C-terminal SOCS box. The expression of CIS, SOCS1, SOCS2 and SOCS3 is induced in response to stimulation by a wide variety of cytokines, and overexpression of these proteins in cell lines results in inhibition of cytokine signalling. Thus, SOCS proteins appear to form part of a classical negative feedback loop (Ishihara and Hirano, 2002).

2.10.9 Interleukin 4 (IL-4)

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Interleukin -4 is a cytokine, with a 15-kD polypeptide. It has multiple effects (pleitropic) on many cell types. It influences the transformation of naive helper T cells (Tho cells) to Th 2 cells (Choi and Reiser 1998). Basophils are the first effector cells that produce IL-4 before its activities on Tho cells. Interleukin-4 has many physiological functions; it brings about B-cells class switching to IgE; it upregulates MHC class 11 production; decreases the production of Th 1 cells, IFN-γ, macrophages, dendritic cells and IL-12. IL 4 stimulates activated B-cells and T- cell proliferation. They also stimulate the transformation of B cells into plasma cells (Paul, 1997). Inappropriate production of IL-4 is associated with allergies and it increases susceptibility to infectious diseases, while the normal response of Th1 response provides body resistance to infectious diseases (Scott, 1996).

There is evidence IL-4 is pro-inflammatory in injured intestines, the inflammatory process is the mechanisms involved at the beginning of infection but alters to anti-inflammatory during tissue resolution (Van Kampen *et al*., 2005). The pro-inflammatory role of IL 4 was considered from its invivo ability to attract Leucocytes, (eosinophils, monocytes and lymphocytes), macrophages, and fibroblast and B cells. Although

neutrophils were however not attracted, IL-4 is functioned in the production of several pro-inflammatory molecules (Ratthe *et al*., 2009).

The pro-inflammatory functions of IL-4 are in its potentials to delay apoptosis of neutrophils (apoptosis of neutrophils by macrophages comes up at termination of its responses) and its ability to influence the production of IL-8 from neutrophils (Girard *et al.,* 1997)

2.10.10 Interferon

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Interferons are signalling proteins or communication molecules among cells. Interferon released by virus-infected cells goes to nearby uninfected cells to establish antiviral defenses.

The classification of interferon depends on the type of receptor they interact.

Type 1 interferon: these are produced by fibroblast and monocytes and they respond to viral infection. There are five types; IFN-α, IFN-β, IFN-∈, IFN-ω, IFN $-k$. They bind to a common cell receptor complex described as IFN-α/β receptor (IFNAR) and are made up of two chains, IFNAR1 and IFNAR2 (Tau and Rothman, 1999).

Type 11 interferon (IFN-γ): they are produced by Th 1 cells but activated by IL-12. They are described as immune interferon. They promote Th 1 responses and inhibit the proliferation of Th 2 immune response. They bind to cell receptors IFNGR that also has two chains, IFNGR 1 and IFNGR 2 (Tau and Rothman, 1999)..

2.10.11 Interferon-gamma IFN-γ

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IFN-γ is an important mediator of inflammatory responses activated primarily by IL-12. In *Plasmodium* infection, IFN-γ is essential in the control of both blood and liver stages of the life cycle of the parasites (King and Lamb, 2015). NK cells secrete IFN-γ in innate responses; CD4+ and CD8+ take over-secretion in adaptive immune responses. Later, CD4+ secretes additional molecules, IL-10 that limit immunopathogenesis of malaria infection. High and early production of IFN-γ during natural malaria infection and even in experimental infection is protective (D'Ombrain *et al.*, 2008). Early and elevated levels of IFN-γ against malaria infection was reported to have significant and strong protectivity among the Fulanis in Mali (McCall et al., 2010) Although, contradictory data could be expected due to varying level of pathogen coinfecting with Plasmodium infection (King and Lamb, 2015) in experimental condition, mice lacking IFN-γ had delayed clearance of blood-stage parasitaemia (Actor *et al.,* 1993).

Interferon-gamma and Pathology during malariaIFN-γ is associated with progression of

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malaria disease by responding to blood-stage of *Plasmodium* infection and targetting infected red blood cells (iRBC) which are sequestrated in various organs. IFN-γ facilitates the expression of adhesion molecules (ICAM) in the endothelial cells in the brain resulting in increased sequestration of parasites and mobilization of activated leucocytes to the brain (Belnoue *et al.,* 2008) Besides, it influences the expression of varieties of chemokines such as CXCL9 and CXCL10, which mobilize CD8+(produces pathological mediators, IFN-γ, perforins and granzyme B) to cerebral tissues during cerebral malaria infections (King and Lamb, 2015). IFN-γ is responsible for the guided movement of pathogenic T- cells in the brain tissues. Mice deficient in IFN-γ have

fewer T cells resulting in reduced accumulation of neutrophils in brain tissues (Belnoue *et al.,* 2008).

2.11. Macrophages Inflammatory Protein (MIP)

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Macrophage inflammatory protein (MIP) is a chemotactic cytokine (chemokine) of the CC group (Rollings, 1997), Produced by many cells, macrophages, dendritic cells and lymphocytes, when stimulated with bacterial endotoxins. IL 1 and TNF can also induce the production of MIP. In humans, MIP-1 exists in two forms, MIP-1α/CCL3 and MIP-1β/CCL4; others are MIP-delta/CCL9-10 and MIP-1γ/CCL-15. Their expression is restricted to haematopoietic cells and fibroblast (homeostatic and proinflammatory in functions) activating the proliferation of granulocytes (neutrophils, eosinophils and basophils) (Kasama *et al*, 1993). Besides, they influence macrophages and fibroblast to produce and release other pro-inflammatory cytokines (IL-1, IL-6 AND TNA-). MIP-1α/CCL3 and MIP- 1β/CCL4 are heterodimer by nature and have antiviral activity against HSV. They share common receptor CCR 5 which is also an obligate receptor in macrophages for HIV, signifying that attachment of MIP -1 to CCR5 can interfere with the entry of HIV into macrophages (Dragic *et al.,* 1996). They act through G -protein-coupled cell surface receptors, CCR 1, 3, 5 expressed by lymphocytes, macrophages, dendritic cells and lymphocytes (Maurer and Stebut, 2004). Both MIP 1α and MIP 1β work in complementary to bring about inflammatory response and sometimes the excess of MIP 1β can antagonise the effect of MIP -1 α. Independently, however, MIP-1α chemoattract CD 8 T lymphocytes while MIP-1β attract naive T helper cells (Scholl *et al.*, 1993) reported that in co-infection of acute *Nippostrongylus brasiliensis* (Nb) and *Mycobacterium tuberculosis* an accelerated increase in neutrophils and alveolar macrophages; and a reduction in colonization of pulmonary mycobacterium colony-

forming units which was linked to early and increased activation of pulmonary CD4 T cells and increased T helper type 1 (Th1) and Th2 cytokine secretion. MIP-β is a potent chemoattractant for macrophages progenitors, induced by inflammatory cytokines; IFN-γ, TNF-α and LPS, thus in inflammatory conditions, MIP-3β functions in the trafficking of macrophage progenitors in and out of bone marrow (Kim *et al.,* 1998). An elevated level of MIP-1β has been associated with placental malaria in pregnant women (Chaisavaneeyakom *et al.,* 2003). Known receptors for MIP-β are CCR1, CCR5, and CCR8 and through CCR 5 receptors CC chemokines are reported to enhance IFN-γ production. In summary, MIP-1 functions as pro-inflammatory molecules, modulate cytokine production and induce pyrogens (fever).

2.12 Eotaxin

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(Synonyms: CCL-26, Eotaxin 3, Macrophage Inflammatory Protein 3-alpha MIP-4α Thymic Stroma chemokine-1-TSC -1 and IMAC)

It is a cytokine of CC chemokines with a strong affiliation for eosinophils and basophils, produced by several tissues; endothelial cells, heart, lungs and ovary upon stimulation by IL-4. They have the common receptor, CCR 3 which they bind to and activate, although with a low level of homology but portray different physiological potentials. The CCR 3 eotaxin receptor is predominantly expressed by the hematopoietic cells involved in allergic responses: eosinophils, basophils, and T helper type 2 cells (Mathew *et al.,* 1998). The functionality of CCR3 is dependent on the microenvironment of the tissues: the local ph and salt concentration such that small alteration can yield disparate results (Dairaghi *et al*., 1997).

In humans, there are three families of eotaxins; CCL 1 (eotaxin 1), CCL 24 (eotaxin 2) and CCL 26 (eotaxin 3). CCL 26 is highly expressed in an individual with Eosinophilic oesophagitis with a strong correlation with tissue eosinophilia and mastocytosis (Blanchard, *et al.,* 2006).

Eotaxin is constitutively expressed in the intestine as well as other organs in the body and has been identified as a specific chemo-attractant for eosinophils in dermatitis in humans and corneal pathology in the mouse (Mathew *et al*., 1998). Besides, eosinophils have been proven in a model to be essential for the clearance of primary infection (not secondary) of *B. malayi* microfilaria infection form tissues and blood system (Rankin *et al*., 2000)

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The communities of choice for this study were Ibelebiri, Otuesega, Immiringi and Otegala, all in Kolo district, Ogbia Local Government Area in Bayelsa State, Nigeria. They are rural communities located longitudinally along the bank of Kolo Creek Latitude: 4° 38' 59.99" N Longitude: 6° 15' 60.00" E Bayelsa State is located in the Niger Delta region, Southern Nigeria.

The vegetation is fresh water swamps of lowland rain forest with much of clay soil such that greater part of the land is flooded during rainy seasons. Although rain falls every month of the year, the heavy downpour is typical of tropical rain during the raining season (April-October). Bayelsa State generally has the heaviest rainfall area in Nigeria with a short dry season (from November to March). It has a uniform mean annual temperature ranging from 25^0C to 31^0C throughout the year. The relative humidity is usually high and slightly higher during the rainy season (Abah and Temple, 2015).

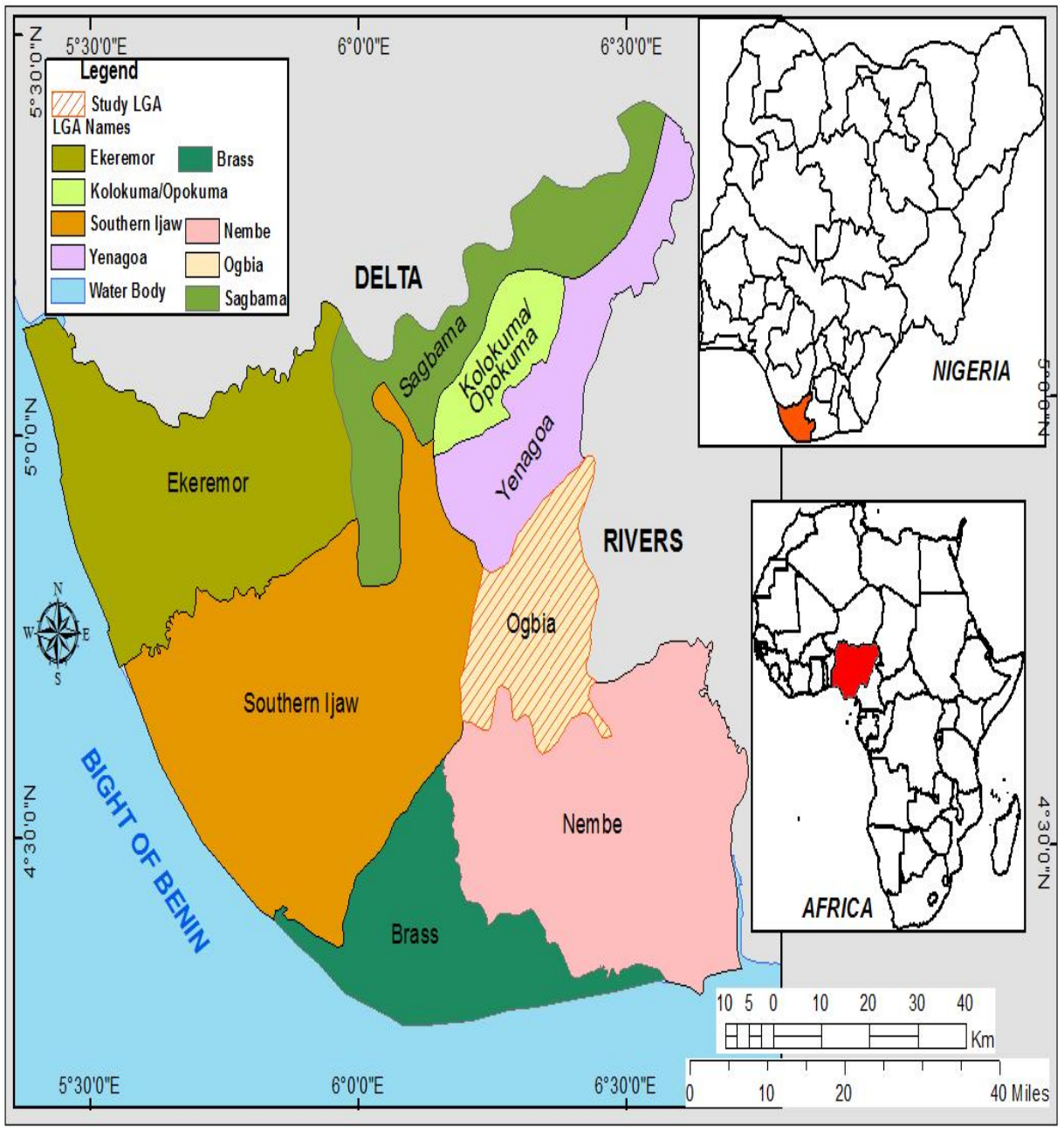

Figure 3.1: Bayelsa State Showing Ogbia Local Government Area

Source: https://latitude.to/articles-by-country/ng/nigeria/65311/ogbia

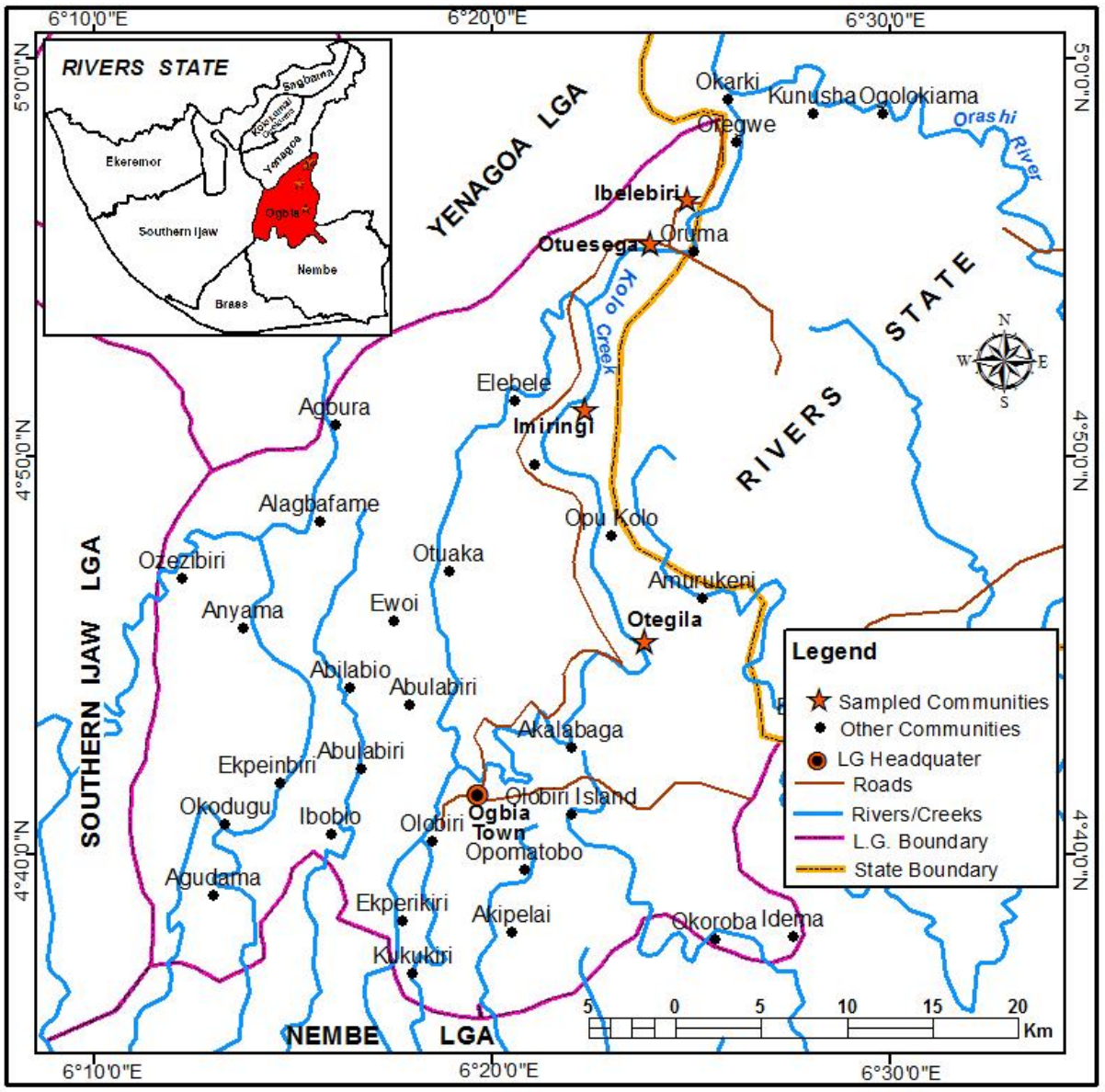

Figure 3.2: Map of Ogbia Local Government Area Showing study areas

Source: https://latitude.to/articles-by-country/ng/nigeria/65311/ogbia

3.2 Ethics and Consents

Permission for this study was given by the Ethics Committee, College of Medicine, University of Benin, Benin City, Edo State, Nigeria. Further approval was obtained from the Ministry of Health, Bayelsa State; The Post Primary Schools Board of Bayelsa State Ministry of Education authorized the study to be conducted among pupils in Secondary Schools in Ogbia Local Government Area. Informed consent was obtained from leaders of the respective communities, Principals of Schools and teachers, parents, and guardians of volunteer children. Written consent was obtained from every subject who participated in the study, explaining the objective and benefits of the study.

3.3 Inclusion Criteria

Volunteers were restricted to residents of the respective communities who had lived in the study area for more than three months.

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3.4 Exclusion Criteria

Volunteers who were less than four years of age were excluded from the study.

3.5 Study Participants and Sample size

The original population of the four communities of study, based on National Census, 1991 were;

Since the population in the study communities was above 1000 people, Daniel (1995) determined the sample size (n) from the statistical formula,

$n= z^2p\ (1-p)/d^2$ where;

n = minimal number of sample size

z = level of confidence of 95% (standard value is 1.96)

p = expected prevalence 50% and

d = margin of error is 5% (0.05)

The overall prevalence in the study area was not known, a 'p' value of 50% was taken.

Design effect, D (n x 2) = 768.

Contingency of 5% was considered

Final sample size, N (768) was divided by the number of clusters (4)

Table 3.1: Population Statistics of Study Areas in Bayelsa State

Locality	Males	Females	Both sexes	1996 Projection
Ebelebiri	455	386	841	995
Otuesega	3,349	2,977	6,326	7,487
Immiringi	2,759	2,360	5,117	6,056
Otegela	212	165	377	446

(National Population Commission, 1991).

Table 3.2: Silent Wards Analysis Template for Monitoring Acute Flacid Paralysisis (AFP) Reporting in Ogbia Local Government Area, Bayelsa State

LGAs	Ward	Total Population
Ogbia	Otuesega (6)	15,461
Ogbia	Immiringi (8)	14,181
Ogbia	Otakeme (12)	19,934
Total		49,576

(WHO, 2017)

3.6 Population of Study

A total of 1441 people were recruited in three seperate studies.

First study: Two surveys were conducted within the same period:

i. School based study 1: this took place in community primary school, Ibelebiri. A total of 75 pupils participated willingly.

ii. Community based study: this involved four communities and over 850 volunteers, aged, 4-80 participated in this study. About 192 participants were recruited from each studied community.

S**econd study:**

iii. School based study 11: a total of 537 pupils participated in the study, with 210 children from each Post Primary Schools in Ibelebiri, Otuesega and Immiringi. Otuegela community had no Secondary School.

3.7 Study Design

The study had two phases; the first was conducted in May to July, 2016 , and the design was longitudinal, and required among others, treatment of infected volunteers and collection of samples 18 days after therapy for a post-treatment evaluation. Samples were examined and infected individuals were treated base on laboratory findings. The second phase of study was carried out in July, 2018. It was cross-sectional and limited to prevalence of infection of malaria parasites and intestinal helminthes in primary school pupils only. Generally our method of investigation was by use of structured questioneers, anthropometric measurement and laboratory analysis of stool, blood, and

serum samples. Anaemia in children was defined as hemoglobin concentration below <11g/dl. BMI was determined considering the age, weight, height, and sex of pupils. The weight in kilograms was divided by the height in meters square and the obtained BMI expressed using percentile growth chart for boys and for girls. Normal BMI values were taken between 5th and 85th percentiles (CDC 2015).

3.8 Collection/Processing of Blood Samples

The cubital vein was cleaned with a swab moistened with 70% v/v alcohol. By vein puncture, about 4 mls (from children) -10 mls (from adults) of blood was taken from each subject and half the volume collected was dispensed into a heparinized bottle for a full blood count. The other half was left in syringes to clot for serum production, which was decanted into clean labeled containers and preserved at -180C for later use in the immunoassay. About 30µl of EDTA blood was spotted on well labeled FTA cards (3 MM) which were allowed to air dry and preserved at room temperature for DNA extraction and PCR.

3.8.1 Thick Blood Films Preparation

Thick blood films were prepared and fixed with 10% alcohol in the field. Using a plastic pipette a large drop of blood was taken from the syringes and was placed at the center of a grease -free and well-labelled microscope slide. The drop of blood was immediately carefully spread. Slides were placed in storage tray at horizontal position, and covered with netted lid to keep the film from insects.

In the laboratory, thick film prepared slides were held in a downward position in a shallow tray. A (10%) Giemsa stain was poured into the shallow tray and process was allowed for 10 minutes. Stains were flushed with clean water from the slides and tray.

Wiping the back, slides were placed on draining rack for air-dry. A drop of immersion oil was placed around the edges of a well-dried film. Slides were read and scanned without coverslip at low magnification of 10X and 40 X objectives are examined at 100 X objective. When a well-stained area was identified, the objective was changed to 100 X and malaria parasites were examined relative to 200 WBCs (Cheesbrough (2005).

Preliminary preparation: according to WHO (2016) before counting parasites 100 fields of thick film preparation were examined first at x 100 oil immersion to detect parasites.

1. The labelled side of the slide was positioned to the left, while placing it on a microscope stage.

2. From the topmost part of the film where a good number of white blood cells are expectedly present, counting of the asexual form of the parasites.

3. Parasites and white blood cells were counted simultaneously, using a multiple type tally counter. Counting process was repeatedly moved from field to field recorded on a worksheet.

4. Parasite density was calculated using an estimated average white cell count of 8000/µL (patient's actual white cell count could be used) .

Expression of parasitemia was by the formula:

Parasites / µL blood =

Number of parasites counted x 8000 white blood cells/µL No. of white blood cells counted

Three experienced microscopists read the slides independently and blindly.

3.8.2 Full Blood Count

This process was carried out within 24 hours of sample collection. Automatic Hematology Analyzer, Abacus 380 was used in the analysis of EDTA preserved blood samples. Variables measured were; total white blood cell count, lymphocytes count, granulocytes, hemoglobin, red blood cell count hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cells distribution width, platelet count, platelet percentage, mean platelet volume and platelet distribution width.

3.9 Process of Measurement of Body Mass Index of Children

A newly purchased scale was used in the measurement of weight. A portable stadiometer was used in the measurement of height. Both types of equipment were calibrated and accurate before being used by a trained measurer.

3.10 Treatment of Infected Patients

All participants were given a single dose of Albendazole. However, participants who were infected with *Plasmodium* or intestinal *Schistosoma* were treated strategically, according to infection. Drugs prescription was done by a Registered Medical Doctor and dispensing of drugs was a registered Pharmacist, both were employees of Bayelsa State Government. The drugs were Albendazole 400 mg STAT dose, Praziquantel (60 mg/kg) in 5 hours apart and Artemether/Lumefantrine (20 mg/120 mg).

3.11 Examination of stool samples

Each participant was given clean and labeled container to bring stool samples, which were analyzed quantitatively by wet preparation and formol ethol methods

(Cheesbrough, 2009). About 1g of well-mixed faeces was collected using emulsified stick into a tube containing about 4mls of 10% formol water. A little more formol water was added to the tube and covered with a cap. Tube content was agitated gently for more thorough mixing. The emulsified faecal samples were sieved with 20 µm mesh stainless steel flat sieve, 200 mm, and the filterate was collected in a beaker, which was transferred into a centrifuge tube and about 3-6 ml of ethyl acetate was added. The tube was stoppered and mixed for 1 minute. The stopper was loosened and centrifuged immediately at 3000 rpm for 1 minute. The tube was slightly inverted to discard the ether, faecal debris, and formol water while leaving the sediment. After mixing the sediment in the tube, it was transferred to a slide and covered with a slip. Specimens were examined using X 10 and X 40 objectives.

