A SURVEY OF DENGUE VIRUS AND MALARIA PARASITE CO-INFECTIONS IN FEBRILE PATIENTS IN KADUNA METROPOLIS, NIGERIA

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BY

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Declaration

I declare that the work in this thesis titled "A SURVEY OF DENGUE VIRUS AND MALARIA PARASITE CO-INFECTIONS IN FEBRILE PATIENTS IN KADUNA METROPOLIS, NIGERIA" was performed by me in the Department of Microbiology under the supervision of Prof. S.A. Ado and Prof. V.J. Umoh.

The information derived from literature has been duly acknowledged in the text and a list of references provided. No part of this thesiswas previously presented for another degree or diploma at this or any other institution.

Matthew Owoicho IDOKO _____ _____

Signature Date

Certification

This thesis entitled "A SURVEY OF DENGUE VIRUS AND MALARIA PARASITE CO-INFECTIONS IN FEBRILE PATIENTS IN KADUNA METROPOLIS, NIGERIA" by Matthew Owoicho IDOKO meets the regulation governing the award of the Master of Science degree in Microbiology of the Ahmadu Bello University and is approved for contribution to knowledge and literary presentation.

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Abstract

Dengue fever and malaria are febrile illnesses and common arthropod-borne diseases in humans.They represent major public health problems. The aim of this work was to survey the occurrence of dengue virus and malaria co-infection among febrile patients in Kaduna Metropolis, Nigeria. Blood specimens were collected from 340 subjects with febrile complaints attending four General hospitals in Kaduna metropolis selected for the research and who gave their consent to participate in the research. Serological determination of dengue virus IgM was carried out using Enzyme Linked Immunosorbent Assay (ELISA) method. Malaria status was determined using microscopy and malaria rapid diagnostic test kit. Structured questionnaires were also administered to obtain data on risk factors, demographic data and symptoms of illness. A total of 6 subjects were positive for dengue virus IgM, giving a prevalence of 1.8%. For malaria, microscopy gave a prevalence of 46.5% and rapid diagnostic test gave a prevalence of 35.6%. There was no statistically significant relationship between the occurrence of dengue virus IgM and the presence of risk factors in the environment. A total of 2 (1.3%) of the malaria positive cases were co-infected with dengue virus. The occurrence of dengue virus in the study population was confirmed. Therefore, as the clinical symptoms associated with dengue virus Infection are indistinguishable from many other febrile illnesses such as malaria, specific diagnostic tests assume critical importance in the identification of dengue virus infection.Long lasting physical transformation of vector habitats should be enhanced. Protective clothing and repellents which are common means of protection against mosquitoes and other biting insects should also be enhanced.

Dedication

In loving memory of my mother-Late Mrs. Victoria Idoko, who started it all with me but is now free from the corruption of this flesh. I will always love you mama.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Dengue fever and malaria are febrile illnesses and common arthropod-borne diseases in humans.They represent major public health problems (Epelboin *etal*., 2012). The early symptoms of dengue fever (High grade fever, headache, fatigue, malaise, nausea, vomiting) mimic that of malaria which is hyper endemic in the environment, thereby making the diagnosis of this viral infection very confusing. In such situations, these infections are quite often misdiagnosed and so, inappropriately treated. Consequently these cases often result in high rate of morbidity, complications and mortality (Baba *et al*., 2009).

The most common viral disease transmitted by arthropods (Rodenhuis-Zybert *et al*., 2010), dengue has a disease burden estimated to be 1600 disability-adjusted life years per million population, which is similar to other childhood and tropical diseases such as tuberculosis (Guzman *et al*., 2010). As a tropical disease dengue is deemed only second in importance to malaria (Ranjit and Kissoon, 2010), though the World Health Organization counts dengue as one of sixteen neglected tropical diseases (WHO, 2009).

There are four antigenically related, but distinct, dengue virus serotypes (DEN-1, DEN-2, DEN-3 and DEN-4), all of which can cause dengue fever (DF), dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS) (Gubler, 1997). All

analyses undertaken to date show that the four serotypes of dengue virus are phylogenetically distinct and often to the same degree as different "species" of flaviviruses (Holmes and Twiddy, 2003).

The first dengue viruses were isolated from soldiers who became ill in Calcutta, India, New Guinea, and Hawaii. Dengue is the most common arthropod transmitted viral infection in the world (Guzman and Kouri, 2002; Mairuhu *et al*., 2004). The geographical distribution of dengue is steadily expanding, and in many areas the epidemiology is changing stratum from epidemic to endemic (Guzman and Kouri, 2002; Gibbons and Vaughn, 2002; Gubler, 2002). Estimations of the incidence and thus the disease burden attributable to dengue are variable. The main reason is the variability of the clinical presentation of dengue virus infections (Gubler, 1998; Guzman and Kouri, 2004). The complications are mainly associated with secondary dengue virus infections. Immunity against dengue virus is determined by production of neutralizing antibodies. The immune response is monotypic; it does not protect against an infection by another serotype. The immune response to secondary infections, which does not neutralize the virus, may even increase the risk of complications (Halstead, 2003). Dengue surveillance is usually based on notification of complicated cases (Gibbons and Vaughn, 2002; Gubler, 2002). This does not reflect the true incidence of the disease. The majority of uncomplicated cases do not get recognition as dengue cases. This leads to substantial under-reporting of dengue in the health information systems of most developing countries, as reporting is usually based on diagnosed cases.

Malaria is a mosquito-borne infectious disease of humans and other animals caused by protozoans of the genus *Plasmodium*. The World Health Organization has estimated

that in 2010, there were 216 million documented cases of malaria. Around 655,000 people died from the disease, many of whom were children under the age of five. The actual number of deaths may be significantly higher, as precise statistics are unavailable in many rural areas, and many cases are undocumented. *Plasmodium falciparum* — responsible for the most severe form of malaria — causes the vast majority of deaths associated with the disease. Malaria is commonly associated with poverty and is a major hindrance to economic development (World Malaria report, 2011).

1.2 Statement of Research Problem

Dengue has emerged in recent decades as a worldwide public problem, particularly in Asia-Pacific and Americas-Caribbean regions (Gubler and Clark, 1995; Gubler, 2004; Halstead, 2008a). In Africa, the epidemiology and public health effects of dengue is not clear (Amarasinghe *et al*., 2011). *Aedes* sp. mosquitoes are widely distributed in Africa and can serve as vectors of dengue virus (Amarasinghe *et al*., 2011). When their distribution is combined with rapid population growth, unplanned urbanization and increase in international travel, extensive transmission of dengue virus is likely in Africa (Gubler, 2004; United Nations, 2009). Over the past five decades, cases of epidemic or sporadic dengue have been reported in sub-Saharan Africa (WHO, 2009). Although sporadic cases of dengue haemorrhagic fever have been reported in a few countries in Africa, no outbreaks have been reported (Gubler and Clark, 1995). However, when compared with the Asia-Pacific and Americas-Caribbean regions, the epidemiology of dengue in Africa has not been defined.

In regions to which malaria is endemic, >70% of febrile illnesses are treated as presumptive malaria, often without proper medical examination and a laboratory diagnosis (Amexo *et al*., 2004; Ndyomugyenyi *et al*., 2007). In a setting where diagnostic testing is conducted, such as the GeoSentinel Surveillance Network, malaria was found to be the predominant cause of systemic febrile illness among travellers returning from sub-Saharan Africa (622/1000 patients) compared with dengue (7/1000) (Freedman *et al*., 2006). This finding is not unexpected because malaria is more endemic in Africa than other febrile illnesses. However, over diagnosis of malaria in areas of low transmission is well documented and overestimation by clinical diagnosis is ≈61% (Amexo *et al*., 2004; Ndyomugyenyi *et al*., 2007). Many patients in Africa with fever are designated as having fever of unknown origin or malaria and remain without diagnosis even if they fail to respond to antimalarial drugs. Under these prevailing practices, there is a real danger of misdiagnosing dengue as malaria.

A study in Nigeria determined the prevalence of flavivirus infection among 1,816 children and adults from urban and rural areas in samples obtained mainly during the early 1970s. Virus-specific haemagglutination inhibition testing showed that the prevalence of immunity was 38% for dengue virus – 1 infection, 45% for dengue virus – 2 infection, 43% for yellow fever virus infection and 49% for West Nile virus infection (Fagbami *et al*., 1977). Serum specimens were also tested by suckling mouse neutralization of dengue virus – 2. The authors concluded that because a high proportion of specimens with antibody to dengue virus – 2 were confirmed by neutralization and because many had only monotypic antibody, the prevalence results were not likely confounded by cross-reactive antibodies to other flaviviruses. Another study by Baba *et al*. (2009) revealed the

prevalence of DEN IgG antibodies in Rain forest and wooded savannah areas of Nigeria to be 69.0%.In addition, the study by Fagbemi *et al.* (1977) showed an increase in prevalence of antibodies against dengue virus with age. These suggest endemic infection.

