ASSESSMENT OF SOME IMMUNOLOGICAL RESPONSE TO HOOKWORM INFECTION IN PATIENTS ATTENDING SELECTED HOSPITALS IN KANO STATE, NIGERIA

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OCTOBER, 2017

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A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULLFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE MASTER OF SCIENCE (M.Sc) IN MICROBIOLOGY

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OCTOBER, 2017.

DECLARATION

I declare that the work in this dissertation entitled "**Assessment of Some Immunological Response to Hookworm Infection in Patients Attending Selected Hospitals in Kano State, Nigeria**" has been performed by me in the Department of Microbiology. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other Institution.

Maryam Donald Vandu _____ _____

CERTIFICATION

This dissertation entitled "ASSESSMENT OF SOME IMMUNOLOGICAL RESPONSE TO HOOKWORM INFECTION IN PATIENTS ATTENDING SELECTED HOSPITALS IN KANO STATE, NIGERIA" by Maryam Donald VANDU meets the regulations governing the award of the degree of Master of Science of the Ahmadu Bello University Zaria, and is approved for its contribution to knowledge and literary presentation.

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ACKNOWLEDGEMENT

I want to appreciate Almighty God for His grace, wisdom, provision and direction to carry out this work.

I want to thank my supervisors, Prof. H.I. Inabo and Dr. E.E. Ella for their guidance, time and energy contributed in ensuring the completion of this research work.

I want to appreciate all my lecturers and Staff of the Department for their help and support. Special thanks to all the Doctors and staff of various hospitals that gave their unreserved support toward the success of this work.

I want to express my profound appreciation to my parents Mr. and Mrs. Donald Vandu, for their love, support and advice: may God richly reward you and cause you to eat from the fruits of your labour. To my wonderful husband Suleiman Yakubu thank you for your love and unending support; God bless you richly.

And to my siblings; Agnes (Mama G), Emeka (Daddy G), Isa, Hasna, Noni and my friends; David Balogun, Stephanie Samuel, Sa'adatu Suleiman, Esther Alonge, Hafsat Musa Magaji and Becky Robert thanks for your love, help and prayers during this work.

ABSTRACT

Hookworm infection is the leading cause of maternal and child morbidity in the tropics and subtropics. The most serious consequence of hookworm infection is anaemia, secondary to loss of iron and protein of gut. The complexity of the hookworm life cycle offers numerous opportunities for the parasite and host to interact at the molecular level. Infection with human hookworms is usually chronic, with adult worms surviving an average of 2–4 years, with a maximum of 18 years and it triggers a series of immune responses in the host. Despite repeated exposure, protective immunity does not seem to develop in humans, so that infections occur in all age groups (depending on exposure patterns) and tend to be prolonged. In this study, the prevalence and the immunological response to hookworm infection were studied among patients attending some selected hospitals in Kano state. A total of four hundred (400) blood and stool samples were collected. Microscopy was carried out to establish the presence of hookworm egg in the stool samples, Enzyme linked immunosorbent assay (ELISA) was used to determine C3b and IgM levels in the patients' serum samples. The PCV of the study population was also determined. Statistical package for social science (SPSS) version 21 was used to test for the associated risk factors for the infection. Twenty one patients representing 5.3% were positive for hookworm and were mainly children. The following results were obtained using questionnaire; age group 6-10 years had the highest prevalence of 17.8% followed by 1-5 years (13.3%) while those within the age group 16-20 years had the least prevalence (2%), hence, the distribution was statistically significant ($P<0.05$). There was increase in IgM level in all the participants but the participants that were infected with hookworm showed slightly higher level (9.8mg/ml). PCV was determined for all participants, 19 (35%) of the positive participants had PCV within the range of 1-20% and 2 (0.57%) participants were within the range 21-above (%), a significant relationship was observed between PCV and hookworm infection ($p<0.05$).

In conclusion, this study revealed that the prevalence of hookworm infection was low (5.3%) among the study participants in the selected hospitals in Kano and were mainly children (21). Hookworm infection was characterized by low PCV (21.67%) and significantly elevated IgM (9.87mg/ml). However, complement C3b a major activation component of the system was not elevated (0.73mg/ml) and falls within the normal range (0.3-1.6mg/ml). Therefore, more work needs to be carried out on the immunology of hookworm infection in order to have a better understanding of the interaction of the parasite and the host immune system.

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LIST OF ABBREVIATIONS

IgM	Immunoglobulin M
C3b	Complement 3b
PCV	Packed Cell Volume
CR1	Complement Receptor 1
STHs	Soil Transmitted Helminthes
NTD	Neglected Tropical Diseases
Th2	T helper type 2
IgE	Immunoglobulin E
TF	Tissue Factor
DALYs	Disability-Adjusted Life Years
IgG	Immunoglobulin G
CP	Classical Pathway
AP	Alternative Pathway
MBL	Mannose-Binding Lectin Pathway
RCAs	Regulators of Complement Activities
MCP	Membrane Cofactor Protein
PCR	Polymerase Chain Reaction
C3	Component 3
BCR	B-cell Receptor

FC Fragment Crystallizable

IgA Immunoglobulin A

IgD Immunoglobulin D

Fab Fragment antigen-binding

FcR Fragment Crystallizable Receptor

TRIM 21 Tripartite motif-containing 21

MASP Mannose binding lectin Associated Serine Proteases

MIX Mixture

EIA Enzyme Immuno Assay

SP Strptavidin Peroxidase

OD Optical Density

CHAPTER ONE

1.0 INTRODUCTION

1.1 Hookworm Infection

Parasitic diseases have contributed immensely in undermining the health status of people and jeopardizing the economic development of nations in the tropics (Adeyeba and Tijjani, 2002). Human hookworm infection causes such parasitic diseases. Human hookworm infection is caused by both *Ancylostoma duodenale* and *Necator americanus*, which are blood feeding nematode (Yulan *et al*., 2009). They live in the small intestine where they attach to the mucosa via teeth or cutting plates and feed on the host mucosa and blood (Hotez *et al*., 2004). They infect over one billion people around the globe mostly in tropical and subtropical climates, especially in communities with low socio-economic status (Furst *et al*., 2012; Fenwick, 2012; Halpenny *et al*., 2013).

Hookworm infection is one of the commonest infections in Nigeria, with most persons having an episode of infestation in their lifetime with far reaching disabling and debilitating effects on the individual victims (Bala and Yakubu, 2010). Hookworm infection thrives in areas where sanitary and environmental conditions favor the development of filariform larvae and infection of the host (Halpenny *et al*., 2013; Abah and Woken, 2016).

The infection of the host is caused by the infective larvae that penetrate the skin of individuals walking bare footed through areas contaminated with faecal matter. The most important medical feature of hookworm infectiom is iron deficiency anaemia and this is as a result of loss of iron and protein in the gut. The extent of iron deficiency anaemia resulting from hookworm is also

connected to the number of hookworm which infects the individual, particularly in people with inadequate iron reserves or intake (Hotez *et al*., 2011).

The most vulnerable and high risk group of individuals to acquiring the infection is children and pregnant women (Liabsuetrakul *et al*., 2009). In addition, heavy infection in children causes stunted growth and impairment of intellectual and cognitive development (Jardim-Botelho *et al*., 2008). Anaemia remains one of the most prevalent causes of morbidity suffered by these individuals in the developing world and is a critical co morbid factor contributing to the excess mortality in these regions (WHO, 2001).

In most developing countries for instance, anaemia in pregnancy has been associated with worm disease, especially hookworm (Hotez, 2009). Women and young children have the lowest iron levels and are therefore most vulnerable to chronic blood loss as the result of hookworm infection (Brooker and Bundy, 2009).

1.2 Hookworm and Host Cell Responses

The complexity of the hookworm life cycle offers numerous opportunities for the parasite and host to interact at the cellular level. Further, natural attrition of larvae at critical barriers, such as during skin invasion, and transit through lung tissues, as well as arrival in the gut and penetration of its mucosa, presents the host with an extensive diversity of antigenic challenge, immune stimulation and, most probably, immune modulation. Hence, host immune responses can be said to be triggered by larval invasion of the skin, larval migration through the circulation and lungs, and worm establishment in the intestine, where adult worms feed on blood and mucosa while injecting various molecules that facilitate feeding and modulate host protective responses (Alex and Paul, 2001).

Despite repeated exposure, protective immunity does not seem to develop in humans, so that infections occur in all age groups (depending on exposure patterns) and tend to be prolonged. Immunoglobulin M (IgM) is a basic antibody that is produced by B cells and the first to appear in response to antigen (Racine *et al*., 2011).

It is an effective neutralizing agent in the early stage of disease. Elevated levels can be a sign of recent infection or exposure to antigen. Due to its polymeric nature, IgM possesses high avidity and is particularly effective at complement activation. By itself, IgM is an ineffective opsonin; however it contributes greatly to opsonisation by activating the complement system (Bours *et al*., 2005).

1.3 The complement Activation System

Resistance to infection and clearance of cell debris in mammals depend on the activation of the complement system, which is an important component of innate and adaptive immunity. Central to the complement system is the activated form of C3, called C3b, which attaches covalently to target surfaces to amplify complement response, label cells for phagocytosis and stimulate the adaptive immune response (Janssen *et al*., 2006).

The complement system can be activated in two main ways. The first means of activation is part of the innate (natural) immune response (such as neither antibodies nor T cell receptors are involved). The second and most potent means occurs in an adaptive immune response when antibodies (IgM or IgG) bind to antigen at the surface of a cell. Activation of complement via innate and adaptive immune system is vital to the body's defenses in fighting infections (Janssen *et al*., 2005).

Activation of component C3 is central to the three complement pathways and results in inflammation and elimination of some self and non-self targets. C3b is the larger of two elements formed by the cleavage of complement component 3. C3b binds covalently to pathogen surfaces to promote phagocytosis. This leads to the production of surface-bound C3-convertase and thus more C3b components. Bound C3b also aids in opsonization of the microbe and eventual phagocytosis by macrophages. Complement receptor 1 (CR1) on macrophages allows the engulfing of C3b covered microbes. C3b also aids in the clearance of immune complexes (antigen-antibody) (Janssen *et al*., 2005).

1.4 Statement of Problem

Intestinal parasites or soil transmitted helminthes (STHs) are the aetiological agents of the most common Neglected Tropical Diseases (NTD) worldwide which continues to cause significant morbidity in Nigeria and other less developed tropical and subtropical countries (Sachs and Hotez, 2006; Odu *et al*., 2013). STHs have been reported as one of the world's most important causes of physical and intellectual growth disturbances of people living in area with poverty in the developing world (Akinseye *et al*., 2017).

Infection with human hookworms is usually chronic, with adult worms surviving an average of 2–4 years, with a maximum of 18 years (Rupert *et al*., 2004). This suggests that infection with hookworms modulate parasite-specific immune responses. Such immuno-modulation is characteristic of infection with tissue-dwelling filarial nematodes, resulting in an antigen-specific suppression of cellular immune responses (Rupert *et al*., 2004). Hookworm infection play a central position as contributory factors in the cause of childhood malnutrition because heavy insidious infections with these worms may worsen malnutrition particularly among already malnourished families from socio-economically underprivileged communities. It is also

recognized as one of the leading causes of child morbidity in the developing countries of the tropics and sub- tropics (Nnachi *et al*., 2015). Despite repeated exposure, protective immunity does not seem to develop in humans, so that infections occur in all age groups (depending on exposure patterns) and tend to be prolonged (Alex and Paul, 2001). Intense exposure to Hookworm results in heavy parasitism which can produce acute gastrointestinal hemorrhage, severe acute anemia, and congestive heart failure (David, 2014).

1.5 Justification of the Study

Hookworms infect almost one billion people and are a major cause of iron-deficiency anaemia in developing countries of the tropics (Moorkoth *et al*., 2015). Despite their prevalence and the morbidity they cause, little is known about the immune response to this complex parasite, their interaction with the immune system and the chronicity of the infection such as how these parasites interact with their hosts, negotiate host anatomy to arrive in the gut via circuitous pathways, and tolerate the complex immune responses generated against them and how (or even if) the immune response has any meaningful effect in terms of host protection or parasite survival (Loukas and Prociv, 2001; Loukas *et al*., 2005). The immunology of human hookworm infection has not received as much focus as that of other helminthes parasite (Mcsorley and Loukas, 2010). Thus, this study is designed to bridge this gap by looking at some of the immunological response to hookworm infection.

1.6 Aim:

The aim of this study was to assess some immunological response to hookworm infection in patients attending selected hospitals in Kano State, Nigeria.

1.7 Objectives:

The specific objectives of this study were to determine the;

1. Prevalence of hookworm infection in a cross section of patients in some hospitals in Kano.
2. Socio-demographic factors associated with hookworm infection among the study population.
3. Total IgM and C3b convertase levels among hookworm infected individuals in some hospitals in Kano.
4. Relationship of Packed Cell Volume (PCV) levels and hookworm in infected individuals in some hospitals in Kano.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Hookworm Infection

Hookworm is a soil-transmitted helminth that is common in many third world and developing countries (WHO, 2012). Hookworm infection in humans is predominantly caused by the nematode parasites *Necator americanus* and *Ancylostoma duodenale*. Hookworm infection is acquired through skin exposure to larvae in soil contaminated by human faeces. Infective filariform larvae are found in the soil following voiding of the ova of hookworm from an infected person or if the faeces from an infected person are used as fertilizer (CDC, 2014). Worldwide, hookworms infect an estimated 576-740 million people (CDC, 2014). Although most of those affected are asymptomatic (de Silva *et al*., 2003), approximately 10% experience anaemia. Hookworms may persist for many years in the host and impair the physical and intellectual development of children and the economic development of communities.

Historically, hookworm infection has disproportionately affected the poorest among the least-developed nations, largely as a consequence of inadequate access to clean water, sanitation and health education. The frequent absence of symptoms notwithstanding, hookworm disease substantially contributes to the incidence of anaemia and malnutrition in developing nations (WHO, 2012). It occurs most commonly in the rural tropical and subtropical areas of Asia, sub-Saharan Africa, and Latin America (de Silva *et al*., 2003).

2.2 Life Cycle of Hookworm

Man is the only host for hookworms no intermediate host is required. Adult worms inhabit the small intestine of man attaching themselves to the mucous membrane by means of their mouth parts. The eggs containing segmented ova are passed out in faeces of infected person (Arora and Arora, 2004). In the warm and moist soil, rhabditform larvae hatch out from the egg in 24-48 hours. These measure 250µm in length and 17µm in maximum diameter. These feed on bacteria and organic debris and moult twice on the third and fifth day and develop into filariform larvae, which no longer feed. They measure 500-700µm in length and are the infective stage of the parasite. Depending on the temperature and moisture content of the soil, filariform larvae can remain infective for up to 6 weeks (Arora and Arora, 2004).

When a person walks barefooted on soil containing the filariform larvae they penetrate the skin, particularly the skin between the toes, the dorsum of the foot and medial aspect of the sole. In farm workers, the larvae may penetrate the skin of the hands. On reaching the subcutaneous tissue the larvae enter into the lymphatics or small venules. Through lymph-vascular system they enter into venous circulation and are carried via the right heart into the pulmonary capillaries. Here they break through the capillary walls and enter into alveolar spaces. From alveoli, the larvae migrate up the bronchii, trachea and larynx, crawl over the epiglottis to the pharynx and are swallowed (Arora and Arora, 2004).