3.12 Second Samples Collection of Blood and Stool

At 18^{th} day after treatment, another round of blood and stool was collected from the participants to ascertain the efficacy of treatment and a repeat process of the thick film, full blood count was carried out. Serum obtained was again stored at -18^{o}C for cytokines assay. Samples collected from participants post treatment were considered for control groups during analysis.

3.12.1 Infection groups, immunoassy and haematology:

Infected participants, n = 88 were investigated for immunoassay and haematology; and were grouped into three; those infected with *Plasmodium* only, n = 48; *Plasmodium*/intestinal helminths, n = 18 and for intestinal helminths infections, n = 22.

3.13 DNA Extraction

3.13.1 Blood DNA extraction

Blood samples from 50 volunteers chosen arbitrarily from samples which were positive by microscopy for Plasmodium parasites, were used for DNA extraction using ZymoBIOMICS™ DNA Miniprep Kit protocols. About 30 - 50 microliters of whole blood was spotted on Whatman filter paper (3 MM chromatography paper) and allowed to dry by air; and were placed singularly on a transparent plastic bag and preserved at room temperature for DNA extraction. In the Laboratory, with a sterile blade, blood spotted area in Whatman filter paper was excised into a tube and soaked in the chilexTM solution for 30 minutes, to produce about 50 microliters of blood.

About 400 microliter of Genomic Lysis Buffer was added to50 microliter blood samples at a ratio of 4:1. Mixture was transferred to a Zymo-Spin IICTM Column in a Collection Tube and was centrifuged at 10,000 x g for 1 minute. Zymo-SpinTM11C Column was transferred to a new collection tube and 200 microliter of DNA Pre-Wash Buffer was added to the spin column and centrifuged at10,000 x g for 1 minute. Wash buffer, 500 microliters of g-DNA was added to the spin column and centrifuged at 10,000 x g for one minute. Spin column was transferred to a clean microcentrifuge tube and about 55 microliter DNA Elution Buffer was added to the spin column. This was incubated at room temperature for 2-5 minutes and centrifuged at top speed (10,000 x g) for 30 seconds to elute the DNA.

3.14 Polymerase Chain Reaction Assay for *Plasmodium* Parasites

Nested PCR assay was employed using Oligonucleotide primers obtained from Zymo Research. Primers were purified by high-performance chromatography and were

designed based on the small subunit ribosomal RNA (ssRNA). Plasmodium genus-specific primers were used to amplify nest 1 and nest 2 and product of nest 1 amplification that was detected positive were reused for species-specific nest 2 amplifications.

3.14.1 Amplification condition of Nest 2

Nest 2 primers and other constituents for nest 2 had same concentration as nest 1 except, except that 0.5 units Tag DNA polymerase was used in nest 2.

The species-specific primers were (rFAL 1 and 2, rMAL 1 and 2, rVIV 1 and 2, and rOVA 1 and 2)

Step 1-2 same as nest 1.

Step 3, annealing temperature was 588C for species- specific primer and 628C for genus-specific primers45

PCR product of nest 2 amplification was run in gel electrophoresis and stained with ethidium bromide.

3.15 Transfer of DNA to FTA Cards

About 80µl of DNA aliquot was aspirated into labeled Whatman filter paper (3 MM chromatography paper), air dried and stored in double zip plastic bags at room temperature for further investigation using PCR in Baylor College of Medicine, Houston Texas, USA. The remaining 20µl is stored at -18OC as back up in NVRI, Vom.

3.16 Determination of Chemokines and Cytokines

Quantification of chemokines and cytokines was by enzyme-linked immunosorbent assays (ELISA) specific for IL-4, IFN-γ, CCL-4/MIP-1β and CCL-24 manufactured by

Peprotech LTD, USA. Mini ELISA Development kit was used and procedure was carried out based on the manufacturer's protocols and according to Prakash *et al.* (2006); The key component of the development kit were required for the quantitative measurement of natural and or recombinant analyte in a sandwich ELISA format within the range of 16-2000pg/ml. Using the ELISA protocol, the recommended microplates, reagents and solutions, the component supplied in the kit was sufficient to assay human samples in approximately 200 ELISA plate wells.

3.16.1 Materials used:

Capture antibody:

Antigen-affinity 6µg purified rabbit anti-human (IFNγ /MIP-1β/Eotaxin-3/IL-4) + 0.5mg D-mannitol. The vial was centrifuged before opening.

Reconstitution was in the 60µl sterile water at a concentration of 100µg/ml.

Detection antibody:

Biotinylated antigens-affinity (6 microgram) purified rabbit anti-human analyte + 0.5mg D mannitol. This was centrifuged before opening and was reconstituted in 60 microliter sterile water for a concentration of 100µl/ml.

Standard:

Recombinant human analyte (1µg) + 2.2 mg BSA + 11.0 mg D-mannitol. This was centrifuged before opening and was reconstituted in 1ml sterile water for a concentration of 1µg/ml

Streptavidin-HRP Conjugate:

This was a 4µl vial which was centrifuged before opening. Conjugate was diluted using 36µl 0f 1Xpbs for a total of 40µl at a concentration of 100µg/ml.

3.16.2 Preparation of ELISA Plate

Capture antibody was diluted with PBS to a concentration of 0.25µg/ml. immediately; 100µl was added to each ELISA plate well. The plate was sealed with transparent cellophane and left at room temperature overnight. The following day, the liquid in the wells were removed by aspiration and plate was washed 4 times using 300µl of wash buffer per well. After the last wash, the plate was inverted to remove residual buffer. Paper towel was finally used to blot the plate. Block buffer (300µl) was added to each well and incubated for 1 hour. Wells were aspirated and the plate washed 4 times.

3.16.3 ELISA Protocol

Standard/ Sample: Standard was diluted from 2000pg/ml to zero.

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Standard/serum sample (100µl) was added to each well and incubated at room temperature for 2 hours.

3.16.4 Detection

ELISA Plate was aspirated and washed 4 times and detection antibody was diluted to a concentration of 0.25µg/ml and (detection antibody 100µl) was added to each well. The plate was left at room temperature for 2 hours.

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3.16.5 Streptavidin-HRP

The well content was aspirated and the plate was washed 4 times.

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Streptavidin-HRP in diluent was diluted to a concentration 0f 0.05µg/ml.

Streptavidin-HRP (100µl) was added to each well.

The plate was left at room temperature for 30 minutes.

3.17 Statistical Analysis

The "R" Programming software (version 2016) was used for statistical analysis of data. The serum concentrations of chemokines during infection and after treatment were analyzed by Wilcoxon rank sum test with continuity correction. Data were log transformed to base 10.To evaluate the effect of therapy on serum concentration of cytokines and chemokines, values in infection and after treatment were analyzed by Student's paired *t*- test. Multivariat statistics was used for the analysis of blood cell parameters before and after treatment. $P < 0.001$ was considered significant.

CHAPTER FOUR

RESULTS

4.1 Stool samples: Prevalence of intestinal helminths in school based study 1

No helminths egg was detected from stool of 75 pupils examined because pupils were dewormed a forthnight before stool samples were collected.

4.1.1 Age Group and Gender distribution of the study population for helminthiasis in four study area in Bayelsa State, Nigeria.

A total of 829 participants were examined for intestinal helminths infection. The population was made of 380 (46%) males and 449 (54%) females. Highest population of 335 (40.4%) was derived from age group 1-10, which progressively declined to a minimum population of 4 (0.4%) obtained from age group 71-80. The population-based on age distribution and sex in each community is shown in Table 4. 1.

4.1.2 Gender-based Prevalence of intestinal helminthiasis in the study population of four rural communities in Bayelsa State

A total of 218 (26.3%) fecal samples examined were infected with intestinal helminths. In Table 4.2, eighty-two males and 136 females were infected, representing a prevalence of (21.5%) and (27.2%) respectively.

4.1.3 Prevalence of intestinal helminthiasis with respect to age groups among the study population in four rural communities of Bayelsa State, Nigeria

The prevalence of intestinal helminths infection with respect to age group is indicated in Table 4.3. Age group 61-70 had the highest prevalence 11 (73%) and least infected group 51- 60 with a prevalence of 4 (15%). The age group 71-80 were exclusively infected with *S. mansoni* 1 (50%) and *S.intercalatum* 1 (50%). Infection of *Schistosoma*

intercalatum 86 (10.4%) was most commonly found followed by *Ascaris lumbricoides* 53 (6.4%) and least by Taenia species 2 (0.2%).

4.1.4 Prevalence of Infection of Intestinal Helminths Based on Sex

In all species of helminthes, the occurrence of *S. intercalatum* was higher in females 57(13.0%) than in males 29(7.6%) as shown in Table 4.4.

4.1.5 Prevalence of Multiple Infections of Intestinal Helminthes Based on Sex

In similar trend, Table 4.5 indicates that multiple infection affected more females 7(63.6) than males 4 (36.4%). A combination of *S.intercalum*/*A.lumbricoides* 6 (0.7%) was higher than *S.mansoni*/hookworm 1(0.1%).

4.1.6 Age Distribution of Multiple Infections of Intestinal Helminthes

In Table 4.6, out of the 11 people that had a double infection, 9 were adults; the age group 41-50 were most affected.

4.1.7. Intestinal helminths in school-base study 11 in Bayelsa State

Pupils in school-base study 11 were not dewormed before stool samples were collected for examination. In Table 4.7, out of 537 school children examined, 162 were tested positive, representing 30.2% prevalence. Children in Ibelebiri community were most affected with 47%, male infection rate and 45% in females. Males in Otuesaga had 44.4% and females, 30.3%. children in Imiringi were least affected; males 9.5% and females 2.6%.

4.1.8 Types of ova of helminth identified in school based study 11

In Table 4.8 Seven worm eggs were identified in stool: *A.lumbricoides* 10.3 %, *S.mansoni* 8 %, *S. intercalatum:* 5 %, *T.trichiura 1.8* %, hookworm 1.6 % , *Taenia* spp 1.3 % and *Strongyloides 1* %

Table 4.1: **Distribution of the study population for helminthiasis in four rural communities of Bayelsa State, Nigeria, with respect to Age Group and Gender**

Parameter	Gender		Total (%)
	Male (%)	Female (%)	
Age group (years)			
1-10	168 (50.1)	167 (49.8)	335 (40.4)
11-20	106 (52.7)	95 (47.2)	201 (24.2)
21-30	40 (39.2)	62 (60.7)	102 (12.3)
31-40	25 (30.8)	56 (69.1)	81 (9.7)
41-50	22 (33.8)	43 (66.1)	65 (7.8)
51-60	10 (38.4)	16 (61.5)	26 (3.1)
61-70	6 (40.0)	9 (60.0)	15 (2.0)
71-80	3 (75)	1 (25)	4 (0.4)
Community			
Otuegela	86 (40.9)	124 (59.0)	210 (25.3)
Ibelebiri	82 (39.0)	128 (61.0)	210 (25.3)
Otuesega	114 (54.4)	95(45.4)	209 (25.2)
Immiringi	98 (49.0)	102 (51.0)	200 (24.1)
Total	380 (45.8)	449 (54.2)	829 (100)

Table 4.2: Prevalence of intestinal helminthiasis based on gender among the study population of four rural communities in Bayelsa State

S/N	COMMUNITY	MALE n (%)	FEMALE n (%)	TOTAL n (%)
1	Otuegela	15 (17.4)	30 (24.2)	45 (21.4)
2	Ibelebiri	19 (23.1)	40 (32.0)	**59 (28.6)**
3	Otuesega	26 (22)	34 (95)	60 (28.7)
4	Immiringi	22 (22.4)	32 (102)	54 (27)
5	Total	82 (37.6)	136 (62.4.)	218 (100)

Table 4.3: Prevalence of intestinal helminthiasis with respect to age groups among the study population in four rural communities of Bayelsa State, Nigeria

Age group (years)	No examined	No positive (%) infected	*S. intercalatum* No (%) infected	*A. lumbricoides* No (%) infected	*S. mansoni* No (%) infected	*T. trichiura* No (%) infected	*Taenia*sp No (%)infected	Hookworm No (%) infected
1-10	335	100 (29.8)	36 (10.7)	29 (8.6)	20 (6.0)	12 (3.6)	-	3 (0.9)
11-20	201	34 (16.9)	17 (8.4)	5 (2.5)	4 (2.0)	5 (2.5)	-	3 (1.5)
21-30	102	24 (23.5)	8 (7.8)	8 (7.8)	1 (0.9)	1 (0.9)	-	6 (5.9)
31-40	81	23 (28.4)	7 (8.6)	7 (8.6)	3 (3.7)	2 (2.5)	1 (1.2)	3 (3.7)
41-50	65	20(30.7)	10 (15.3)	4 (6.1)	1 (1.5)	1 (1.5)	1 (1.5)	3 (4.6)
51-60	26	4 (15.4)	4 (15.4)	-	-	-	-	-
61-70	15	11 (73)	3 (20)	-	5 (33.3)	1 (6.7)	-	2 (13.3)
71-80	4	2 (50)	1 (50)	-	1 (50)	-	-	-
Total	829	218 (26.3)	86 (10.4)	53(6.4)	35(4.2)	22 (2.6)	2 (0.2)	20 (2.4)

Keys: *S=Schistosoma, A=Ascaris, T=Trichuris*

Table 4.4 Prevalence of Infection of Intestinal Helminths Based on Sex

Sex	No. examined	*S.intercalatum* n (%)	*A.lumbricoides* n (%)	*S.mansoni* n (%)	*T. trichiura* n (%)	Taenia sp n (%)	Hookworm n (%)
Male	380	29 (7.6)	25 (2.4)	12 (3.2)	9 (2.3)	2 (0.5)	3 (0.8)
Female	449	57 (13)	28 (5.7)	23 (5.1)	12 (2.7)	-	17(5.2)
Total	829	86 (10.4)	53 (6.4)	35 (4.2)	21(2.5)	2 (0.24)	20 (2.4

Table 4.5 Prevalence of Multiple Infections of Intestinal Helminthes Based on Sex

Helminths types	Male (N= 380) n (%) infected	Female (N= 449) n (%) infected	Total n (%) infected
S.intercalatum and hookworm	1 (0.3)	3 (0.7)	4 (0.5)
S.intercalatum/A.lumbricoides	2 (0.5)	4 (1.0)	6 (0.7)
S.mansoni/hookworm	1 (0.3)		1 (0.1)
	4 (36.4)	7 (63.6)	11 (1.3)

Key: S = *Schistosoma*

Table 4.6: Age Distribution of Multiple Infections of Intestinal Helminthes

Age (years)	*S.intercalatum*/Hookworm n (%) infected	*S.intercalatum/A.lumbricoides* n(%) infected	*S. mansoni*/Hookworm n(%) infected
≤ 10	0	0	0
11-20	1(25)	0	1(100)
21- 30	0	2 (33.3)	0
31- 40	0	1 (17)	0
41- 50	2 (50)	2 (33.3)	0
51- 60	0	0	0
61- 70		1 (17)	0
71- 80	1(25)	0	0
Total	4	6	1

Key: S = ***Schistosoma***

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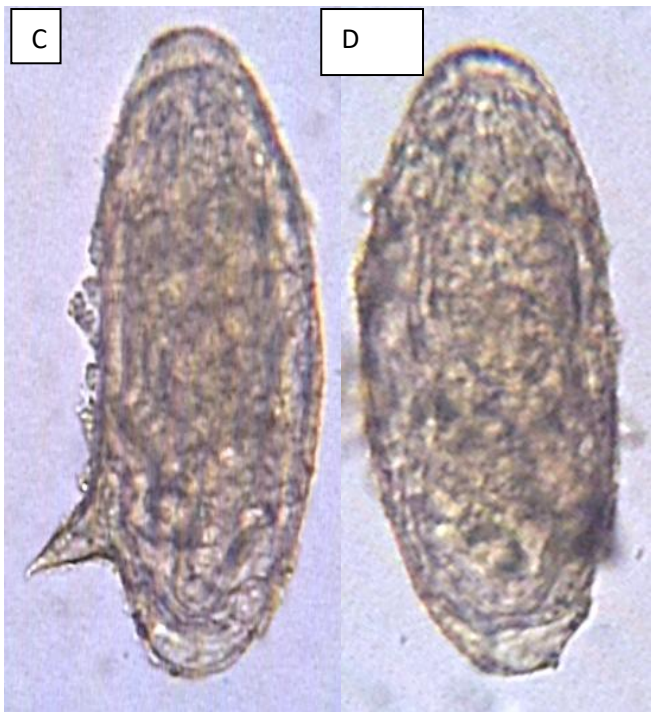

Plate 4.1: Ova of helminth parasites recovered from stool samples from of individuals from Ibelebiri community of Ogbia LGA, Bayelsa State. (A). *Schistosoma haematobium*; (B). *S.intercalatum*; (C). *S. mansoni*; (D). Unidentified ova Scale bars = 0.05mm

Table 4.7: Prevalence of intestinal helminths in school based study 11 in Bayelsa State

School	Sex	Number Examined	Number Infected	Prevalence
Ibelebiri	Males	70	33	47
	Females	113	51	45
Otuesega	Males	89	27	30.3
	Females	81	36	44.4
Immiringi	Males	84	8	9.5
	Females	97	7	2.6
Total	Males	243	68	28
	Females	294	94	32
	Sum total	537	162	30.2%

Table 4.8: Types of helminth species recovered in school based study 11 in Bayelsa State

School	Sex	NE	*A. lumbricoides*	*S. mansoni*	*S. intercalatum*	*T. trichuria*	*Hook worm*	*Taenia Spp*	*Strongy-Loides*
Ibelebiri	Males	70	12	9	7	3	2	-	-
	Females	113	20	16	8	4	3	-	-
Otuesega	Males	89	7	7	5	2	1	2	3
	Females	81	10	10	6	1	3	5	1
Immiringi	Males	84	5	-	-	2	-	-	1
	Females	97	7	-	-	-	-	-	-
Total	Males	243	24 (10)	16 (7)	13 (5.3)	7 (2.0)	3 (1.2)	2 (0.8)	4 (1.6)
	Females	294	37 (12.6)	26 (9)	14 (5.0)	5 (2.0)	6 (2.0)	5 (2.0)	1 (0.3)
	Sum total	537	61 (10.3)	42(8)	27 (5.0)	10 (1.8)	9 (1.6)	7 (1.3)	5 (1.0)

4.1.9 Intensity of infection of helminths in school-based study 11

In Table 4.9 below, the number of larvae and eggs identified in normal saline preparation were reported according to Chessbrough (2009), scanty: 1-3 per preparation; few: 4-10 per preparation; moderate: 11-20 per preparation; many: 21- 40 per preparation. Below, *Ascaris lumbricoides* had the highest percentage of light infection (83%), moderately infected, 11.5%. Only 5% of those infected with *A.lumbricoides* had heavy infection. 59% of the population infected by *S.intercalatum* had scanty load. Those moderately infected were 26%. *S.mansoni* had the highest population of student infected in moderate (26%) and heavy (15%) intensity. The intensity of infection with Strongyloides was all light. Only 9 students were infected with hookworm; 6 representing 67% had light infection, 2 (22%) were moderately infected; while just a student (11%) was heavily infected. All population infected by tapeworm were of light intensity. Of the population infected by *T.trichiuria*, 75% were of light intensity, and the rest, 25% had moderate infection.

4.1.10 Infection pattern of intestinal helminthiasis in school based study 11

Of the infected 162 population 145 (89.5%) had single helminths infection while 17 (10.5%) had double infection. There was no case of multiple infection. More females (32%) were infected than males (28%). See Table 4.10.

Table 4.9: Frequency of intensity of infection in school based study 11 in Bayelsa State

Parasites	Intensity	Male	Female	Total infection
	Light	18	33	61
	Moderate	3	4	
A. lumbricoides	Heavy	3	-	
	Light	16	26	42
	Moderate	-	-	
S. mansoni	Heavy	-	-	
	Light	4	5	12
	Moderate	3	-	
T. trichiuria	Heavy	-	-	
	Light		6	9
	Moderate	2		
Hookworm	Heavy	1		
	Light	9	7	27
	Moderate	2	5	
S. intercalatum	Heavy	2	2	
	Light	2	5	7
	Moderate			
Tapeworm	Heavy			
	Light	4	1	5
	Moderate	-	-	
Strongyloides	Heavy	-	-	

Table 4.10: Infection pattern of intestinal helminthes in schools based study 11 in Bayelsa State

School	Sex	No. Examined	No. Infected	Single Infection	Double infection
Ibelebiri	Males	70	33	30	3
	Females	113	51	48	3
Otuesega	Males	89	27	23	4
	Females	81	36	30	6
Immiringi	Males	84	8	7	1
	Females	97	7	7	-
Total	Males	243	68(28%)	60(25%)	8(3.2%)
	Females	294	94(32%)	85(29%)	9(3.1%)
	Sum total	537	162 (30.2%)	145 (89.5%)	17 (10.5%)

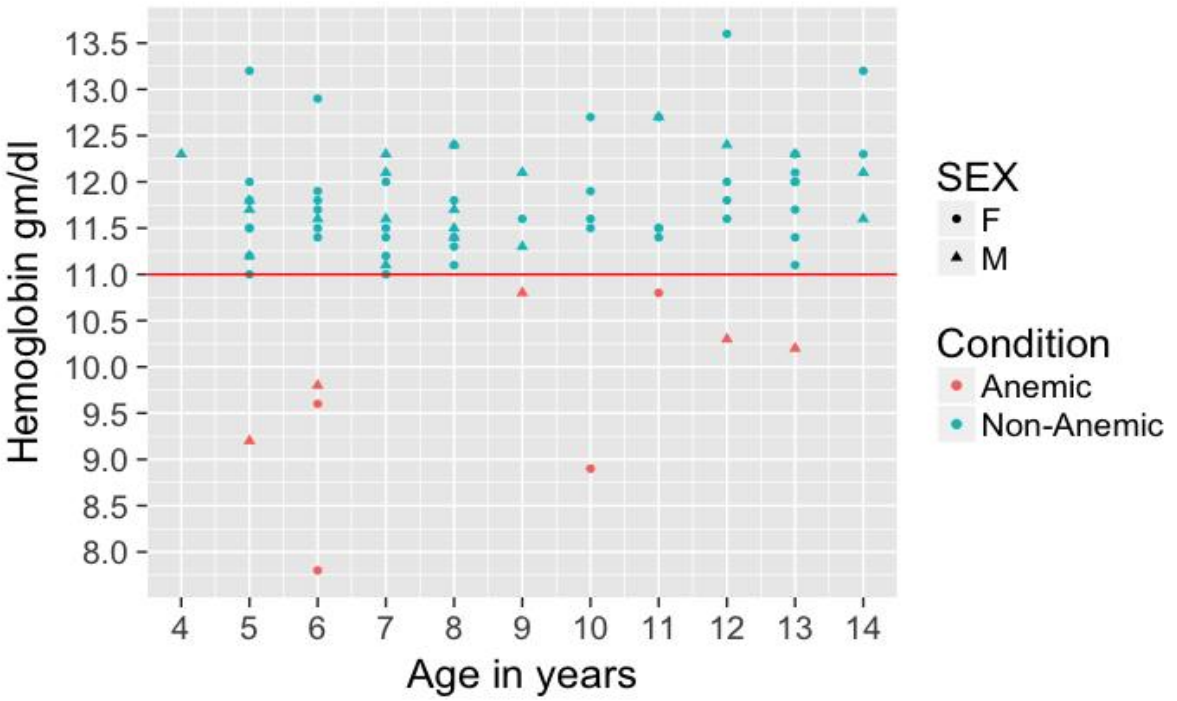

Figure 4.1 Plasmodium infection and anaemia levels in Children in first school-based study in Bayelsa State

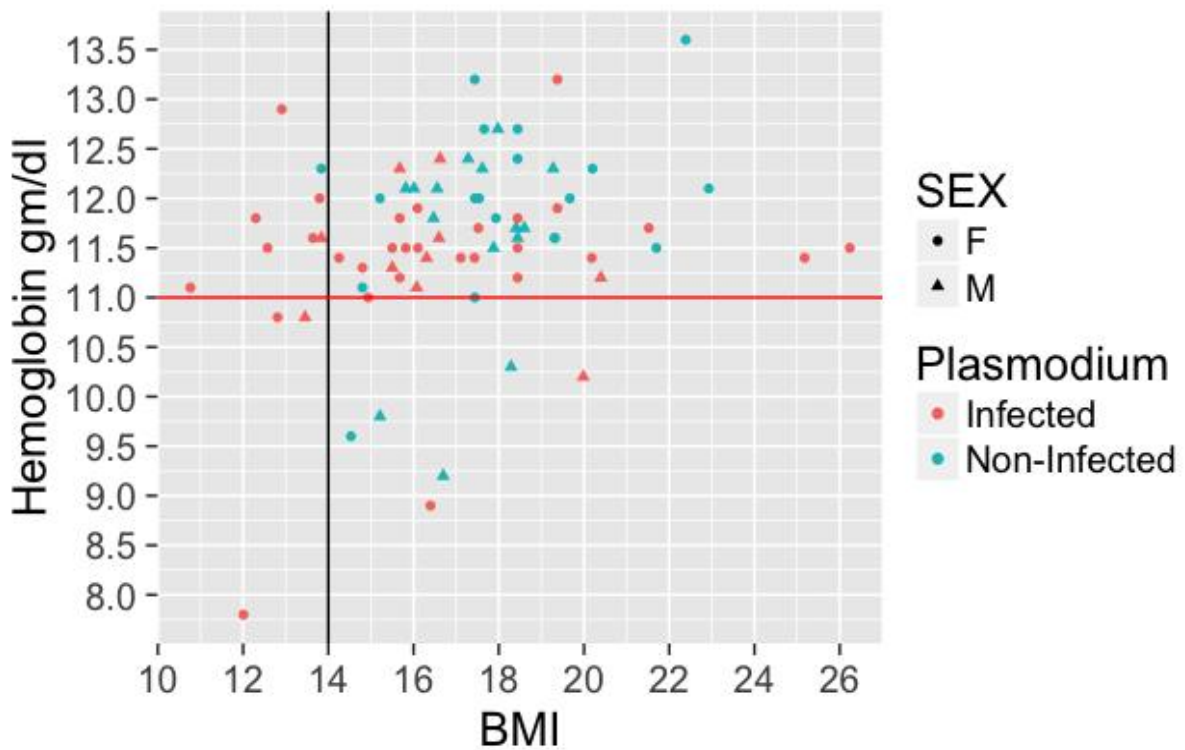

Figure 4.2 Body Mass Index (BMI) and haemoglobin level in children infected/non-infected with *Plasmodium*

Figure 4.1 anaemia affected both males and females, and all age groups.