1.3 Justification

Reported incidence of dengue has increased worldwide in recent decades, but little is known about its incidence in Africa. During 1960 – 2010, a total of 22 countries in Africa reported sporadic cases or outbreaks of dengue; 12 other countries in Africa reported dengue only in travellers. The presence of disease and high prevalence of antibody to dengue virus in limited serologic surveys suggests endemic dengue virus infection in all or many parts of Africa. Dengue is likely under-recognized and underreported in Africa because of low awareness by health care providers, other prevalent febrile illnesses and lack of diagnostic testing and systematic surveillance. Other hypothesis to explain low reported numbers of cases include cross-protection from other endemic flavivirus infections, genetic host factors protecting against infection or disease and low vector competence and transmission efficiency. Therefore, population based studies of febrile illnesses are needed to determine the epidemiology and true incidence of dengue in Africa (Amarasinghe *et al*., 2011).

1.4 Aim and Objectives

The aim of this research is to survey the occurrence of Dengue viruses (DENs) and malaria co-infection among febrile patients Kaduna metropolis, Nigeria and to identify the risk factors associated with the diseases.

The objectives of the research are:

1. To determine the occurrence of dengue virus infection in febrile patients using the ELISA technique.
2. To detect malaria parasites in the febrile patients by microscopy.
3. To determine co-infection between malaria parasite and dengue virus in the study area.
4. To identify some of the risk factors (use of mosquito bed nets, insecticides, presence of ditches or bush around residential area, sleeping in the afternoon and using insecticide treated bed nets/insecticides when sleeping in the afternoon, gender, highest educational level attained, marital status and occupation) which may be associated with the diseases.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 History of Dengue Virus

The first record of a case of probable dengue fever is in a Chinese medical encyclopaedia from the Jin Dynasty (265–420 AD) which referred to it as "water poison" associated with flying insects (Gubler, 1998). There have been descriptions of epidemics in the 17th century, but the most plausible early reports of dengue epidemics are from 1779 and 1780, when an epidemic swept Asia, Africa and North America. From that time until 1940, epidemics were infrequent (Gubler, 1998).

In 1906, transmission by the *Aedes* mosquitoes was confirmed, and in 1907 dengue was the second disease (after yellow fever) that was shown to be caused by a virus (Henchal and Putnak, 1990). Further investigations by John Burton Cleland and Joseph Franklin Siler completed the basic understanding of dengue transmission (Henchal and Putnak, 1990).

The marked spread of dengue during and after the Second World War has been attributed to ecologic disruption. The same trends also led to the spread of different serotypes of the virus to new areas, and to the emergence of dengue haemorrhagic fever. This severe form of the disease was first reported in the Philippines in 1953; by the 1970s, it had become a major cause of child mortality and had emerged in the Pacific and the Americas (Gubler, 1998). Dengue haemorrhagic fever and dengue shock syndrome were

first noted in Central and South America in 1981, as DENV-2 was contracted by people who had previously been infected with DENV-1 several years earlier (Gould and Solomon, 2008).

2.2 Etymology of Dengue Virus

The origins of the word "dengue" are not clear, but one theory is that it is derived from the Swahili phrase *Ka-dinga pepo*, which describes the disease as being caused by an evil spirit. The Swahili word *dinga* may possibly have its origin in the Spanish word *dengue*, meaning fastidious or careful, which would describe the gait of a person suffering the bone pain of dengue fever (Harper, 2001). However, it is possible that the use of the Spanish word was derived from the similar-sounding Swahili. Slaves in the West Indies having contracted dengue were said to have the posture and gait of a dandy, and the disease was known as "dandy fever" (Halstead, 2008b).

The term "break-bone fever" was first applied by physician and Founding Father Benjamin Rush, in a 1789 report of the 1780 epidemic in Philadelphia. In the report he uses primarily the more formal term "bilious remitting fever" (Barrett and Stanberry, 2009). The term dengue fever came into general use only after 1828 (Halstead, 2008b). Other historical terms include "breakheart fever" and "la dengue" (Halstead, 2008b). Terms for severe disease include "infectious thrombocytopenic purpura" and "Philippine", "Thai", or "Singapore haemorrhagic fever" (Halstead, 2008b).

2.3 Epidemiology of Dengue Virus

Most people with dengue recover without any ongoing problems (WHO, 2009). The mortality is 1–5% without treatment (Ranjit and Kissoon, 2010), and less than 1% with adequate treatment (WHO, 2009); however severe disease carries a mortality of 26% (Ranjit and Kissoon, 2010). Dengue is endemic in more than 110 countries (Ranjit and Kissoon, 2010). It infects 50 to 100 million people worldwide a year, leading to half a million hospitalizations (Whitehorn and Farrar, 2010) and approximately 12,500–25,000 deaths (WHO, 2009; Varatharaj, 2010).

The incidence of dengue increased 30 fold between 1960 and 2010 (WHO, 2009). This increase is believed to be due to a combination of urbanization, population growth, increased international travel, and global warming (Whitehorn and Farrar, 2010). The geographical distribution is around the equator with 70% of the total 2.5 billion people living in endemic areas from Asia and the Pacific (WHO, 2009). In the United States, the rate of dengue infection among those who return from an endemic area with a fever is 2.9–8.0% (CDC, 2010), and it is the second most common infection after malaria to be diagnosed in this group (Chen and Wilson, 2010).

Until 2003, dengue was classified as a potential bioterrorism agent, but subsequent reports removed this classification as it was deemed too difficult to transfer and only caused haemorrhagic fever in a relatively small proportion of people (WHO, 2009).

Like most arboviruses, dengue virus is maintained in nature in cycles that involve blood-sucking vectors and vertebrate hosts. The viruses are maintained in the forests of

Southeast Asia and Africa by transmission from female *Aedes* mosquitoes of species other than *A. aegypti* to her offspring and to lower primates. In rural settings the virus is transmitted to humans by *A. aegypti* and other species of *Aedes* such as *A. albopictus*. In towns and cities, the virus is primarily transmitted to humans by *A. aegypti*, which is highly domesticated. In all settings the infected lower primates or humans greatly increase the number of circulating dengue viruses. This is called amplification (Gubler, 2010). The urban cycle is the most important to infections of humans and dengue infections are primarily confined to towns and cities (Gubler, 2010). In recent decades, the expansion of villages, towns and cities in endemic areas, and the increased mobility of humans have increased the number of epidemics and circulating viruses. Dengue fever, which was once confined to Southeast Asia, has now spread to Southern China, countries in the Pacific Ocean and America (Gubler, 2010), and might pose a threat to Europe (Reiter, 2010).

2.4 Dengue Virus Infections

Dengue is an acute febrile disease caused by any one of four related flaviviruses known as dengue types 1, 2, 3, and 4. Dengue fever is the most commonly diagnosed form of dengue virus infection and is characterized by the sudden onset of fever lasting between two to seven days, accompanied by severe headache, gastrointestinal symptoms, muscle, joint and bone pain, and a rash (WHO, 1997; Nishiura and Halstead, 2007). The classic form is self-limited and usually results in complete recovery.

The most severe form of dengue infection is dengue haemorrhagic fever. Although it represents a small proportion of all dengue illnesses, it is the only form of severe dengue that has been defined by standardized criteria (WHO, 1997). Dengue haemorrhagic fever is

characterized in its early stages by the signs and symptoms of dengue fever described above, followed by haemorrhaging and/or increased vascular permeability in its latter stages. This increased vascular permeability may lead to vascular collapse (also known as dengue shock syndrome) and death (WHO, 2008).

2.5 Clinical Course of Dengue Virus Infections

The clinical outcomes of Dengue virus infection could vary from asymptomatic infection to mild febrile dengue fever (DF) to severe and life threatening dengue haemorrhagic fever (DHF)/dengue shock syndrome (DSS) (Gunther *et al*., 2007).

Typically, people infected with dengue virus are asymptomatic (80%) or only have mild symptoms such as an uncomplicated fever (WHO, 2009; Whitehorn and Farrar, 2010). Others have more severe illness (5%), and in a small proportion it is life-threatening (Whitehorn and Farrar, 2010; Reiter, 2010). The incubation period (time between exposure and onset of symptoms) ranges from 3–14 days, but most often it is 4–7 days (Gubler, 2010). Therefore, travellers returning from endemic areas are unlikely to have dengue if fever or other symptoms start more than 14 days after arriving home (Ranjit and Kissoon, 2010). Children often experience symptoms similar to those of the common cold and gastroenteritis (vomiting and diarrhoea) (Varatharaj, 2010) but are more susceptible to the severe complications (Ranjit and Kissoon, 2010).

The characteristic symptoms of dengue are sudden-onset fever, headache (typically located behind the eyes), muscle and joint pains, and a rash. The alternative name for dengue, "break-bone fever", comes from the associated muscle and joint pains (Whitehorn

and Farrar, 2010; Chen and Wilson, 2010). The course of infection is divided into three phases: febrile, critical, and recovery (WHO, 2009).