During migration or on reaching the oesophagus they undergo third moulting. Thereafter they reach

small intestine, undergo fourth moulting and develop into adult worms. They attach themselves to the mucous membrane of small intestine by means of their mouth parts. In about six weeks, from time of infection, adult worms become sexually mature. Male fertilizes female and the

latter lays eggs which are passed in faeces and the cycle is repeated (Arora and Arora, 2004). Larval *A. duodenale* may pass from the mother to foetus in uterus. In addition, infection by *A. duodenale* may also occur by the oral and transmammary route (Arora and Arora, 2004).

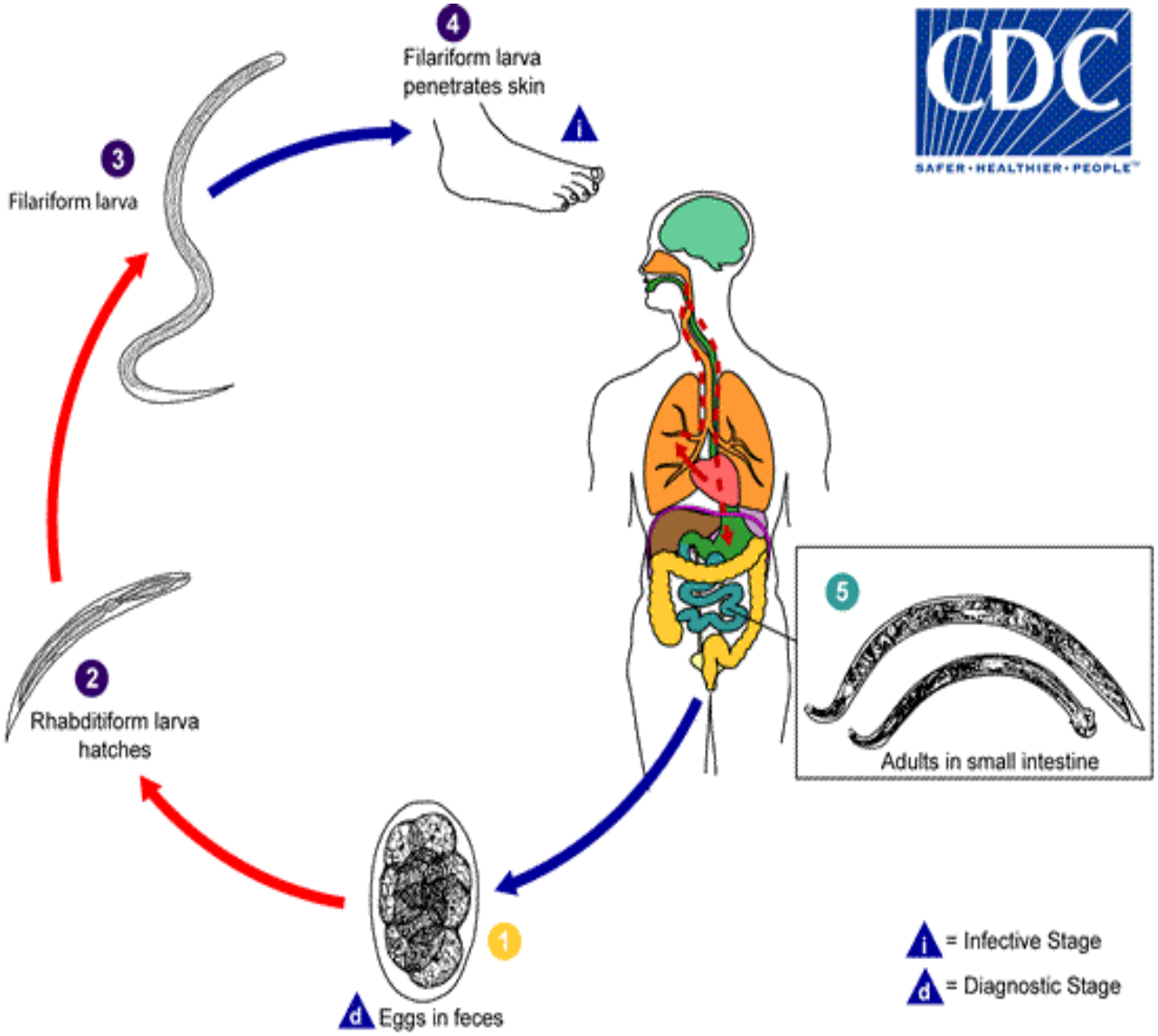


Figure 2.1: The Life Cycle of Hookworm (CDC, 2016).

2.3 Types of Hookworm Infection

Hookworm infection gives rise to the following three clinical manifestations in humans:

Classic hookworm infection - This is a gastrointestinal infection characterized by chronic blood loss that leads to iron-deficiency anaemia and protein malnutrition; it is caused primarily by *N americanus* and *A duodenale* and less commonly by the zoonotic species *A ceylonicum*

Cutaneous larva migrans - This is an infection whose manifestations are limited to the skin; it is most commonly caused by *A braziliense*, whose definitive hosts include dogs and cats

Eosinophilic enteritis - This is a gastrointestinal infection characterized by abdominal pain but no blood loss; it is caused by the dog hookworm *A caninum*

In cutaneous larva migrans, the infective larvae of zoonotic species such as *A braziliense* do not elaborate sufficient concentrations of hydrolytic enzymes to penetrate the junction of the dermis and epidermis. The larvae thus remain trapped superficial to this layer, where they migrate laterally at a rate of 1-2 cm/day and create the pathognomonic serpiginous tunnels associated with this condition. Larvae can survive in the skin for about 10 days before dying (David, 2014).

In eosinophilic enteritis, *A caninum* larvae typically enter a human host by penetrating the skin, though infection by oral ingestion is also possible. These larvae probably remain dormant in skeletal muscles and create no symptoms. In some individuals, larvae may reach the gut and mature into adult worms (Blair and Diemert, 2015).

Some individuals sustain *A caninum* development and then respond with a severe localized allergic reaction but the reason for this is largely unknown. Adult worms secrete various potential allergens into the intestinal mucosa. Some patients have been reported to experience increasingly severe recurrent abdominal pain, which may be analogous to a response to repeated insect stings (Blair and Diemert, 2015).

2.4 Epidemiology of Hookworm Infection

It was estimated that 576–740 million individuals are infected with hookworm (Bethony *et al*., 2006; Fenwick, 2012, CDC, 2014). Of these infected individuals, about 80 million are severely affected (Gasser *et al.*, 2009). The major etiology of hookworm infection is *Necator americanus* which is found in the Americas, Sub-Saharan Africa, and Asia (Hotez *et al.*, 2005). *Ancylostoma duodenale* is found in more scattered focal environments, namely Europe and the Mediterranean. Most infected individuals are concentrated in sub-Saharan Africa and East Asia/the Pacific Islands with each region having estimates of 198 million and 149 million infected individuals, respectively. Other affected regions include: South Asia (50 million), Latin America and the Caribbean (50 million), South Asia (59 million), Middle East/North Africa (10 million) (Bethony *et al*., 2006).

2.4.1 Prevalence of Hookworm Infection in Nigeria

Previous works had placed Nigeria as one of the hookworm endemic area of the world. A high prevalence had been reported across the country: 50% (Biu and Kofur, 2010) in Borno state, North-East Nigeria; 47.2% (Alade and Ibemologi, 2017) in Kwara state, South-West, Nigeria; 41% (Damen *et al*., 2014) in Plateau state, North-Central Nigeria; 39.6% (Eke *et al*., 2014) in Nasarawa state, North-Central Nigeria; 35.8% (Alli *et al*., 2011) in Oyo state, South-West, Nigeria; 34.2% (Houmsou *et al*., 2009) in Benue state, North-Central Nigeria and 26.7% (Otu-Bassey *et al*., 2017) in Calabar state, South-South, Nigeria . Odinaka *et al*. (2015) observed that hookworm ranked highest (94.2%) of all soil-transmitted helminthes in Imo state, South-East Nigeria and Alli *et al*. (2011) reported that hookworm ranked the second (34.5%) of intestinal nematodes among pregnant women in Oyo state, South-West Nigeria.

Recent studies in Nigeria show that hookworm infection is more prevalent in pre-school and school children than adults as reported by Bassey and Loko. (2017) in Bayelsa state, South-South Nigeria; Odo *et al*. (2016) in Enugu state, South-East Nigeria; Tyoalumun *et al*. (2016) in Benue state, North-Central Nigeria; Omitola *et al*. (2016) in Ogun state, South-West, Nigeria and Awobode *et al*. (2016) in Oyo state, South-West, Nigeria;

A majority of these infected individuals live in poverty-stricken areas with poor sanitation. Hookworm infection is most concentrated among the worlds poorest who live on less than $2 a day (Hotez *et al*., 2005). While hookworm infection may not directly lead to mortality, its effects on morbidity demand immediate attention. When considering disability-adjusted life years (DALYs), neglected tropical diseases, including hookworm infection, rank among diarrheal diseases, ischemic heart disease, malaria, and tuberculosis as one of the most important health problems of the developing world (Bethony *et al*., 2006).

2.5 Risk Factors Associated with Hookworm Infection

Warm climates and adequate moisture, lack of personal or environmental hygiene, sanitation, and education, walking barefooted and poor health could increase the risk of hookworm infection (Vandemark *et al*., 2010; Alemu *et al*., 2011).

Hookworm infection is highly prevalent among school-aged children. The infection occurs due to penetration of the skin by the larvae of the parasite; as a result, children walking barefooted on the soil contaminated with faecal matter which exposes them to the infective larval stages of the parasite, and also their habits of not washing hands before eating is associated with increased odds of the infection (Degarege *et al*., 2014).

2.6 Pathology of Hookworm Infection

Hookworm infection is generally considered to be asymptomatic. There are general symptoms that an individual may experience soon after infection. Itching, which is an allergic reaction at the site of parasitic penetration and entry, is common in patients infected with *N. americanus* (Markell *et al*., 2006). Additionally, cough and pneumonitis may result as the larvae begin to break into the alveoli and travel up the trachea. Then once the larvae reach the small intestine of the host and begin to mature, the infected individual will suffer from diarrheoa and other gastrointestinal discomfort (Markell *et al*., 2006).

Major morbidity associated with hookworm infection is caused by intestinal blood loss, iron deficiency anaemia and protein malnutrition (Bethony *et al*., 2006). These result mainly from adult hookworms in the small intestine ingesting blood, rupturing erythrocytes and degrading hemoglobin in the host (Hotez *et al*., 2005). This long-term blood loss can manifest itself physically through facial and peripheral oedema; eosinophilia and eating disorder caused by iron deficiency anaemia are also experienced by some hookworm infected patients (Markell *et al*., 2006).

More attention has been given to other important outcomes of hookworm infection that play a large role in public health. It is now widely accepted that children who suffer from chronic hookworm infection can suffer from growth retardation as well as intellectual and cognitive impairments (Bassey and Loko, 2017). Additionally, research has focused on the potential of adverse maternal-foetal outcomes when the mother is infected with hookworm during pregnancy (Hotez *et al*., 2005).

2.7 Clinical Manifestation of Hookworm Infection

Intestinal blood loss secondary is the major clinical manifestation of hookworm infection (Hotez *et al*., 2005). In fact, hookworm infection historically refers to the childhood syndrome of iron deficiency anaemia, protein malnutrition, growth and mental retardation with lethargy resulting from chronic intestinal blood loss (David, 2014).

Hookworms ingest and digest some of the blood from the injured mucosa by means of a multienzyme cascade of metallohemoglobinases. Each *Necator* worm ingests 0.03 mL of blood daily, whereas each *Ancylostoma* worm ingests 0.15-0.2 mL of blood daily. Inhibition of host coagulation system due to a series of anticoagulants directed against factor Xa and the factor VIIa–tissue factor (TF) complex, as well as against platelet aggregation, further exacerbates blood loss (WHO, 2012).

The amount of blood loss and the degree of anaemia are positively correlated with the worm burden, whereas hemoglobin, serum ferritin, protoporphyrin levels are significantly and negatively correlated with the number of worms. Threshold worm loads for anaemia differ nationally, with as few as 40 worms producing anaemia in countries with low iron consumption (Brooker and Bundy, 2009).

Generally, the extent of hookworm infection may be categorized as follows: Light (< 100 worms); Moderate (100-500 worms) and Heavy (>500 worms) (CDC, 2016). People who develop an initial heavy infection seem to reacquire heavy infection, and individuals who are lightly infected reacquire light infections. Since each adult worm is the molt of a single infective larva, this suggests continuing exposure to highly contaminated environments with little amnestic immunity in the host. Individuals with light infection have minimal blood loss and may

have infection but not disease, especially if iron intake or reserves are adequate to compensate for the blood loss. Moderate-to-heavy infections cause iron-deficiency anaemia (CDC, 2012).

In addition, because *A duodenale* consumes more blood per worm than *N americanus* does, the severity of anaemia may differ as a factor of the hookworm species that is causing the infection. Severe anaemia affects intellectual and physical development in children and cardiovascular performance in adults (Hotez *et al*., 2005).

Hypoproteinaemia may develop due to significant blood loss and the worm's ingestion of serum proteins, which is clinically manifested as weight-loss, anasarca, and oedema. This results in stunted growth, as well as an increased susceptibility to infections such as malaria and gastrointestinal infections with enteric bacteria, viruses, and protozoa (Bhatia *et al*., 2010).

2.7.1 Anaemia Caused by Hookworm Infection

The major clinical manifestation of human hookworm infection is intestinal blood loss (Crompton, 2001). Large scale of hookworm infections results in iron deficiency anaemia (Smith and Brooker, 2010). Blood loss occurs when the worms attach themselves to the intestinal mucosa and sub mucosa and contract their muscular esophagi to create negative pressure (del Valle *et al*., 2003 Hotez *et al*., 2004). In the gut, the adult worms feed by sucking clumps of villi into their buccal capsules, effectively burying their anterior ends into the mucosa or deeper (Loukas *et al*., 2005). Their large anterior glands secretes various products, including proteases and anti-coagulant peptides into the worm’s buccal cavity and oesophagus, as well as into the attachment site to predigest host mucosal tissues (Williamson *et al*., 2003). The buccal capsule contains teeth (*Ancylostoma species*) or cutting plates (*Necator americanus*), which probably help to anchor the worm and facilitate host tissue maceration (Loukas *et al*., 2005).

Worms are thought to rely mostly on blood for nutrition (*A. duodenale* can cause in excess of 0.05ml blood loss per worm a day), intact erythrocytes are ingested (Bungiro and Cappello, 2004) and lysed in parasite's intestine by haemolytic proteins (Don *et al*., 2004). Haemoglobin is then released from lysed erythrocytes and digested by semi-ordered pathway of proteolysis (Williamson *et al*., 2004). After feeding, adult worms mate and females produced about 10,000 eggs per worm a day. In heavily infected people who can harbour several hundred worms at one time, iron-deficiency anaemia develops (Hotez *et al*., 2004).

Pregnant women and children are at greatest risk for hookworm associated iron deficiency anaemia due to low iron stores resulting from diets insufficient to meet demands. The development of hookworm related iron deficiency anaemia depends on the level of an individual's iron stores, the intensity of infection, and the infecting species as *A. duodenale* causes a greater blood loss than *N. americanus*. Iron-deficiency anaemia resulting from chronic intestinal blood loss due to hookworm infection often causes long term morbidity (Steketee, 2003).