Figure 4.2 shows that majority of the children have normal BMI and Hemoglobin levels. 85% had normal BMI. The underweight children were 11(75) out of which, 10 were infected with Plasmodium parasites. The 3 anaemic and Plasmodium infected pupils (2 feamles and a male) had low BMI. A few anaemic and infected population had normal BMI values.

4.2 Prevalence of malaria infection in some communities in Bayelsa State

In Table 4.11, the overall prevalence in community study was 42% with 345 infected out of 829 people examined. In Otuegela community, 97 out of 210 examined were positive for malaria infection (46.2%). Adult females, 71%; adult males, 44%; female children, 39.2% and male children 37.0%. The prevalence rate of malaria infection in Ibelebiri was 53%; out of 210 people examined. Adult females, 71%; adult males, 64%, male children, 53% and female children, 36% respectively. A prevalence rate of 29% was recorded in Otuesega community; male children had the least infection rate, 17% and the highest was obtained from adult females, 52%. In Immiringi, a total prevalence of 38.5% was recorded. Adult male and females had higher infection of 50% and 46%. Children, male and females had 31.1% and 37.0% respectively. Generally, the highest population infected with malaria parasites was Ibelebiri with 53%. The least infected community was Otuesega, 29 %.

4.2.1 School-based study II for Malaria Parasites

In table 4.12 the total population of pupils examined were 190; population infected: 61, percentage prevalence: 32.1%, male pupils: 27%, females' pupils: 37%.

Table 4.11: Prevalence of malaria in relation to sex in school-based study 11 in Bayelsa State

S/N	Community	Adult M n(%)	Adult F n(%)	Children M n(%)	Children F n(%)
1.	Otuegela	14(32)	32(45)	20(54)	31(79)
2.	Ibelebiri	16 (25)	38(53)	30 (57)	27(75)
3.	Otuesega	10(45)	30(58)	12(69)	8(37)
4.	Immiringi	11 (24)	21(42)	23(74)	22 (60)
	Total	51(126)	121(198)	85(254)	88(251)

Table 4.12: Prevalence of malaria in relation to sex in school-based study 11 in Bayelsa State

School	Sex	No.positive (No. examined)
Otuegela	Males	11(36)
	Females	12(39)
Ibelebiri	Males	10(35)
	Females	15(28)
Otuesega	Males	7(32)
	Females	6(20)
Immiringi	Males	28(103)
	Females	33(87)
	Total	61(190)

+ive samples:1, 12, 15, 22

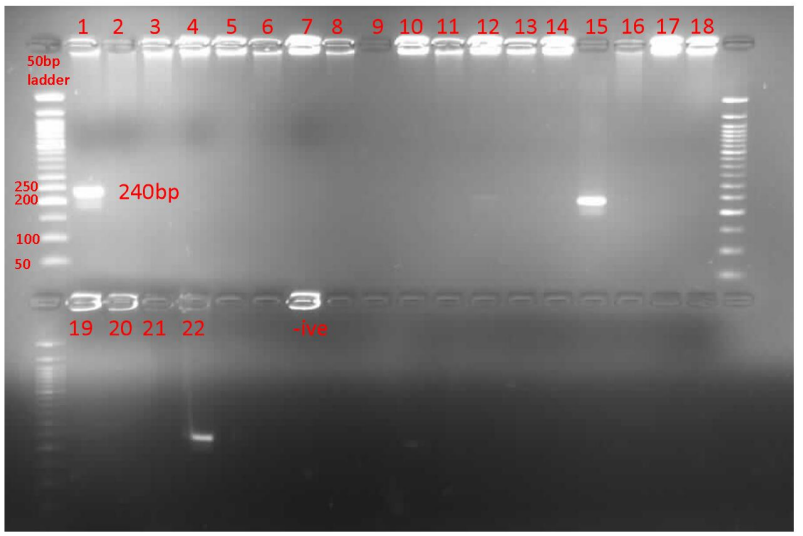

Figure 4.3 Nested polymerase reaction for identification of ova of intestinal helminths, lane: 1-22

+ive samples: 24, 29, 34, 36, 37, 38, 39, 40

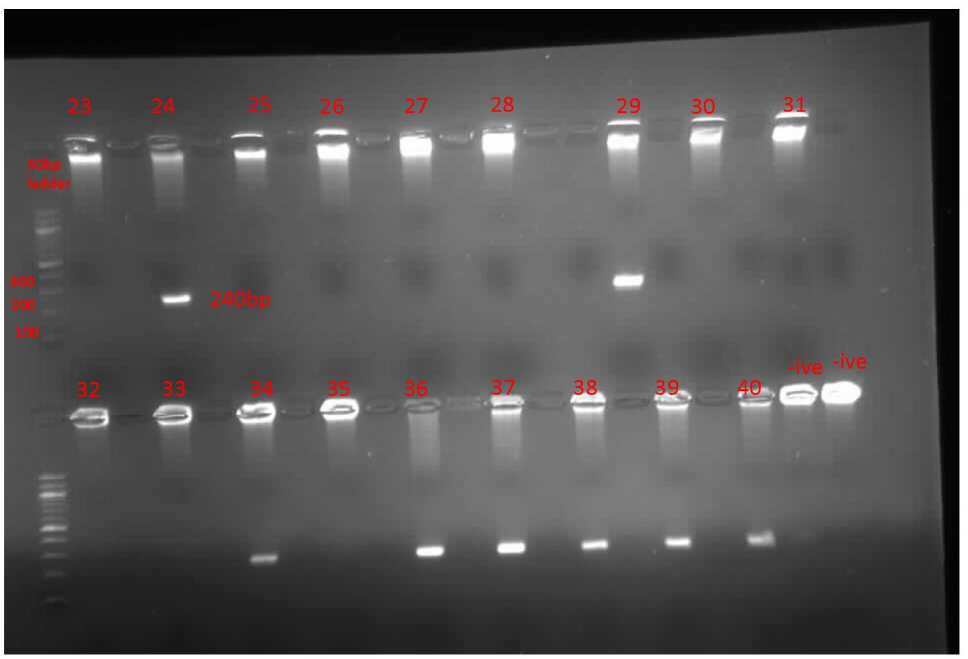

Figure 4.4 Nested polymerase reaction for identification of intestinal helminths, lane 23 -40

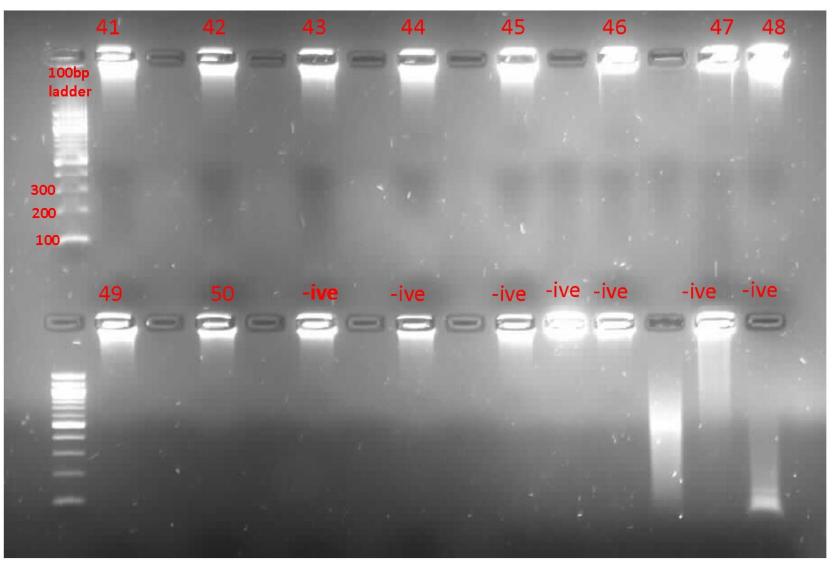

Figure 4.5 Nested polymerase reaction for the identification of helminth ova, lane 41-50

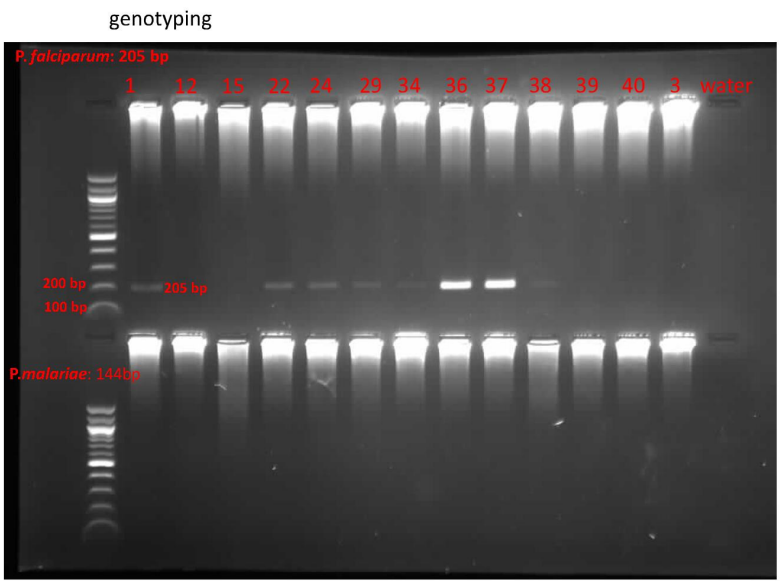

Figure 4.6: Nested polymerase chain reaction for the identification of ova of intestinal helminths; genus specific at 205 bp

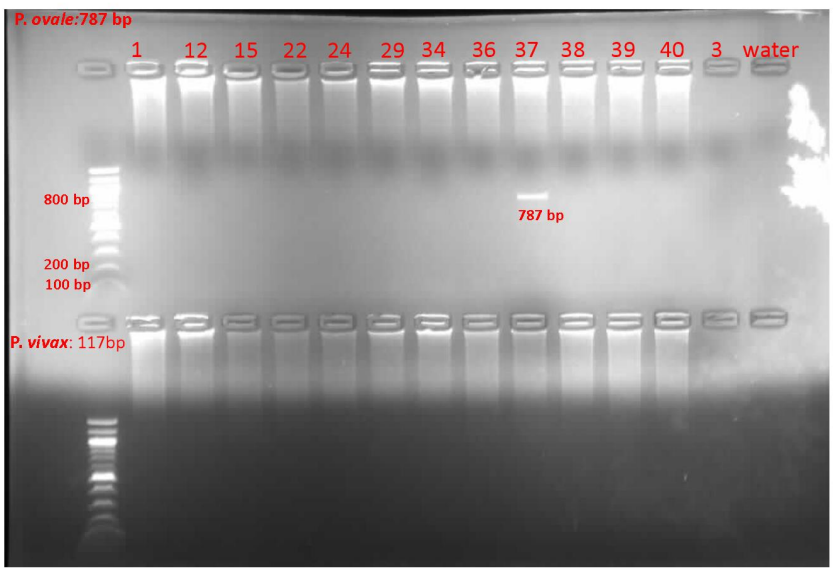

Figure 4.7 Nested polymerase chain reaction; genus specific at 787 bp

Figure 4.3: determining the - genus of malaria parasites using molecular technology, the Nested (qualitative) polymerase chain reaction (PCR). Genus specific primers used for the amplification of both nest 1 and 2. The PCR products of nest 1 amplifications with DNA templates prepared from 50µl blood spots on FTA card were positive for Plasmodium *spp* at 240bp in the following lanes: 1, 12,15 and 22. Molecular size markers (500-basepair [bp] ladder) are shown in first lane.

Figure 4.4: determining the genus of malaria parasites using molecular technology, the Nested (qualitative) polymerase chain reaction (PCR): shows the Nested (qualitative) polymerase chain reaction (PCR). Genus specific primers used for the amplification of both nest 1 and 2. The PCR products of nest 1 amplifications with DNA templates prepared from 50µl blood spots on FTA card were positive for Plasmodium at 240bp in the following lanes: 24,29,34 and 36, 37, 38, 39 and 40. Molecular size markers (500-basepair [bp] ladder) are shown in first lane .

Figure 4.5: determining the genus of malaria parasites using molecular technology, the Nested (qualitative) polymerase chain reaction (PCR): shows the Nested (qualitative) polymerase chain reaction (PCR). Genus specific primers used for the amplification of both nest 1 and 2. The PCR products of nest 1 amplifications with DNA templates prepared from 50µl blood spots on FTA card were negative for Plasmodium parasites in samples 41-50. Molecular size markers (500-basepair [bp] ladder) are shown in first lane.

Figure 4.6 shows the Nested (qualitative) polymerase chain reaction (PCR). Genus specific primers used for the amplification of both nest 1 and 2. The PCR products of nest 2 amplifications with DNA templates prepared from 50µl blood spots on FTA card were positive for *Plasmodium falciparum* at 205 bp.

Figure 4.7: determining the species of malaria parasites using molecular technology, the Nested (qualitative) polymerase chain reaction (PCR): shows the nested (qualitative) polymerase chain reaction (PCR). Genus specific primers used for the amplification of both nest 1 and 2. The PCR products of nest 2 amplifications with DNA templates prepared from 50µl blood spots on FTA card were positive for *Plasmodium ovale* at 787 bp in lane: 37. Molecular size markers (800-basepair [bp] ladder) are shown in first lane.

4.3 Co-infection of intestinal helminths and malaria parasites in communities

In table 4.13, the prevalence of co-infection of *Plasmodium* parasites and intestinal helminths was 18%. Adult females had the highest prevalence of 32.3%, followed by female children, 19%; male children had 9.4% and adult male 8%. In the communities, Otuegela had the highest number of people infected (56%) with both intestinal helminths and malaria parasites. Ibelebiri, Immiringi and Otuesega had 17%, 15% and 12% infection rates. Adult females had the highest prevalence in Otuesega, 53%, Immiringi, 33% and 30.2% in Ibelebiri. However, in Otuesega, the population of female children examined had the highest rate of infection, 27%, compared to 17% for adult females, 9% for male children. There was no case of mixed infection in adult males in Otuesega commuinity only.

4.4 Co-infection of helminths and malaria parasites

In table 4.14, a total of 17 students were co-infected, with 88% involving *Ascaris lumbricoide* and 12% for *S.intercalatum* and *T.trichiura.* The number of males infected were 10 (58%) and 7 (42%) females.

4.5 Frequency of co-infection of intestinal helmiths in school study 11.

Occurrences of co-infection were highest in the ova of A.lumbricoides and Hoolworm, identified in 17 students of both sexes. Least occurrence of paired infection were between A.lumbricoides and *Taenia* species, where only 2 students were infected. See figure 4.15.

4.6 Examination of participants after treatment

Infected participants, n = 88 were examined 18 days post treatment. With a second post treatment laboratory examination, no helminth eggs nor *Plasmodium* parasites was found.

4.7 Mean Concentration of cytokines and chemokines before Treatment

In table 4.16, the mean concentration was highest in MIP-β, followed by eotaxin-3, then IFN-γ and the least mean concntration was observed in IL-4 in participants infected with *Plasmodium* only. IL-4 is poorly expressed even at upper limit.

4.8 Mean concentration of cytokines and chemokines after treatment

After treatment, in table 4.17 below, there was reduction in concentration; highest mean concentration in MIP-β, followed by eotaxin-3, then IFN-γ and the least mean concentration was observed in IL-4.

Table 4.13: Prevalence of Co- infection of Malaria and Intestial helminths in the study community

S/N	Community	Adult male n(examined)	Adult female n(examined)	Children male n(examined)	Children female n(examined)	Total n(examined)
1.	Otuegela	6(32)	24 (45)	8 (54)	16(79)	54 (210)
2.	Ibelebiri	2 (25)	16(53)	6 (57)	12 (75)	36 (210)
3.	Otuesega	(45)	10(58)	6(69)	10(37)	26 (209)
4.	Immiringi	2(24)	14(42)	4 (74)	10 (60)	30 (200)
	Total	10 (126)	64(198)	24 (254)	48(251)	146 (829)

***n= number positive**

Table 4.14: Prevalence of co-infection of intestinal helminths and *Plasmodium* parasites in school based study 11

School	Sex	No.positive (No. examined)
Otuegela	Males	4(36)
	Females	2(39)
Ibelebiri	Males	5(35)
	Females	4(28)
Otuesega	Males	3(32)
	Females	2(20)
Immiringi	Males	12(103)
	Females	8(87)
		20(190)

Table 4.15: Frequency of co-infection of intestinal helminths in school bases study 11

Parasites	Males	Females	Total co infection
	10	7	17
A.lumbricoides Hookworm	4	3	7
A.lumbricoides S.mansoni	3	2	5
A.lumbricoides Tapeworm	1	1	2
S.intercalatum T.trichiuria	*2*	1	3

Table 4.16: Serum concentration of cytokines and chemokines *before* treatment (pg/ml) in Plasmodium infection only

	IL-4	IFN-γ	Eotaxin 3	MIP-β
Mean	206	685.5	2332	3180
Lower	89.59	308.3	216.8	2271
Upper	406.7	1190	6301	4299

Table 4.17: Serum concentration of cytokines and chemokines *after* treatment (pg/ml) in Plasmodium infections only

	IL-4	IFN-γ	Eotaxin 3	MIP-β
Mean	213.6	470.8	596.6	3136
Lower	57.73	123.5	196.8	2383
Upper	439.1	962.2	1124	3960

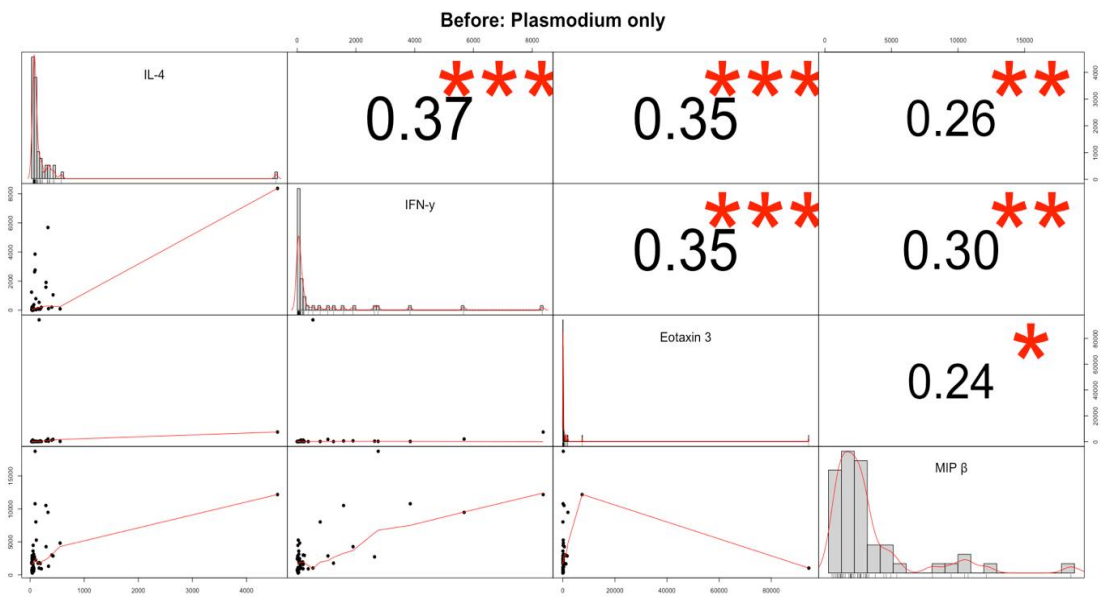

Figure 4.8 Correlation plot for Plasmodium infected population before treatment

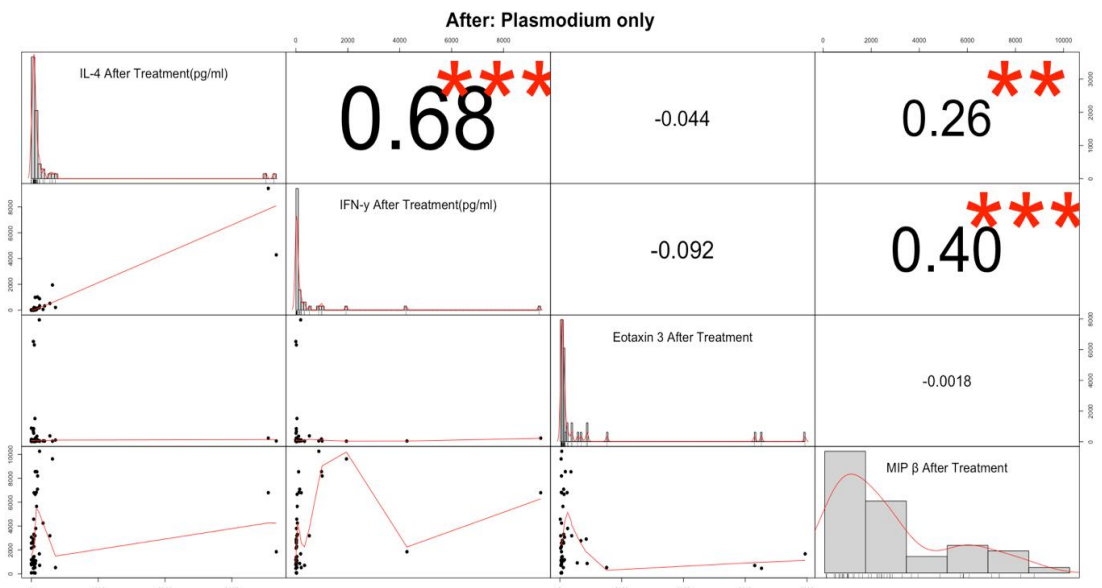

Figure 4.9 Correlation plot for *Plasmodium* infection only after treatment with Artemether/Lumefantrine.