The febrile phase involves high fever, often over 40°C (104 °F), and is associated with generalized pain and a headache; this usually lasts two to seven days (WHO, 2009; Chen and Wilson, 2010). At this stage, a rash occurs in approximately 50–80% of those with symptoms (Wolff and Johnson, 2009; Chen and Wilson, 2010). It occurs in the first or second day of symptoms as flushed skin, or later in the course of illness (days 4–7), as a measles-like rash (Wolff and Johnson, 2009; Knoop *et al.,* 2010).Some petechiae (small red spots that do not disappear when the skin is pressed, which are caused by broken capillaries) can appear at this point (WHO, 2009), as may some mild bleeding from the mucous membranes of the mouth and nose (Chen and Wilson, 2010; Ranjit and Kissoon, 2010). The fever itself is classically biphasic in nature, breaking and then returning for one or two days, although there is wide variation in how often this pattern actually happens (Gould and Solomon, 2008; Knoop *et al.*, 2010).

In some people, the disease proceeds to a critical phase, which follows the resolution of the high fever and typically lasts one to two days (WHO, 2009). During this phase there may be significant fluid accumulation in the chest and abdominal cavity due to increased capillary permeability and leakage. This leads to depletion of fluid from the circulation and decreased blood supply to vital organs (WHO, 2009). During this phase, organ dysfunction and severe bleeding, typically from the gastrointestinal tract, may occur (WHO, 2009; Ranjit and Kissoon, 2010). Shock (dengue shock syndrome) and haemorrhage (dengue haemorrhagic fever) occur in less than 5% of all cases of dengue

(Ranjit and Kissoon, 2010), however those who have previously been infected with other serotypes of dengue virus ("secondary infection") are at an increased risk (Ranjit and Kissoon, 2010; Rodenhuis-Zybert *et al*., 2010).

The recovery phase occurs next, with resorption of the leaked fluid into the bloodstream (WHO, 2009). This usually lasts two to three days (Ranjit and Kissoon, 2010). The improvement is often striking, but there may be severe itching and a slow heart rate (WHO, 2009; Ranjit and Kissoon, 2010). During this stage, a fluid overload state may occur; if it affects the brain, it may cause a reduced level of consciousness or seizures (Ranjit and Kissoon, 2010).

2.6 Virology of Dengue Virus

Dengue fever virus (DENV) is an RNA virus of the Family *Flaviviridae*; Genus *Flavivirus*. Other members of the same family include yellow fever virus, West Nile virus, St. Louis encephalitis virus, Japanese encephalitis virus, tick-borne encephalitis virus, Kyasanur forest disease virus, and Omsk haemorrhagic fever virus (Gould and Solomon, 2008). Most are transmitted by arthropods (mosquitoes or ticks), and are therefore also referred to as arboviruses (arthropod-borne viruses) (Gould and Solomon, 2008).

The dengue virus genome (Fig 2.1a and b) contains about 11,000 nucleotide bases, which code for the three different types of protein molecules (C, prM and E) that form the virus particle and seven other types of protein molecules (NS1, NS2a, NS2b, NS3, NS4a, NS4b, NS5) that are only found in infected host cells and are required for replication of the virus (Guzman *et al*., 2010; Rodenhuis-Zybert *et al*., 2010). There are four strains of the

virus, which are called serotypes, and these are referred to as DENV-1, DENV-2, DENV-3 and DENV-4 (WHO, 2009). All four serotypes can cause the full spectrum of disease (Rodenhuis-Zybert *et al*., 2010). Infection with one serotype is believed to produce lifelong immunity to that serotype but only short term protection against the others (WHO, 2009; Chen and Wilson, 2010).

The severe complications on secondary infection occurs particularly if someone previously exposed to serotype DENV-1 then contracts serotype DENV-2 or serotype DENV-3, or if someone previously exposed to type DENV-3 then acquires DENV-2 (Guzman *et al*., 2010).

2.7 Transmission of Dengue Viruses

Dengue virus is primarily transmitted by *Aedes* mosquitoes, particularly *A. aegypti* (WHO, 2009). These mosquitoes bite primarily during the day. Other *Aedes* species that transmit the disease include *A. albopictus*, *A. polynesiensis* and *A. scutellaris*(WHO, 2009). Humans are the primary host of the virus,but it also circulates in nonhuman primates (WHO, 2009). An infection can be acquired via a single bite (CDC, 2010). A female mosquito that takes a blood meal from a person infected with dengue fever becomes itself infected with the virus in the cells lining its gut. About 8–10 days later, the virus spreads to other tissues including the mosquito's salivary glands and is subsequently released into its saliva. The virus seems to have no detrimental effect on the mosquito, which remains infected for life. *Aedes aegypti* prefers to lay its eggs in artificial water containers, to live in close proximity to humans, and to feed off people rather than other vertebrates (Gubler, 2010).

Dengue can also be transmitted via infected blood products and through organ donation (Wilder-Smith *et al*., 2009). In countries such as Singapore, where dengue is endemic, the risk is estimated to be between 1.6 and 6 per 10,000 transfusions (Teo *et al*., 2009).Vertical transmission (from mother to child) during pregnancy or at birth has been reported (Wiwanitkit, 2010).

2.8 Predisposition to Dengue Viruses

Severe disease is more common in babies and young children, and in contrast to many other infections it is more common in children that are relatively well nourished (Ranjit and Kissoon, 2010). Women are more at risk than men (Guzman *et al*., 2010). Dengue can be life-threatening in people with chronic diseases such as diabetes and asthma (Guzman *et al*., 2010).

Polymorphisms (normal variations) in particular genes have been linked with an increased risk of severe dengue complications. Examples include the genes coding for the proteins known as TNFα, mannan-binding lectin (Whitehorn and Farrar, 2010),CTLA4, TGFβ (Rodenhuis-Zybert *et al*., 2010),DC-SIGN, and particular forms of human leukocyte antigen (Guzman *et al*., 2010). A common genetic abnormality in Africans, known as glucose-6-phosphate dehydrogenase deficiency, appears to increase the risk (Byron*et al*., 2009). Polymorphisms in the genes for the vitamin D receptor and FcγR seem to offer protection against severe disease in secondary dengue infection (Guzman *et al*., 2010).

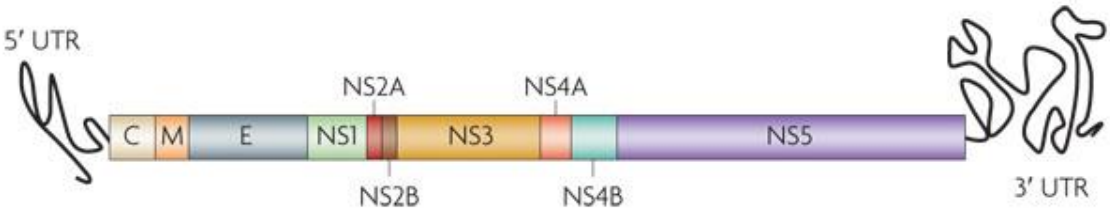


Figure 2.1a: Dengue Virus Genome(Guzman *et al*., 2010)

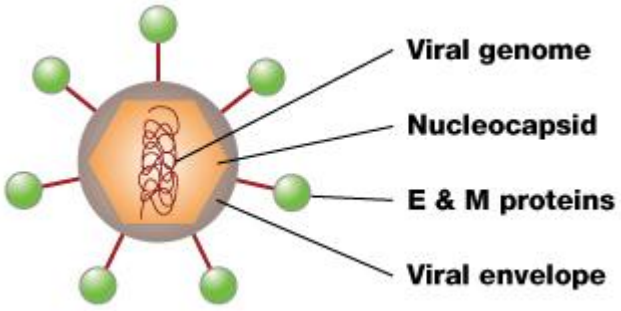


Figure 2.1b: Structure of Dengue Virus
(http://www.nature.com/scitable/content/dengue-virus-structure-22401481. Accessed 13-10-2015).

2.9 Dengue Virus Reproduction

Once inside the skin, dengue virus binds to Langerhans cells (a population of dendritic cells in the skin that identifies pathogens) (Byron *et al*., 2009). The virus enters the cells through binding between viral proteins and membrane proteins on the Langerhans cell, specifically the C-type lectins called DC-SIGN, mannose receptor and CLEC5A (Rodenhuis-Zybert *et al*., 2010). DC-SIGN, a non-specific receptor for foreign material on dendritic cells, seems to be the main point of entry (Guzman *et al*., 2010). The dendritic cell moves to the nearest lymph node. Meanwhile, the virus genome is replicated in membrane-bound vesicles on the cell's endoplasmic reticulum, where the cell's protein synthesis apparatus produces new viral proteins, and the viral RNA is copied. Immature virus particles are transported to the Golgi apparatus, the part of the cell where some of the proteins receive necessary sugar chains (glycoproteins). The now mature new viruses bud on the surface of the infected cell and are released by exocytosis (Fig 2.2). They are then able to enter other white blood cells, such as monocytes and macrophages(Rodenhuis-Zybert *et al*., 2010).

The initial reaction of infected cells is to produce interferon, a cytokine that raises a number of defences against viral infection through the innate immune system by augmenting the production of a large group of proteins mediated by the Janus Kinase/Signal Transducers and Activators of Transcription (JAK-STAT) pathway. Some serotypes of dengue virus appear to have mechanisms to slow down this process. Interferon also activates the adaptive immune system, which leads to the generation of antibodies against the virus as well as T cells that directly attack any cell infected with the virus

(Rodenhuis-Zybert *et al*., 2010). Various antibodies are generated; some bind closely to the viral proteins and target them for phagocytosis (ingestion by specialized cells) and destruction, but some bind the virus less well and appear instead to deliver the virus into a part of the phagocytes where it is not destroyed but is able to replicate further (Rodenhuis-Zybert *et al*., 2010).