2.8 Immune Responses to Hookworm Infection

The sophistication of the hookworm life cycle ensures that antigenic challenges occur at numerous anatomic site including the skin, lungs and intestinal mucosa (Verjovski-Almeida *et al*., 2003). To add to the complexity, hookworms secrete immunomodulatory molecules to selectively skew or dampen immune responses to promote their long-term survival (Loukas and Prociv (2001).

In many respects, hookworms are typical of gastrointestinal nematodes in the types of immune response they generate in definitive hosts. Antibody isotypes and subclasses, as well as cellular

responses, fit loosely within the framework of a T helper type 2 (Th2) immune response. People infected with *N. americanus* produce elevated levels of parasite-specific and total IgE, this is accompanied by eosinophilia, both systemic and localised to the lungs (Loukas *et al*., 2005).While human hookworm infections exhibit some of the hallmark features of Th2 responses (IgE and local and systemic eosinophilia), these immune responses clearly fail to protect most infected people. The reason for the observed failure of Th2 cells to mount effective anti-hookworm responses remains unknown, although several findings suggest the presence of immune cross-regulatory mechanisms (Loukas *et al*., 2005).

2.8.1 Immunoglobulins

Immunoglobulins (Ig) or antibodies are glycoproteins belonging to the immunoglobulin superfamily (Maverakis *et al*., 2015). They are secreted by B cells of the adaptive immune system, mostly by differentiated B cells called plasma cells. Antibodies can occur in two physical forms, a soluble form that is secreted from the cell to be free in the blood plasma, and a membrane-bound form that is attached to the surface of a B cell and is referred to as the B-cell receptor (BCR). The BCR is found only on the surface of B cells and facilitates the activation of these cells and their subsequent differentiation into either antibody factories called plasma cells or memory B cells that will survive in the body and remember that same antigen so the B cells can respond faster upon future exposure (Borghesi and Milcarek, 2006).

The antibody recognizes a unique molecule of the harmful agent, called an antigen, via the Fab's variable region (Philips *et al*., 2015). Each tip of the "Y" of an antibody contains a paratope (analogous to a lock) that is specific for one particular epitope (similarly analogous to a key) on an antigen, allowing these two structures to bind together with precision. Using this binding mechanism, an antibody can *tag* a microbe or an infected cell for attack by other parts of the

immune system, or can neutralize its target directly (for example, by blocking a part of a microbe that is essential for its invasion and survival) (Janeway *et al*., 2001).

The antibodies are typically made of basic structural units each with two large heavy chains and two small light chains. There are several different types of antibody heavy chains that define the five different types of fragment crystallizable region (Fc) that may be attached to the antigen-binding fragments. The five different types of Fc regions allow antibodies to be grouped into five isotypes (IgA, IgD, IgE, IgG and IgM). Each Fc region of a particular antibody isotype is able to bind to its specific Fc Receptor except for IgD, which is essentially the B cell receptors (BCR), thus allowing the antigen-antibody complex to mediate different roles depending on which FcR it binds (Maverakis *et al*., 2015).

2.8.2 Types of Immunoglobulin

Immunoglobulin A (IgA)

It is an antibody that plays a crucial role in the immune function of mucous membranes. It is found in mucosal areas, such as the gut, respiratory tract ant urogenital tract. The amount of IgA produced in association with mucosal membranes is greater than all other types of antibody combined (Fagarasan and Honjo, 2003). It prevents colonization by pathogens. It is also found in saliva, tears and breast milk.

Immunoglobulin D (IgD)

It is an antibody isotype that makes up about 1% of proteins in the plasma membranes of immature B-lymphocytes where it is usually co-expressed with another cell surface antibody called IgM. IgD is also produced in a secreted form that is found in very small amounts in blood serum, representing 0.25% of immunoglobulins in serum (Ohta and Martin, 2006).

It functions mainly as an antigen receptor on B cells that have not been exposed to antigens (Geisberger *et al*., 2006). It has been shown to activate basophils and mast cells to produce antimicrobial factors (Chen *et al*., 2009).

Immunoglobulin E (IgE)

It is a type of antibody that has only been found in mammals. IgE is synthesised by plasma cells. M IgE's main function is immunity to parasites such as helminthes. It binds to allergens and triggers histamine release from mast cells and basophils, and is involved in allergy (Erb, 2007)

Immunoglobulin G (IgG)

Immunoglobulin G is the main type of antibody found in blood and extracellular fluid allowing it to control infection of body tissues, by binding many kinds of pathogens such as viruses, bacteria, and fungi. IgG also plays an important role in antibody-dependent cell-mediated cytotoxicity (ADCC) and intracellular antibody-mediated proteolysis, in which it binds to tripartite motif-containing 21 (TRIM21) (the receptor with greatest affinity to IgG in humans) in order to direct marked virions to the proteasome in the cytosol (Mallery *et al*., 2010).

Immunoglobulin G has two antigen binding sites representing approximately 75% of serum antibodies in humans, IgG molecules are created and released by plasma B cells. It provides the majority of antibody-based immunity against invading pathogens. It is the only antibody capable of crossing the placenta to give passive immunity to the fetus (Maverakis *et al*., 2015).

2.8.3 Immunoglobulin M (IgM)

Immunoglobulin M is a basic antibody that is produced by B cells. IgM is by far the largest antibody in the human circulatory system. It is the first antibody to appear in response to initial exposure to antigen. The spleen is the major site of specific IgM production in adults (Racine *et al*., 2011).

2.8.4 Structure and function of IgM Antibodies

IgM forms polymers where multiple immunoglobulins are covalently linked together with disulfide bonds, mostly as a pentamer but also as a hexamer. IgM has a molecular mass of approximately 970 kDa (in its pentameric form). Because each monomer has two antigen binding sites, a pentameric IgM has 10 binding sites. Typically, however, IgM cannot bind 10 antigens at the same time because the large size of most antigens hinders binding to nearby sites (Bours *et al*., 2005).

The J chain is found in pentameric IgM but not in the hexameric form, perhaps due to space constraints in the hexameric complex. Pentameric IgM can also be made in the absence of J chain. At present, it is still uncertain what fraction of normal pentamer contains J chain, and to this extent it is also uncertain whether a J chain-containing pentamer contains one or more than one J chain (Wiersma *et al*., 1998).

IgM cannot diffuse well, and is found in the interstitium only in very low quantities because it is a large molecule. IgM is primarily found in serum; however, because of the J chain, it is also important as a secretary immunoglobulin (Johansen *et al*., 2000). Due to its polymeric nature, IgM possesses high avidity, and is particularly effective at complement activation. By itself, IgM is an ineffective opsonin; however it contributes greatly to opsonization by activating complement and causing C3b to bind to the antigen (Bours *et al*., 2005).

2.8.5 Expression of IgM

In germline cells, the gene segment encoding the μ constant region of the heavy chain is positioned first among other constant region gene segments. For this reason, IgM is the first

immunoglobulin expressed by mature B cells. It is also the first immunoglobulin expressed in the fetus (around 20 weeks) and phylogenetically the earliest antibody to develop (Jayasekera *et al*., 2007).

2.8.6 Clinical Significance of IgM Antibodies

IgM antibodies appear early in the course of an infection and usually reappear, to a lesser extent, after further exposure. IgM antibodies do not pass across the human placenta (only isotype IgG). These two biological properties of IgM make it useful in the diagnosis of infectious diseases. Demonstrating IgM antibodies in a patient's serum indicates recent infection, or in a neonate's serum indicates intrauterine infection (such as congenital rubella) (McAlister *et al*., 2004).

IgM in normal serum is often found to bind to specific antigens, even in the absence of prior immunization. For this reason IgM has sometimes been called a "natural antibody" (Jayasekera *et al*., 2007). This phenomenon is probably due to the high avidity of IgM that allows it to bind detectably even to weakly cross-reacting antigens that are naturally occurring. For example the IgM antibodies that bind to the red blood cell A and B antigens might be formed in early life as a result of exposure to A- and B-like substances that are present on bacteria or perhaps also on plant materials (Jayasekera *et al*., 2007).

IgM antibodies are mainly responsible for the clumping (agglutination) of red blood cells if the recipient of a blood transfusion receives blood that is not compatible with their blood type (McAllister *et al*., 2004).

2.8.7 The Role of IgM in the Immune Response

Immunoglobulin M is built as the first antibody during an immune response and is responsible for agglutination and cytolytic reactions since its pentameric structure gives it 10 free antigen

binding sites in theory and it possesses a high avidity. Due to conformational constraints among the 10 Fab portions, IgM only has a valence of 5. Additionally, IgM is not as versatile as IgG, but it is of vital importance in complement activation and agglutination (Stavnezer *et al*., 2008).

IgM is predominantly found in the lymph fluid and the blood and is a very effective neutralizing agent in the early stages of disease. Elevated levels can be a sign of recent infection or exposure to antigen (Boes, 2000).

2.8.8 Complement System

The complement system is a biochemical cascade of the immune system that helps or complements the ability of antibodies to clear pathogens or mark them for destruction by other cells. It is a major player in innate immunity, and because complement is present in tissue fluids and in the blood, it encounters parasites immediately upon introduction into the host organism (Merle *et al*., 2015).

The complement system comprises more than 30 plasma and membrane-associated proteins that interact in a cascade through the sequential activation of many proenzymes that catalyze the activation of other enzymes (Dunkelberger and Song, 2010). The proteins work to trigger the recruitment of inflammatory cells; "tag" pathogens for destruction by other cells by opsonizing or coating the surface of the pathogen and rid the body of neutralized antigen-antibody complexes. Elements of the complement cascade can be found in many non-mammalian species including plants, birds, fish and some species of invertebrates (Janeway *et al*., 2005).

Several complement activation pathways have been described. The Classical (CP), Alternative (AP) and Mannose-binding lectin (MBL) pathways lead to the formation of the C3 convertase, which cleaves C3 into C3b and C3a (Gros *et al*., 2008).

In the classical pathway, the microbial pathogen is coated in antibodies (IgM or IgG) released by B cells. The C1 complement complex binds to these antibodies resulting in its activation via cross proteolysis. This activated C1 complex cleaves C4 and C2 forming a C4bC2a complex that covalently bonds to the surface of the microbe and functions as a C3 convertase, binding and cleaving C3 into C3a and C3b. Binding of a C3b molecule to the C4bC2a complex (C4b2a3b) results in the formation of a C5 convertase, which cleaves C5 into C5a and C5b. C5b associates with C6, C7, C8, and C9, all of which form a complex that results in a pore through the antigen's membrane. This pore disrupts the ionic and osmotic balance provided by the antigen's membrane, and leads to the death of the antigen cell (Liszewski and Atkinson, 2015; Merle *et al*., 2015).

In the alternative pathway, C3, present in the blood stream, spontaneously cleaves at low rates into C3b and C3a. If a microbe is present, the C3b component will covalently bind to the surface of the foreign invader. It then associates with Factor B, which is cleaved by Factor D into Factor Bb. This C3bBb convertase is stabilized by properdin (Factor P) preferentially on microbial surfaces over normal host surfaces, and is now able to cleave many more C3 molecules thus amplifying the signal(Liszewski and Atkinson, 2015). Incorporation of an additional C3b into the C3bBb C3 convertase leads to the formation of C3Bb3b C5 convertase (Merle *et al*., 2015).

The lectin pathway is activated when mannose-binding lectin (MBL) binds to mannose on the pathogen surface. This pathway is similar to the classical pathway but acts in the absence of antibody. MBL, or alternatively ficolin, activates MBL-associated serine proteases 1 and 2 (MASP-1 and MASP-2). These MASPs cleave C4 and C2 similarly to C1r and C1s in the classical pathway, leading to the formation of the C3 convertase C4b2a (Fujita *et al*., 2004).

2.8.9 Complement 3b

C3b is the larger of two elements formed by the cleavage of complement component 3, and is considered an important part of the innate immune system (Janssen *et al*., 2005). It plays a role in forming a C3 convertase when bound to Factor B (C3bBb complex), or a C5 convertase when bound to C4b and C2a (C4b2a3b complex) or when an additional C3b molecule binds to the C3bBb complex (C3bBb3b complex) (Liszewski and Atkinson, 2015).

The cleavage of C3 leaves C3b with an exposed thioester bond, allowing C3b to effectively coat and tag foreign cells by covalently binding to hydroxyl (-OH) and amine (-NH2) groups on foreign cell surfaces (Merle *et al*., 2015).

2.8.10 Functions of Complement 3b

*Opsonization and pathogen clearance***:**

Once cleaved, C3b can either generate C3 or C5 convertase, as mentioned above, or else covalently bind to the microbial surface, tagging the cell for phagocytosis in a process known as opsonization. Additionally, C3b molecules can attach to the Fc regions of antigen-bound antibodies leading to phagocytosis or movement to the liver, where the C3b-tagged immune complex is then destroyed. In both cases C3b interacts with the C3b receptor, complement receptor 1 on phagocytic cells, such as macrophages and neutrophils, allowing for engulfment of the pathogen. Furthermore, erythrocytes with Cr1 on their surface bind and deliver the immune complexes to the mononuclear phagocyte system via interactions with C3b (Tausk and Gigli, 1990).

*Regulation***:**

The key to the success of the complement system in clearing antigens is regulating the effects of C3b to pathogens alone and not healthy, host cells. This is done through several different

mechanisms. One mechanism is the stabilization of the C3bBb convertase by properdin preferentially on microbial surfaces, not on host surfaces, a step necessary for formation of a functional C3 convertase. Furthermore, if C3b does bind to a host-cell surface, regulators of complement activity (RCAs), a group of genetically, structurally, and functionally related proteins, inactivate the complement component. Given that C3 is constantly being turned over in the alternative pathway and its ability to rapidly amplify a signal via this pathway, these proteins are important in regulating the temporal and spatial effects of C3b to infected tissues. An example of RCA is membrane cofactor protein (MCP; CD46), which is ubiquitously expressed and plays a critical role in protecting host cells from damage by the C3b (Liszewski and Atkinson, 2015).

C3b's ability to perform these important functions derives from its ability to covalently bind to the surface of invading pathogens within an organism's body via the various pathogen recognition receptors (Merle *et al*., 2015).

2.9 Diagnosis of Hookworm Infection

Hookworm infection is confirmed with a microscopic examination of the stool to look for ova and parasites in the person who is seen to manifest symptoms of hookworm infection (Gasser *et al*., 2009). Complete blood count to check for anaemia and differential count to look for eosinophilia is done to evaluate the level of infection. High white blood cell count indicates the presence of infection. Because hookworm eggs are often indistinguishable from other parasitic eggs, PCR assays could serve as a molecular approach for accurate diagnosis of hookworm in the feaces (Yong *et al*., 2007; Gasser *et al*., 2009).

Rarely, endoscopy is done that would reveal the presence of adult worms in the intestine. This is done for the purpose of identification of the species that has infected the person (Venkatakrishnan, 2016).

2.9.1 Packed Cell Volume (PCV) as a tool for determining Anaemia in Hookworm Infection

Packed cell volume (PCV) or hematocrit is the volume expressed as percentage (vol %) of red blood cells in blood. PCV test is used to measure the amount of cells in the blood: blood is made up of cells and plasma. The amount of cells in the blood is expressed as a percentage of the total volume of blood, for example; a PCV measurement of 50% means that there are 50 millilitres of cells per 100 millilitres of blood.The PCV measurement may increase or decrease depending on the individual's health: if they are dehydrated, the measurement will rise and the measurement will decrease if the individual has a condition such as anaemia (Purves *et al*., 2004).