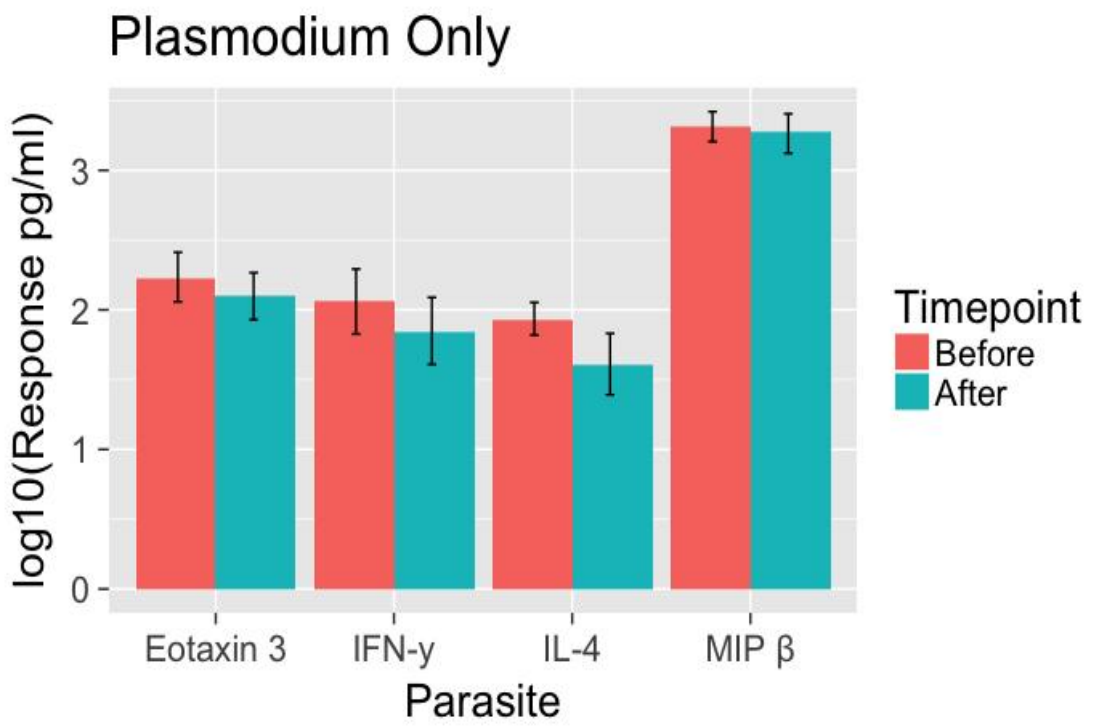

Figure 4.10 Concentration for IFN-γ, IL-4 Eotaxin 3 and MIP β, before and after treatment

In Figure 4.8, a plot correlation for plasmodium before treatment showed that all analytes were positively associated to one another : IL-4/ IFN-γ (0.37), IL4/Eotaxin (0.35) IL-4/ MIP-β (0.26). weak positive correlation amongs the immune molecules before treatment. Weak positive association was observed between Eotaxin 3/MIP-β (0.24), Eotaxin 3/IFN-γ (0.35). Between MIP-β/IFN-γ was a weak positive correlation. Correlation value below 0.5 is progressively week.

Figure 4.9 shows that however, after treatment positive correlation was observed between IL-4/IFN-γ (0.68), IFN-γ/ MIP-β (0.40) and a weaker positive association between IL-4/ MIP-β (0.26). After treatment, Eotaxin was negatively correlated with IL-4 (-0.044), IFN-γ (0.092) and MIP-β (0.0018). Stonger correlation (0.68) was observed between IFN-γ /IL-4 after antiparasitic treatment than before (0.37); and between IFN-γ / MIP-β,a correlation value 0.40 was obtained after treatment compared to 0.26 before treatment. There was no statistical significant difference in the value of individual immune molecule before and after treatment; IL-4 (P = 0.06147), IFN-γ (P = 0.1132), Eotaxin (P = 0.2899) and MIP-β (P = 0.5117).

Figure 4.10: shows the concentration chart of immune molecules for Plasmodium only before and after treatment. There was reduction in concentration in all immune molecules after treatment, though no significant difference was recorded in individual immune molecule before and after treatment of participants.

4.9 Before treatment in co-infected population in the study

Before treatment in co-infected groups, the mean concentration of IFNγ (387.6 pg/ml), IL-4 (91.36pg/ml) and Eotaxin (344.1 pg/ml were low compared to MIP 5718pg/ml).

See Table 4.18

Table 4.18: Mean concentration of immune molecules (pg/ml) before treatment in co-infection of Plasmodium and intestinal helminths

	IL-4	IFN-γ	Eotaxin 3	MIP-β
Mean	91.36	387.6	344.1	5718
Lower	60.84	182.1	125.4	2852
Upper	131	663.7	642.4	10420

4.10 After treatment in co-infection: concentration of immune molecules

In Table 4.19, after treatment in co-infection MIP-β (5725pg/ml) had the highest mean concentration and the least concentration was obtained from IL-4 75.07pg/ml.

4.11 Helminths Infection only: Concentration of immune molecules

In Table 4.20, the mean concentration of MIP-β, 2167pg/ml was the highest and least was IL-4, 70.8pg/ml. Eotaxin and MIP-β had mean values of 245.2 and 666.8pg/ml respectively.

4.12 Helminth infection only. Mean concentration of immune molecules after treatment

In Table 4.21. IFN-γ had the least mean concentration value,49.32pg/ml after treatment of helminths infections. MIP-β had the highest, 2059pg/ml; IL-4,64.34pg/ml and Eotaxin 520.6pg/ml.

Table 4.19: Mean concentration of immune molecules (pg/ml) after treatment in co-infection of Plasmodium and intestinal helminth infections

	IL-4	IFN-γ	Eotaxin 3	MIP-β
Mean	75.07	225.1	642.6	5725
Lower	37.81	25.9	177.9	1985
Upper	127.4	594.7	1221	11523

Table 4.20: Mean Concentration of immune molecules (pg/ml) before treatment in helminths infections only

	IL-4	IFN-γ	Eotaxin 3	MIP-β
Mean	70.88	245.2	666.8	2167
Lower	37.62	74.86	163.7	1336
Upper	125.5	469.9	1563	3185

Table 4.21: Mean Concentration of immune molecules (pg/ml) after treatment in helminths infections only

	IL-4	IFN-γ	Eotaxin 3	MIP-β
Mean	64.34	49.32	520.6	2059
Lower	45.61	33.59	116.4	1525
Upper	86.48	68.1	1145	2626

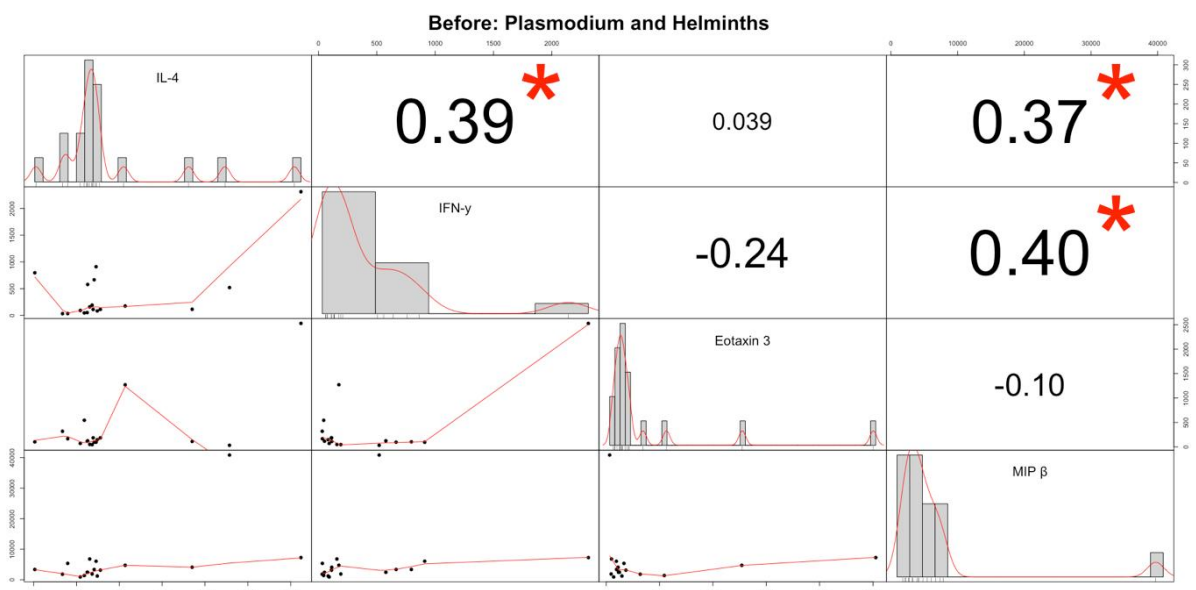

Figure 4.11 Correlation plot for population infected with Plasmodium and intestinal helminths infection before treatment

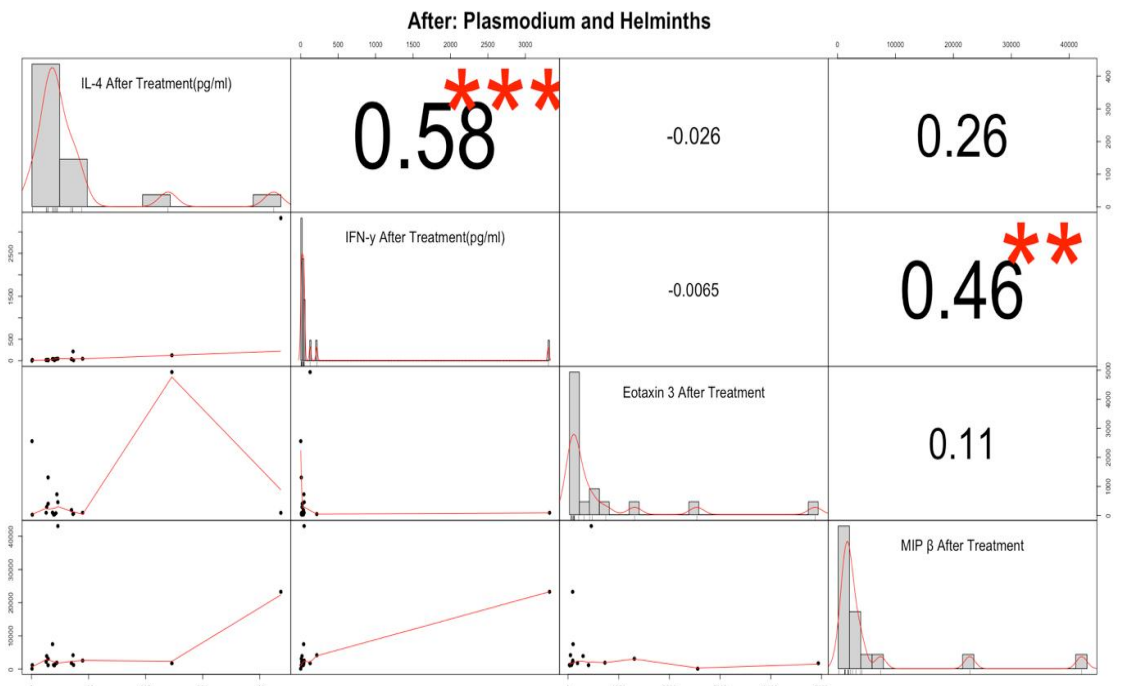

Figure 4.12 Correlation plot for population infected with Plasmodium and Intestinal helminths infection after treatment

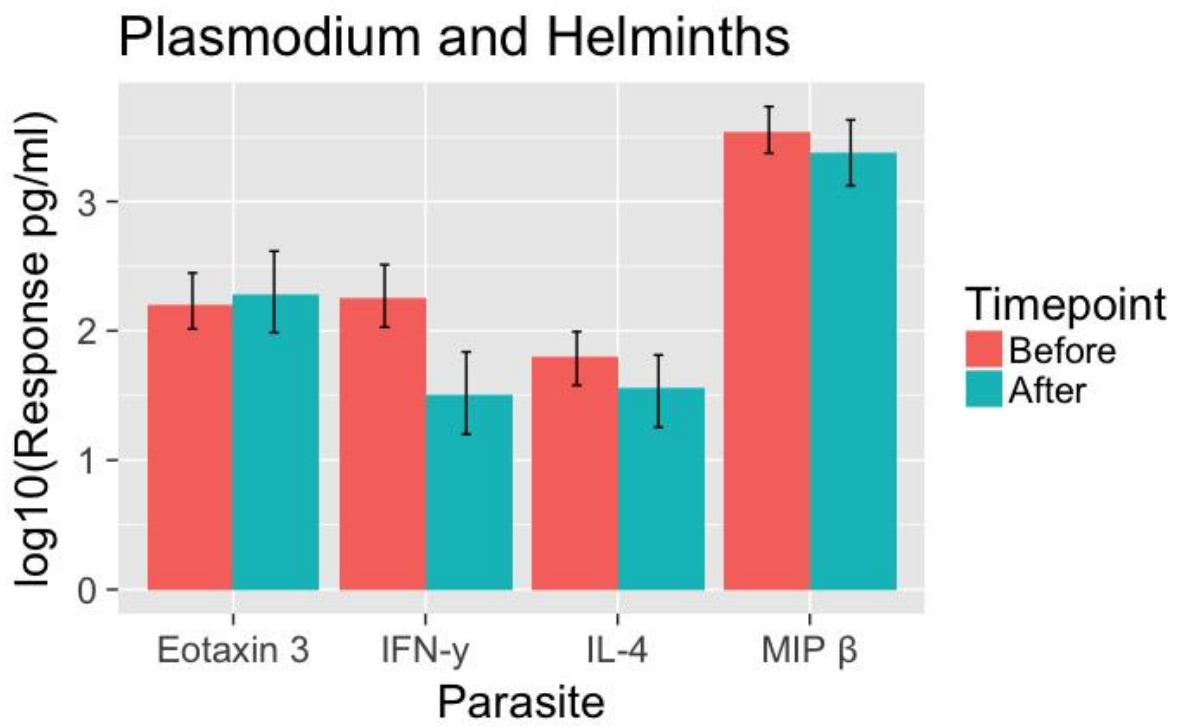

Figure 4.13: Co-infection: Concentration for IFN-γ, IL-4 Eotaxin 3 and MIP β, before and after treatment

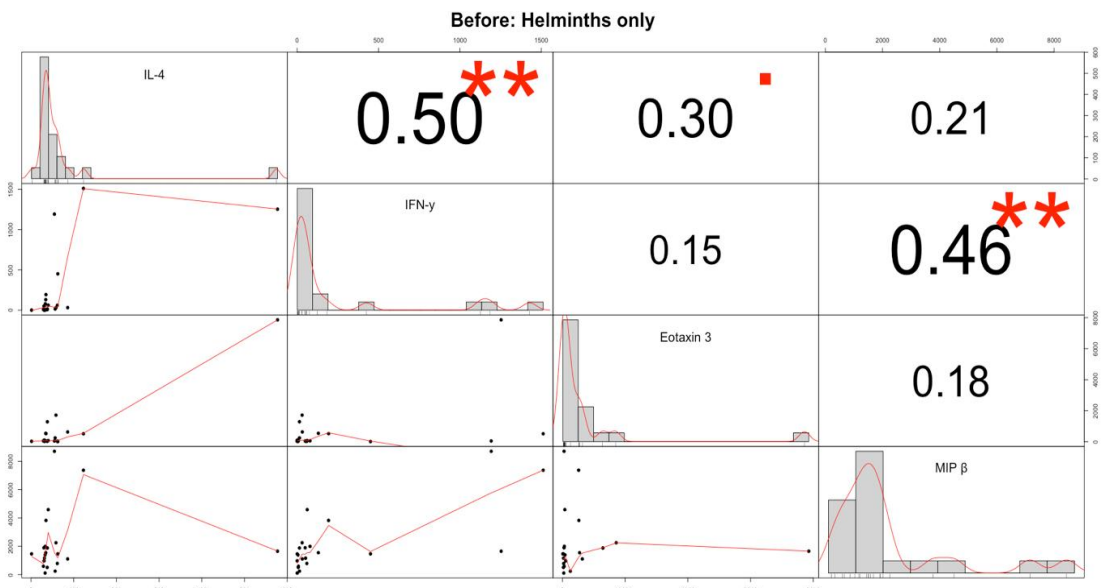

Figure 4.14 Correlation plot for population infected with helmiths only before treatment

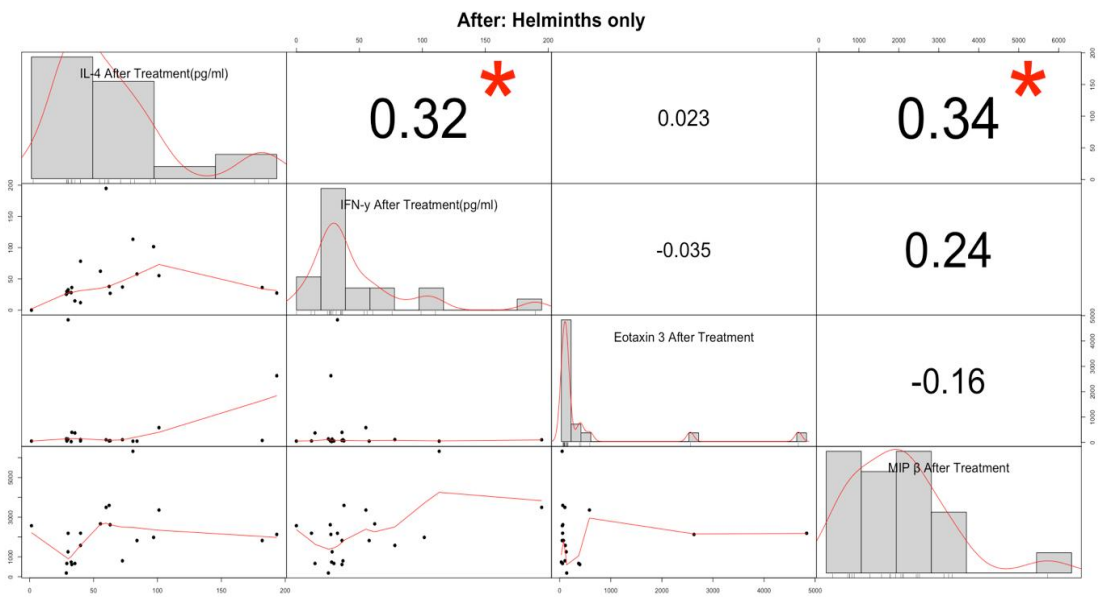

Figure 4.15 Correlation plot for population infected with helminths only and had been treated.

Figure 4.11 shows the correlation plot for Plasmodium/intestinal helminth before treatment. Weak correlation of immune molecules with infections of Plasmodium and intestinal helminth. Before treatment there was positive correlation in IL4/ IFN-γ (0.39), IL-4/ MIP-β (0.37) . A positive correlation value of 0.4 was found between IFN-γ / MIP-β. Eotaxin had no correlation with IL-4 before treatment (0.039) but was negatively correlated with (IFN-γ) (- 0.24) and MIP-3 (-0.10).

Figure 4.12 shows the correlation plot for Plasmodium/intestinal helminths infection after treatment; stronger positive correlation after treatment with IL-4/IFN-γ. A stronger positive relationship was observed after treatment between IL-4/IFN-γ (0.58) than before treatment (0.39). MIP-β/ IFN-γ also had stronger correlation after treatment (0.46) than before treatment (0.40) but the correlation between MIP-3/IL-4 after treatment was weak (0.26). Eotaxin had negative correlation with IL-4 (-0.026) and IFN-γ (-0.0065). Weak association of 0.11was found between Eotaxin/ MIP-β after treatment. There was no statictical significant difference before and after treatment for IL-4 (P = 0.1297), Eotaxin (0.5798) and MIP-β (0.5791). However, there was satistiscal difference, P = 0.001045 in the concentration of IFN-γ

Figure 4.13 shows the concentration chart of analytes for Plasmodium/intestinal helminth before and after treatment. The concentration of all molecules dropped after treatment but that of eotaxin rather increased.

Figure 4.14 shows the correlation plot for helminth infection only before treatment. Before treatment in helminths infections was positive correlation (0.50) between IL-4/ IFN-γ and between IL-4/Eotaxin (0.30) and between IL-4/ MIP-β (0.21). MIP-β had positive correlation with IFN-γ (0.46) and positive correllation with IL-4 (0.21) and with Eotaxin (0.18).

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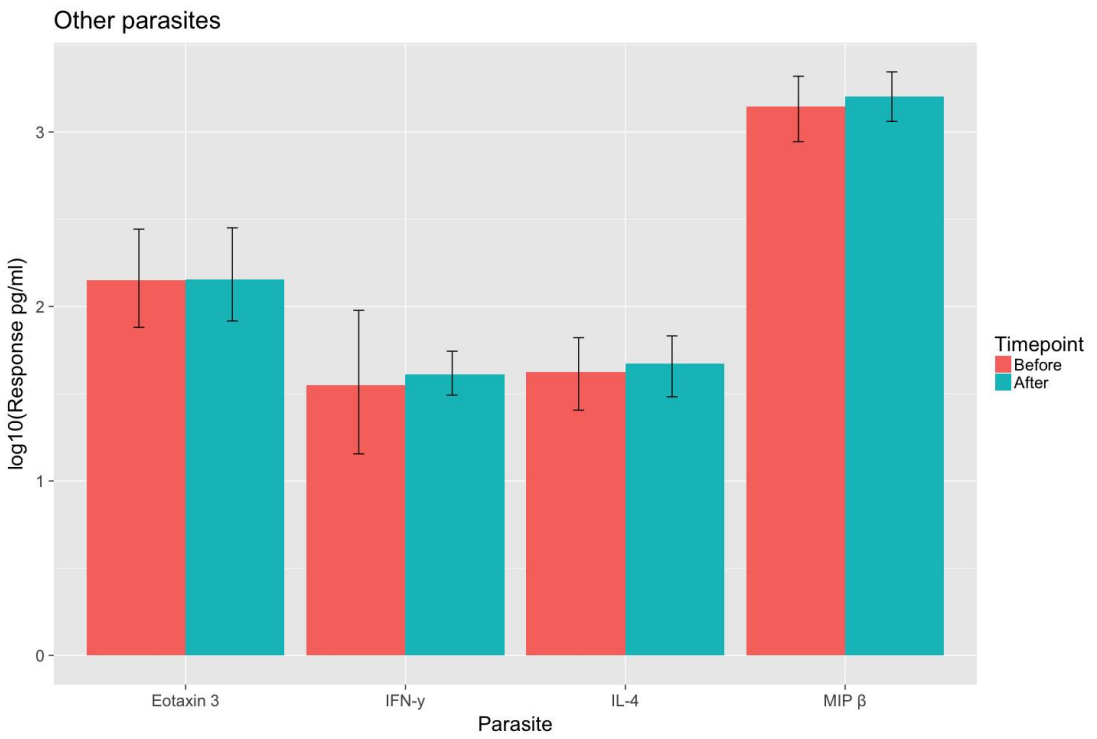


Figure 4.16: Helminths infection concentration for IFN-γ, IL-4 Eotaxin 3 and MIP β before and after treatment;

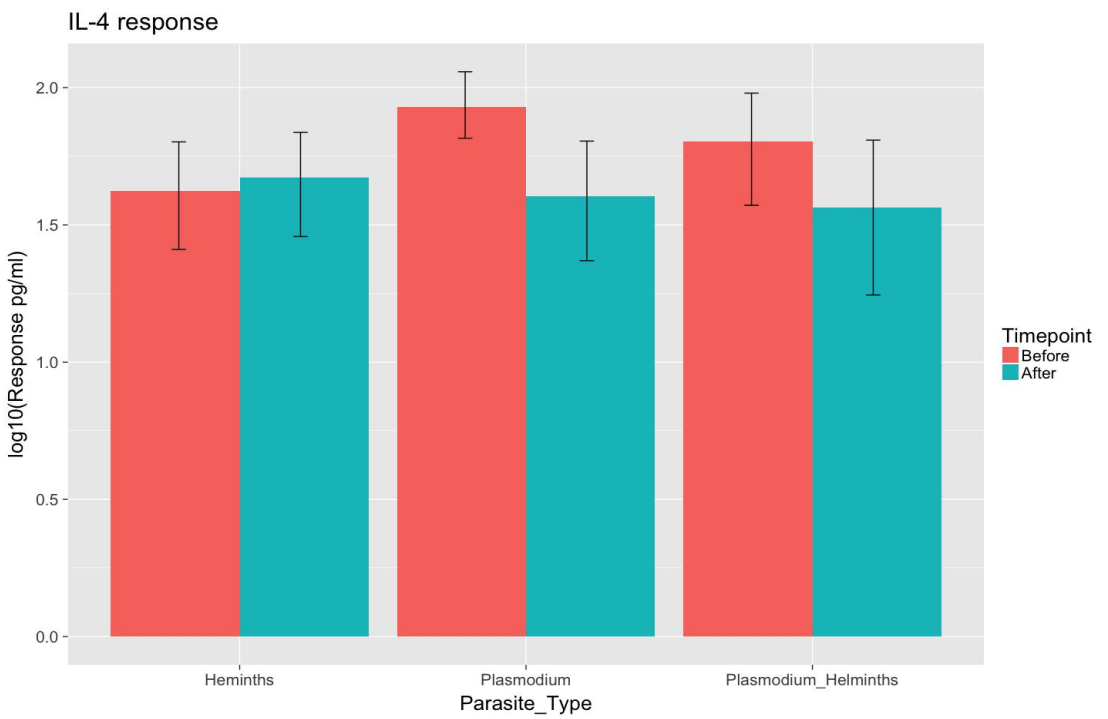


Figure 4.17 IL-4 response in all categories of infection (helminths, *Plasmodium* and *Plasmodium* –helminths) and after treatment