2.10 Diagnosis of Dengue Virus using MAC ELISA

Anti-dengue IgM detection using enzyme-linked immunosorbent assay (ELISA) represents one of the most important advances and has become an invaluable tool for routine dengue diagnosis. Specifically, MAC-ELISA (IgM antibody capture ELISA) diagnosis is based on detecting dengue-specific IgM antibodies in the test serum by capturing them using anti-human IgM antibody previously bound on a solid phase. In general, 10% false negative and 1.7% false positive reactions have been observed (Guzman and Kouri, 2004). In dengue infections, the IgM monotypic response is not correlated with the serotype isolated from patients, and for this reason, MAC-ELISA cannot be used for viral identification. There are several commercial kits available for dengue diagnosis by IgM-capture, including those made by well-known research institutes, such as the Oswaldo Cruz and Pedro Khouri Institutes. MAC-ELISA has been a valuable tool for the surveillance of dengue and DHF/DSS. During epidemics, MAC-ELISA has the advantage of fast detection of the propagation of transmission. In areas where dengue is endemic, MAC-ELISA can be used as a valuable tool in the evaluation of a great number of clinical samples, with relative ease (De Paula and Fonseca, 2004).

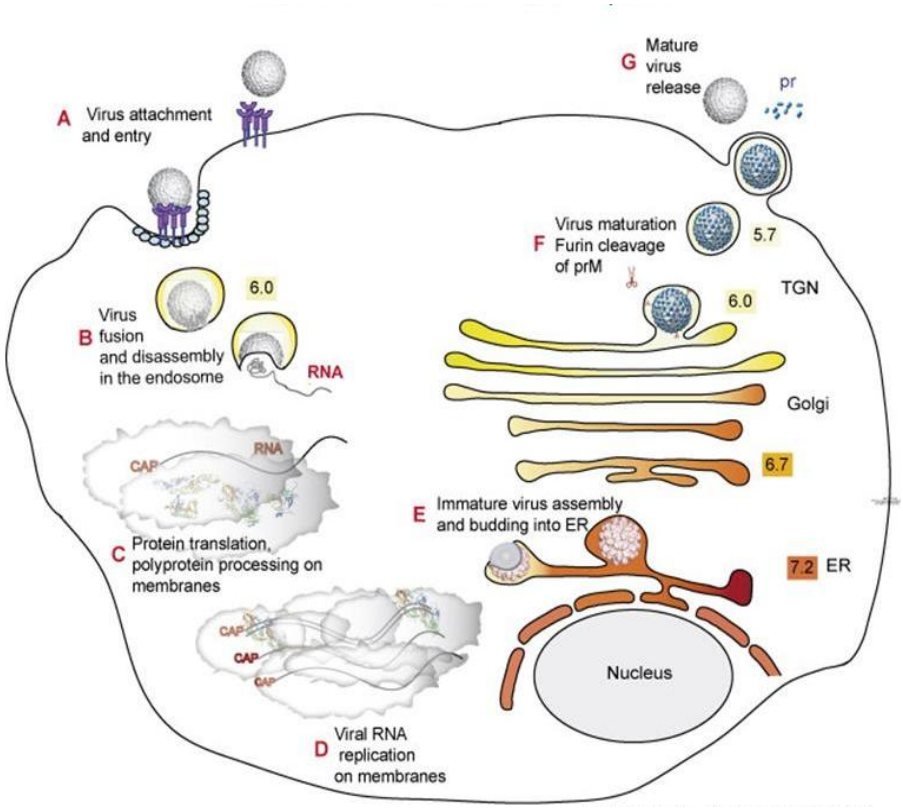


Figure 2.2: Reproduction of Dengue Virus(Venere,2008).

2.11Chemotherapy and Chemoprophylaxis of Dengue Virus

There are actually no known antiviral drugs or injections available for the cure of dengue. However, the disease can be treated with plenty of supportive care and maintaining proper fluid balance (Simmons *et al*., 2012). Treatment depends on the symptoms (WHO, 2009). Those who are able to drink, are passing urine, have no "warning signs" and are otherwise healthy can be managed at home with daily follow up and oral rehydration therapy. Those who have other health problems, have "warning signs" or who cannot manage regular follow up should be cared for in hospital. In those with severe dengue care should be provided in an area where there is access to an intensive care unit (WHO, 2009).

Intravenous hydration, if required, is typically only needed for one or two days. The smallest amount of fluid required to achieve this is recommended. Invasive medical procedures such as nasogastric intubation, intramuscular injections and arterial punctures are avoided, in view of the bleeding risk (Ranjit and Kissoon, 2011). Paracetamol (acetaminophen) is used for fever and discomfort while non-steroidal anti-inflammatory drugs such as ibuprofen and aspirin are avoided as they might aggravate the risk of bleeding (WHO, 2009). Blood transfusion is initiated early in people presenting with unstable vital signs in the face of a *decreasing hematocrit*, rather than waiting for the hemoglobin concentration to decrease to some predetermined "transfusion trigger" level. Packed red blood cells or whole blood are recommended, while platelets and fresh frozen plasma are usually not.

During the recovery phase intravenous fluids are discontinued to prevent a state of fluid overload (Ranjit and Kissoon, 2011). If fluid overload occurs and vital signs are stable, stopping further fluid may be all that is needed. If a person is outside of the critical phase, a loop diuretic such as furosemide may be used to eliminate excess fluid from the circulation (WHO, 2009).

2.12 Malaria

Malaria is a mosquito-borne infectious disease of humans and other animals caused by protists (a type of microorganism) of the genus *Plasmodium*. The protists first infect the liver, then act as parasites within red blood cells, causing symptoms that typically include fever and headache, in severe cases progressing to coma or death. The disease is widespread in tropical and subtropical regions in a broad band around the equator, including much of Sub-Saharan Africa, Asia, and the Americas (WHO, 2011).

Five species of *Plasmodium* can infect and be transmitted by humans. Severe malaria is largely caused by *P. falciparum* while the disease caused by *P. vivax, P. ovale,* and *P. malariae* is generally a milder form that is rarely fatal. The zoonotic species *P. knowlesi*, prevalent in Southeast Asia, causes malaria in macaques but can also cause severe infections in humans. Malaria is prevalent in tropical regions because the significant amounts of rainfall, consistently high temperatures and high humidity, along with stagnant waters in which mosquito larvae readily mature, provide them with the environment they need for continuous breeding. Disease transmission can be reduced by preventing mosquito bites by distribution of mosquito nets and insect repellents, or with mosquito-control measures such as spraying insecticides and draining standing water (WHO, 2011).

The World Health Organization has estimated that in 2010, there were 216 million documented cases of malaria. Around 655,000 people died from the disease, many of whom were children under the age of five (WHO, 2011). The actual number of deaths may be significantly higher, as precise statistics are unavailable in many rural areas, and many cases are undocumented. *P. falciparum* – responsible for the most severe form of malaria – causes the vast majority of deaths associated with the disease.

2.13 History of Malaria

Malaria has infected humans for over 50,000 years, and *Plasmodium* may have been a human pathogen for the entire history of the species (Da Silva*et al*., 2003). Close relatives of the human malaria parasites remain common in chimpanzees. Some new evidence suggests that the most virulent strain of human malaria may have originated in gorillas (Prugnolle *et al*., 2011).

References to the unique periodic fevers of malaria are found throughout recorded history, beginning in 2700 BC in China (Cox, 2002). Malaria may have contributed to the decline of the Roman Empire, (Sallares and Gomzi, 2001) and was so pervasive in Rome that it was known as the "Roman fever" (Sallares, 2002). Several regions in ancient Rome were considered at-risk for the disease because of the favourable conditions present for malaria vectors. This included areas such as southern Italy, the island of Sardinia, the Pontine Marshes, the lower regions of coastal Etruria and the city of Rome along the Tiber River. The presence of stagnant water in these places was preferred by mosquitoes for breeding grounds. Irrigated gardens, swamp-like grounds, runoff from agriculture, and

drainage problems from road construction led to the increase of standing water (Hays, 2005).

The term malaria originates from Medieval-Italian: *mala aria* - "bad air"; the disease was formerly called *ague* or *marsh fever* due to its association with swamps and marshland (Reiter, 2000). Malaria was once common in most of Europe and North America (Lindemann, 1999), where it is no longer endemic (Gratz and World Health Organization, 2006), though imported cases do occur (Webb, 2009).