The normal PCV is 40-45% for men, 38-42% for women and 30-40% for children, though it varies with age and sex. It is considered an integral part of a person's complete blood count results, along with hemoglobin concentration, white blood cell count, and platelet count. Because the purpose of red blood cells is to transfer oxygen from the lungs to body tissues, a blood sample's hematocrit (the red blood cell volume percentage) can become a point of reference of its capability of delivering oxygen. Additionally, the measure of a subject's blood sample's hematocrit levels may be used as an indication of diseases conditions in the subject (Purves *et al*., 2004).

Anaemia refers to an abnormally low PCV, as opposed to polycythemia, which refers to an abnormally high PCV. For a condition such as anaemia that goes unnoticed, one way it can be

diagnosed is by measuring the PCV of the blood. Both are potentially life-threatening disorders (Purves *et al*., 2004).

2.10 Treatment

The most common drug of choice for the treatment of hookworm is benzimidazoles, specifically albendazole and mebendazole. Benzimidazoles kill adult worms by binding to the nematode's β-tubulin and subsequently inhibiting microtubule/ polymerization within the parasite (Gasser *et al*., 2009). In certain circumstances, levamisole and pyrantel pamoate may be used (Hotez *et al*., 2005). A 2008 review found that the efficacy of single-dose treatments for hookworm disease is as follows: 72% for albendazole, 15% for mebendazole, and 31% for pyrantel pamoate. This substantiates prior claims that albendazole is much more effective than mebendazole for Hookworm infection (Keiser and Utzinger, 2008).

Also of note is that the World Health Organization does recommend anthelmintic treatment in pregnant women after the first trimester (Gasser *et al*., 2009). It is also recommended that if the patient also suffers from anaemia that ferrous sulfate (200 mg) be administered three times daily at the same time as anthelmintic treatment; this should be continued until hemoglobin values return to normal which could take up to 3 months (Markell *et al*., 2006). Hookworm infection can be treated with local cryotherapy when the hookworm is still in the skin (Albanese *et al*., 2001). Albendazole is effective both in the intestinal stage and during the stage the parasite is still migrating under the skin (Albanese *et al*., 2001).

In case of anaemia, iron supplement can cause relief of symptoms of iron deficiency anaemia. However, as red blood cell levels are restored, shortage of other essentials such as folic acid or vitamin B12 may develop, so these might also need to be supplemented (Gasser *et al*., 2009).

Other important issues related to the treatment of hookworm are reinfection and drug resistance. It has been shown that reinfection after treatment can be extremely high. Some studies even showed that 80% of pre-treatment hookworm infection rates can be seen in treated communities within 30–36 months. While reinfection may occur, it is still recommended that regular treatments be conducted as it will minimize the occurrence of chronic outcomes (Gasser *et al*., 2009).

There are also increasing concerns about the issue of drug resistance. Drug resistance has emerged to front-line anthelmintics used for livestock nematodes. Generally human nematodes are less likely to develop resistance due to longer reproducing times, less frequent treatment, and more targeted treatment. Nonetheless, the global community must be careful to maintain the effectiveness of current anthelmintic as no new anthelmintic drugs are in the late-stage development (Gasser *et al*., 2009).

2.11 Prevention of Hookworm Infection

The infective larvae develop and survive in an environment of damp dirt, particularly sandy and loamy soil. The main lines of precaution are those dictated by good hygiene behaviors such as defaecation in the open should be discouraged; untreated human excreta or raw sewage should not be used as fertilizer; walking on barefooted in known faecally contaminated area should be avoided and pet dogs and cats should be dewormed regularly (Hotez *et al*., 2005).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was conducted in Kano State. Kano is located in North-Western Nigeria of Latitude: 12°00′00″ N and Longitude: 8°31′00″ E with a total area of 20,131km^2 within the savannah region. It has a rainy season spanning from May to October and the cold dry season is within the months of November to April. Kano State consists of forty-four (44) Local Government Areas (Duwa and Oyeyi, 2009) (Figure 3.1).

The study was carried out in the following hospitals; Mohammed Abdullahi Wase Specialist Hospital located at Nassarawa local government area, Murtala Mohammed Specialist Hospital located at Kano Municipal, Sir Mohammed Sanusi General Hospital located at Nassarawa local government area, Sheik Mohammed Jidda General Hospital located at Fagge local government area, Infectious Diseases Hospital (IDH) located at Fagge local government area, Waziri Shehu Gidado General Hospital located at Ungogo local government area, Wudil General Hospital located at Wudil local government area and Kura General Hospital located at Kura local government area, Kano, from April to August 2016.

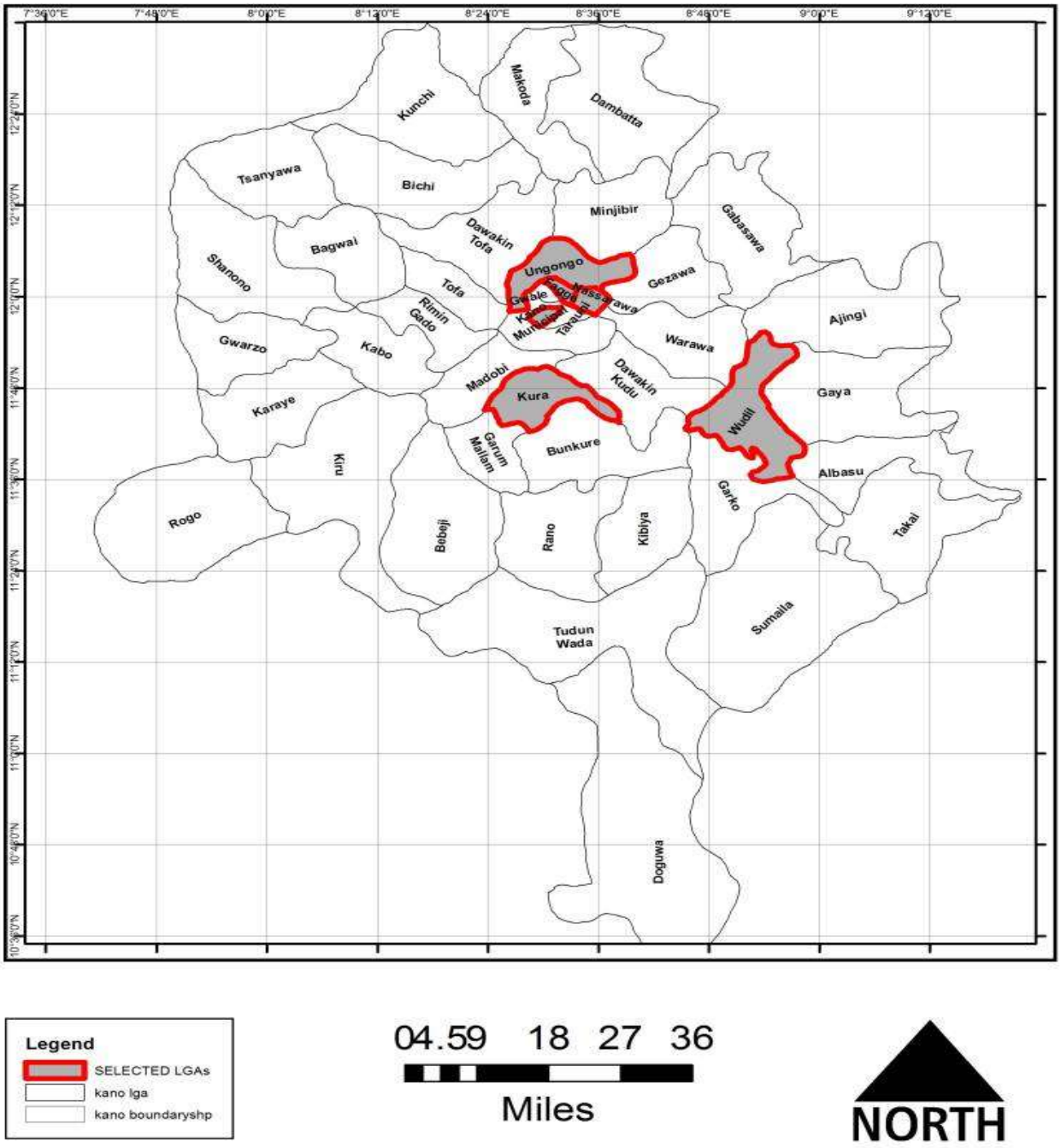


Figure 3.1: Map of Kano State showing the sampling points.

3.2 Study population

The study population comprised of men, women and children of all age groups attending the Outpatient department of the various hospitals, presenting with symptoms of Hookworm infection; these include: diarrhoea, vomiting, dizziness, abdominal pain, abdominal discomfort, headache, fever, shortness of breath and blood in stool.

3.3 Study Design

This study was a cross-sectional and hospital-based survey involving patients presenting symptoms of hookworm infection in some hospitals in Kano state. The administration of questionnaire (Appendix III) to participants was carried out, and equal numbers of blood and stool sample were collected from the patients.

3.4 Ethical Approval / Consent Form

Ethical approval was obtained from the ethical committee of the Kano State Hospital Management Board and also from the ethical committee of the selected hospitals (Appendix I). Consent was obtained from all patients after careful explanation of the research objectives to them (Appendix II).

3.5 Inclusion and Exclusion Criteria

Inclusion criteria for this study were those suspected for hookworm infection presenting symptoms such as diarrhoea, vomiting, dizziness, abdominal pain, abdominal discomfort, headache, fever, shortness of breath and blood in stool and have voluntarily agreed to participate in the study by endorsing the Research Consent form. Those who did not give their consent were excluded from the research.

3.6 Sample Size

The sample size for this study was determined using the formula by Naing *et al*., 2006 at 95% confidence level and a prevalence of 50% which was used for both the prevalence of hookworm and complement since there was not similar work that was currently conducted.

$$N = \frac{Z^2pq}{d^2}$$

Where, N= Sample size

Z= Statistics for a level of 95% confidence interval= 1.96

P= prevalence = 50%

d = level of significance (allowable error) = 5% or 0.05

q= 1-p

$$\text{Thus, } N = \frac{(1.96^2) \times 0.5 \times (1-0.5)}{(0.05)^2}$$

$$= \frac{3.8416 \times 0.5 \times 0.5}{0.0025}$$

$$= 384.16 \approx 400$$

For this study, a total of 400 samples were collected from patients suspected with hookworm infection; 400 blood and 400 stool samples respectively.

3.7 Specimen Collection

The following samples were obtained from each patient included in the study:

3.7.1 Blood Collection

Five milliliters (5mls) of venous blood was drawn from each patient aseptically by venipuncture and dispensed into sterile labeled anticoagulant free container; for the detection of Immunoglobulin M and C3b convertase level. The samples were allowed to stand for one hour to clot and then centrifuged at 1500 rpm for 10 minutes to separate the serum. The sera samples were then transferred into sterile properly labeled screw capped vials and stored at -20^{o}C until required for analysis.

3.7.2 Stool Collection

Subjects were provided with clean wide mouth screw cap containers in which stool specimens was collected and properly covered and labeled. After collection, stool samples were stored in a cool box containing ice packs and then transferred to the laboratory.

3.8 Laboratory Analysis

All the blood samples were assayed for the IgM and C3b levels and the PCV level was also determined, while the stool samples were examined for the parasite ova.

3.8.1 Assay for Total Immunoglobulin M (IgM)

Assay for IgM was carried out according to manufacturer's instruction (Assaypro LLC, USA).

Assay Procedure

All reagents were brought to room temperature (28ºC) before use. The controls were prepared by reconstituting 260 ng of Human IgM standard with 2.6ml of the Mixture (MIX) diluent to generate 100 ng/ml of the standard stock solution. The standard was allowed to stand for 10 minutes; the serial dilution of the standard stock was carried out and 50, 25, 12.5, 6.25, 3.125 and 1.563 ng/ml solutions were produced. The Mixture (MIX) diluent was served as the zero standards (0 ng/ml).

The wash buffer was diluted twenty times (20x) by diluting with distilled water to make a volume of 1.2litre in a measuring cylinder. 1:20 dilutions of the test samples were prepared. A total of 50µl controls and diluted serum sample was transferred into the appropriate well strip coated with polyclonal antibody against IgM, leaving the first well as blank. The microtitre plate holder was gently tapped to remove air bubbles from the dispensed liquid.

The well strips were covered well with sealing tapes and incubated for two (2) hours at room temperature (28ºC). After incubation, the liquid was vigorously shaken out of the wells and the wells were washed four (4) times with the diluted wash buffer. This was done by filling the wells with wash buffer and vigorously shaking out the liquid each time. The wells were blot-dried and inverted on absorbent tissue paper. Thereafter, 50µl of Biotinylated Human IgM antibody solution was added to each well and the wells were covered and incubated at room temperature for one (1) hour, followed by another round of washing and blotting as previously described. Streptavidin-Peroxidase conjugate (SP Conjugate) solution (50µl) was added to each well and incubated for thirty (30) minutes at room temperature, after which the wells were washed as before and blot-dried. This was followed by addition of 50µl Chromogen substrate solution to each well and incubated for twenty five (25) minutes at room temperature. Finally,stop solution

(50µl) was added to each well and gently shaken. Each well's optical density (OD) was read with a microtitre-plate reader, the reader was set at wavelength 450nm.

3.8.2 Assay for Complement Component 3b (C3b).

Assay for C3b was carried out according to manufacturer's instruction (Assaypro LLC, USA).

Assay Procedure

All reagents were brought to room temperature (28ºC) before use. The controls were prepared by reconstituting 20 ng of Human Complement C3b standard with 2ml of the Enzyme Immunoassay (EIA) diluent to generate 10 ng/ml of the standard stock solution. The standard was allowed to sit for 10 minutes; the serial dilution of the standard stock was carried out and 5, 2.5, 1.25, 0.625, 0.313 and 0.156 ng/ml solutions were produced. The Enzyme Immunoassay (EIA) diluent was served as the zero standards (0 ng/ml).

The wash buffer was diluted twenty times (20x) by diluting with distilled water to make a volume of 1.2litre in a measuring cylinder. 1:20 dilutions of the test samples were prepared. A total of 50µl controls and diluted serum sample was transferred into the appropriate well strip coated with polyclonal antibody against C3b, leaving the first well as blank. The microtitre plate holder was gently tapped to remove air bubbles from the dispensed liquid.

The well strips were covered well with sealing tapes and incubated for two (2) hours at room temperature (28ºC). After incubation, the liquid was vigorously shaken out of the wells and the wells were washed four (4) times with the diluted wash buffer. This was done by filling the wells with wash buffer and vigorously shaking out the liquid each time. The wells were blot-dried and inverted on absorbent tissue paper. Thereafter, 50µl of Biotinylated Human Complement C3b

antibody solution was added to each well and the wells were covered and incubated at room temperature for one (1) hour, followed by another round of washing and blotting as previously described. Streptavidin-Peroxidase conjugate (SP Conjugate) solution (50µl) was added to each well and incubated for thirty (30) minutes at room temperature, after which the wells were washed as before and blot-dried. This was followed by addition of 50µl Chromogen substrate solution to each well and incubated for ten (10) minutes at room temperature. Finally, stop solution (50µl) was added to each well and gently shaken. Each well's optical density (OD) was read with a microtitre-plate reader, the reader was set at wavelength 450nm.