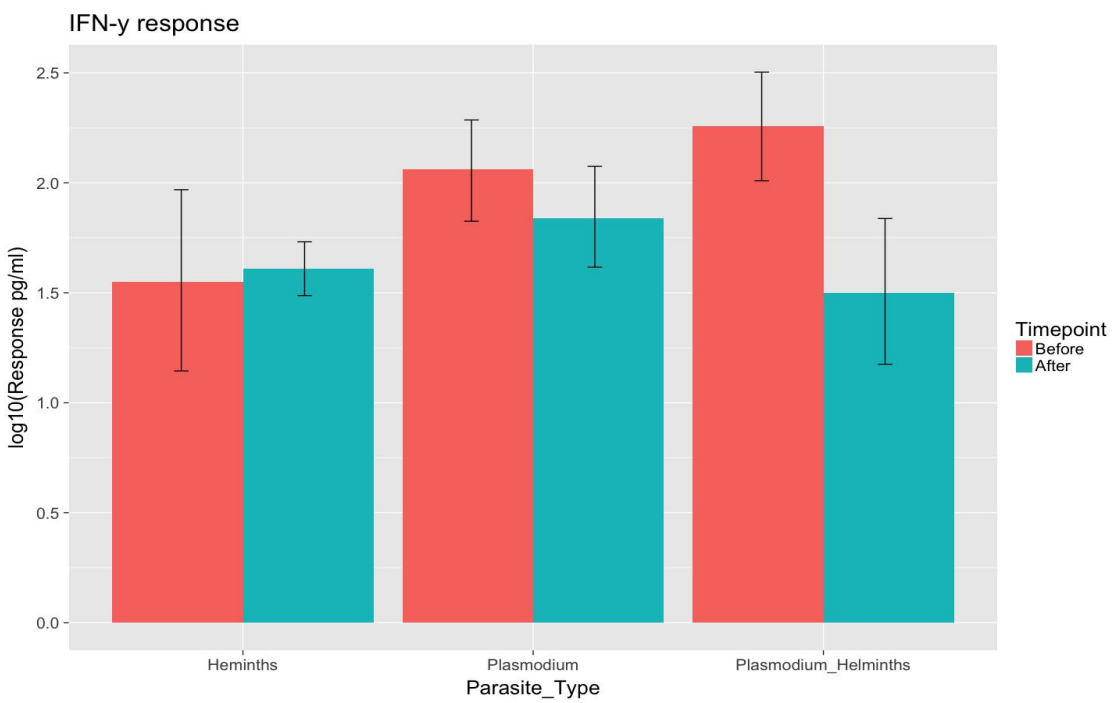


Figure 4.18: IFN-γ response in all categories of infection (helminths, *Plasmodium* and *Plasmodium* –helminths) and after treatment

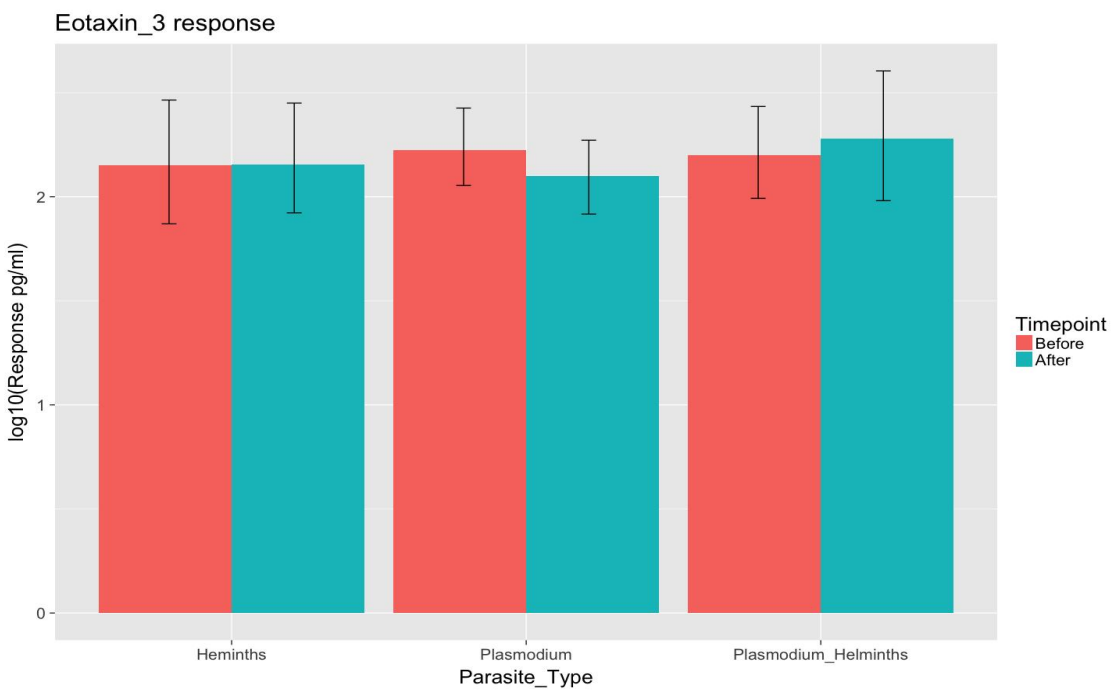


Figure 4.19 Eotaxin 3 responses in all categories of infection (helminths, *Plasmodium* and *Plasmodium* –helminths) and after treatment

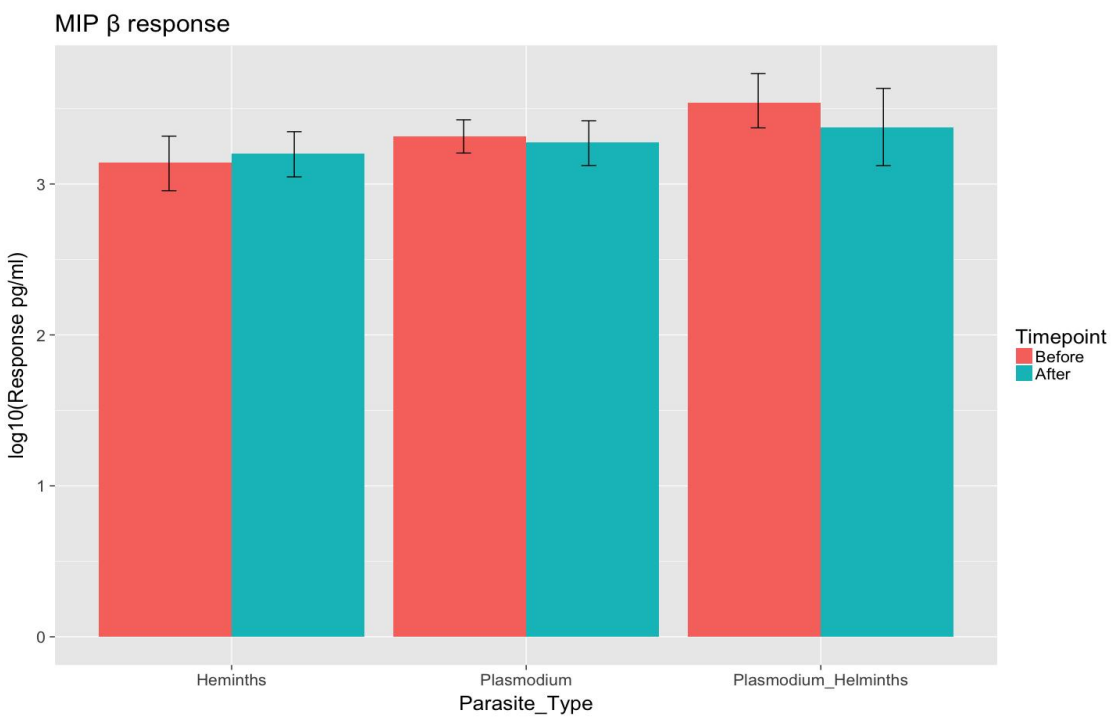


Figure 4.20: MIP-β response in all categories of infection (helminths, *Plasmodium* and *Plasmodium* –helminths) and after treatment:

Figure 4.15 shows the correlation plot for helminth infection only after treatment. Correlation were weaker after expulsion of worms. After treatment, correlation between IL-4/ IFN-γ was reduced to 0.32 compared to 0.52 before treatment. IL-4 had positive correlation (0.34) with MIP-β but had no relationship (0.023) with Eotaxin after treatment. Eotaxin had negative correlation -0.16 with MIP-β but no correlation-0.035 with IFN-γ

Figure 4.16 shows the concentration chart of analytes for helminth infection only before and after treatment. No difference in concentration after expulsion of worms.

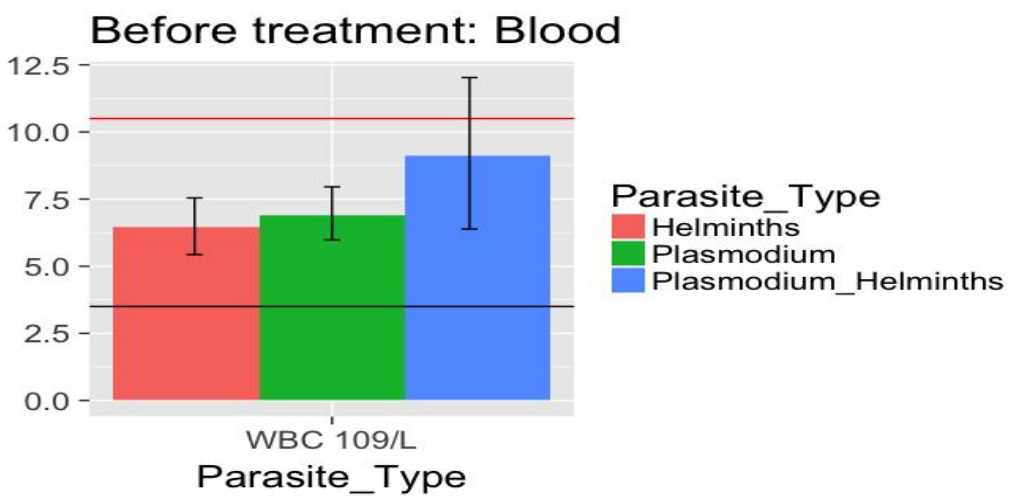
Figure 4.17 shows the concentration chart for IL-4 response in all infections before and after treatment. The concentration of IL-4 is highest in Plasmodium infections and least in helminthiasis. The concentration remains the same after treatment in all categories of infections.

Figure 4.18 shows the concentration chart for IFN-γ response in all infections before and after. The concentration was highest in co-infection and least in helminths sole infections. After treatment, concentration was reduced in category with co-infections only.

Figure 4.19 shows the concentration chart for Eotaxin-3 response in all infections before and after treatment. The concentration of eotaxin remained insignificantly high in all categories of infections and after treatment.

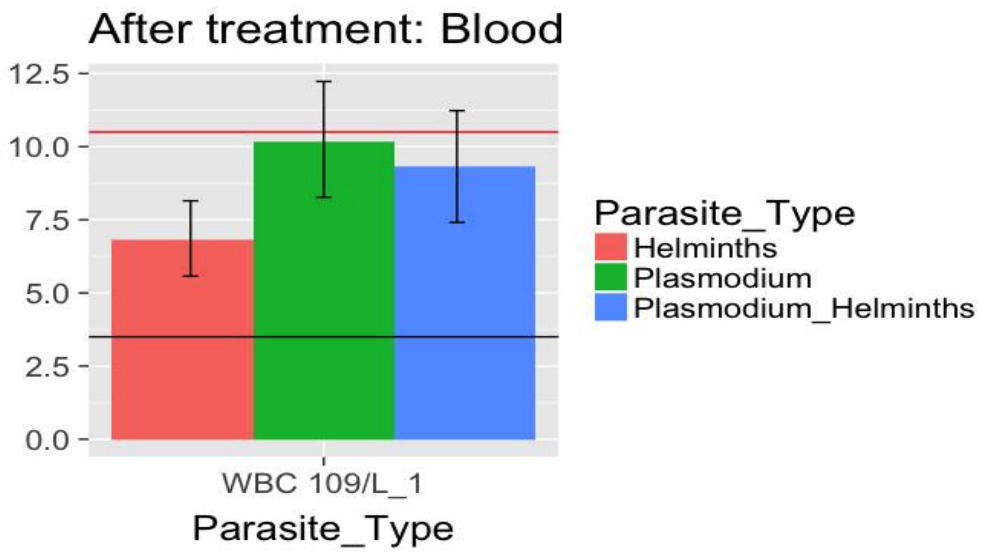
Figure 4.20 shows the concentration chart for MIP-β response in all infections before and after treatment.The concentration of MIP-β was very high in all categories of infection and did not fall any significanctly after treatment.