Malaria was the most important health hazard encountered by U.S. troops in the South Pacific during World War II, where about 500,000 men were infected (Bray 2004). According to Joseph Patrick Byrne, "Sixty thousand American soldiers died of malaria during the African and South Pacific campaigns" (Byrne, 2008). Scientific studies on malaria made their first significant advance in 1880, when a French army doctor working in the military hospital of Constantine in Algeria named Charles Louis Alphonse Laveran observed parasites for the first time, inside the red blood cells of people suffering from malaria. He therefore proposed that malaria is caused by this organism, the first time a protist was identified as causing disease (Laveran, 1907). For this and later discoveries, he was awarded the 1907 Nobel Prize for Physiology or Medicine. The malarial parasite was called *Plasmodium* by the Italian scientists Ettore Marchiafava and Angelo Celli (Valkiūnas, 2005). A year later, Carlos Finlay, a Cuban doctor treating people with yellow fever in Havana, provided strong evidence that mosquitoes were transmitting disease to and from humans (Tan and Sung, 2008). This work followed earlier suggestions by Josiah

C. Nott (Chernin, 1983), and work by Sir Patrick Manson, the "father of tropical medicine", on the transmission of filariasis (Chernin, 1977).

In April 1894, a Scottish physician Sir Ronald Ross visited Sir Patrick Manson at his house on Queen Anne Street, London. This visit was the beginning of four years of collaboration and fervent research that culminated in 1898 when Ross, who was working in the Presidency General Hospital in Calcutta, proved the complete life-cycle of the malaria parasite in mosquitoes. He thus proved that the mosquito was the vector for malaria in humans by showing that certain mosquito species transmit malaria to birds. He isolated malaria parasites from the salivary glands of mosquitoes that had fed on infected birds (Ross, 1902). For this work, Ross received the 1902 Nobel Prize in Medicine. After resigning from the Indian Medical Service, Ross worked at the newly established Liverpool School of Tropical Medicine and directed malaria-control efforts in Egypt, Panama, Greece and Mauritius (CDC, 2012). The findings of Finlay and Ross were later confirmed by a medical board headed by Walter Reed in 1900. Its recommendations were implemented by William C. Gorgas in the health measures undertaken during construction of the Panama Canal. This public-health work saved the lives of thousands of workers and helped develop the methods used in future public-health campaigns against the disease (Simmons, 1979).

The first effective treatment for malaria came from the bark of cinchona tree, which contains quinine. This tree grows on the slopes of the Andes, mainly in Peru. The indigenous peoples of Peru made a tincture of cinchona to control malaria. The Jesuits noted the efficacy of the practice and introduced the treatment to Europe during the 1640s,

where it was rapidly accepted (Kaufman and Rúveda, 2005). It was not until 1820 that the active ingredient, quinine, was extracted from the bark, isolated and named by the French chemists Pierre Joseph Pelletier and Joseph Bienaimé Caventou (Pelletier and Caventou, 1820; Kyle and Shampe, 1974). Quinine became the predominant malarial medication until the 1920s, when other medications began to be developed. In the 1940s, chloroquine replaced quinine as the treatment of both uncomplicated and severe falciparum malaria until resistance supervened, first in Southeast Asia and South America in the 1950s and then globally in the 1980s (Achan *et al*., 2011). Artemisinins, discovered by Chinese scientists in the 1970s, are now the recommended treatment for falciparum malaria, administered in combination with other antimalarials as well as in severe disease (Hsu, 2006).

2.14 Epidemiology of Malaria

Based on documented cases, the WHO estimates that there were 216 million cases of malaria in 2010 resulting in 655,000 deaths (WHO, 2011). An estimate in *The Lancet*, based on a systematic analysis of all available mortality data combined with empirical methods for estimating causes of death, places the number of deaths in 2010 at 1.24 million (Murray *et al*., 2012; Olver, 2012). The majority of cases occur in children under five years old (Greenwood *et al*., 2005); pregnant women are also especially vulnerable. Despite efforts to reduce transmission and increase treatment, there has been little change in which areas are at risk of this disease since 1992 (Hay, 2004). Indeed, if the prevalence of malaria stays on its present upwards course, the death rate could double in the next twenty years (Breman, 2001). Precise statistics are unknown because many cases occur in rural areas

where people do not have access to hospitals or the means to afford health care. As a consequence, the majority of cases are undocumented (Breman, 2001).

Malaria is presently endemic in a broad band around the equator, in areas of the Americas, many parts of Asia, and much of Africa; however, it is in sub-Saharan Africa where 85–90% of malaria fatalities occur (Layne, 2006). The geographic distribution of malaria within large regions is complex, and malaria-afflicted and malaria-free areas are often found close to each other (Greenwood and Mutabingwa, 2002). Malaria is prevalent in tropical regions because of the significant amounts of rainfall, consistent high temperatures and high humidity, along with stagnant waters in which mosquito larvae readily mature, providing them with the environment they need for continuous breeding (Prothero, 1999). In drier areas, outbreaks of malaria have been predicted with reasonable accuracy by mapping rainfall (Abeku, 2007). Malaria is more common in rural areas than in cities; this is in contrast to dengue fever where urban areas present the greater risk (Jansen and Beebe, 2010). For example, several cities in Vietnam, Laos and Cambodia are essentially malaria-free, but the disease is present in many rural regions (Trung *et al*., 2004). By contrast, malaria in Africa is present in both rural and urban areas, though the risk is lower in the larger cities (Machault *et al*., 2011). The Welcome Trust, UK, has funded the Malaria Atlas Project to map global endemic levels of malaria, providing a more contemporary and robust means with which to assess current and future malaria disease burden (Guerra *et al*., 2007). This effort led to the publication of a map of *P. falciparum* endemicity in 2010 (Gething *et al*., 2011). As of 2010, countries with the highest death rate per 100,000 populations are Cote d'Ivoire with (86.15), Angola (56.93) and Burkina Faso (50.66) – all in Africa (Provost, 2011).

2.15 Signs and Symptoms of Malaria

The signs and symptoms of malaria typically begin 8–25 days following infection (Mandell *et al*., 2010). However, symptoms may occur later in those who have taken antimalarial medications as prevention (Nadjm and Behrens, 2012). The presentation may include fever, shivering, arthralgia (joint pain), vomiting, haemolytic anaemia, jaundice, haemoglobinuria, retinal damage (Beare *et al*., 2006), and convulsions. Approximately 30% of people however will no longer have a fever upon presenting to a health care facility (Nadjm and Behrens, 2012).

The classic symptom of malaria is cyclical occurrence of sudden coldness followed by rigor and then fever and sweating lasting about two hours or more, occurring every two days in *P. vivax* and *P. ovale* infections, and every three days for *P. malariae*. *P. falciparum* infection and can cause recurrent fever every 36–48 hours or a less pronounced and almost continuous fever (Fernando *et al*., 2001). For reasons that are poorly understood, but that may be related to high intracranial pressure, children with malaria frequently exhibit abnormal posturing, a sign indicating severe brain damage (Idro *et al*., 2005). Cerebral malaria (encephalopathy specifically related to *P. falciparum* infection) is associated with retinal whitening, which may be a useful clinical sign in distinguishing malaria from other causes of fever (Beare *et al*., 2011).

Severe malaria is usually caused by *P. falciparum*, and typically arises 6–14 days after infection (Trampuz *et al*., 2003). Non-*falciparum* species have however been found to be the cause of ~14% of cases of severe malaria in some groups (Nadjm and Behrens, 2012). Consequences of severe malaria include coma and death if untreatedyoung children

and pregnant women are especially vulnerable. Splenomegaly (enlarged spleen), severe headache, cerebral ischemia, hepatomegaly (enlarged liver), hypoglycaemia, and haemoglobinuria with renal failure may occur. Renal failure is a feature of blackwater fever, where haemoglobin from lysed red blood cells leaks into the urine (Trampuz *et al*., 2003).

2.16 Complications of Malaria

There are a number of serious complications of malaria. Among these is the development of respiratory distress which occurs in up to 25% of adults and 40% of children with falciparum malaria (Taylor *et al*., 2012). The causes of this problem are diverse and include respiratory compensation of metabolic acidosis, non-cardiogenic pulmonary oedema, concomitant pneumonia and severe anaemia. Acute respiratory distress syndrome (ARDS) may develop in 5–25% in adults and up to 29% of pregnant women but is rare in young children (Taylor *et al*., 2012).

2.17 Causes of Malaria

Malaria parasites are from the genus *Plasmodium* (phylum Apicomplexa). In humans, malaria is caused by *P. falciparum*, *P. malariae*, *P. ovale*, *P. vivax* and *P. knowlesi* (Mueller *et al*., 2007; Collins, 2012). Among those infected, *P. falciparum* is the most common species identified (~75%) followed by *P. vivax* (~20%) (Nadjm and Behrens, 2012). *P. falciparum* accounts for the majority of deaths (Snow *et al*., 2005). *P. vivax* proportionally is more common outside of Africa (Mendis *et al*., 2001). There have been documented human infections with several species of *Plasmodium* from higher apes; however, with the exception of *P. knowlesi*-a zoonotic species that causes malaria in

macaques (Collins, 2012), these are mostly of limited public health importance (Collins and Barnwell, 2009).