3.8.3 Estimation of Packed Cell Volume (PCV)

Hepanirized capillary tube was filled to about 3/4th length with blood. One end of the tube was sealed with a sealant about 2 mm deep. The filled tube was then placed in the microhematocrit centrifuge, with the plugged end away from the center of the centrifuge and centrifuge at 12,000 rpm for 5 minutes. The tube was then placed on the microhematocrit reader and the hematocrit was read by following the manufacturer's instruction on the microhematocrit reading device.

3.9 Stool Analysis

The stool was examined using two methods as follows:

3.9.1 Direct Microscopic Examination

A drop of normal saline was placed on a clean slide. Using an applicator stick, a small amount of stool specimen approximately one gram was emulsified in saline solution. The preparation was then covered with a cover slip and examined under the microscope for the presence of parasite ova using X10 and X40 objectives respectively. The recovered eggs were identified using Atlas by Cheesbrough (Cheesbrough, 2004).

3.9.2 Concentration Method (Formol-Ether)

A total of 7ml of formalin solution was dispensed into a 10ml test-tube, after which about 1g of the stool sample was emulsified in it using an applicator stick. Subsequently, 3ml of ether was added and properly mixed. It was then centrifuge at 1500 rpm for 5 minutes. A smear of the sediment was prepared and covered with a cover slip for microscopic examination. The X10 and X40 objective was used to examine the whole area under the cover slip for parasite ova. The recovered eggs were identified using Atlas by Cheesbrough (Cheesbrough, 2004).

3.10 Statistical Analysis

The data collected were analyzed using the Statistical Package for Social Sciences (SPSS version 21.0) software. Percentage prevalence was calculated, differences between qualitative parameters were evaluated using the chi-square test, Odds ratio (OR) and the corresponding 95% confidence interval (CI) to measure the strength of association. Means were calculated using analysis of variance (ANOVA), p-values ≤ 0.05 were regarded as statistically significant. Data were summarized into tables and figures as appropriate.

CHAPTER FOUR

4.0 RESULTS

Of the total 400 stool samples collected in the study area, 21 representing 5.3% were positive for hookworm infection by microscopy while 379 (94.7%) were negative (Figure 4.1). Eight (38%) of the positive samples were obtained using direct microscopy while thirteen (61.9%) were obtained using concentration method.

The prevalence of hookworm infection among patients in relation to selected hospitals was shown in Figure 4.2. A high prevalence of 26% (13/50) was recorded in Kura general hospital, followed by the prevalence of 16% (8/50) recorded in Wudil general hospital. A prevalence of 0.0% was recorded from other hospitals visited in this study.

With respect to age of the patients as shown in Table 4.1, the participants were categorized into seven different age groups with class size of 5. A total of 30, 73, 62, 50, 50, 53 and 82 participants fell within the age group of 1-5, 6-10, 11-15, 16-20, 21-25, 26-30 and ≥31 years respectively. Those within the age group 6-10 years had the highest prevalence of 17.8% followed by 1-5 years (13.3%) while those within the age group 16-20 years had the least prevalence (2%). There was no identified case of the infection among the other age groups and the distribution was statistically significant (P= 0.000*).

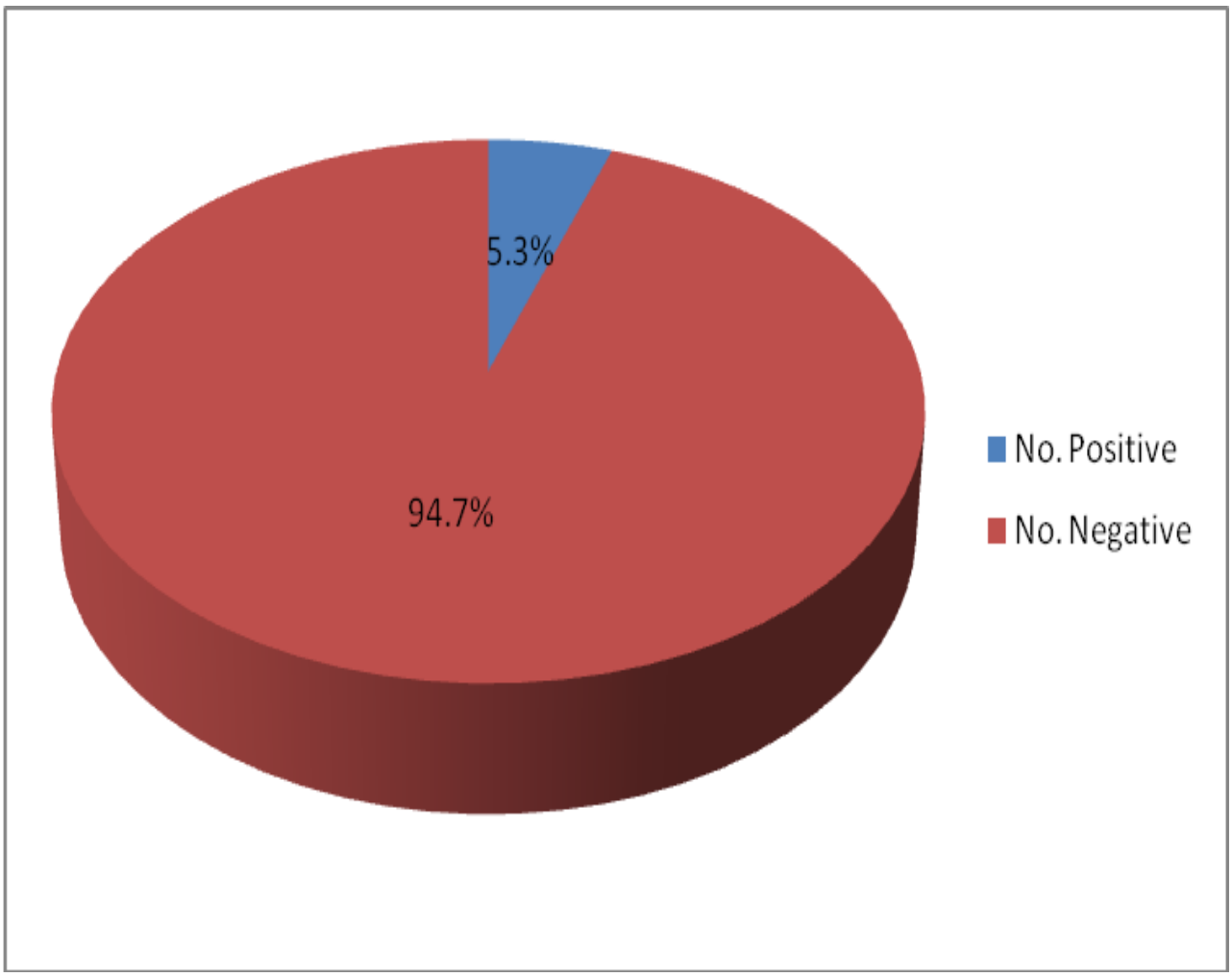


Figure 4.1: The Prevalence of Hookworm infection

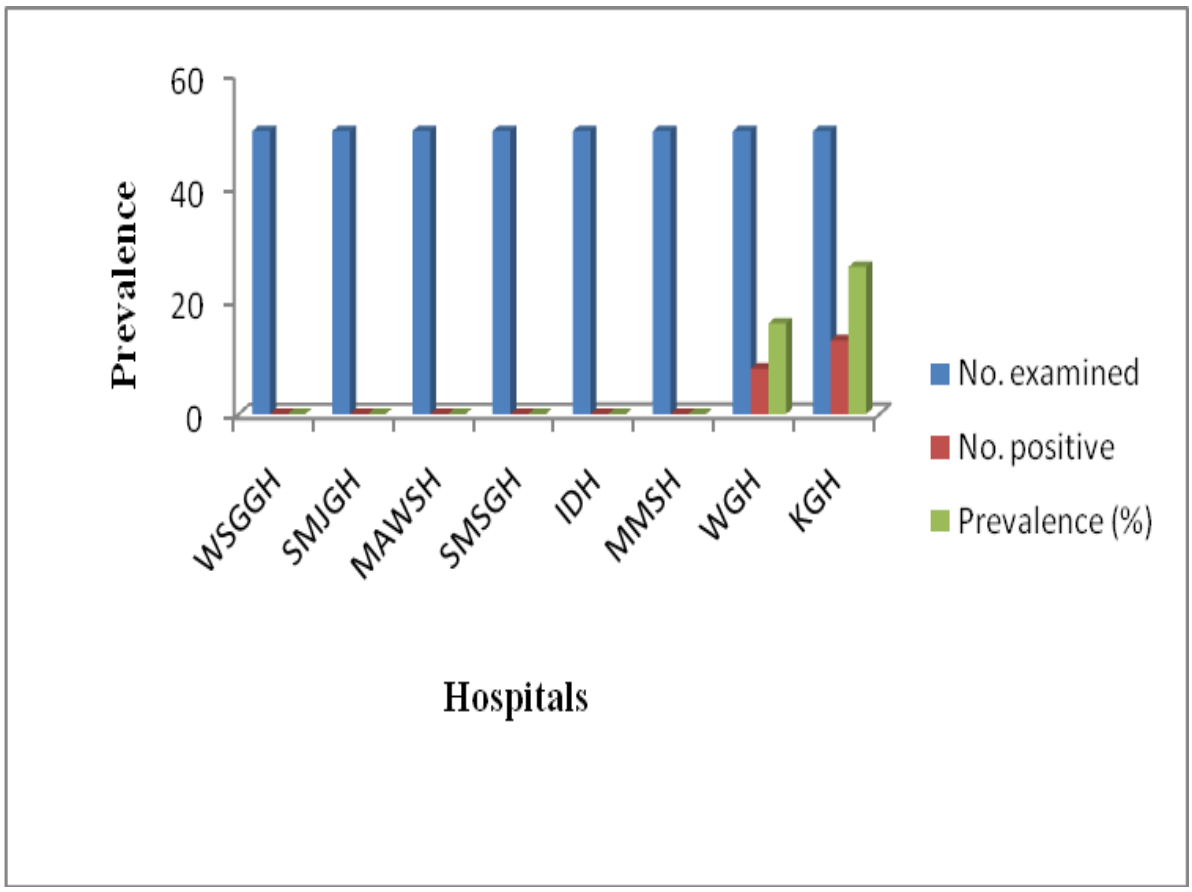


Figure 4.2: Prevalence of Hookworm Infection in some Hospitals in Kano.

Key:

WSGGH- Waziri Shehu Gidado General Hospital

SMJGH- Sheik Mohammed Jidda General Hospital

MAWSH- Mohammed Abdullahi Wase Specialist Hospital

SMSGH- Sir Mohammed Sanusi General Hospital

IDH- Infectious Diseases Hospital

MMSH- Murtala Mohammed Specialist Hospital

WGH- Wudil General Hospital

KGH- Kura General Hospital

Table 4.1: Prevalence of Hookworm Infection in Relation to Age Group.

Age group	No. examined	No. positive (%)	χ^2	P-value
1-5	30	4 (13.3)		
6-10	73	13 (17.8)		
11-15	62	3 (4.8)		
16-20	50	1 (2.0)	36.053	0.000*
21-25	50	0 (0.0)		
26-30	53	0 (0.0)		
31- above	82	0 (0.0)		

χ^2 = chi square, p= level of significance, *= significant association exist (p<0.05)

From the symptoms presented by the participants in Table 4.2; headache (OR=1.210), shortness of breath (OR=1.320) and blood in stool (OR=1.121) are likely associated with hookworm infection while abdominal discomfort, abdominal pain, vomiting, dizziness, diarrhoea and fever are less likely associated with the infection. However, the association between hookworm infection and the symptoms presented was not statistically significant.

Table 4.3 shows the socio demographic characteristics of the study participants in Kano. Among the study population a prevalence of 20.1% (21/104) was established in children while 0.0% prevalence was recorded in men, pregnant women and non pregnant women and it was statistically significant (P-value=0.000*). Using education as a factor in relation to hookworm infection showed that a prevalence of 6.9% (16/230) was obtained among pre/primary school pupils and 2.9% (5/170) was recorded among post primary students. Though, there was no significant association between hookworm and education (Table 4.3). A prevalence of 9.4% (21/222) was obtained among the students, and a prevalence of 0.0% each among civil servants, business men/women, plumber and farmers was obtained when considering occupation in relation to hookworm infection. This was not statistically significant (Table 4.3).

There was no significant association between footwear and hookworm infection as a prevalence of 5.7% (18/316) was obtained among patients who use footwear compared to a prevalence of 3.6% (3/84) among those who do not use footwear (Table 4.3).

The analysis of results obtained in this study in relation to type of toilet, revealed no significant association with the prevalence of Hookworm infection ($p>0.05$). A prevalence of 5.4% (3/56) among patients that use water cistern was observed compared to a prevalence of 5.2% (18/344) among patients who use pit latrine (Table 4.3).

The mean levels of PCV, C3b and IgM in patients with hookworm infection were shown in Table 4.4. The PCV was determined to be 21.67% in positive participants while 29.5% was recorded in participants that tested negative for the infection. 0.73mg/ml of C3b level was observed in both the positive and negative participants. There was increase in IgM level in all the participants but the participants that were infected with hookworm shows slightly higher level (9.8mg/ml).

Table 4.5 showed that there was no association between IgM and C3b (p-value=0.875), the level of IgM does not affect the level of C3b.

The PCV values of the infected participants were shown in Table 4.6. Nineteen (35%) of the positive participants had PCV within the range of 1-20% and two (0.57%) participants were within the range 21-above (%). A significant relationship was observed between PCV and hookworm infection ($p<0.05$).

Table 4.2: Hookworm Infection in Relation to Symptoms Presented.

Factor	No examined	No positive	Odds ratio	χ^2	p-Value
Dizziness					
Yes	115	4(3.5)	0.565(0.157-1.726)	1.019	0.458
No	285	17(6.0)			
Headache					
Yes	88	4(1.0)	1.210(0.397-3.614)	0.113	1.000
No	312	17(4.3)			
Shortness of Breathe					
Yes	30	2(6.7)	1.320(0.292-5.955)	0.131	0.665
No	370	19(5.1)			
Abdominal discomfort					
Yes	87	3(3.4)	0.585(0.168-2.035)	0.726	0.587
No	313	18(5.8)			
Abdominal pain					
Yes	87	3(3.4)	0.585(0.168-2.035)	0.726	0.587
No	313	18(5.8)			
Diarrhea					
Yes	138	5(3.5)	0.546(0.196-1.522)	1.376	0.356
No	252	16(6.6)			
Vomiting					
Yes	171	7(4.1)	0.655(0.259-1.661)	0.803	0.498
No	229	14(6.1)			
Blood					
Yes	100	10(10.00)	1.121(0.785-2.019)	0.911	0.254
No	300	11(3.7)			
Fever					
Yes	191	7(3.7)	0.365(0.259-1.654)	0.789	0.291
No	209	14(6.7)			

χ^2 = Fisher exact, p= level of significance

Table 4.3: Prevalence of Hookworm Infection in Relation to Socio Demographic and Risk Factors.