4.13 Blood cells profiles



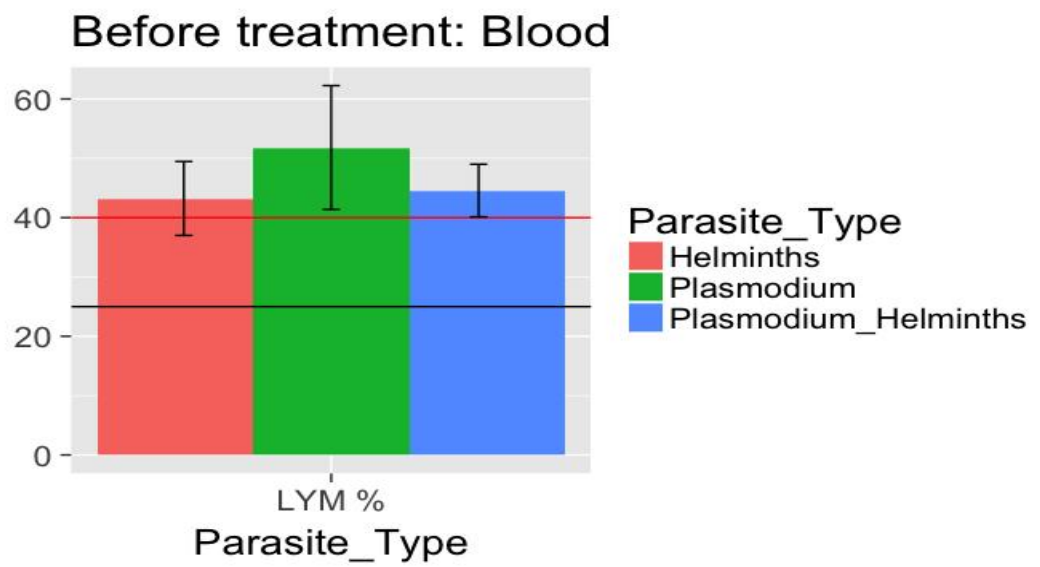
WBC - White blood cells

Figure 4.21: Blood cells profile before treatment: White Blood Cell



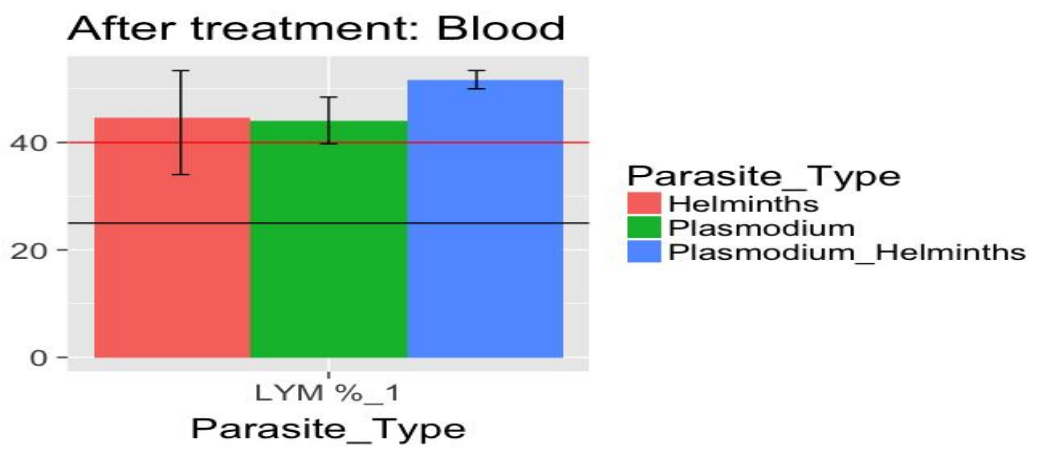
WBC - White blood cells

Figure 4.22 Blood cells profile after treatment: White Blood Cell



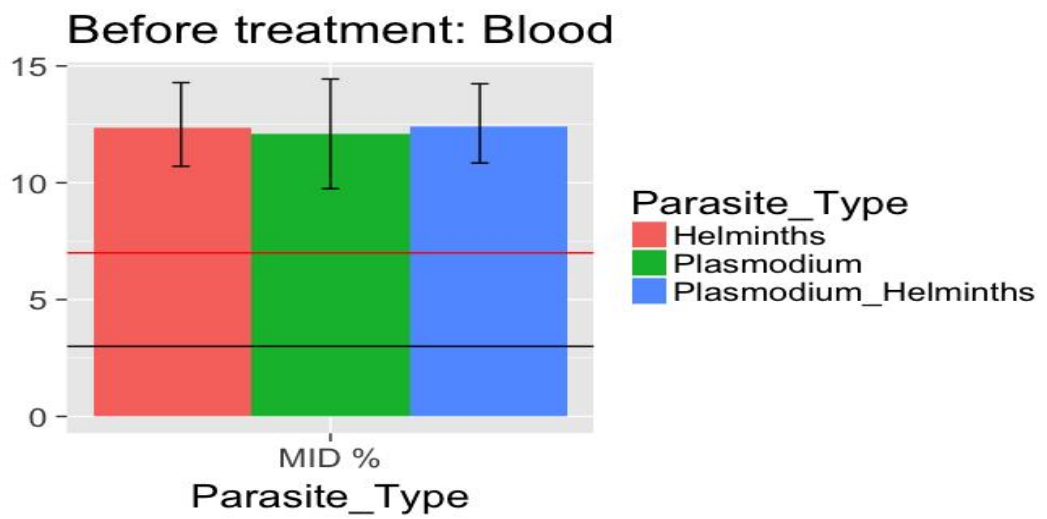
LYM - Lymphocytes

Figure 4.23: Blood cells profile before treatment: Lymphocytes



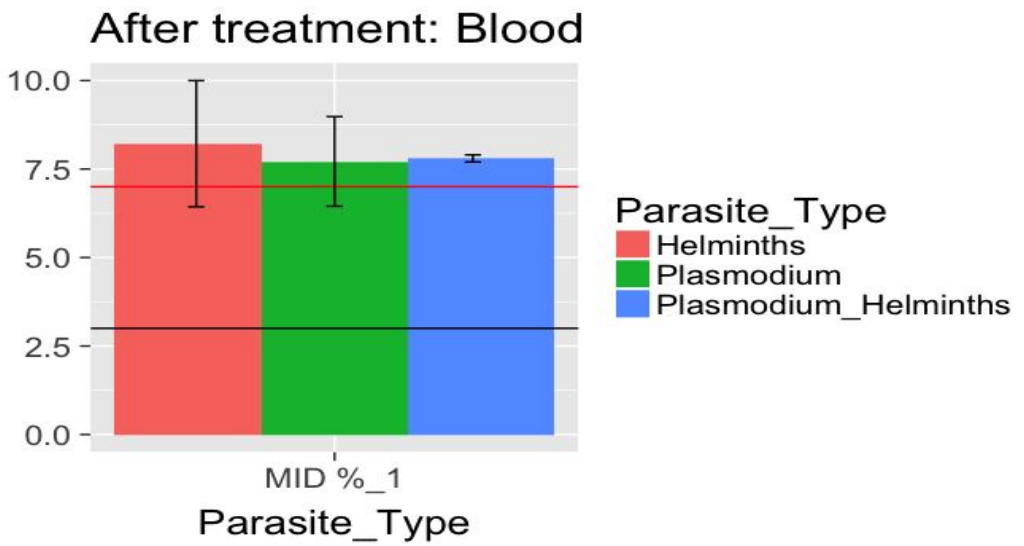
LYM - Lymphocytes

Figure 4.24: Blood cells profile after treatment: Lymphocytes



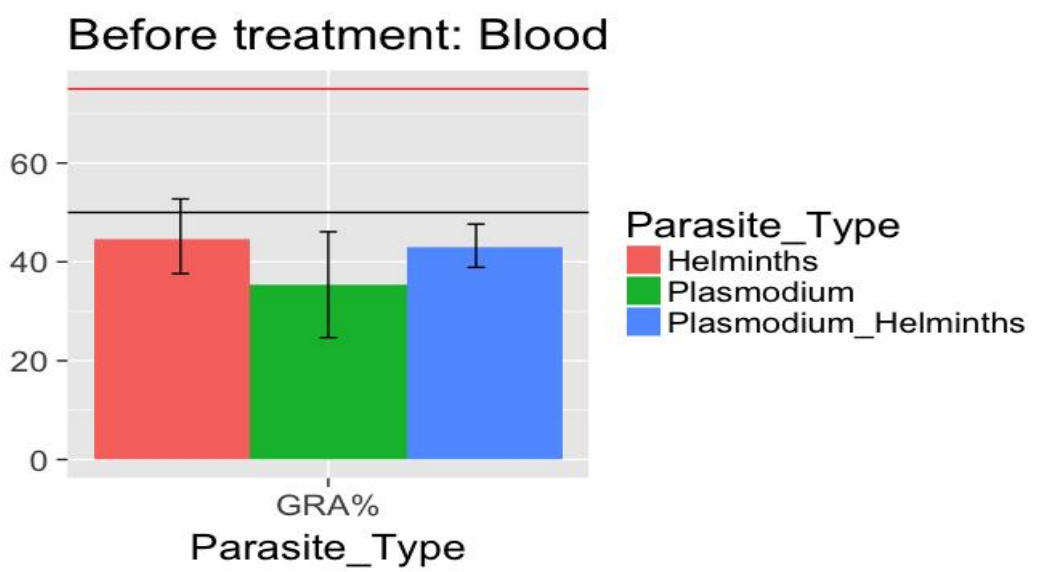
MID - Monocytes

Figure 4.25: Blood cells profile before treatment: Monocytes



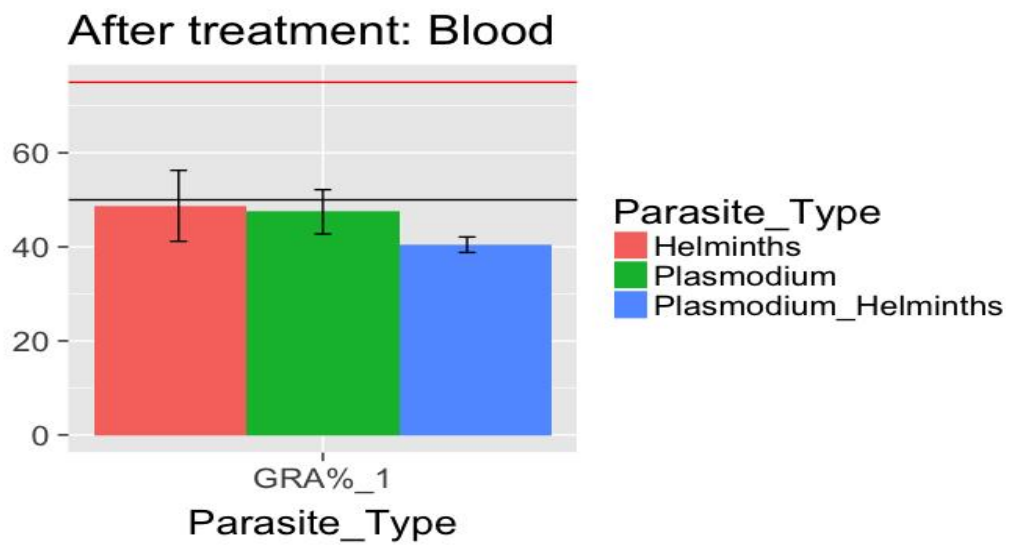
MID - Monocytes

Figure 4.26: Blood cells profile after treatment: Monocytes



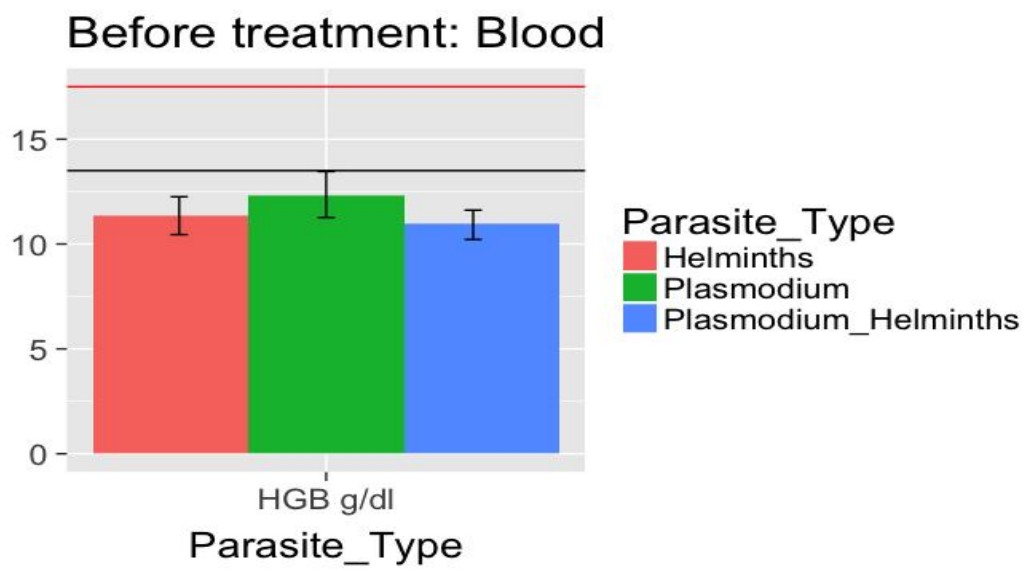
GRA - Granulocytes

Figure 4.27: Blood cells profile before treatment: Granulocytes



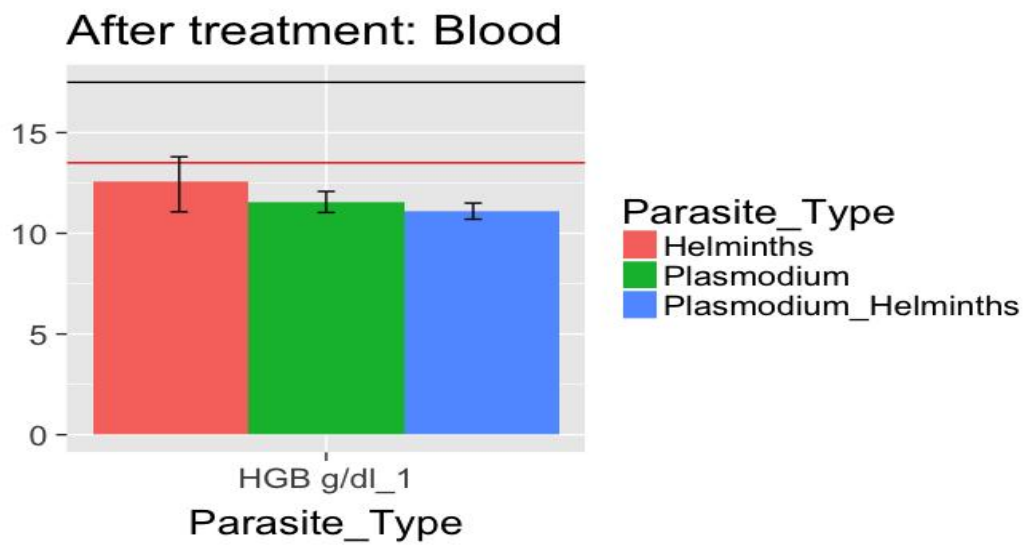
GRA - Granulocytes

Figure 4.28: Blood cells profile after treatment: Granulocytes



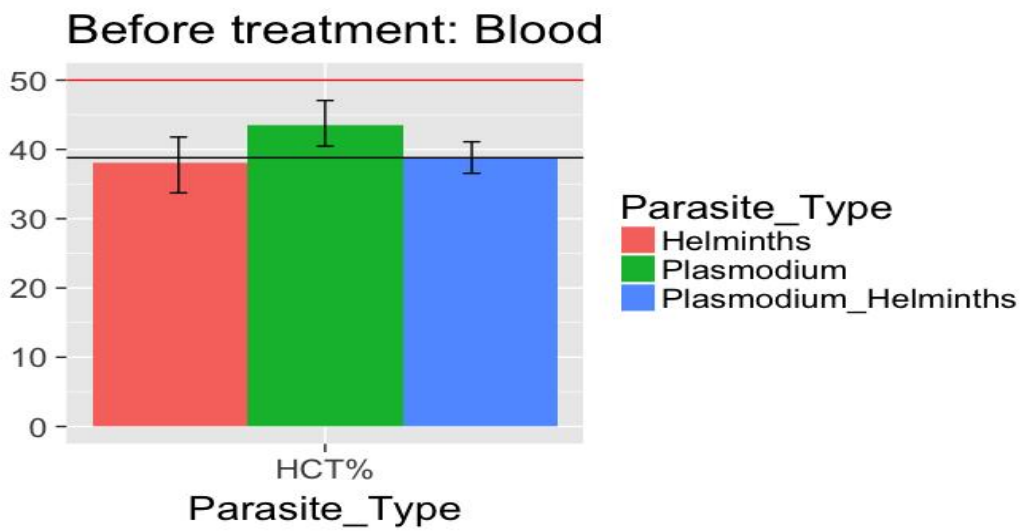
HGB - Hemoglobin

Figure 4.29: Hemoglobin concentration before treatment



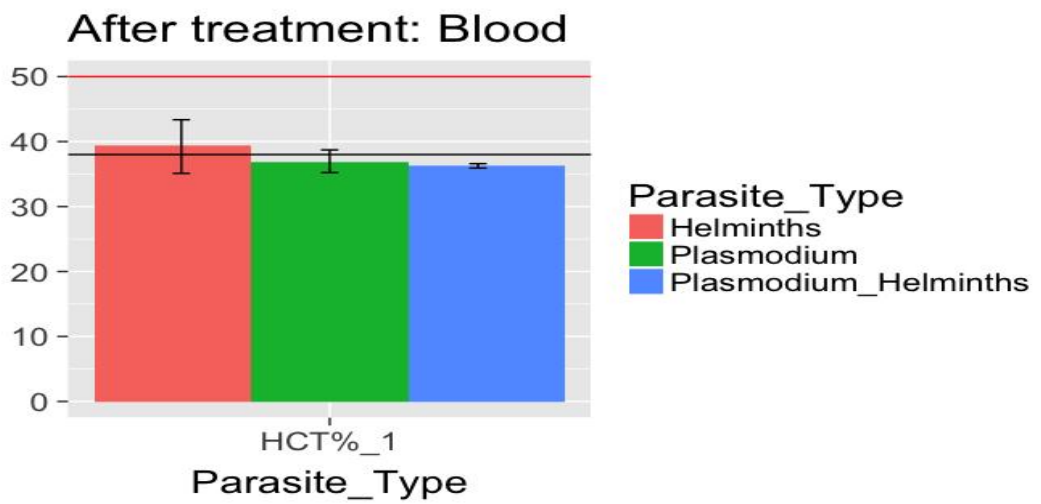
HGB - Hemoglobin

Figure 4.30: Hemoglobin concentration after treatment



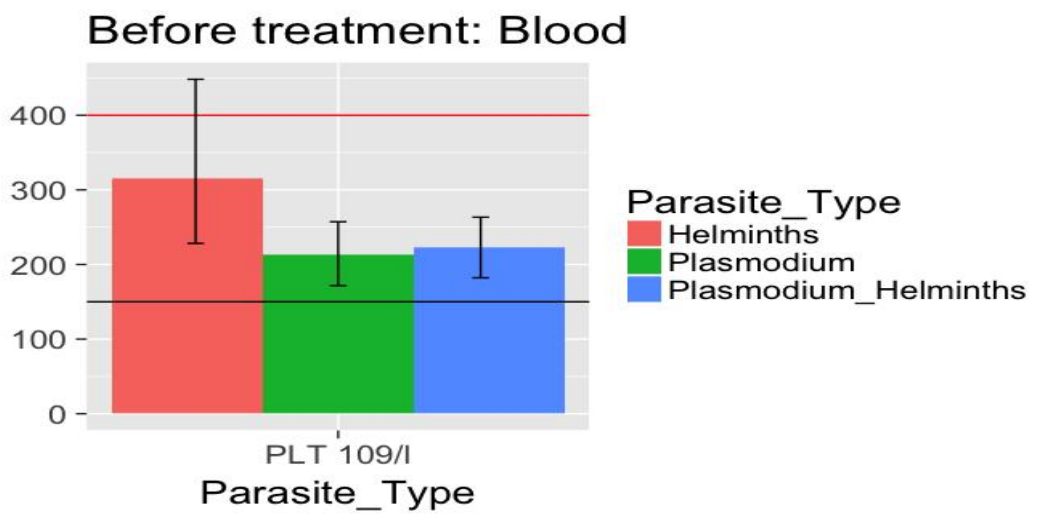
HCT - Hematocrit

Figure 4.31: Hematocrit value before treatment



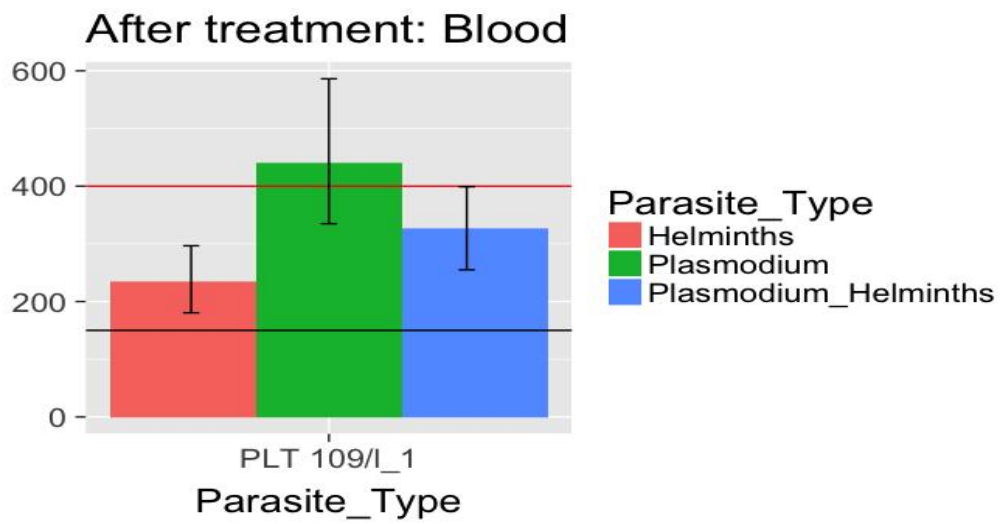
HCT - Hematocrit

Figure 4.32: Hematocrit value after treatment



PLT - Platelets

Figure 4.33: Platelets values before treatment



PLT - Platelets

Figure 4.34 Platelets value after treatment

Figure 4.21 shows all categories of infections have normal values, though lowest values were obtained in helminths infection

Figure 4.22 shows the improvement in WBCs values in all treated groups; highest response was obtained after elimination of *Plasmodium* parasites.

Figure 4.23 shows increase in count of lymhocytes in all infected groups; a highest value was obtained in *Plasmodium* infection before treatment.

Figure 4.24 shows that lymphocytes were slow to drop to normal ranges18 days ater treatment; there was reduction in values compared to infected value.

Figure 4.25 shows the uncontrolled increase in MID in all categories of infections

Figure 4.26 shows the slow reduction to normal ranges 18 days after treatment;

Figure 4.27 shows that Granulocytopenia in all categories of infections, most affected was *Plasmodium* infection.

Figure 4.28 shows the improvement in granulocytes compared to infected values.

Figure 4.29 shows Anemia in all categories of infectioms

Figure 4.30 shows the slow response in improvement of haemoglobin values after treatment.

Figure 4.31 shows that the Hematocrit values were below normal in helminths and in co-infections; normal HCT values was obtained in Plasmodium infections.

Figure 4.32 shows that the HCT values in helminths infections were improved to normalcy. After treatment, HCT values of Plasmodium dropped below normal

Figure 4.33 shows the reduction in Platelets count in Plasmodium and in co-infection. Infection in helminth had no negative impact on platelets count

Figure 4.34 shows Thrombocytopenia observed after treatment of helminths infections and also, Thrombocytosis after treatment of Plasmodium infection. Absence of intestinal helminths and malaria parasites in co-infections stabilized platelets levels.

4.14 Pg/ml values of immune molecules before and after treatment in all categories of infections

Median serum levels of IL-4 and IFN-γ as shown in Table 4.22: Serum values of IL-4 and IFN-γ were determined from 82 volunteers using ELISA method on a 2- times samples collected before and after administration of drugs. The values of both molecules were higher in all categories of infection, before treatment.

4.15 Correlation between IFN-y and IL-4 for all groups before treatment

In Table 4.23, there was a positive correlation in all groups before treatment. However, significant level in correlation between IL-4/IFN-γ was observed in group infected with Plasmodium only.

4.16 Correlation between IFN-y and IL-4 for all groups after treatment

After treatment, in Table 4.24, positive correlation was observed in all groups; with significance difference in groups infected with Plasmodium and in co-infection.

Table 4.22: Pg/ml values of all immune related molecules during infection and after treatment of participants

Groups	Immune Molecules	Treatment Group	Median value pg/ml
Helminths	IFN-y	Before	59.8
Helminths	IFN-y	After	36.2
Helminths	IL-4	Before	40.3
Helminths	IL-4	After	59.8
None	IFN-y	Before	31.9
None	IFN-y	After	32.7
None	IL-4	Before	32.25
None	IL-4	After	45.4
Plasmodium	IFN-y	Before	84
Plasmodium	IFN-y	After	39
Plasmodium	IL-4	Before	61
Plasmodium	IL-4	After	44.6
Plasmodium and Helminths	IFN-y	Before	135.6
Plasmodium and Helminths	IFN-y	After	35.9
Plasmodium and Helminths	IL-4	Before	68.8
Plasmodium and Helminths	IL-4	After	41.55

Table 4.23: Correlation between IFN-y and IL-4 for the three groups of infection

Groups <chr>	estimate <chr>	statistic <chr>	p.value <chr>	alternative <chr>
Helminths	0.5092286	2.8447058	0.004	two.sided
None	-0.3333333	2	0.75	two.sided
Plasmodium	0.3703707	3.7500464	< 0.001*	two.sided
Plasmodium and Helminths	0.3856209	106	0.026	two.sided

*p <0.001 is significant in *Plasmodium* infected population

Table 4.24: Correlation between IFN-y and IL-4 for all groups

Groups <chr>	Estimate <chr>	statistic <chr>	p.value <chr>	Alternative <chr>
Helminths	0.3111111	1.7330227	0.083	two.sided
None	0.3333333	4	0.75	two.sided
Plasmodium	0.6779854	6.6208086	< 0.001*	two.sided
Plasmodium and Helminths	0.5770523	3.3356314	< 0.001*	two.sided

* $p < 0.001$ is significant for *Plasmodium* infection and for co-infection

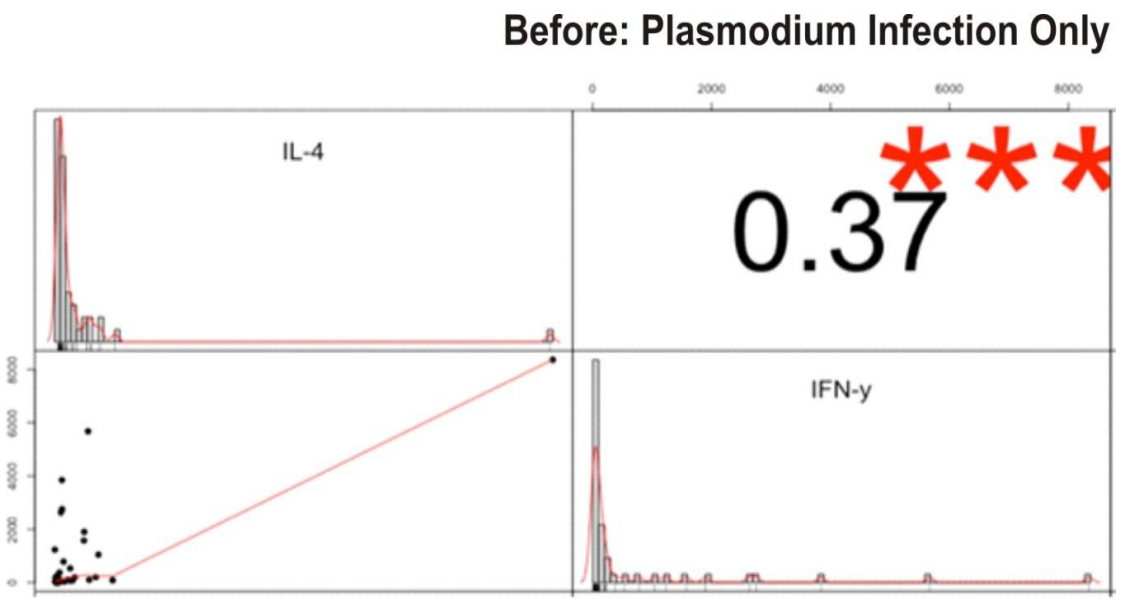


Figure 4.35 Correlation plot for Plasmodium before treatment

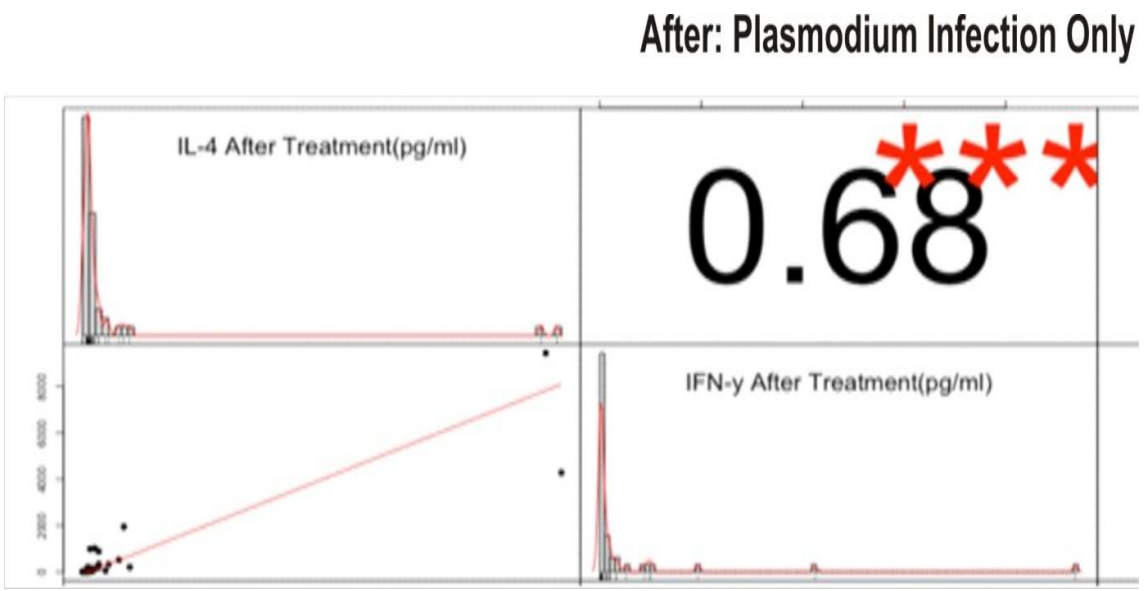


Figure 4.36: Correlation plot for Plasmodium infection after treatment

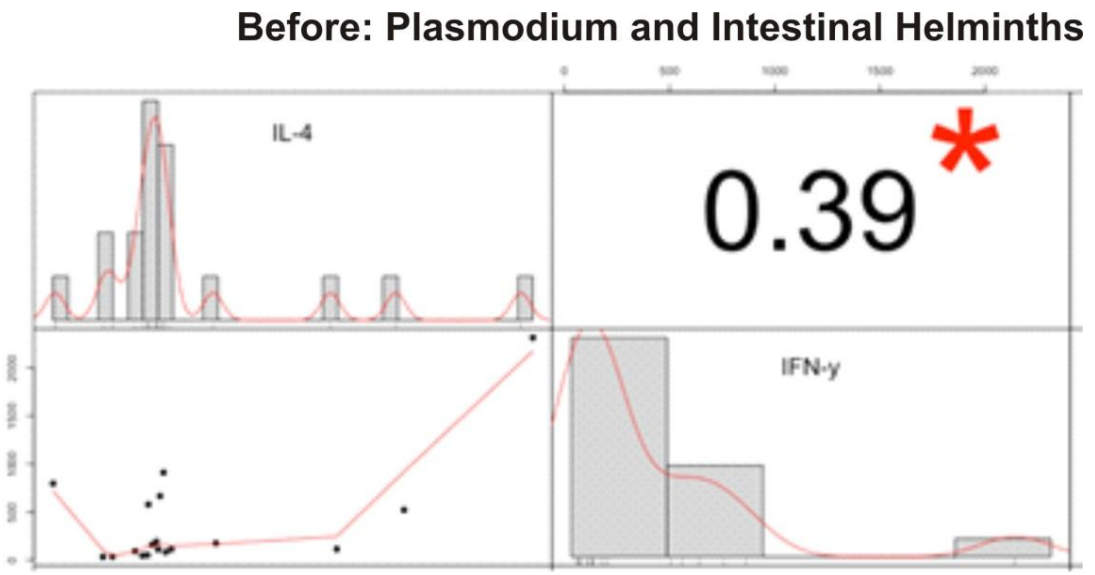


Figure 4.37: Correlation Plot for Plasmodium/Intestinal Helminths Infection before treatment

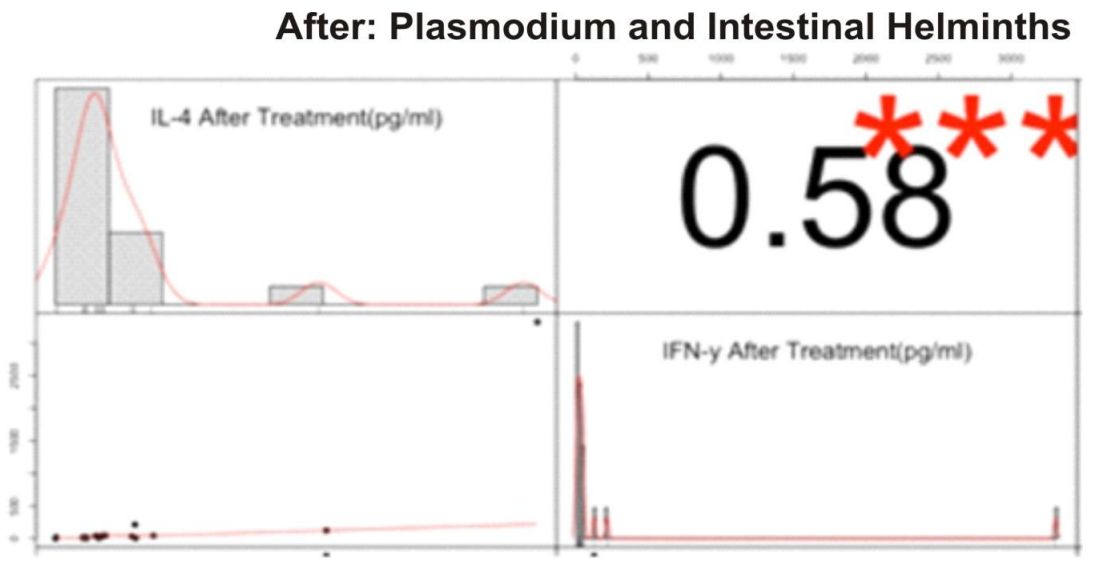


Figure 4.38: Correlation Plot for Plasmodium/Intestinal Helminths Infection after treatment

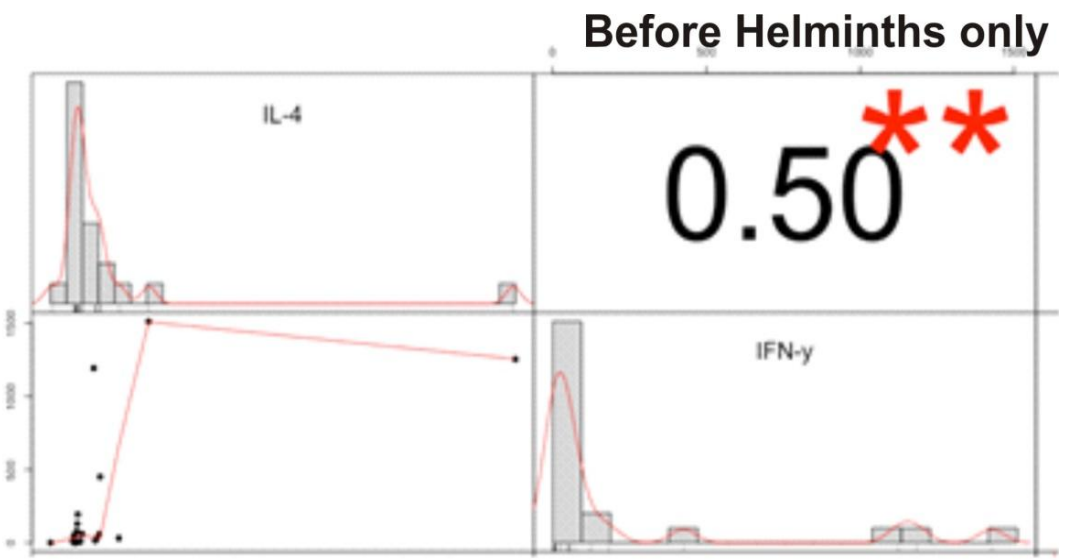


Figure 4.39: Correlation plot for helminth infection before treatment

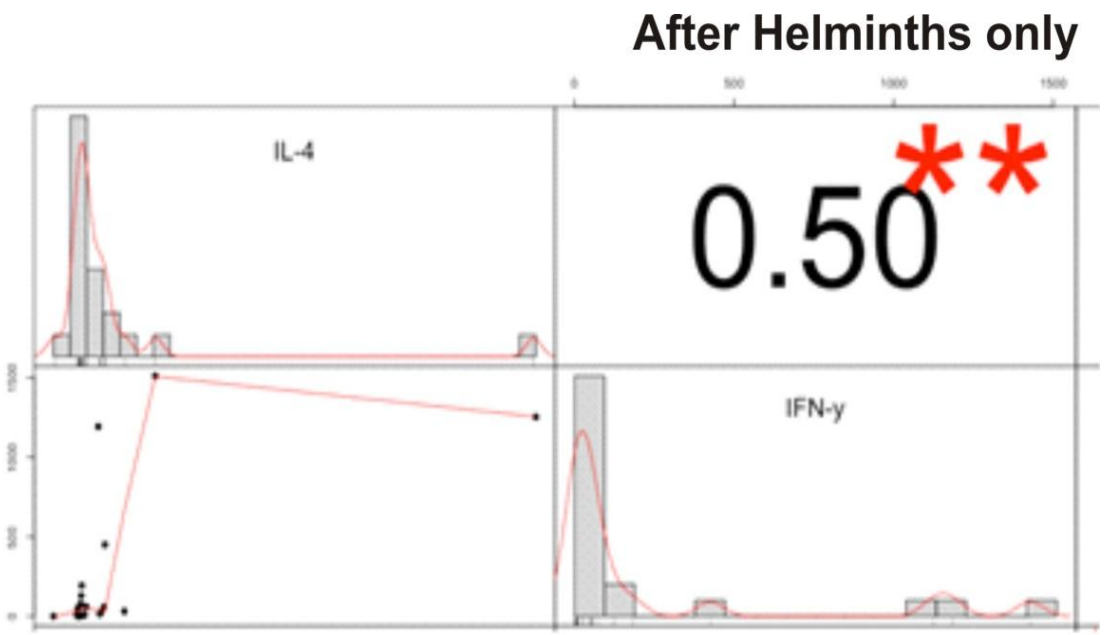


Figure 4.40 Correlation plot for helminth infection after treatment

Figure 4.37 shows the correlation plot for Plasmodium before treatment: positive correlation of immune molecules before treatment.