2.18 Life Cycle of *Plasmodium* Species

The definitive hosts for malaria parasites are female mosquitoes of the *Anopheles* genus, which act as transmission vectors to humans and other vertebrates, the secondary hosts. Young mosquitoes first ingest the malaria parasite by feeding on an infected vertebrate carrier and the infected *Anopheles* mosquitoes eventually carry *Plasmodium*sporozoites in their salivary glands. A mosquito becomes infected when it takes a blood meal from an infected vertebrate. Once ingested, the parasite gametocytes taken up in the blood will further differentiate into male or female gametes and then fuse in the mosquito's gut. This produces an ookinete that penetrates the gut lining and produces an oocyst in the gut wall. When the oocyst ruptures, it releases sporozoites that migrate through the mosquito's body to the salivary glands, where they are then ready to infect a new human host. The sporozoites are injected into the skin, alongside saliva, when the mosquito takes a subsequent blood meal. This type of transmission is occasionally referred to as anterior station transfer (Talman *et al*., 2004).

Only female mosquitoes feed on blood; male mosquitoes feed on plant nectar, and thus do not transmit the disease. The females of the *Anopheles* genus of mosquito prefer to feed at night. They usually start searching for a meal at dusk, and will continue throughout the night until taking a meal (Arrow *et al*., 2004). Malaria parasites can also be transmitted by blood transfusions, although this is rare (Owusu-Ofori *et al*., 2010).

2.19 Recurrent Malaria

Malaria recurs after treatment for three reasons. Recrudescence occurs when parasites are not cleared by treatment, whereas reinfection indicates complete clearance with new infection established from a separate infective mosquito bite; both can occur with any malaria parasite species. Relapse is specific to *P. vivax* and *P. ovale* and involves re-emergence of blood-stage parasites from latent parasites (hypnozoites) in the liver (Nadjm and Behrens, 2012). Describing a case of malaria as cured by observing the disappearance of parasites from the bloodstream can, therefore, be deceptive. The longest incubation period reported for a *P. vivax* infection is 30 years (White, 2011). Approximately one in five of *P. vivax* malaria cases in temperate areas involve overwintering by hypnozoites, with relapses beginning the year after the mosquito bite (White, 2011).

2.20 Pathogenesis of Malaria

Malaria develops via two phases: one that involves the liver or hepatic system (exoerythrocytic), and one which involves red blood cells, or erythrocytes (erythrocytic). When an infected mosquito pierces a person's skin to take a blood meal, sporozoites in the mosquito's saliva enter the bloodstream and migrate to the liver where they infect hepatocytes, multiplying asexually and asymptomatically for a period of 8–30 days (Bledsoe, 2005). After a potential dormant period in the liver, these organisms differentiate to yield thousands of merozoites, which, following rupture of their host cells, escape into the blood and infect red blood cells to begin the erythrocytic stage of the life cycle (Bledsoe, 2005). The parasite escapes from the liver undetected by wrapping itself in the cell membrane of the infected host liver cell (Vaughan *et al*., 2008).

Within the red blood cells, the parasites multiply further, again asexually, periodically breaking out of their hosts to invade fresh red blood cells. Several such amplification cycles occur. Thus, classical descriptions of waves of fever arise from simultaneous waves of merozoites escaping and infecting red blood cells (Bledsoe, 2005).

Some *P. vivax* sporozoites do not immediately develop into exoerythrocytic-phase merozoites, but instead produce hypnozoites that remain dormant for periods ranging from several months (6–12 months is typical) to as long as three years. After a period of dormancy, they reactivate and produce merozoites. Hypnozoites are responsible for long incubation and late relapses in *P. vivax* infections, although their existence in *P. ovale* is uncertain (Richter *et al.*, 2010).

The parasite is relatively protected from attack by the body's immune system because for most of its human life cycle it resides within the liver and blood cells and is relatively invisible to immune surveillance. However, circulating infected blood cells are destroyed in the spleen. To avoid this fate, the *P. falciparum* parasite displays adhesive proteins on the surface of the infected blood cells, causing the blood cells to stick to the walls of small blood vessels, thereby sequestering the parasite from passage through the general circulation and the spleen (Chen *et al.*, 2000). The blockage of the microvasculature causes symptoms such as in placental and cerebral malaria. In cerebral malaria the sequestrated red blood cells can breach the blood–brain barrier possibly leading to coma (Grau and Craig, 2012).

Although the red blood cell surface adhesive proteins (called PfEMP1, for *P. falciparum* erythrocyte membrane protein 1) are exposed to the immune system, they do

not serve as good immune targets, because of their extreme diversity; there are at least 60 variations of the protein within a single parasite and even more variants within whole parasite populations (Chen *et al.,* 2000). The parasite switches between a broad repertoire of PfEMP1 surface proteins, thus staying one step ahead of the pursuing immune system (Maier *et al*., 2009).

Some merozoites turn into male and female gametocytes. If a mosquito pierces the skin of an infected person, it potentially picks up gametocytes within the blood. Fertilization and sexual recombination of the parasite occurs in the mosquito's gut. New sporozoites develop and travel to the mosquito's salivary gland, completing the cycle. Pregnant women are especially attractive to the mosquitoes, and malaria in pregnant women is an important cause of stillbirths, infant mortality and low birth weight (Hartman *et al*., 2010), particularly in *P. falciparum* infection, but also in other species infection, such as *P. vivax* (Rijken *et al*., 2012).

2.20.1 Genetic resistance to malaria

Due to the high levels of mortality and morbidity caused by malaria – especially the *P. falciparum* – it is thought to have placed the greatest selective pressure on the human genome in recent history. Several diseases may provide some resistance to it including sickle cell disease, thalassemia, glucose-6-phosphate dehydrogenase deficiency as well as the presence of Duffy antigens on the subject's red blood cells (Kwiatkowski, 2005; Hedrick, 2011).

The impact of sickle cell anaemia on malaria immunity is of particular interest. Sickle cell anaemia causes a defect to the haemoglobin molecule in the blood. Instead of retaining the biconcave shape of a normal red blood cell, the modified haemoglobin S molecule causes the cell to sickle or distort into a curved shape. Due to the sickle shape, the molecule is not as effective in taking or releasing oxygen, and therefore malaria parasites cannot complete their life cycle in the cell. Individuals who are homozygous for sickle cell anaemia seldom survive this defect, while those who are heterozygous experience immunity to the disease. Although the potential risk of death for those with the homozygous condition seems to be unfavourable to population survival, the trait is preserved because of the benefits provided by the heterozygous form (Barnes, 2005).

2.20.2 Malarial hepatopathy

Hepatic dysfunction as a result of malaria is rare and is usually a result of a coexisting liver condition such as viral hepatitis and chronic liver disease (Bhalla *et al*., 2006). Hepatitis, which is characterized by inflammation of the liver, is not actually present in what is called malarialhepatitis; the term as used here invokes the reduced liver function associated with severe malaria. While traditionally considered a rare occurrence, malarial hepatopathy has seen an increase in malaria endemic areas, particularly in Southeast Asia and India. Liver compromise in people with malaria correlates with a greater likelihood of complications and death (Bhalla *et al*., 2006).

2.21 Diagnosis of Malaria

Malaria is typically diagnosed by the microscopic examination of blood using blood films or using antigen-based rapid diagnostic tests (Abba *et al.,* 2011; Kattenberg *et al*., 2011). Rapid diagnostic tests that detect *P. vivax* are not as effective as those targeting *P. falciparum* (malERA Consultative Group on Drugs, 2011). They also are unable to tell how many parasites are present (Nadjm and Behrens, 2012). Areas that cannot afford laboratory diagnostic tests often use only a history of subjective fever as the indication to treat for malaria (Perkins and Bell, 2008). Polymerase chain reaction based tests have been developed, though these are not widely implemented in malaria-endemic regions as of 2012, due to their complexity (Nadjm and Behrens, 2012).

2.22 Prevention of Malaria

Methods used to prevent malaria include medications, mosquito eradication and the prevention of bites. The presence of malaria in an area requires a combination of high human population density, high mosquito population density and high rates of transmission from humans to mosquitoes and from mosquitoes to humans. If any of these is lowered sufficiently, the parasite will eventually disappear from that area, as happened in North America, Europe and much of the Middle East. However, unless the parasite is eliminated from the whole world, it could become re-established if conditions revert to a combination that favours the parasite's reproduction (WHO, 1958). Many countries are seeing an increasing number of imported malaria cases owing to extensive travel and migration.

Many researchers argue that prevention of malaria may be more cost-effective than treatment of the disease in the long run, but the capital costs required are out of reach of many of the world's poorest people. There is a wide disparity in the costs of control (i.e. maintenance of low endemicity) and elimination programs between countries. For example, in China – whose government in 2010 announced a strategy to pursue malaria elimination in the Chinese provinces – the required investment is a small proportion of public expenditure on health. In contrast, a similar program in Tanzania would cost an estimated one-fifth of the public health budget (Sabot *et al*., 2010).

2.22.1 Vector control

Efforts to eradicate malaria by eliminating mosquitoes have been successful in some areas. Malaria was once common in the United States and southern Europe, but vector control programs, in conjunction with the monitoring and treatment of infected humans, eliminated it from those regions. In some areas, the draining of wetland breeding grounds and better sanitation were adequate. Malaria was eliminated from most parts of the USA in the early 20th century by such methods, and the use of the pesticide Dichlorodiphenyl trichloroethane(DDT) and other means eliminated it from the remaining pockets in the South by 1951 (US Centres for Disease Control and Prevention, 2010).