Factor	No. Examined	No. Positive	χ^2	p-value
Study population				
Men	90	0 (0.0)		
Children	104	21 (20.1)	22.793	0.000*
Pregnant women	92	0 (0.0)		
Non Pregnant	114	0 (0.0)		
Education				
Pre/Primary	230	16 (6.9)		
Post Primary	170	5 (2.9)	1.739	0.691
Occupation				
Civil servant	62	0 (0.0)		
Business	88	0 (0.0)	4.674	0.06
Plumber	10	0 (0.0)		
Students	222	21 (9.5)		
Farmer	18	0 (0.0)		
Foot wear				
Yes	316	18 (5.7)		
No	84	3 (3.6)	0.438	0.322
Toilet type				
Water cistern	56	3 (5.4)		
Pit latrine	344	18 (5.2)	0.969	0.585

χ^2 = chi square, p= level of significance, *= significant association exist (p<0.05)

Table 4.4: Mean levels of PCV, C3b and IgM in Patients with Hookworm Infection.

Hookworm	No. positive (%)	Mean PCV	Mean C3b	Mean IgM
Positive	21 (5.3)	21.67±1.19	0.73±0.00	9.87±1.49
Negative	379 (94.6)	29.53±0.41	0.73±0.00	8.96±0.17
P-value		0.143	0.985	0.049*
Total	400	28.53±0.41	0.73±0.00	9.36±0.18

Normal range: PCV=30-40%; C3b=0.3-1.6mg/ml; IgM=0.4-2.3mg/ml

Table 4.5: Relationship between IgM and C3b levels among Patients with Hookworm Infection.

Parameters	**Mean** (mg/ml)	**R**	**p-value**
IgM	9.87	0.038	0.875
C3b	0.73		

R= Pearson correlation, P-value=0.875 (association does not exist)

Table 4.6: PCV levels among Patients with Hookworm Infection.

PCV (%)	No. examined	No. (%) positive	χ^2	p-value
1-20	54	19 (35.18)	115.056	0.000*
21-above	346	2 (0.57)		

χ^2=chi square, *= statistically significant p-value (where p≤0.05)

CHAPTER FIVE

5.0 Discussion

In this study, the prevalence of hookworm infection, PCV, IgM, C3b and the risk factors associated with hookworm infection in patients attending some hospitals in Kano State, Nigeria were examined. The study showed an overall prevalence of 5.3% (n=21) for hookworm infection among the study population. The low prevalence in this study means that the worm burden is low and heavy infection is rare. This finding is in agreement with Ihesiulor *et al*. (2013) who reported a prevalence of 5.3% in Kano State, North-West Nigeria. However, this finding differs from other studies across the country who reported a high prevalence of hookworm infection such as; 26.7% (Otu-Bassey *et al*., 2017) in Calabar state, South-South, Nigeria and 47.2% (Alade and Ibemologi, 2017) in Kwara state, South-West, Nigeria.

In this study, the urban hospitals (Mohammed Abdullahi Wase Specialist Hospital, Murtala Mohammed Specialist Hospital, Sir Mohammed Sanusi General Hospital, Sheik Mohammed Jidda General Hospital, Infectious Diseases Hospital (IDH) and Waziri Shehu Gidado General Hospital) recorded zero case of the infection while the rural hospitals (Wudil General Hospital and Kura General Hospital) showed prevalence of hookworm. This may be be due to the fact that both Wudil and Kura are farming communities, the moist soil is suitable for the development of the infective filariform larvae (Brooker *et al*., 2004) and also, it might be because study was carried out during the rainy season (April to August) which is the period where farming activities are intensive and individuals are at high risk of being infected. Hookworm infection transmission has been found to be highly seasonal and occur mostly during the rainy season when their eggs hatch and make quick penetration into the host skin (Ogomaka *et al*., 2012). Most of

parasitological survey of common parasitic infections such as hookworm infection in Nigeria has been confined to rural villages where poor sanitation and hygiene as well as a general ignorance of the infection provide optimal environment for transmission (Damen *et al*., 2010).

The patients' socio demographic/risk factors data were assessed in relation to hookworm. In this study, the entire positive cases where children (21/400). This may be due to the fact that children engage in outdoor activities such as helping on the farm, playing at the backyard on moist soil that may be contaminated with filariform larvae there making the children to be more at risk. As a rule, children carry the heaviest worm and egg burdens also because of their defecation practices; they are principal disseminators of infection. This should be worrisome to the public because a range of studies has shown that iron deficiency can undermined the growth, appetite and physical fitness of children and may impair their educational performance (Elom *et al*., 2012). This observation agrees with existing findings that prevalence of hookworm is higher among children than adults (Omitola *et al*., 2016; Awobode *et al*., 2016; Bassey and Loko, 2017).

By their educational status, pre/primary school recorded a prevalence of 6.9% while post primary school recorded a prevalence of 2.9%. The prevalence of the infection decreases as the age of the children increased. Since, helminthes infection is attributed to poor personal hygiene, improvement of the hygienic status of the individuals could aid in the reduce prevalence of the infection among the post primary students. Hence, the level of education affects infection with hookworm. However, these differences were not statistically significant ($p>0.05$).

In relation to occupation, hookworm infection in this study was found to be prevalent among the rural participants whose occupation are mainly farming, therefore, the prevalence of 9.4% among the students (which are all children) could be as a result of the fact that the children go to farm

with their parents where they play around barefoot. The observed difference was not statistically significant ($p>0.05$).

We observed no significant association between hookworm infection and use of footwear as a risk factor in this study. Prevalence of those that wear footwear is high (5.7%) compared to those that do not (3.6%); hence, the infection in this study could be attributed to playing with soil by the children even though they wore foot wears since the parasite can also penetrate the hand.

Contrary to what has been observed in most studies, no association was found to exist between toilet system and hookworm infection ($p=0.858$). However, the use of both water-closet and pit-latrine could constitute transmission routes if proper personal and environmental hygiene were not applied. Toilet facilities are important tools integrated in gastrointestinal health in general populations. When not properly cleaned in a routine and periodic manner, it can serve as a major source of microbial transmission and act as a hidden source of infection (Vernon *et al*., 2005; Livinov *et al.*, 2010).

The age group 6-10 years was the most infected followed by 1-5 years. This finding could be attributed to the fact that hookworm is a common geo-helminth, which is easily transmitted to children, especially due to their habit of geophagy (Chigozie *et al*., 2007). Moreover, the complete absence of infection among the other age groups could be attributed to improved health habit (Ibrahim and Zubairu, 2010). The prevalence in children can be attributed to age immunity. This is in agreement with Oguanya *et al*., (2012); Amaechi *et al*. (2013); Eke *et al*. (2014) whose studies shows the prevalence of hookworm infection in children within this age groups. There was significant association between age and hookworm infection ($p<0.05$).

Some of the symptoms presented by the infected participants are associated with anaemia such as headache and shortness of breath. Diarrhoea causes loss of fluid and electrolytes and the presence of blood in stool are caused by the parasite and lead to decrease in the PCV and occurrence of anaemia (Yahya *et al*., 2014). Though, there was no significant difference between symptoms presented and hookworm infection, this study shows that hookworm infection can be present even in the absence of many symptoms, but symptom suggestive to anaemia such as blood in stool, shortness of breath and headache show the most association.

The IgM levels were elevated in all the participants but slightly higher in the positive subjects where the mean prevalence was revealed to be 9.87mg/ml. Adult hookworms induce the production of IgM and the level of this immunoglobulin returns to normal after treatment. The high elevation of IgM in positive subjects could be attributed to an intense antigenic stimulus by the worm; also the increased IgM level indicates current infection with hookworm (Pritchard *et al*., 1992). This finding agrees with a similar study conducted in Zaria by Musa and Onyemelukwe, (2000).

The mean C3b level of 0.73mg/ml was found to be within the normal range (0.3-1.6) in all the subjects. The role of complement system in parasitic infection is to control the infection by inducing the killing of the parasites, but the complement system fails to be activated in this study. Studies examining the role of complement in parasitic infections have shown that the complement system fails in many ways to exert the intended function of parasite destruction. The reason for this failure is the development of efficient mechanisms by the parasites to evade complement attack (Goto and Sanchez, 2013). Helminths usually evade the effects of complement by inhibiting complement action or increased local consumption of complement factors (Carroll and Robert, 2011).

The mean PCV of 21.67% in the infected subjects' falls below the normal range (30-40) and could be an indication that the participants are likely anaemic (Maguire *et al*., 2007). Hookworm infection is known to result in anaemia due to chronic blood loss. The relationship between parasite infestation and anaemia is a pathogenic-physiologic type. It is recognized that certain factors play an important role, they include the strain and number of the parasite, the size and site, metabolic process of the parasite, particularly the nature of waste products, age and level of immunity at the time of infestation or presence of co-existing condition which reduce immune responses, malnutrition and the life style of the person infested (Muoneke and ChidiIbekwe, 2011). The low PCV value in children in this study might be due to the fact that hookworm is more prevalent in children than adults. The interactions between hookworm and anaemia were demonstrated in children living in Tanzania (Tatala *et al*., 2008) and India (Osei *et al*., 2010).

CHAPTER SIX

6.0 Conclusion and Recommendations

6.1 Conclusion

In conclusion, this study revealed that the prevalence of hookworm infection was low (5.3%) among the study participants in the selected hospitals in Kano which are mainly children (21), and it is characterized by low PCV (21.67%) and significantly elevated IgM (9.87mg/ml). However, complement C3b a major activation component of the system was not elevated (0.73mg/ml) and falls within the normal range (0.3-1.6mg/ml). The prevalence of the infection in children in this study shows that children are still among the most vulnerable and high risk group of acquiring hookworm infection. Hence, there is need for urgent intervention and more research work needs to be carried out on the immunology of hookworm infection in order to have a better understanding of the interaction of the parasite and the host immune system.

6.2 Recommendation

1. Public enlightenment on health education in the rural areas to improve sanitary conditions and to promote key environmental and personal hygiene practices especially among children should be sustained, so as to eradicate the prevalent of hookworm infection.

2. There should be toilet facilities to discourage indiscriminate defecation in public places, drugs and diagnostic facilities in the hospitals for the diagnosis and treatment of infected individuals and to prevent spread of infections.

3. There is need to carry out more research work on the immunological aspect of hookworm infection so as to get a better understanding of the complex interaction of the parasite with the host immune system.

4. Surveillance and intervention on the factors and infections such as hookworm which may lead to anaemia should be given priority in high-risk populations, especially young children.

REFERENCES

Adeyeba, O.A. and Tijani, B.D. (2002). Intestinal Helminthiasis among Malnourished School Age Children in Peri-Urban Area of Ibadan, Nigeria. *African Journal of Clinical Experimental Microbiology*, 3: 24-28.

Akinseye F. J., Egbebi A. O., Fadareo. S. (2017). Challenges of Soil- Transmitted Helminthiasis in Some Communities in Ondo State, Nigeria. *International Journal of Advanced Research in Biological Sciences*, 4(3): 164-171.

Albanese, G., Venturi, C. and Galbiati, G. (2001). Treatment of Larva Migrans Cutanea (creeping eruption): A comparison between Albendazole and Traditional Therapy. *International Journal of Dermatology*, 40 (1): 67–71.

Alade, T.O. and Ibemologi, A. (2017). Relationship between Intestinal Helminthiasis and Haemoglobin Level in Ilorin South Local Government Area of Kwara State. *International Journal of Current Microbiology and Applied Sciences*, 6(3): 1456-1460.

Alemu, A., Atinafu, A., Addis, Z., Shiferaw, Y., Teklu, T., Mathewos, B., Birhan, W., Gebretsadik, S. and Gelaw, B. (2011). Soil transmitted helminthes and *Schistosoma mansoni* infections among school childrens in Zarima town, Northwest Ethiopia. *BMC Infectious Diseases*, 11:189–196.

Alex, L. and Paul, P. (2001). Immune Responses in Hookworm Infections. *Clinical Microbiology*, 14(4): 689–703.

Alli, J.A., Okonko, I.O., Kolade, A.F., Nwanze, J.C., Dada, V.K. and Ogundele, M. (2011). Prevalence of Intestinal Nematode Infection among Pregnant Women Attending Antenatal Clinic at the University College Hospital, Ibadan, Nigeria. *Advances in Applied Science Research*, 2 (4): 1-13.

Amaechi, E.C., Ohaeri, C.C. and Ukpai, O.M. (2013). Prevalence of Helminthiasis among School Children in Some Rural Communities of Abia State, Nigeria. *Animal Research International*, 10(3): 1817 – 1825.

Arora, D.R. and Arora, B.B. (2004). Medical Parasitology. 2nd ed. CBS Publishers and Distributors Limited, Darya Ganj, New Delhi. pp 186-188.

Awobode, H.O., Okunlola, D.O., Oyekunle, A.O., Adekeye, T.A. (2016). Prevalence of Schistosoma and other Parasites among Female Residents of Some Communities in Oyo State, Nigeria. *Journal of Public Health and Epidemiology*, 8(3): 38-44.

Bassey, S.E. and Loko, G.T. (2017). A Survey of Helminth Infections in Amakakala, Ogbia Local Government Area, Bayelsa State, Nigeria. *Biotechnological Reserch*, 3(2):39-42.

Bhatia, V., Das, M.K., Kumar, P. and Arora, N.K. (2010). Infantile Hookworm Disease. *Indian Pediatrics*, 47(2):190-192.

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

Biu, A.A. and Kofur, I. (2010). Prevalence of Human Gastro-Intestinal Parasites in Biu, Borno State, Nigeria. *Animal Research International*, 7(2): 1223-1225.

Blair, P. and Diemert, D. (2015). Update on Prevention and Treatment of Intestinal Helminth Infections. *Current Infectious Diseases Reports*; 17 (3):465.

Boes, M. (2000). Role of Natural and Immune IgM Antibodies in Immune Responses. *Molecular Immunology*, 37: 1141–1149.

Borghesi, L. and Milcarek, C. (2006). From B cell to plasma cell: regulation of V(D)J recombination and antibody secretion. *Immunologic Research*, 36 (13): 27–32.

Bours, J., Reitz, C., Strobel, J. and Breipohl, W. (2005). Detection of Secretory IgM in Tears during Rhino-Conjunctivitis. *Graefe's Archive for Clinical and Experimental Ophthalmology*, 243 (5): 456–463.

Brooker, S., Bethony, J. and Hotez, P.J. (2004). Human Hookworm Infection in the 21st Century. *Advances in Parasitology*, 58:197–288.

Bungiro, R. and Cappello, M. (2004). Hookworm Infection: New Developments and Prospects for Control. *Current Opinion in Infectious Diseases*, 17: 421-426.

Brooker, S and Bundy, D.A.P. (2009). Soil-transmitted Helminths (Geohelminths). Cook GC, Zumla AI, eds. *Manson's Tropical Diseases*. 22nd ed. Philadelphia, PA: WB Saunders pp 1515-1548.

Carroll, V. and Robert, B.S. (2011). Complement in health and disease. *Advanced Drug Delivery Reviews*, 63(12): 965-975.

Centers for Disease Control and Prevention. (2014). Hookworm Infection. Available at https://www.cdc.gov/parasites/hookworm/gen_info/faqs.html.

Centers for Disease Control and Prevention. (2016). Hookworm Infection. Available at https://www.cdc.gov/dpdx/hookworm/index.html.

Cheesbrough, M. (2004). District Laboratory Practice, Part I. Cambridge University Press, Cambridge, United Kingdom. pp 194-197.