Figure 4.38 shows the correlation plot for Plasmodium infection after treatment: stronger positive correlation between IL-4/IFN-γ after treatment. Figure 4.39 shows the correlation plot for Plasmodium/intestinal helminth before treatment: weak positive c **Figure 3.40** shows the correlation plot for Plasmodium infection only after treatment: stronger positive correlation between IL-4/IFN-γ after treatment.

Figure 4.41 shows the correlation plot for helminth infection only before treatment. There was strong positive correlation between IL-4/IFNγ.

Figure 4.42 shows the correlation plot for helminth infection only after treatment. Correlation remained positive after expulsion of worms.

CHAPTER FIVE

DISCUSSION

5.1 Discussion

Identified in this study were ova of 6 species of intestinal helminths:

S.*mansoni*, *A. lumbricoides, T. trichiura*, *Taenia* sp, Hookworm*, and* stool samples. This distribution differs from a commonly reported triad of occurrences of intestinal helminths, involving *A.lumbricoides*, Hookworm, and *T. trichiura* (Salawu and Ughele, 2015). The species of helminths identified by authors vary depending on environmental factors such as temperature, rainfall, humidity, soil moisture; and others such as personal hygiene and level of contamination of the environment (Karshima, 2018). In the south-west, the commonest species of helminths were *A. lumbricoide* and *T. trichiura* while hookworms and *S. stercoralis* were the highest prevalence obtained in north-east and north-central regions. Among primary school children in Aniocha, south, southern Nigeria, a report was given of three helminths species; *Ascaris lumbricodes*, *Trichuris trichiura*, and hookworm species (Mordi *et al* 2018). However, across Nigeria, the highly prevalent species of helminths are *Ascaris lumbricoides*, *Strongyloides stercoralis*, *Trichuris trichiura*, and hookworms (Karshima, 2018). In a study of school-age children in Kwara State, four species of intestinal helminths were recovered from stool samples and these were hookworm, *Hymenolepis nana*, *Schistosoma mansoni*, and *Enterobius vermicularis* (Adedoja *et al*., 2015)].

The occurrence of all species of helminths followed a similar trend, the highest prevalence 11/15 (73%) was derived from 61 - 70 years age group. This is contrary to other reports where a higher prevalence was obtained in younger children and was

attributed to poor personal hygiene practices such as eating with unwashed hands, spending more time playing out-door without adult supervision (Adanyi *et al*., 2011). The school age children (< 18 years) were dewormed in schools, about 5 weeks before this study. This could account for a much lower occurrence in children less than 10 years old (100/335, 29.8%) than in adults. Generally, low helminths infection in adults could be attributed to the relative consciousness of good hygiene practice, with less contact with the dirty environment (Adanyi *et al*. 2011). *A. lumbricoides* was 53 (6.4%) and was much lower than what was reported in Edo 15.0% (Aisien *et al*., 2001) and Osun State 13.1% (Asaolu *et al.,* 2002). Hookworm infection was low 20 (2.4%) affecting more adults, and had been acquired by people that go barefooted. Hookworm larvae actively penetrate the exposed skin. The presence of adequate moisture and optimal temperature in the study allows for larval activity and migration (Stromberg, 1997). Nevertheless, too much rainfall characteristic of the studied area may have carried infective larvae away into a runoff, which was responsible for the low infectivity of hookworm in the studied area. The prevalence of Taeniasis was 2 (0.2%) affecting only adults. Low tapeworm infection could be attributed to the consumption of beef/pork that is not properly cooked. With the availability of fresh-fish and snails and the peasantry life of the people, roasted "suya" was not consumed in the studied area.

Most studies reported *Ascaris lumbricoides* as the most prevalent intestinal helminths in the world (Karshima, 2018). This is different in this study where the occurrence of *S. intercalatum* 86(10.4%) was more than *A.lumbricoides* 53 (6.4%) and *S. mansoni* 35(4.2%). This study identified three species of *Schistosoma* predominantly; *S. intercalatum*, *S. mansoni* and *S. haematobium*. Reported was the presence of all three species of *Schistosoma* in Rivers State, which is an adjourning environment to the studied area (Ekpo *et al*., 2013). Intestinal schistosomiasis was identified in all age

groups, reflecting the regularity of swimming activities of participants in snail infested water bodies in the environment. *Schistosoma hematobium* was identified in a stool sample of an eight-year old male (Plate 4.1). The aberrant presence of species of *Schistosoma* is not uncommon. In a study of urinary and intestinal schistosomiasis among 1,709 children (5-15 years) in Port Harcourt, Nigeria the ova of *S. intercalatum* was identified in urine samples only, although with a low prevalence of 5.7%; neither *S. mansoni* nor *S. haematobium* was diagnosed in both stool and urine samples (Arene *et al.,* 1989). Eggs of *Schistosoma* or parasite migration can be lodged in strange sites such as the CNS thereby compressing the area of the lodge, with associated clinical presentation that may include pyrexia, fever, headache, vomiting, blurred vision, and Jacksonian epilepsy (Ross *et al*., 2012).

In Nigeria, the geographical distribution of *Schistosoma* infection depends on the availability of the right vector; freshwater snails. *S. mansoni* needs freshwater *Biomphalaria* spp. while *S. haematobium* needs *Bulinus* spp. as vectors for transmission (Colley, 2014).

The most common occurrence of multiple infections involved *A. lumbricoides*/*S. intercalatum*, followed by *S. intercalatum*/hookworm. Parents in the study areas were peasant farmers and spent less time at home to take care of their children who indulge in poor hygiene practices such as failure to wash hands after defecation or after handling stools; this could promote helminth infection. Besides, children with less adult supervision spent more time recreating in snail infested water bodies. Adults pass stool and clean up with water from the stream. Besides, because cleaning with water is a common practice, adults who defecate in the environment make use of the availability of cheap sachet water of 500ml sold at N10.00 per sachet and this may account for the low fecal-oral transmission of intestinal helminths in adults. This study accepts

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observation that contact with infective ova of helminths depends on the hygienic-behavioral disposition of the individual such as handwashing habit, nail hygiene, and foot-wear wearing habit (Rosanty, 2015).

Our observation of Kolo Creek showed that it was shallow and slow-flowing, invariably stagnant with dense vegetation which favors the breeding of snail intermediate host and the proliferation of miracidium. Although the sanitary condition of the study area was typical of a rural community in a developing country, it is most probable that human activities in snail infested water bodies are responsible for sustained infection with intestinal Platyhelminthes, despite ongoing chemoprophylaxis. This is in agreement with previous work which showed that persistent transmission of Schistosomiasis had the cultural affiliation with the use of river water for "drinking" and swimming, meaning that behavioral changes are needed to reduce the transmission of Schistosomiasis (Utzinger *et al.,* 2003). The primary objective of any control program is at the least, to reduce morbidity (Barakat, 2003). The study observed a lopsided control strategy, where chemoprophylaxis with praziquantel was the only measure adopted by the health intervention program. Praziquantel was reported to be effective for adult Schistosoma parasites only (Utzinger *et al.,* 2003); hence we recommend a combination of anti-malarial agents such as the artemisinin derivatives (e.g. Artemether) whose potency have been proven to eliminate young stage Schistosoma infection (Utzinger *et al.,* 2003).

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The co-infection of malaria and intestinal helminths modulates the outcome of clinical malaria and a good understanding is necessary for the design strategy for the control of malaria (Degarege and Erko 2016). In community study (Table 4.13), we recorded a prevalence of 18 % in the co-infected population; malaria had a higher percentage, 42% than intestinal helminths, 25.0%. The prevalence in adult females was

32.3%, and 19% in female children. In south-west Nigeria, a co-infection of malaria and intestinal helminths, 20.9% was reported, with soil-transmitted helminths having rather, a higher percentage, 64.6% than malaria, 19.7% (Dada-Adegbola *et al*., 2013). Our report was slightly higher than 14.4% obtained by (Adedoja *et al*. 2015) in a co-infection study of school children in Kwara State. In Illero southwest Nigeria, febrile children were investigated for malaria infection and intestinal helminths infection, cases of malaria by age <5, 6-10 and11-15 years were 23.2%, 2.2% and 4.7%; and only 18.3% were infected with intestinal helminths (Harhay *et al*. 2010).

The prevalence of 53% obtained in school-based study 1 , 42% in community study and 32.1% (Table 4.12) in school-based study 11 for malaria could be considered moderate to high taking into account, previous reports on a spatial model of malaria risk which estimated a variation in prevalence of malaria disease in Nigeria from less than 20% to more than 70%. The highest population of people susceptible to malaria disease in the Niger Delta, Nigeria could be found in Bayelsa and Rivers States (Onyiri, 2015). In Table 4.11, adult females were most infected in all study sites. In overall, 121 out of 198 (61%) females examined were infected with malaria parasites. The distribution of malaria infection is not influenced by sex/gender, and in a poor rural population, the prolonged exposure time to bites of mosquitoes in females arises from nocturnal economic activities of snail hunting and fishing in the creeks. Our report agrees with a study done by Tela *et al*. (2015) which documented females' dominance in infection rate, 54.75% females than 45.28% males. On the contrary, a study in Bayelsa State reported a higher infection rate of malaria parasites in males, 56.8% than females, 46.2% (Abah and Temple 2015). Singh *et al.* (2014) similarly reported a male dominance of 63.6% and 36.4% females in Kebbi State and in Kaduna State, (Umaru and Uyaiabasi, 2015) reported a male dominance. Others reported no difference in

prevalence rate based on gender clarifying that the distribution risk of malaria is heterogeneous (Dawaki *et al.* 2016).

Malaria disease is endemic in Nigeria such that fever and other primary symptoms of malaria are not specific and positive laboratory results for malaria parasites are common among apparently healthy individuals. A prevalence of 42% (Table 4.11) of malaria parasites obtained from healthy individuals in this study is relatively low in southern Nigeria compared to high prevalence of 81.5% in Abeokuta, South West Nigeria (Okonko *et al*., 2009), 80.4% in Aba and 74.40% in Umahia southern Nigeria (Kalu *et al.,* 2012) and 67.5% in Port Harcourt, south-south Nigeria (Wariso and Oboro, 2015). The vegetation in southern Nigeria is characteristics of tropical rainforest with an average rainfall of 1600 mm providing an adequate environment for the proliferation of mosquitoes and consequent spread of malaria disease. For instance, in Northern Nigeria, the period of rainfall is short, June to September and a lower prevalence of 32.5% of malaria parasites was reported in Sokoto (Zama *et al*., 2013).

The WHO recommends preventive therapies of malaria; an artemisinin-based drug combination, the use of insecticides treated net (ITNs), and the application of insecticide residual spray as effective for the control of malaria disease (Salam *et al*., 2014). Evidence from community-based intervention by seasonal chemoprophylaxis in the prevention and control of malaria in the studied area may be effective and could account for a reduced incidence and prevalence of malaria and anaemia (Salam *et al.,* 2014). In table 4.10, a high prevalence of malaria parasites (53%) among school pupils despite ongoing routine rural health intervention suggest constant reinfection. Children play outdoors at night resulting in regular bite by infected mosquitoes. Parents

of these children are peasants and much of their economic activities are nocturnal. Besides, mosquitoes are known to feed on living organic matters and can adaptively breed in fresh and brackish water and in any small collection of water such as tree holes, cans, exposed vats, banana leaves and axils of different plants such pineapples and cocoyam, an environment, typical of the study areas. Moreso, in malaria infections, anaemia is the primary clinical manifestation in children (Pathak and Ghosh, 2016).

Anaemia which is a measure of poor health and poor nutrition has multiple causal factors. Malnutrition and acute falciparum malaria contribute to anaemia from the destruction of both parasitized and uninfected red blood cells accounting for the high prevalence of 80.3% of anaemia in Cameroon (Sumbele *et al.,* 2013). The haemoglobin levels for boys and girls were similar in this study (Figures 4.1 and 4.2), although Hb level was lower in females than in males which might be attributed to a few older females who may be in their menstrual cycle within the period of sample collection. However, a contrary report was made in Ghana by (Owusu-Agyei *et al*., 2002) where anaemia was observed higher in males than in females without significant difference, anyway. A similar investigation carried out in a close community, Angiama Bayelsa State, a higher prevalence of 63.30% of malaria parasites was obtained from primary school children who were apparently healthy, although the pupils did not receive the routine preventive therapy like our study groups did **(**Abah and Temple, 2015).

It is reported that weight in children correlates significantly with haemoglobin concentration (Sumbele *et al*., 2013). Our anthropometric and haematological indices showed (figure 4.2) that the majority of the children had normal BMI and haemoglobin levels, with no association between malaria infection and anaemia for all children. Although the parents and guardians of the pupils were subsistence farmers, their stable

food was plantain, banana, cassava, cocoyam, potatoes and much of freshwater fishes which could account for good nutrition. Thus, with the improved nutritional status of the children combined with routine IPT, the morbidity due to malaria parasites was reduced.

Our identification of *Plasmodium* was by microscopy and by nested Polymerase Chain Reaction (PCR). The confirmation of *Plasmodium falciparum* was at 205bp and *Plasmodium ovale* at 787 bp. *P.ovalae* was identified in a mixed infection with *P.falciparum* (see figures: 4.6 and 4.7). According to (Lim *et al*., 2010) the frequency of sole infection of *P.ovale* is usually low even in an endemic area but could be high in mixed-species infection. Yet, by microscopy, it is difficult to diagnose *P. ovale* in a scanty and mixed-species infection, maybe, through suppression hypothesis mediated by the competition of parasites for host cells (Richie, 1988). Moreso, low parasite density is a feature of *P.ovale* malaria, making detection by microscopy difficult (Zaw and Lin, 2015). *Plasmodium ovale* exist as two different species: *P.o.curtisi* and *P.o. wallikeri* (Zaw and Lin, 2015). Our study, however, did not involve molecular discrimination of the two species. *P.ovale* upon infection in humans undergo latent phase in the liver resulting in asymptomatic infections (Collins and Jeffery, 2007). After treatment of primary infection, relapse in ovale malaria could occur between 17- 255 days and up to 5 years after infections (Rojo-Marcos *et al.* 2011). Two mechanisms can explain the occurrence of *P. falciparum infection*; reinfection and recrudescence. Reinfection is possible within 14 days of treatment in the area of endemicity, however, is a result of incomplete clearance of parasites in the blood due to drug resistance or incomplete treatment; when different strains of parasites are involved in infection, more so, variation in antigenicity, recrudescence can occur (Omonuwa and Omonuwa, 2002)

Eighty-eight infected patients were strategically treated for malaria and intestinal helminthiasis. Eighteen days post-treatment no parasites were detected by microscopy. For malaria therapy, an artemisinin-based combination is widely recommended (Phillips *et al*. 2017). In an evaluation of recurrent parasitaemia (RP) after treatment of uncomplicated malaria in children (Woodring *et al*., 2010) recorded a median RP of 37 days (36-38). In his study, there was no detectable parasite in the population by day 14, 71% by the 28th day and 41% by the 42nd day. Antimalarial causes destruction of asexual malaria parasites (young ring stage) to limit the pathological effect of the parasites. Erythrocytes infected by *Plasmodium* parasites are usually deformed, bound to antibodies, and are cleared through splenic pitting (White, 2017). However, a decline in the density of drugs following treatment could affect the therapeutic response. Some *Plasmodium*-infected erythrocytes may evade splenic clearance through cytoadherence/sequestration and return to circulation, although with deformities and short lifespan. As the Parasites in the red blood cells that have returned to circulation matures, *P. vivax*, infected erythrocytes become more deformed but less deformable in *P falciparum* (World Health Organization Tropical Medicine and International Health, 2014).

Taking into account that every participant for this study was healthy, and were beneficiaries of an ongoing Mass Drugs Administration program (MDA), the prevalent rate of 42% for malaria parasites for community study; and exclusively 53% for the school-based study 1 could be considered high. The prevalence of intestinal helminths and co-infections are acceptably low for a rural community where endemicity of these diseases is expected. It goes to support the effectiveness of the community-based health intervention programme for malaria and intestinal helminths infections in public Primary Schools in the studied area. Alteration in blood cells profiles is common in

infectious diseases. In figure 4.21, the levels of white blood cells in the three categories of infections (malaria, helminths and co-infections) were low but within normal values. In malaria infection, WBC count ranged from low to normal, not related to depletion rather to re-localization of leucocytes to the spleen and other marginal pools (McKenzie *et al*. 2005). There was an improvement in the value of white blood cells after elimination of parasites by therapy in all categories of infections (Figure 4.22).

Lymphocytosis was recorded in all infected studied groups (Figure 4.23) and it was most pronounced in the group infected by malaria parasites. This is contrary to results obtained in the Thailand-Myanmar border, in which the value of lymphocytes was significantly lower in *Plasmodium*-infected population (Kotepui *et al.,* 2014). According to Hviid and Kemp (2000), lymphopenia is an established trend in malaria disease, however, an increase in lymphocytes count is obtained following antimalaria treatment which returns to normal in a couple of days. The slowness of the values of lymphocytes to drop to normal after treatment may suggest underlying inflammatory cause other than malaria and intestinal helminths (Figure 32). High lymphocyte count ordinarily is a transient harmless condition which can occur after an illness. Nonetheless, in a rural environment where both personal and environmental hygiene is poor, a bacterial infection could occur concurrently with malaria and intestinal helminths increasing lymphocytes count beyond normal (Thorley *et al.,* 1977).

The clearance of malaria parasites in circulation requires the recruitment and activation of monocytes and macrophages (Chua *et al*., 2013). Monocytes exhibit plasticity and heterogeneous in function (can differentiate into either inflammatory or anti-inflammatory subsets). In infection, monocytes migrate into tissues where they transform into macrophages and dendritic cells which produce cytokines, function as

phagocytes and as antigen-presenting cells. Before treatment in the three categories, monocytes count was very high as seen in figure 4.25. However, following parasite elimination in figure 4.26, the values of monocytes returned to normalcy. Kotepui *et al.* (2014) reported low count of monocytes in malaria infections.

Granulocytes (neutrophils, eosinophils and basophils) play specific roles in host protection in helminths infection (Makepeace *et al*., 2012). Granulocytopenia was observed in helminths, malaria and co-infections (Figure 36) and an improved count was recorded 18 days after treatment (Figure: 4.27) expectedly, increasing to normal values within more days post-treatment, without re-infection. In malaria infections, the results of the study agree with reports that neutrophils and eosinophils are significantly reduced (Kotepui *et al*., 2014). Eosinophils are mobilised in helminthiasis, viral and allergic reaction. They have surface receptors for chemokines, cytokines, immunoglobulins and serine proteins, which facilitate its mobilization to sites of infections, where they discharge the granules in their system. The discharged granules have tissue destructive cationic protein as well as antiparasitic effects (Shamri *et al*., 2011). The mobilization of neutrophils to the site of inflammation prevents cell apoptosis, ensuring prolong lifespan (Makepeace *et al.,* 2012). Basophils, like eosinophils, contain granules and surface receptors for IgE, chemokines, cytokines and complement (Stone *et al*. 2010).

In community study, all infected participants were anaemic; consideration was for normal ranges for adults. In figure 4.28, the infected populations were all anaemic. Restoration of haemoglobin concentration to normal values was slow following helminths and malaria infection. The study, however, observed a steady increase after 18 days of therapy. We defined anaemia as Hb levels below 12.0 g/dL for women;

below 13.0 g/dL for men. Severe anaemia was defined as Hb level below 7 g/dL. No participant was severely anaemic in infection and after treatment, as seen in figures 4.29/4.29 but the response was faster in categories treated for helminths infection only (figure: 4.29). In a study of malaria, helminths co-infections among children in Cameroun Njua-Yafi *et al.* (2016) children infected with malaria parasites alone were vulnerable to anaemia while helminths infected children were protective to anaemia. Suggesting that helminth co-infection with malaria was protective against anaemia. A contrary report, however, were cases of malaria-helminth co-infection which had a higher prevalence of anaemia compared to those infected with malaria only (Degarege *et al*., 2010). Malaria anaemia is a result of clinical cases as Kotepui *et al* (2015) reported a decrease in Hb concentration in malaria-infected population. In asymptomatic cases as our study, maleric anaemia could occur through haemolysis, increase splenic clearance and cytokine-induced des erythropoiesis. Other factors that can cause anaemia include direct blood loss from hookworm infection; nutritional theft by tapeworm species and reduced appetite due to immunological factors. The infection of *Ascaris lumbricoides* could result in the deficiency of vitamin A which may increase susceptibility to malaria infections in case of co-infections (Naing *et al*., 2013). The Pact cell volume (PCV) is used as an alternative measure to haemoglobin concentration in the determination of anaemia in malaria infection (Lee *et al*., 2008). To equate this measure, a standard three-fold conversion is required: Hb = Haematocrit/3. The haematocrit values in our study were although low, it was normal in all groups of infection, highest in a *Plasmodium*-infected group (figure 40). After treatment in figure 4.32, the values of Haematocrit in helminths infections were improved to normalcy while values of *Plasmodium*-infected group dropped below normal. Hookworm infection is known to cause anaemia. Before deworming, (Watthanakulpanich *et al*.,

2011) reported low values in haemoglobin, haematocrits, red blood cells compared to values obtained from a non-infected group. Within 2 months after deworming, the level of Hb, Hct in hookworm-infected children were the same as the uninfected group.

This study observed a similar rebound of Hct level to normal 18 days post-therapy. Thrombocytopenia as a result of phagocytosis and anaemia is a common haematological disorder in falciparum malaria (Coelho *et al.,* 2013). The value of platelets in infection of malaria and co-infection of malaria/intestinal helminths was low, though within the normal range. Thrombocytopenia is an established feature in malaria disease (Kotepui *et al*., 2014). The value of platelets in helminths infections was normal as seen in Figure 4.33. Following deworming, we observed reduced platelets count in helminth-infected groups (Figure 4.34). Interestingly, after treatment for antimalaria thrombocytosis was discovered in Plasmodium-infected groups. Thus thrombocytopenia is a haematological feature of malaria infection but was replaced by thrombocytosis few days after onset of chemotherapy, expectedly to return to normalcy in more days.

We investigated correlation before and after treatment between blood cells and cytokines and results shown in figures 4.35 and 4.36. In infection, IFN-γ was positively and weakly correlated with Hb (0.12) and Hct (0.16) but no correlation was observed after treatment. The low concentration of Hb and low level of Hct (figures 4.28-4.32) in the study correlates positively with low expression of IFN-γ during infections. Considering the protective relevance of IFN-γ during infection, anaemic individuals are rather more susceptible to infectious agents. The relationship between the expression of cytokines and blood cells as seen in figure 4.35, IFN-γ was negatively associated with platelets value (-0.25). The participants had thrombocytopenia (figure 4.33) especially

in malaria infection, suggesting that the low platelet value recorded in our study was associated with a rise in the concentration of IFN-γ (685.5pg/ml) as seen in table 4.17.