Before DDT, malaria was successfully eradicated or controlled in tropical areas like Brazil and Egypt by removing or poisoning the breeding grounds of the mosquitoes or the aquatic habitats of the larva stages, for example by applying the highly toxic arsenic compound Paris Green to places with standing water. This method has seen little application in Africa for more than half a century **(**Killeen *et al*., 2002).

A more targeted and ecologically friendly vector control strategy involves genetic manipulation of malaria mosquitoes. Advances in genetic engineering technologies make it possible to introduce foreign DNA into the mosquito genome and either decrease the lifespan of the mosquito, or make it more resistant to the malaria parasite (Raghavendra *et al*., 2011). Sterile insect technique is a genetic control method whereby large numbers of sterile males mosquitoes are reared and released. Mating with wild females reduces the wild population in the subsequent generation; repeated releases eventually eradicate the target population. Progress towards transgenic, or genetically modified, insects suggest that wild mosquito populations could be made malaria resistant. Successful replacement of current populations with a new genetically modified population relies upon a drive mechanism, such as transposable elements to allow for non-Mendelian inheritance of the gene of interest. Although this approach has been used successfully to eradicate some parasitic diseases of veterinary importance, technological problems have hindered its effective deployment with malaria vector species (Raghavendra *et al*., 2011).

2.22.2 Indoor residual spraying

Indoor residual spraying (IRS) is the practice of spraying insecticides on the interior walls of homes in malaria-affected areas. After feeding, many mosquito species rest on a nearby surface while digesting the bloodmeal, so if the walls of dwellings have been coated with insecticides, the resting mosquitoes will be killed before they can bite another victim and transfer the malaria parasite (Enayati and Hemingway, 2010).

The first pesticide used for IRS was DDT (US Centres for Disease Control and Prevention, 2010). Although it was initially used exclusively to combat malaria, its use quickly spread to agriculture.

The World Health Organization advises the use of 12 insecticides in IRS operations, including DDT as well as alternative insecticides (such as the pyrethroids permethrin and deltamethrin) (Pates and Curtis, 2005). This public health use of small amounts of DDT is permitted under the Stockholm Convention on Persistent Organic Pollutants (POPs), which prohibits the agricultural use of DDT (van den Berg, 2009). However, because of its legacy, many developed countries previously discouraged DDT use even in small quantities (Rosenberg, 2004).

Amidst staggering mortality and morbidity rates due to malaria in the African continent, African Heads of State and Government have adopted the use of DDT as the means of eradicating malaria in the continent. Nigeria's Minister of Health, Prof. Onyebuchi Chukwu, in 2014, emphasized that the World Health Organization has cleared use of DDT in countries where mosquitoes are resistant to other insecticide, noting that the manner of usage is what matters(Ovuorie, 2013).

One problem with all forms of IRS is insecticide resistance via evolution. Mosquitoes that are affected by IRS tend to rest and live indoors, and due to the irritation caused by spraying, their descendants tend to rest and live outdoors, meaning that they are not as affected – if affected at all – by the IRS, which greatly reduces its effectiveness as a defence mechanism (Pates and Curtis, 2005).

2.22.3 Mosquito nets

Mosquito nets help keep mosquitoes away from people and significantly reduce infection rates and transmission of malaria. The nets are not a perfect barrier and they are often treated with an insecticide designed to kill the mosquito before it has time to search for a way past the net. Insecticide-treated nets (ITNs) are estimated to be twice as effective as untreated nets and offer greater than 70% protection compared with no net (Raghavendra *et al*., 2011). Although ITNs are proven to be very effective against malaria, only about 13% of households in sub-Saharan countries own them (Miller *et al*., 2007). Since the *Anopheles* mosquitoes feed at night, the preferred method is to hang a large "bed net" above the centre of a bed to drape over it completely (Schlagenhauf-Lawlor, 2008).

2.22.4 Other methods

Community participation and health education strategies promoting awareness of malaria and the importance of control measures have been successfully used to reduce the incidence of malaria in some areas of the developing world (Lalloo *et al*., 2006). Recognizing the disease in the early stages can stop the disease from becoming fatal. Education can also inform people to cover over areas of stagnant, still water, such as water tanks that are ideal breeding grounds for the parasite and mosquito, thus cutting down the risk of the transmission between people. This is generally used in urban areas where there are large centres of population in a confined space and transmission would be most likely in these areas (Mehlhorn and Armstrong, 2001).

Other interventions for the control of malaria include mass drug administrations (malERA Consultative Group on Drugs, 2011) and intermittent preventive therapy (Grobusch *et al*., 2007).

2.23 Medications

Several drugs, most of which are used for treatment of malaria, can be taken to prevent contracting the disease during travel to endemic areas. Chloroquine may be used where the parasite is still sensitive (Jacquerioz and Croft, 2009). However, due to resistance one of three medications-mefloquine (*Lariam*), doxycycline (available generically), or the combination of atovaquone and proguanil hydrochloride (*Malarone*)—is frequently needed (Jacquerioz and Croft, 2009). Doxycycline and the atovaquone and proguanil combination are the best tolerated; mefloquine is associated with higher rates of neurological and psychiatric symptoms (Jacquerioz and Croft, 2009).

The prophylactic effect does not begin immediately upon starting the drugs, so people temporarily visiting malaria-endemic areas usually begin taking the drugs one to two weeks before arriving and should continue taking them for four weeks after leaving (with the exception of atovaquone proguanil that only needs to be started two days prior and continued for seven days afterwards). Generally, these drugs are taken daily or weekly, at a lower dose than is used for treatment of a person who contracts the disease. Use of prophylactic drugs is seldom practical for full-time residents of malaria-endemic areas, and their use is usually restricted to short-term visitors and travellers to malarial regions. This is due to the cost of purchasing the drugs, negative adverse effects from long-term use, and because some effective anti-malarial drugs are difficult to obtain outside of wealthy nations

(Freedman, 2008). The use of prophylactic drugs where malaria-bearing mosquitoes are present may encourage the development of partial immunity (Turschner and Efferth, 2009).

2.24 Treatment of Malaria

The treatment of malaria depends on the severity of the disease; whether people can take oral drugs or must be admitted depends on the assessment and the experience of the clinician.

2.24.1 Uncomplicated malaria

Uncomplicated malaria may be treated with oral medications. The most effective strategy for *P. falciparum* infection is the use of artemisinins in combination with other antimalarials (known as artemisinin-combination therapy) (Kokwaro, 2009). This is done to reduce the risk of resistance against artemisinin (Kokwaro, 2009). These additional antimalarials include amodiaquine, lumefantrine, mefloquine or sulfadoxine/pyrimethamine. Another recommended combination is dihydroartemisinin and piperaquine (WHO, 2010). In the 2000s (decade), malaria with partial resistance to artemisins emerged in Southeast Asia (Dondorp *et al*., 2010; Phyo *et al*.,2012).

2.24.2 Severe malaria

Severe malaria requires the parenteral administration of antimalarial drugs. Until the mid-2000s the most used treatment for severe malaria was quinine, but artesunate has been shown to be superior to quinine in both children (Dondorp*et al*., 2010) and adults

(Dondorp and Day, 2007; Achan *et al*., 2011). Treatment of severe malaria also involves supportive measures that are optimally performed in a critical care unit, including management of high fevers (hyperpyrexia) and the subsequent seizures that may result from it, and monitoring for respiratory depression, hypoglycemia, and hypokalemia (Sarkar *et al*., 2009). Infection with *P. vivax*, *P. ovale* or *P. malariae* is usually treated on an outpatient basis (while a person is at home). Treatment of *P. vivax* requires both treatment of blood stages (with chloroquine or ACT) as well as clearance of liver forms with primaquine (Waters and Edstein, 2012).

2.25 Malaria Prognosis

Severe malaria can progress extremely rapidly and cause death within hours or days (Trampuz *et al*., 2003). In the most severe cases of the disease, fatality rates can reach 20%, even with intensive care and treatment (Nadjm and Behrens, 2012). Over the longer term, developmental impairments have been documented in children who have suffered episodes of severe malaria (Fernando *et al*., 2010). It causes widespread anaemia during a period of rapid brain development and also direct brain damage. This neurologic damage results from cerebral malaria to which children are more vulnerable (Fernando *et al*., 2010). When properly treated, people with malaria can usually expect a complete recovery (Fernando *et al*., 2010).

Co-infection with HIV and malaria does increase mortality, although this is less of a problem than with HIV/tuberculosis co-infection, due to the two diseases usually attacking different age ranges, with malaria being most common in the young and active tuberculosis most common in the old (Korenromp *et al*., 2005). Although HIV/malaria co-

infection produces less severe symptoms than the interaction between HIV and TB, HIV and malaria do contribute to each other's spread. This effect comes from malaria increasing viral load and HIV infection increasing a person's susceptibility to malaria infection (Barnabas *et al*., 2011).