Chen, K., Xu, W., Wilson, M., He, B., Miller, N.W., Bengtén, E., Edholm, E.S., Santini, P.A., Rath, P., Chiu, A., Cattalini, M., Litzman, J.B., Bussel, J., Huang, B., Meini, A., Riesbeck, K., Cunningham-Rundles, C., Plebani, A., Cerutti, A. (2009).

Immunoglobulin D enhances immune surveillance by activating antimicrobial, proinflammatory and B cell-stimulating programs in basophils. *Nature Immunology*, 10 (8): 889–898.

Chigozie, J.U., Kelvin, O. E., Patrick, G. N., Welson, C.A. and Emmanuel, A. (2007). Helminthosis among Primary School Children in Zaria; *Journal* of *Third World Medicine,* 4*(*1): 20-25.

Crompton, D.W. (2001). How Much Human Helminthiasis is there in the World? *Journal of Parasitology*, 85(3): 397-403.

Damen, J.G., Lar, P., Mershak, P., Mbaawuga, E.M. and Nyary, B.W. (2010). A Comparative Study on the Prevalence of Intestinal Helminthes in Dewormed and Non-Dewormed Students in a Rural Area of North-Central Nigeria. *LabMedicine,* 41:585-589.

David, R.H. (2014). Hookworm Disease Clinical Presentation. *Medscape Drugs & Diseases***.**

Degarege, A., Animut, A., Medhin, G., Legesse, M. and Erko, B. (2014). The Association between Multiple Intestinal Helminth Infections and Blood Group, Anaemia and Nutritional Status in Human Populations from Dore Bafeno, Southern Ethiopia. *Journal of Helminthology*, 88(2):152–159.

de Silva, N.R., Brooker, S., Hotez, P.J., Montresor, A., Engels, D. and Savioli, L. (2003). Soil-Transmitted Helminth Infections: Updating the Global Picture. *Trends in Parasitology*, 19(12):547-551.

del Valle, A., Jones, B.F. and Cappello, M. (2003). Isolation and Molecular Cloning of a Secreted Hookworm Platelet Inhibitor from Adult *A. caninum*. *Molecular and Biochemical Parasitology*, 129: 167-177.

Don, T.A., Jones, M.K., Smyth, D., O'Donoghue, P., Hotez, P. and Loukas, A. (2004). A Pore Forming Haemolysin from the Hookworm, A. caninum. *International Journal of Parasitology*, 34: 1029-1035.

Dunkelberger, J.R. and Song, W.C. (2010). Complement and its Role in Innate and Adaptive Immune Responses. *Cell Research*, 20(1):34-50.

Duwa, M.R. and Oyeyi, T.I. (2009). The Role of Jakara Dam in the Transmission of Schistosomiasis. *Bayero Journal of Pure and Applied Science*, 2: 58-63.

Eke, S.S., Omalu, I.C. J., Otuu, C.A., Hassan, S.C., Ibrahim, S. and Boyi, A.A. (2014). Hookworm Infection among Humans in Panda, Panda Development Area, Karu Lga of Nasarawa State, Nigeria. *International Journal of Applied Biological Research*, 6(1): 66–73.

Elom, M.O., Alo, M.N. and Anyim, C. (2012). Intestinal Helminthiases in Two Rural Communities in South-Eastern Nigeria. *Advances in Applied* Science *Research*, 3 (3):1413-1417.

Erb, K.J. (2007). Helminths, allergic disorders and IgE-mediated immune responses: where do we stand? *European Journal of Immunology*, 37 (5): 1170–1173.

Fagarasan, S. and Honjo, T. (2003). Intestinal IgA Synthesis: Regulation of Front-line Body Defenses. *Nature Reviews Immunology*, 3 (1): 63–72.

Fenwick, A. (2012). The Global Burden of Neglected Tropical Diseases. *Public Health*, 126 (3): 233–236.

Fujita, T., Matsushita, M. and Endo, Y. (2004). The Lectin-Complement Pathway: Its role in Innate Immunity and Evolution. *Immunological Reviews*, 198:185-202.

Furst, T., Keiser, J. and Utzinger, J. (2012). Global Burden of Human Food-Borne Trematodiasis: A Systematic Review and Meta-Analysis. *The Lancet Infectious Diseases*, 12: 210-221.

Gasser, R.B., Cantacessi, C. and Campbell, B.E. (2009). Improved Molecular Diagnostic Tools for Human Hookworms. *Expert Review of Molecular Diagnostics*, 9 (1): 17–21.

Geisberger, R.., Lamers, M. and Achatz, G. (2006). The riddle of the dual expression of IgM and IgD. *Immunology*, 118 (4): 889–898.

Goto, H. and Sanchez, M.C.A. (2013). Does the Complement System Work for or Against the Host during Parasite Infections? *International Trends in Immunity*, 1(2): 2326-3121.

Gros, P; Milder, F.J. and Janssen, B.J. (2008). Complement Driven by Conformational Changes. *Natural Reviews in Immunology*, 8(1): 48-58.

Halpenny, C.M., Paller, C., Valdes, V.E. and Scott, M.E. (2013). Regional, Household and Individual Factors that Influence Soil Transmitted Helminth Reinfection Dynamics in Preschool Children from Rural Indigenous Panama. *PLoS Neglected Tropical Diseases*, 7: e2070.

Houmsou, R., Amuta, E. and Olusi, T. (2009). Prevalence of Intestinal Parasites among Primary School Children in Makurdi, Benue State-Nigeria. *The Internet Journal of Infectious Diseases*, 8(1):1-5.

Hotez, P.J., Brooker, S., Bethony, J.M., Bottazzi, M.E., Loukas, A. and Xiao, S. (2004). Hookworm Infection. *New England Journal of Medicine*, 351 (8): 799–841.

Hotez, P.J., Bethony, J., Bottazzi, M.E., Brooker, S and Buss, P. (2005). Hookworm: The Great Infection of Mankind. *PLoS Medicine*, 2 (3): 67.

Hotez, P.J. (2009). Mass Drug Administration and Integrated Control for the World's High Prevalence Neglected Tropical Diseases. *Clinical Pharmacology,* 85:659-664.

Hotez, P.J., Mistry, N., Rubinstein, J. and Sachs, J.D. (2011). Integrating Neglected Tropical Diseases into AIDS, Tuberculosis, and Malaria Control. *New England Journal of Medicine,* 364(22): 2086–2089.

Ibrahim, S. and Zubairu, M.L. (2010). Prevalence of Hookworm Infection among Patients Attending Aminu Kano Teaching Hospital Kano, Nigeria. *Bayero Journal of Pure and Applied Sciences,* 3(2): 84 – 86.

Ihesiulor, G.U., Kashibu, E., Akande O.A. and Imoru, M. (2013). Helminths of the Gastrointestinal Tract among Children in Kano, Northern Nigeria, *Asian Journal of Biological and Life Sciences,* 2(2):122-126.

Janssen, J.C.B., Huizinga, E.G., Raaijmakers, H.C.A., Roos, A., Daha, M.R., Ekdahl, K.N; Nilsson, B. and Gros, P. (2005). Structures of Complement Component C3 Provide Insights into the Function and Evolution of Immunity. *Nature,* 437: 505-511.

Janssen, B.J., Christodoulidou, A., McCarthy, A., Lambris, J.D. and Gros, P. (2006). Structure of C3b Reveals Conformational Changes that Underlie Complement Activity. *Nature,* 9:213-216.

Janeway, C.A., Travers, P., Walport, M, Shlomchik, M.J. (2001). *Immunobiology.* (5th ed.). Garland Publishing. pp 29.

Janeway, C.A., Traver, P., Walport, M. and Shlomchik, M.J. (2005). *Immunobiology*. 6th Edition. New York: Garland Science. pp 59.

Jardim-Botelho, A., Raff, S., Rodrigues, R.A., Hoffman, H.J., Diemert, D.J., Corrêa-Oliveira, R., Bethony, J.M. and Gazzinelli, M.F. (2008). Hookworm, *Ascaris lumbricoides*

Infection and Polyparasitism Associated with Poor Cognitive Performance in Brazilian School Children. *Tropical Medicine and International Health*, 13: 994-1004.

Jayasekera, J.P., Moseman, E.A. and Carroll, M.C. (2007). Natural Antibody and Complement Mediate Neutralization of Influenza Virus in the Absence of Prior Immunity. *Journal of Virology*, 81 (7): 3487-3494.

Johansen, F.E., Braathen, R. and Brandtzaeg, P. (2000). Role of J Chain in Secretory Immunoglobulin Formation. *Scandinavian Journal of Immunology*, 52 (3): 240–248.

Keiser, J. and Utzinger, J. (2008). Efficacy of Current Drugs against Soil-Transmitted Helminth Infections: Systematic Review and Meta-Analysis. *Journal of the America Medical Association*, 299 (16): 1937–1948.

Liabsuetrakul, T., Chaikongkeit, P., Korviwattanagarn, S., Petrueng, C., Chaiya, S., Hanvattanakul, C., Kongkitkul, P., Sinthuuthai, C., Kalong, N., Ongsawang, D., Ungsathapornpon, S., Ameeroh, A., Bavonnarongdet, P. and Buadung, A. (2009). Epidemiology and the Effect of Treatment of Soil-Transmitted Helminthiasis in Pregnant Women in Southern Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health*, 40:211–222.

Liszewski, M. K. and Atkinson, J.P. (2015). Complement Regulator CD46: Genetic Variants and Disease Associations. *Human Genomics*, 9(1):7.

Livinov, I. V., Sugatha, P. and Cohen, B. A. (2010). Recognizing and Treating Toilet-Seat Contact Dermatitis in Children. *Pediatrics*, 125:419-422.

Loukas, A. and Prociv, P. (2001). Immune Responses in Hookworm Infections. *Clinical Microbiology Reviews*, 14(4):689-703.

Loukas, A., Constant, S.L. and Bethony, J.M. (2005). Immunology of Hookworm Infection. *FEMS Immunology and Medical Microbiology*, 43:115-124.

Maguire, J.L., deVeber, G. and Parkin, P.C. (2007). Association between Iron-Deficiency Anemia and Stroke in Young Children. *Pediatrics*, 120(5):1053-1057.

Mallery, D.L., McEwan, W.A., Bidgood, S.R., Towers, G.J., Johnson, C.M., James, L.C. (2010). Antibodies mediate intracellular immunity through tripartite motif-containing 21 (TRIM21). Proceedings of the National Academy of Sciences of the United States of America, 107 (46): 19985–19990.

Markell, E.K., John, D.C., Petri,W.H. (2006). Markell and Voge's medical parasitology (9th ed.). St. Louis, Mo: Elsevier Saunders. pp 25-31.

Maverakis, E., Kim, K., Shimoda, M., Gershwin, M., Patel, F., Wilken, R., Raychaudhuri, S., Ruhaak, L.R., Lebrilla, C.B. (2015). Glycans in the immune system and the Altered Glycan Theory of Autoimmunity. *Journal of Autoimmunity*, 57 (6): 1–13.

McAlister, C.C., Gao, Z.H. and McAlister, V.C. (2004). Protective Anti-donor IgM Production after Crossmatch Positive Liver-Kidney Transplantation. *Liver Transplantation*, 10 (2): 315-319.

McSorley, H.J. and Loukas, A. (2010). The Immunology of Human Hookworm Infections. *Parasite Immunology*, 32(8):549-559.

Merle, N.S., Noe, R., Halbwachs-Mecarelli, L., Fremeaux-Bacchi, V. and Roumenina, L.T. (2015). Complement System Part II: Role in Immunity. *Frontiers in Immunology*, 6(2):257.

Moorkoth, A.P., Balakrishnan, S.M., Thayyilekandi, J.P., George, K. (2015). Hookworm infection: An unusual presentation. *Journal of the Academy of Clinical Microbiologists*; 17:121-123.

Muoneke, V.U and ChidiIbekwe, R. (2011) Prevalence and Aetiology of Severe Anaemia in Under-5 Children in Abakaliki South Eastern Nigeria. *Pediatric and Therapeutic*, 1:107-111.

Musa, B.O.P. and Onyemelukwe, G.C. (2000). Secretory IgA and Complement Levels in Patients with Hookworm Infection in Zaria. *African Journal of Medicine and Medical Sciences*, 29:111-114.

Naing, L., Winn, T. and Rusli, B.N. (2006). Practical Issues in Calculating the Sample Size for Prevalence Studies. *Archives of Orofacial Sciences*, 1: 9-14.

Nnachi, I.A., Odikamnoro, O.O., Nnachi, A.U., Ani, O.C., Uhuo, C.A. and Egwuatu, C.C. (2015). Epidemiology of Hookworm Infection and the Influence of Some Epidemiological Factors on their Prevalence in Some Farming Communities in Ebonyi State, Nigeria. *International Journal of Tropical Disease and Health*, 10(4): 1-10.

Odinaka, K.K., Nwolisa, E.C., Mbanefo, F., Iheakaram, A.C. and Okolo, S. (2015). Prevalence and Pattern of Soil-Transmitted Helminthic Infection among Primary School Children in a Rural Community in Imo State, Nigeria. *Journal of Tropical Medicine*, 2015: 349439.

Odo, G.E., Agwu, J.E., Ekeh, F.N., Ezea, C.O., Aguoru, G.C., Anya, C., Omeje, K.O., Ubachuku, P.O. (2016). Prevalence of Intestinal Parasite among School Children in Uzo-Uwani Local Government Area of Enugu State. *International Journal of Research Studies in Microbiology and Biotechnology*, 2(2): 7-14.

Odu, N.N., Elechi, V. and Okonko, O. (2013). Prevalence of Intestinal Helminthes Infection among Primary School Children in Urban and Semi-Urban Areas in Rivers State, Nigeria. *World Rural Observations*, 5(1):52-61.

Ogomaka, I. A., Nwoke, B. E. B., Ukaga, C. N., Nwokeji, C. M., Ajero, C. M. U. and Nwachukwu, M. I. (2012). Prevalence of soil transmitted Helminths among primary school pupils in Owerri West Local Government Area in Imo state, Nigeria. *Nigerian Journal of Parasitology*, 33(1):37-43.

Oguanya, F.C., Okogun, G.R.A., Akhile A.O., Eloka C.C.V., Okoro C.J. and Okpe A.C. (2012). Prevalence f Soil-Transmitted Helminths Infections among Public Primary School Pupils in Ekpoma, Edo State, Nigeria. *International Journal of Community Research*, 1 (1): 30 – 34.

Ohta, Y. and Martin, F. (2006). IgD like IgM is a primordial immunoglobulin class pertuated in most jawed vertebrates. *Proceedings of the National Academy of Sciences*. 103 (28): 10723–10728.

Omitola, O.O., H.O. Mogaji., Alabi, M.O., Adeniran., A.A., Oluwole, A.S. and Ekpo, U.F (2016). Geohelminthes infections and Nutritional Status of Pre-School Aged Children in a Peri-Urban Settlement of Ogun State. *Scientifica*. 2016: 1-9.

Otu-Bassey, I.B., Udofia, I.E., Akpan, P.A. (2017). Intestinal Parasitosis, Haemoglobin and Eosinophil Levels of Patients Attending University of Calabar Teaching Hospital, Calabar, Nigeria. *International Blood Research & Reviews*, 7(2): 1-8.

Osei, A., Houser, R., Bulusu, S., Joshi, T. and Hamer, D.(2010). Nutritional Status of Primary School Children in Garhwali Himalayan Villages of India. *Food and Nutrition Bulletin*, 31:221–233.