Platelets value was normal in helminths infection (figure 4.33) but the value of IFN-γ (49.32pg/ml) was reduced (see table 4.21). Before treatment IL-4 was associated positively with WBC (0.22) and granulocytes (0.2). This is in agreement with results of a previous investigation which showed proliferation of leucocytes following treatment with IL-4 in murine pouch model (Bouchard *et al*., 2004). The negative correlation of IL-4 with platelets in our study could be explained in the data; high platelet values in helminths sole infection had a corresponding low expression of IL-4 (mean value, 70.88pg/ml) compared to the highest limit of detection, 2000pg/ml. After treatment, IL-4/IFN-γ had a positive association with granulocytes. A positive association was expected between neutrophils and IFN-γ since the former is the source of the later (Spees *et al.,* 2014). Our results did not show any correlation between Eotaxin 3, MIP-β/ blood cells. With this lack of association and the arbitrary values obtained with eotaxin 3 and MIP-β, we considered that both immune molecules were not very relevant in the immunology of malaria and helminths infections. Thus, a closer look was given to using median value IL-4 and IFN-γ.

This study involved exclusively cases of low parasitemia of *Plasmodium falciparum* and scanty infection of intestinal helminths. The serum concentration of studied molecules was low and after treatment, there was a decline in the concentration of IFN-γ /IL-4 in the three infections groups, suggesting that the presence of infectious parasites stimulated the production of more immune molecules. Both molecules were most expressed in the co-infected population and least in the helminth-infected group. The presence of intestinal helminths in the co-infected group was expected to modify

the immune response to malaria infection (Hartgers *et al*., 2009): rather, we observed a simultaneous dependency in response of both immune molecules to stimulation by their respective antigen with a significant difference in correlation in the Plasmodium-infected group only as in figures 4.37 with the absence of pathogens, we observed a simultaneous decrease of both molecules with a significant difference in groups infected with *Plasmodium* and in co-infection after treatment (fig 4.40). Expectedly, the expression of IFN-γ in serum is essential in the protectivity against Plasmodium infection (Meding *et al*., 1990) and the presence of IL-4 is likewise required in helminths infections (Guo *et al.,* 2015).

Although, there was a difference in the decline in the concentration of both immune molecules after treatment, in the co-infected population the concentration of IFN-γ was significantly low after anti-parasitic administration (fig.26).The study did not ascertain if the drop in the concentration of IFN-γ after treatment was drug-induced or was it a result of the absence of stimulating antigen. A study has shown that the concentration of IFN-γ in patients with repeated/ previous exposure to malaria infection is usually low compared to primo-infection patients (Medina *et al*., 2011).

IFN-γ and IL-4 are counter-inflammatory with Th 1 and Th 2 respective immune responses (Anthony *et al.,* 2007). IFN-γ is needed to be expressed early in infection of *Plasmodium* parasites for it to be protective (D'Ombrain *et al*., 2008). The inflammatory properties of IFN-γ are achieved in a high concentration such that low doses have anti-inflammatory effect invivo in an asthma model, with a consequent downregulation of Th 2 cytokines (Flaishon *et al*., 2002). This agrees with our study, where the expression of IL-4 was suppressed (see table 4.22-4.24). In our findings, treatment of *Plasmodium* parasites did not reduce the concentration of IL-4 to a

significant difference. However, we recorded a marked decrease of IFN-γ after antihelminthic treatment in co-infection group, suggesting that the administration of antimalaria and anti-helminthics in this study depleted seral concentration of IFN-γ which might significantly affect the individual's ability to fight *Plasmodium* parasites and other infections such as the virus. It has been demonstrated that in-ivivo depletion of IFN-γ with monoclonal antibodies against IFN-γ decreases resistance to infection of *Plasmodium chabaudi chabaudi* in mice (Meding *et al.,* 1990). In a study, the Fulani tribe in Mali had a natural resistance to Plasmodium infections and this was positively correlated with elevated IFN-γ (Mccall *et al*., 2010). The low concentration of IL-4 recorded could result in a delay in activation of Th 2 (IL-4) in *Plasmodium* infection, allowing IFN-γ to respond to the inflammatory need to eliminate *Plasmodium* parasites. Early activation of IL-4 is a result of high grade/chronic infection of intestinal helminths (Kuchtey *et al*., 2010).

These findings need elaboration in mice model. It is our observation that low-grade infection of malaria parasites and intestinal helminths were associated with low concentration and positive correlation of IFN-γ/IL-4. The serum values of studied molecules were higher in infection than after treatment. More so, treatment of cases of co-infections with antiparasitic agents resulted in significant depletion of serum value of IFN-γ, which could increase an individual's susceptibility to malaria parasites and other infectious agents.

5.2 Conclusions

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The study determined the prevalence of the three infections groups; malaria parasites, intestinal helminths, co-infection with malaria and intestinal helminths. For intestinal helminths only two study periods was carried out; the prevalence that involved

both children and adults in the rural population was 25% while in the second school-based study was 30.2%. In malaria infections, three study periods was conducted; the first study was school-based and the prevalence was 53%. The second study involved both adults and children and the prevalence was 42% and in the second school-based study, 32.1%. Children in the school-based study were enrolled in the Government health intervention programme for the prevention of neglected tropical diseases. They were dewormed and treated quarterly for malaria parasites. Our data showed similarity in the prevalence of infection between helminths (30.2%) and malaria (32.1%) in a school-based study.

However, in the rural population involving both adults and children, malaria infections rate was higher 42% than intestinal helminths 25%. This has given an answer to the first question that in natural condition without monitored preventive therapy, the infection and re-infection rate of malaria could be higher than intestinal helminths. In the second specific objectives of this study, 206 participants (children and adults) were infected with different species of intestinal helminths. The prevalence of single infections was 95% and 5.3% was for double infection. Eighty-two per cent of paired infection involved *Schistosoma intercaltum/A. lumbricoides* (54.5%) and *Schistsosoma intercalatum* /hookworm (27.3%). There was no case of multiple infections in the study that concerns children and adults. In the second school-based study 11, the prevalence of single infection was 89.5% and double infection was 10.5%. Similar to the previous study, there was no case of multiple helminths infections in a school-based study 1. In answer to question 2, the predominant species of intestinal helminths in single and double infections were *Schistosoma intercalatum*, *Ascaris lumbricoides*. Two species of *Plasmodium* were identified in this study. Complementing microscopy with PCR techniques, *P.ovale* was identified in a mixed infection with *P.falciparum*.

Seventy-five school children were recruited for a study on malaria disease and their body mass index. The population of school children with normal body mass index was 85.3%. The underweight population was 15%. Ninety per cent of the underweight children were infected with Plasmodium. The values of blood cells in malaria disease and infection with intestinal helminths were determined: The level of WBC was low though, normal in all infections but with a slight increase after treatment.

Lymphocytosis and monocytosis were observed in all infections. After treatment, we recorded a gradual decline to normalcy. Granulocytopenia was the case in all infections. There was an improvement after treatment. All cases of infections were anaemic. Restoration of Hb level to normalcy was slow after treatment. The level of Hct was low in helminths and co-infections but normal in *Plasmodium*-infected cases. Following treatment, there was a decrease in the level of Hct in *Plasmodium*-infected groups while the level was improved in groups infected by helminths and co-infected group. The platelets count was not affected by helminths infection. Low platelet count was observed in *Plasmodium-infected* group and co-infection. Interestingly, after treatment, thrombocytosis was observed in *Plasmodium*-infected group only.

The blood cells/parameters most affected in malaria infections were low WBC count, granulocytopenia, thrombocytopenia, low concentration of haemoglobins and hct. In helminths infections, platelets value was normal but declined after treatment. Other blood parameters affected by helminths infections were low WBC count, granulocytopenia, low hct value and haemoglobin concentration. To answer our test question, platelets were the only blood cells affected negatively after treatment. Thus a sharp abnormal rise in platelets value was recorded following antimalaria therapy. The relationship between cytokine and blood parameter was determined. IL-4 had a positive

relationship with WBC in infection and no correlation after treatment. A negative correlation was observed between IL-4 and platelets values, before and after treatment. Before treatment IFN-γ was negatively related to platelet and positive with Hct and Hb concentration.

5.3 Findings

The following are the findings in this study:

1. This was the first report where *Schistososoma intercalatum* was the predominant (10.4%) intestinal helminths, compared to *A.lumbricoides* (6.5%) which is commonly reported as the most prevalent helminths in most part of the world.
2. The presence of *S haematobium* in stool samples confirmed aberrant location of *Schistosomes* parasites.
3. The correlation between IL-4/IFN-γ was pro-inflammatory: lymphocytosis and granulocytopenia were encountered: suggesting the supression of IL-4 (produced by granulocytes) and expression of lymphocytes, the major source of IFN-γ.
4. The clinical consequence of IL-4 suppression is the disability in class switch: antibody production is suppressed, resulting in increase in susceptibility to infectious diseases.
5. The study confirmed that the use of Aspirin tablet to control fever in malaria disease should be discouraged; Thrombocytopenia was associated in malaria infection.
6. The identification of *Plasmodium ovale* is novel in the study area. Sporozoites of *P.ovale* undergo dormancy in hepatocytes, relapse infection. The *P.ovale is a* key player in malaria transmission in the Niger Delta. Its presence as co-infection is significant for epidemiology and control of malaria disease.

7. The study diagnosed unidentified ovum, more like *Schistosoma* but with no protruding spine, suggestive of trematode's.

5.4 Contribution to Knowledge

1. This is the first report that; in sub-clinical infection at low concentration of IL-4/IFN-γ, their counter-inflammatory properties were altered. They rather depend on each other positively.

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Appendix 1

Deleted[Akanji. A]:

Performing Malaria Parasite count on Thick film and Calculation of Parasites density

Materials:

- Compound microscope with paired 10x oculars (eyepieces), 10x, 40x and 100x objectives.
- Two-key tally counters, one to count malaria parasites and one to count white blood cells;
- Giemsa-stained blood slides.
- Immersion oil, type A,
- Lens paper.
- Pen and pencil
- Calculator.

Process for Measuring Weight

- The scale was set on zero reading.
- Pupils were asked to remove shoes, heavy jacket and empty pockets of any object.
- Pupils were asked to step on the platform of the scale with both feet and with arms hanging at sides of the body while looking forward.
- Weights were read to the nearest 0.1kilogram.
- Values were recorded.
- The scale was ensured to return to zero position before use on the next pupils.

Process for Measuring Height

- The child was asked to remove shoes, cap, braids etc.
- The child was asked to stand on a flat floor, bringing the legs together and allowed the back- heels, buttocks, upper back and head touched against the stadiometer rule., arms were straight and shoulder was relaxed.
- The head was uprightly straight.
- The child was asked to take in a breath and take out.
- The headpiece was lowered until it touches the head.
- With the eyes of the measures positioned parallel with the headpiece, measurement was read to the nearest 0.1cm.
- A second measurement following the previous procedure, an average of the two measures was accepted.
- Using the gender-correct growth chart, the student age on the horizontal axis and the BMI on the vertical, the point of intersection was considered as the BMI for - age percentile.

Materials for Nested PCR Assay

The reaction mixture for nest I amplifications (50-ml) contained

DNA template, 5 ml.

Primer (rPLU 1 and rPLU 5) 250 Nm.

MgCL2, 4mM.

PCR buffer (50mM KCL, 10mM Tris-HCL).

Deoxynucleoside triphosphate, 200mM.

Tag DNA polymerase, 1.25 units.

Amplification Condition of Nest 1

Genus-specific primers (rPLU 3 and 4) - 948C for 4 mins.

Step 2- denaturation - 984C for 30 sec.

Step 3- Annealing - 558C for 1 min.

Step 4- Extension - 728C for 1min.

Step 2-4 was repeated 3-5 times.

Step 4 was repeated for 4 min.

Two microliters was derived and it served as the DNA template for each of 20 ml nest 2 amplification.

TMB Liquid Substrate:

TMB substrate was left at room temperature before use.

The well content was aspirated and the plate was washed 4 times.

Substrate (100µl) was added to each well.

The plate was left to incubate at room temperature for 20 minutes for discoloration.

HCL 1M (100µl) Stop solution was added to each well.

Colour development was detected with an ELISA plate reader at 450 nm with wavelength correction set at 620nm.

The lower limit of detection (LOD) for all analytes was 31.3pg/ml. The concentration of the sample was obtained by the sample dilution factor. Samples which had OD values below or above the standard range were re-analyzed at appropriate dilutions.

Figure 4.35: Correlation between blood cells and cytokines concentration before treatment

Keys: Lym; lymphocytes; Hct: haematocrit; Wbc: White blood cells; Hb: Haemoglobin; GRA: granulocytes; MID: monocytes; RBC; red blood cells, PLT: platelets

Figure 4.35 indicates that there was negative correlation (-0.30) value between age of participants and WBC; and negative correlation (-0.19) between age and platelets. The association between IFN-γ and platelets value was negative (-0.25) and positive with Hb (0.12) and Hct (0.16). A positive correlation was observed between age and hematocrit (0.19) values; and age and hemoglobin concentration (0.22). IL-4 had a positive association (0.19) with WBC and negative association with platelet (-0.19) value.

All immune molecules were negatively correlated with age of participants.

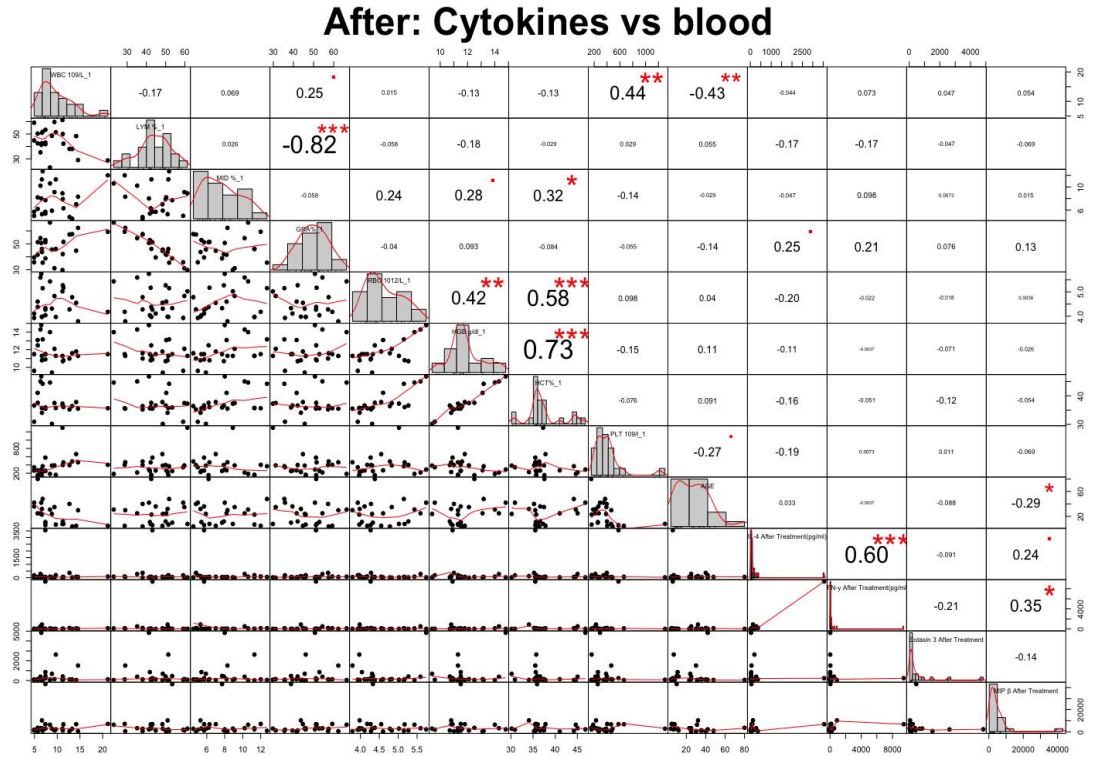


Figure 4.36: Correlation between blood cells and cytokines concentration after treatment

Figure 4.36 indicates that lymphocytes was strongly, negatively associated with granulocytes (-0.82). The value (0.44) of platelets and WBC were positively associated.

Monocytes/Hct was positively correlated (0.32).

Monocytes and Hb were positively correlated (0.28).

Monocytes and rbc were positively associated (0.24)

No relationship between rbc/wbc/lymphocytes

Positive correlation was observed in the following: Hb/Hct (0.73), Hb/rbc (0.42), Hb/monocytes (0.28).

Negative association was recorded in; Hb/lymphocytes (-0.18), Hb, WBC (0.13).

There was no association in the following; Hb/granulocytes, Hct/lymphocytes.

Age was negatively associated with wbc (-0.43) and with platelets (-0.27).

Wbc and platelets were positively associated (0.44).

IL-4 had positive corerelation (0.2) with granulocytes.

There was a positive relationship between age and MIP-β but no association with other molecules studied

Appendix 2

Deleted[Akanji. A]:

From left: Orutugu Langley, Ebube Odoya and Layefa Ayemi during Presurveillance visit to Ibelebiri community in Ogbia Local Government Area.

Presentation of drinks by culture to community Chiefs during Pre-surveillance meeting in Ibelebiri Community

Town crier proclaiming arrival of surveillance team in Otuegela

Tonye Orutugu sorting materials for sample collection

Cold boxes and materials ready for field work

Some Supporting field staff. From left: Minalab Okpu (Laboratory Technologist), Mash, A, and Layefa anyiemi (Environmental Health Technologists) and Tonye Orutugu (Laboratory Technologist).

Volunteers waiting for documentation

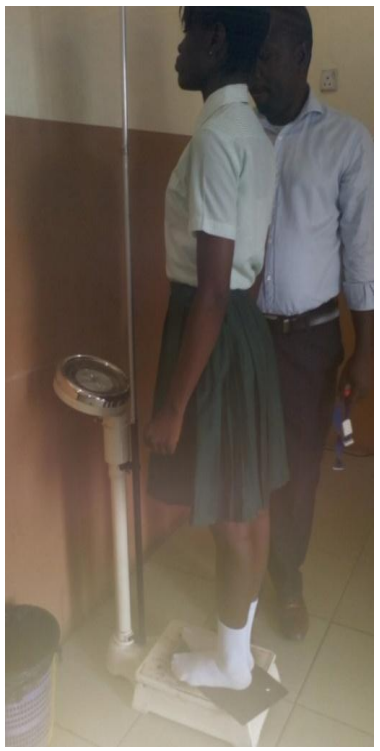
Documentation of volunteers and sample collection in Otuegela community

Taking meal after a long day work in the laboratory

Collection of blood samples

3.15a:

3.15b:



3.15c:

a, b & c: Anthropometric measurement of children

3.16a:

3.16b

3.16c:

Pharmacist, Dr. B Orubor dispensing drugs to infected volunteers

Community-based study Otuesega Town Hall

School-based study Community Secondary School, Otuesega

Appendix 3

RESEARCH AND ETHICS COMMITTEE

COLLEGE OF MEDICAL SCIENCES
UNIVERSITY OF BENIN, BENIN CITY, NIGERIA

Chairman: Prof. E. I. Unuigbe
MB.BS, DPH, FMCP, FWACP
08023374640, 08033382881

P.M.B. 1154, Benin City
email: researchethics@gmail.com

Our Ref: **CMS/REC/01/VOL.3/021**

DATE: 23rd May, 2017

Name of Principal Investigator: **Odoya Ebube Manfred,**
Department of Animal and Environmental Biology,
University of Benin, Benin City.

Dear Mr. Manfred,

Re: Proposal titled "Haemoparasitosis and Intestinal Helminthiasis and their Plasma Cytokine response in Humans in Bayelsa State, Nigeria".

REC Approval No: **CMS/REC/2017/016**

This is to inform you that the research described in the submitted proposal, the informed consent forms, participant information materials and other documents have been reviewed and approved by the College Research Ethics Committee, University of Benin.

This approval dates from 23rd May 2017 to 24th May 2018. In multi-year research, endeavor to submit your annual report to the REC early in order to obtain renewal of your approval and avoid disruption of your research.

The National Code of 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 REC. No changes are permitted in the research without prior approval by REC except in circumstances outlined in the code. REC reserves the right to conduct compliance visit to your research site without prior notice.

Thank you.

Professor E. I. Unuigbe
Chairman: Research Ethics Committee

Promoting Best Ethical & Scientific Standard for Research in Nigeria

GOVERNMENT OF BAYELSA STATE OF NIGERIA

MINISTRY OF HEALTH

Fax: 089 – 490257
Telephone: 089-490257,49035

7th March, 2018

BAYELSA STATE HEALTH RESEARCH ETHICAL COMMITTEE (BSHREC)
"NOTICE OF FULL APPROVAL"

Re: HAEMOPARASITOSIS AND HELMINTHIASIS AND THEIR PLASMA CYTOKINES RESPONSES IN HUMANS IN BAYELSA STATE

To:M. E. Odoya
Faculty of Life Science
University of Benin
Date of receipt of valid application: 27th February, 2018
Date of final determination of research for approval: 7th March, 2018

The Bayelsa State Health Research Ethics Committee (BSHREC) has considered it worthy to grant you ***Full Ethical*** **Approval** to conduct research on, "Haemoparasitosis and Helminthiasis and their Plasma Cytokines Responses in Humans in Bayelsa State". The approval is sequel to approved guidelines.

The effective date of this approval is today, 7th **March, 2018 and expires in 12 months from this date**. If there is reason for delay in starting and/or completing the research project within the time frame, please inform the BSHREC to accommodate the necessary adjustment that may arise thereof. Note also that no participant accrual or activity related to this research may be conducted outside of the approved date. All informed consent forms used in this study must be within the BSHREC approved duration of the study.

However, in case of Multi-Year Research as a result of inevitable delay, effort must be made to submit your annual report to the BSHREC early to obtain renewal of your approval to avoid disruption of your research.

The Bayelsa State Health Research Ethics Committee (BSHREC) wishes to request that you comply with all institutional guidelines, rules, regulations and the tenets of the code of conduct of research ethics. ***You are further requested to submit a copy of the final report of your research whenever it is ready to the BSHREC.*** No changes are permitted in this research without prior approval by the BSHREC. The BSHREC reserves the right to conduct compliance visit to your research site without prior notification.

Congratulations!!!

Please accept my best wishes,

Alabo Ateigbanyo
Head of Research, (PRS)/
Secretary, BSHREC

Administrative Headquarters, Secretariat Complex P.M.B. 24 Yenagoa, Tel: 089 490358, 490458, Fax: 089-490257

BAYELSA STATE POST PRIMARY SCHOOLS BOARD

BYSPPSB/P/308/VOL.I/14

Our Ref:.....

Your Ref:.....

P.M.B. 20
Yenagoa,
Bayelsa State

Date: 30th April. 2018.

E. M. Odoya
Faculty of Life Sciences,
University of Benin
Edo State.

APPROVAL TO CONDUCT HEALTH STUDY IN SECONDARY SCHOOLS IN OGBIA LOCAL GOVERNMENT AREA.

I am directed to refer to your application dated 12th March, 2018 on the above subject matter and hereby convey the Board's approval to conduct the Health Study in the Secondary Schools in Ogbia Local Government Area.

The Board wishes you well in your educational pursuits.

DICKSON, J. A. (FCAI)
Director, HRM
for: Permanent Secretary

INFORMED CONSENT FORM

COMMUNITY/SCHOOL BASED SCREENING AND TREATMENT OF MALARIA DISEASE AND WORM INFECTIONS IN OGBIA LOCAL GOVERNMENT AREA BAYELSA STATE NIGERIA

You are invited to participate in this study which is aimed at screening for malaria parasites and intestinal worms. Everyone that is bitten by mosquitoes can be infected with malaria without necessarily falling sick, but rather, you become an agent of a spread of the disease to others. You may not fall sick with worm infections but this can reduce the ability of the body to fight other diseases.

To reduce the rate of these two diseases, we are going to carry out a test on anyone who accepts to participate in this study. Infected person will be treated with drugs to be provided by the Bayelsa State Ministry of Health (MDG-programme).

To carry out this test, we'll collect from your arm 3 ml of blood. A minor pain and bleeding may occur which will stop in few minutes. The test result will be ready in 3 days and will be made available to you personally.

The benefit of this study to you is that everyone who tested positive would receive free treatment form the Medical Team of Ministry of Health.

The decision to be a part of this study is absolutely voluntary. No money will be paid to you for participating in this study. If you have any doubt, you are at will to say NO and decline from participation.

In case of any questions, please contact the Principal Investigator, Dr. E. M. Odoya- 08036479148.

If you accept to be a participant, having understood the content above, please complete the following:

I Mr/Mrs/Miss..... ..... voluntarily accept to take part in this study.

Study No . ..... Name of witness .....
Designation .....

Signature ..... Phone No.....

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HAEMOPARASITOSIS AND HELMINTHIASIS AND THEIR PLASM...

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