Phillips, A.C., Carroll, D., Drayson, M.T. and Der, G. (2015). Salivary Immunoglobulin A Secretion Rate Is Negatively Associated with Cancer Mortality: The West of Scotland Twenty-07 Study. *PLoS ONE*, 10(12): 1-11.

Pritchard, D.I., Walsh, E.A., Quinell, R.J., Raiko, A., Edmonds, P. and Keymer, A.E. (1992). Isotypic Variation in Antibody Responses in a Community in Papua New Guinea to Larval and Adult Antigens during Infection, and Following Reinfection with the Hookworm, *Necator americanus*. *Parasite Immunology*, 14:617-631.

Purves, W.K., Sadava, D., Orians, G.H. and Heller, H.C. (2004). Life: The Science of Biology (7th ed.). Sunderland, Mass: Sinauer Associates. pp 954.

Racine, R., McLaughlin, M. and Jones, D.D. (2011). IgM Production by Bone Marrow Plasmablasts Contributes to Long-Term Protection Against Intracellular Bacterial Infection. *Journal of Immunology*, 186 (2): 1011–1021.

Rupert, J.Q., David, I.P., Andrew, R., Alan, P.B. and Shaw, M.A. (2004). Immune Responses in Human Necatoriasis: Association between Interleukin-5 Responses and Resistance to Reinfection. *Journal of Infectious Diseases*, 190 (3): 430-438.

Sachs, J.D. and Hotez, P.J. (2006). Fighting Tropical Diseases. *Science*, 311:1521-1527.

Stavnezer, J., Guikema, J.E. and Schrader, C.E. (2008). Mechanism and Regulation of Class Switch Recombination. *Annual Review of Immunology*, 26: 261–292.

Smith, J.L. and Brooker, S. (2010). Impact of Hookworm Infection and Deworming on Anaemia in Non-pregnant Populations: A Systematic Review. *Tropical Medicine International Health*, 15: 776-795.

Steketee, R.W. (2003). Pregnancy, Nutrition and Parasitic Diseases. *Journal of Nutrition*, 133(5):1661–1667.

Tatala, S.R., Kihamia, C.M., Kyungu, L.H. and Svanberg, U. (2008). Risk Factors for Anaemia in Schoolchildren in Tanga Region Tanzania. *Tanzanian Journal of Health Research*, 10:189–202.

Tausk, F. and Gigli, I. (1990). The Human C3b Receptor: Function and Role in Human Diseases. *The Journal of Investigative Dermatology*, 94(6): 141–145.

Tyoalumun, K., Abubakar, S., Christopher, N. (2016). Prevalence of Intestinal Parasitic Infections and their Association with Nutritional Status of Rural and Urban Pre-School Children in Benue State, Nigeria. *International Journal of MCH and AID*, 5(2):146-152.

Vandemark, L.M., Jia, T.W. and Zhou, X.N. (2010). Social Science Implications for Control of Helminth Infections in Southeast Asia. *Advances in Parasitology*, 73:137–170.

Venkatakrishnan, H. (2016). Hookworm Infection. *India Network for Health*.

Vernon, S., Lundbald, B. and Hellstrom,, A. L. (2005). Children's Experience of School Toilets Present Risk to their Physical and Psychological Health. *Child Care Health development*, 29:47-53.

Verjovski-Almeida, S., DeMarco, R., Martins, E.A., Guimaraes, P.E., Ojopi, E.P., Paquola, A.C., Piazza, J.P., Nishiyama Jr., M.Y., Kitajima, J.P., Adamson, R.E., Ashton, P.D., Bonaldo, M.F., Coulson, P.S., Dillon, G.P., Farias, L.P., Gregorio, S.P., Ho, P.L., Leite, R.A., Malaquias, L.C., Marques, R.C., Miyasato, P.A., Nascimento, A.L., Ohlweiler, F.P., Reis, E.M., Ribeiro, M.A., Sa, R.G., Stukart, G.C., Soares, M.B., Gargioni, C., Kawano, T., Rodrigues, V., Madeira, A.M., Wilson, R.A., Menck, C.F., Setubal, J.C., Leite, L.C. and Dias-Neto, E. (2003) Transcriptome analysis of the acoelomate human parasite Schistosoma mansoni. *Nature Genetics*, 35: 148–157.

Wiersma, E.J., Collins, C., Fazel, S. and Shulman, M.J. (1998). Structural and Functional Analysis of J Chain-Deficient IgM. *Journal of Immunology*, 160 (12): 5979–5989.

Williamson, A.L., Brindley, P.J., Knox, D.P; Hotez, P.J. and Loukas, A. (2003). Digestive Proteases of Blood Feeding Nematodes and other Parasites. *Trends in Parasitology*, 19: 417-423.

World Health Organization. (2001). Iron Deficiency Anaemia: Assessment, Prevention and Control. Geneva, Switzerland. Pp 4.

World Health Organization (2012). Parasitic Diseases. Geneva, Switzerland. pp 20.

Yahya, T.D., Khalid, M.D. and Hanaa, D.K.A. (2014). Study the Infection with Intestinal Parasites (*Entamoeba histolytica*) and its Effect on the Blood Nature in Children from Thi-Qar Province. *Al-Qadisiya Medical Journal*, 10:18-25.

Yong, T.S., Lee, J.H., Sim, S., Lee, J., Min, D.Y., Chai, J.Y., Eom, K.S., Sohn, W.M., Lee, S.H., Rim, H.J. (2007). Differential Diagnosis of Trichostrongylus and Hookworm Eggs via PCR using ITS-1 Sequence. *The Korean Journal of Parasitology*, 45 (1): 69–74.

Yulan, W., Xiao, S., Xue, J., Utzinger, J. and Elaine, H. (2009). Systems Metabolic Effects of a *N. americanus* Infection in Syrian Hamster. *Journal of Proteome Research*, 8: 5442-5450.

APPENDICES

APPENDIX I: Ethical Approval

KANO STATE
HOSPITALS MANAGEMENT BOARD
BOARD HEADQUARTER
P.M.B 3340, POST OFFICE ROAD, KANO

HMB/GEN/488/VOL I 03/02/2015

Vandu Maryam Donald
Dept. of Microbiology,
Ahmadu Bello University Zaria

PROVISIONAL ETHICAL CLEARANCE

Sequel to conduct a research titled "**Determination of Total IgM and C3b Convertase Levels in Patients with Hookworm Infection Attending Some Hospitals in Kano**". In the light of the above, I am mandated to convey provisional clearance to proceed on your study based on the following conditions.

i. That the consent of all participants must be obtained by filling in consent forms.
ii. That you should liaise with the Management of the facility for appropriate guidance.
iii. That any publication related to the study should be brought to the knowledge of the Ethical Committee for approval.
iv. That a copy of your finding should be submitted for documentation, record and final approval, please.

Best regards.

MAIMUNA KHALID A.
ASST. SEC. II
FOR: EXECUTIVE SECRETARY

CC:
The Chief Medical Directors,
MMSH, MAWSH, SMSSH, Zone I (IDH)
WSGGH, SMJGH, Kura, and Wudil General Hospital,
Kano.
Above is for your information and further necessary action, please.

MAIMUNA KHALID A.
ASST. SEC. II
FOR: EXECUTIVE SECRETARY

APPENDIX II: Informed Consent Form

DEPARTMENT OF MICROBIOLOGY
SCHOOL OF POSTGRADUATE STUDIES
AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA.

INFORMED CONSENT FORM (ICF)

Serial No. …… Hospital No. …… Age……. Phone No……..……

This Informed Consent Form is basically for patients suspected with Hookworm infection, attending some selected hospitals in Kano. We are inviting you to participate in this research work titled **" Assessment of Some Immunological Response to Hookworm Infection in Patients Attending Selected Hospitals in Kano State, Nigeria"**. Hookworm infection is the leading cause of maternal and child morbidity in the tropics and subtropics. The most serious consequence of hookworm infection is anaemia, secondary to loss of iron and protein of gut and infection with Hookworm triggers a series of immune responses in the host. The research will involve the collection of blood and stool samples of participants. The results obtained will be used in sensitizing government and other stakeholders interested in disease prevention and control towards taking appropriate measures before the situation goes out of hand. Individual data will be treated with strict confidentiality. Participation is strictly voluntary and refusal to participate in the research study will not in any way affect your right and benefit in this clinic/hospital.

CERTIFICATE OF CONSENT

I…….of……

do hereby consent to participate in this study. The full procedures of the test/study have been explained to me by the investigator. I understand that blood sample will be taken for the test. I therefore give this consent voluntarily without being subjected to any pressure.

Name of Participant ……..…

Signature/right thumb print of participant ……..

Date …….

Statement by Witness:

I have witnessed the accurate reading of the consent form and detail explanation of the study to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Name of witness……

Signature of witness…….….

Date …….

Statement by the Researcher/Person Taking Consent

I confirm that sufficient information, including about risks and benefits, to make an informed decision have been fully explained to the participant. The participant was given an opportunity to ask questions about the study, and all the questions asked by participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

Name of Researcher …….

Signature ……...

Date.……

APPENDIX III: Research Questionnaire

AHMADU BELLO UNIVERSITY ZARIA

DEPARTMENT OF MICROBIOLOGY

FACULTY OF LIFE SCIENCES

A RESEARCH QUESTIONNAIRE ON ASSESSMENT OF SOME IMMUNOLOGICAL RESPONSE TO HOOKWORM INFECTION IN PATIENTS ATTENDING SELECTED HOSPITALS IN KANO STATE, NIGERIA.

Note: All given information will be treated confidentially.

Date .....

Name of hospital .....

(1). Personal information

a. Name .....

b. Men ☐ Children ☐ Women: Pregnant ☐ None-pregnant ☐

d. Age .....

(2). Socio- economic factors

a. Educational level:

Pre/ Primary School ☐ Post Primary School ☐

b. Occupation: Civil servant ☐

Business ☐

Driver ☐

Farmer ☐

☐

Plumber

Students ☐

(3). Risk factors

a. Wearing of foot wears: YES ☐ NO ☐

b. Do you have a toilet in your house? YES ☐ NO ☐

If yes, what type of toilet? Water cistern ☐

Pit latrine ☐

c. Washing of hands after using of toilet: YES ☐ NO ☐

(4). Symptoms presented by the patients:

Abdominal discomfort ☐

Abdominal pain ☐

Diarrhoea ☐

Vomiting ☐

Blood in stool ☐

Fever ☐

Dizziness ☐

☐

Headache

Shortness of breath ☐

APPENDIX IV: IgM OD Result

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.076	0.548	0.442	0.373	0.703	0.425	1.126	0.896	0.833	1.45	1.296	1.067
B	0.943	1.306	1.205	0.965	1.122	0.511	0.975	1.045	1.349	1.142	1.138	1.03
C	1.109	1.086	0.816	0.971	0.706	0.855	0.879	0.877	0.915	0.849	0.883	0.708
D	1.051	0.91	0.988	0.953	1.091	1.222	0.881	0.936	0.96	0.853	0.936	0.823
E	0.709	0.969	0.943	0.988	0.967	0.509	0.858	0.487	1.031	0.889	0.915	1.091
F	0.82	1.031	1.172	1.018	0.876	0.915	0.618	0.901	0.909	0.882	1.061	1.213
G	0.908	0.76	0.909	0.86	0.717	1.018	0.892	0.679	0.897	0.832	0.894	0.736
H	0.778	0.669	0.872	0.869	0.968	0.959	0.953	0.854	0.649	0.854	0.906	0.842

APPENDIX V: Complement 3b OD Result

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.069	1.586	1.745	1.696	1.732	2.085	2.257	2.028	1.395	2.213	2.12	2.184
B	2.283	2.319	2.375	2.05	2.223	1.986	1.975	2.25	2.046	2.177	2.141	2.245
C	2.273	2.433	2.375	2.288	2.334	2.099	2.288	2.317	2.151	2.144	2.209	2.227
D	2.008	2.386	2.391	2.122	2.221	2.347	2.169	2.232	2.152	2.188	2.178	1.863
E	2.251	2.168	2.237	2.221	2.129	2.168	2.119	1.75	2.091	2.254	2	1.798
F	2.263	2.365	2.35	2.307	2.032	2.161	2.058	2.138	2.083	2.199	2.068	1.945
G	2.277	2.147	2.306	2.159	2.108	2.143	2.156	2.205	2.014	2.079	2.061	2.109
H	2.228	2.001	2.234	2.154	2.252	2.189	2.024	0.167	2.128	2.057	2.141	2.172

APPENDIX VI: PCV Data of the Participants

	Hospital 1	Hospital 2	Hospital 3	Hospital 4	Hospital 5	Hospital 6	Hospital 7	Hospital 8
S/No	**PCV Levels**							
1	36	38	38	23	40	21	19	30
2	38	23	40	21	36	34	34	31
3	40	21	36	34	26	26	33	27
4	36	34	26	26	20	24	18	29
5	26	26	20	24	26	40	23	20
6	20	24	26	40	19	43	26	31
7	26	40	19	43	30	41	17	40
8	19	43	30	41	31	22	18	43
9	30	41	31	22	27	23	34	41
10	31	22	27	23	29	24	33	22
11	27	23	29	24	20	23	31	23
12	29	24	20	23	31	21	32	24
13	20	23	31	21	45	22	23	23
14	31	21	45	22	44	22	24	21
15	45	22	44	22	24	36	25	22
16	44	22	24	36	38	38	26	22
17	24	36	38	38	23	40	27	36
18	38	38	23	40	21	36	16	38
19	23	40	21	36	34	26	17	40
20	21	36	34	26	26	20	23	36
21	34	26	26	20	24	26	44	26
22	26	20	24	26	40	19	17	20
23	24	26	40	19	43	30	21	26
24	40	19	43	30	41	31	17	16
25	43	30	41	31	22	27	23	19
26	41	31	22	27	23	29	28	31
27	22	27	23	29	24	20	10	13
28	23	29	24	20	23	31	33	29
29	24	20	23	31	21	45	12	20
30	23	31	21	45	22	44	26	31
31	21	45	22	44	22	24	25	18
32	22	44	22	24	36	38	12	28

33	22	24	36	38	38	23	40	29
34	36	38	38	23	40	21	43	18
35	38	23	40	21	36	34	41	23
36	40	21	36	34	26	26	22	15
37	36	34	26	26	20	24	23	23
38	26	26	20	24	26	40	24	21
39	20	24	26	40	19	43	23	22
40	26	40	19	43	30	41	21	17
41	19	43	30	41	31	22	22	23
42	30	41	31	22	27	23	22	14
43	31	22	27	23	29	24	36	20
44	27	23	29	24	20	23	38	25
45	29	24	20	23	31	21	40	26
46	20	23	31	21	45	22	36	15
47	31	21	45	22	44	22	26	26
48	45	22	44	22	24	23	20	19
49	44	22	24	36	38	25	26	30
50	24	36	38	38	23	32	19	12

Key:

Hospital 1- Waziri Shehu Gidado General Hospital

Hospital 2- Sheik Mohammed Jidda General Hospital

Hospital 3- Mohammed Abdullahi Wase Specialist Hospital

Hospital 4- Sir Mohammed Sanusi General Hospital

Hospital 5- Infectious Diseases Hospital

Hospital 6- Murtala Mohammed Specialist Hospital

Hospital 7- Wudil General Hospital

Hospital 8- Kura General Hospital