2.26 Malaria Eradication Efforts

Several notable attempts are being made to eliminate the parasite from sections of the world, or to eradicate it worldwide. In 2006, the organization 'Malaria No More' set a public goal of eliminating malaria from Africa by 2015, and the organization plans to dissolve if that goal is accomplished (Strom, 2011). Several malaria vaccines are in clinical trials, which are intended to provide protection for children in endemic areas and reduce the speed of transmission of the disease. As of 2012, The Global Fund to Fight AIDS, Tuberculosis and Malaria has distributed 230 million insecticide-treated nets intended to stop mosquito-born transmission of malaria. According to director Inder Singh, the U.S.-based Clinton Foundation has significantly reduced the cost of drugs to treat malaria, and is working to further reduce the spread of the disease. Other efforts, such as the Malaria Atlas Project focus on analysing climate and weather information required to accurately predict the spread of malaria based on the availability of habitat of malaria-carrying parasites (Guerra *et al*., 2007).

Malaria has been successfully eradicated in certain areas. The Republic of Mauritius, a tropical island located in the western Indian Ocean, considered ecological connections to malaria transmission when constructing their current plan for malaria control. To prevent mosquitoes from breeding in aquatic areas, DDT is used in moderate

amounts. Additionally, larvae-eating fish are placed in water sources to remove the malaria vectors before they become a threat to the human population. Obstructions are also removed from these sources to maintain water flow and reduce stagnant water. Similarly, marsh or swamp-like environments are drained and filled to diminish mosquito breeding grounds. These actions have produced positive results. The program has cut infection and death rates tremendously, and is cost effective, only requiring $1USD per head each year. This success is a clear indication that responses to adverse environmental conditions can decrease rates of disease (Roberts, 2004).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

Kaduna State occupies part of the Central position of the Northern part of Nigeria (with Kaduna as its capital) and shares boundaries with Niger State to the west, Zamfara, Katsina and Kano states to the north, Bauchi and Plateau states to the east and FCT Abuja and Nasarawa State to the south. Kaduna State lies at latitude 10°20′ north and longitude 7°45′ east. The State occupies an area of approximately 48,473.2 square kilometres and has a population of more than 6 million people (KGIS, 2008).

Some hospitals were selected from Kaduna metropolis and used for the study. These included Barau Diko Specialist Hospital, Ungwar Rimi; Yusuf Dan Tsoho Memorial Hospital, Tudun Wada; General Hospital, Kawo and Gwamna Awan General Hospital, Kakuri.These hospitals were selected because they receive large number of patients every day and their spatial distribution from one another will give representative samples from Kaduna metropolis (Fig 3.1).

3.2 Study Population

Patients with febrile complaints sent to the laboratories of the selected hospitals for malaria test at the time of the research were enrolled for the study. Such febrile complaints include symptoms of fever, headache, muscle and joint pains, cold, abdominal discomfort, diarrhoea, vomiting and rash.

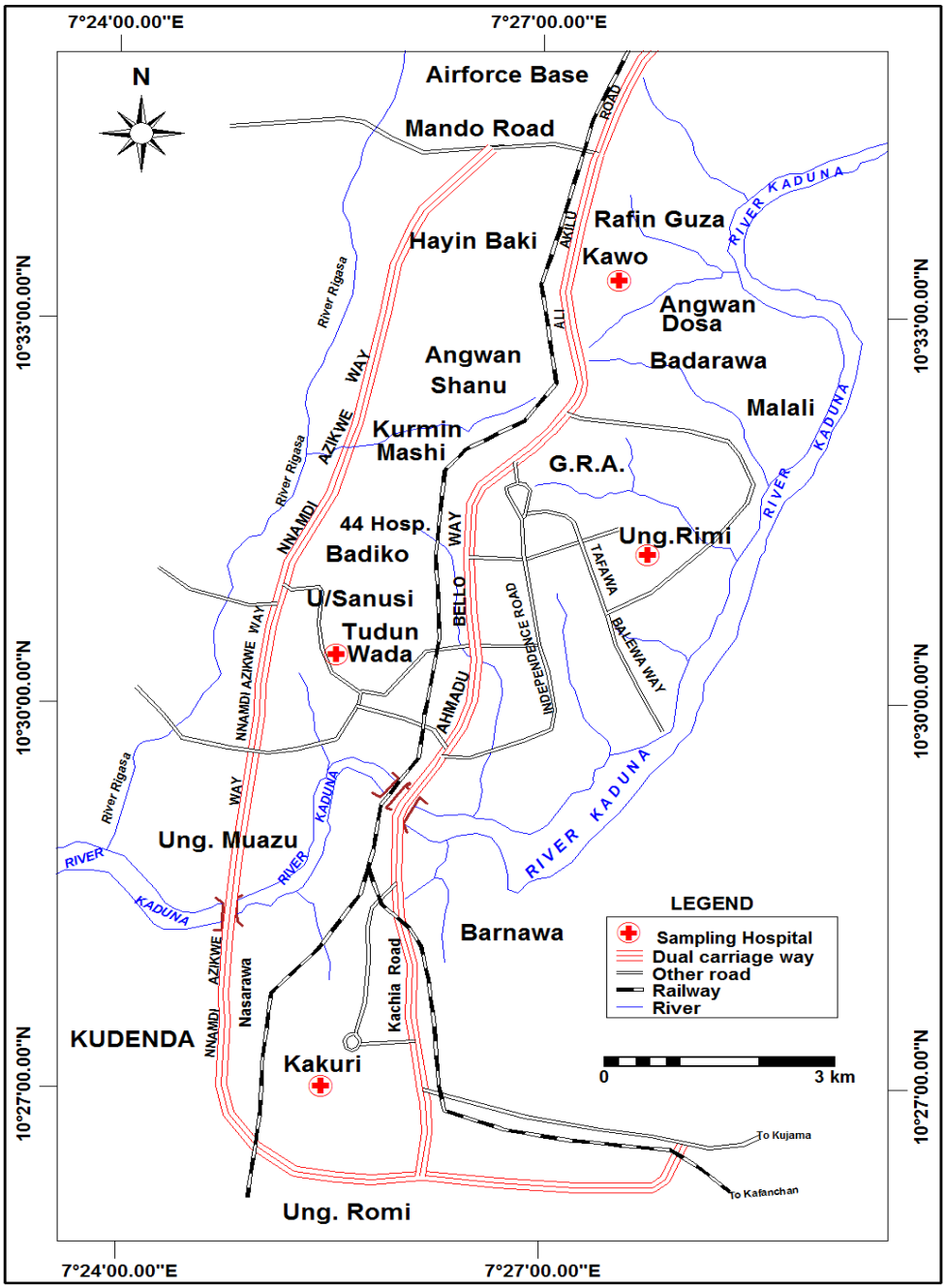


Figure 3.1: Map of study area showing sampling sites(Adapted and modified from Kaduna Metropolis map and digitized at GIS Laboratory, Department of Geography, A.B.U., Zaria).

3.3 Ethical Clearance

Ethical consent was sought and obtained from the ethical committee of Kaduna State Ministry of Health and that of the various hospitals used for the research (Appendix I).

3.4 Inclusion Criteria

Patients of all age, sex, residential area, attending the hospital during the period of the research who are referred to the hospital laboratory for malaria test and who gave their consent to partake in the research (Appendix II and III).

3.5 Exclusion Criteria

Non febrile patients and patients referred for malaria test who did not give their consent to participate in the study.

3.6 Sampling Plan

The sample size was determined using the following equation as described by Naing *et al*., (2006).

$$n = \frac{Z^2 p(1-p)}{d^2}$$

Where n = sample size

z = statistics for a level of 95% confidence interval = 1.96

p = prevalence of 2.2% (0.022) from Dawurung *et al*., (2010).

d = precision (allowable) = 5% = 0.05

Therefore,

$$n = \frac{1.96^2 \times 0.022\ (1 - 0.022)}{0.05^2}$$

n = 33.06

The sample size n, was approximated to 340 to enhance the reliability of the result.

3.7 Sample Collection

A total of 340 serum samples (85 from each hospital) were collected from febrile patients attending these hospitals during the period of the research. About 5ml of blood was collected by venipuncture from febrile patients. The blood was allowed to clot at room temperature and the serum was carefully collected after centrifugation at 2,000 rpm for 10 minutes and stored at –4ºC for further analysis.

3.8 Detection of IgM Antibodies against Dengue Virus:

An IgM capture ELISA (MAC– ELISA) technique (Appendix IV) as previously described by Vorndam and Kuno (1977) was used for the detection of IgM antibodies against DENs. The kit used for this test is Dengue Virus IgM ELISA kit obtained from Diagnostic Automation, Inc., 2391 Craftsman Road, Suite D/E/F, Calabasas, California, USA.

3.9 Determination of Risk Factors Associated with Dengue Virus Infection

A standard questionnaire (Appendix VI) was used to collect demographic data and clinical history of the patients. The questionnaire was divided into three sections. Section A required biodata such as age, gender, highest educational level attained, marital status and occupation from the patients. Section B required some clinical data such as symptoms of fever, headache, muscle and joint pains, cold, abdominal discomfort, diarrhoea, vomiting and rash. Section C attempted to uncover the exposure of the patients to risk factors such as use of mosquito nets, use of insecticides, presence of ditches or bushes close to residential area, sleeping in the afternoon, use of mosquito nets when sleeping in the afternoon, leaving of doors open in the early mornings and evenings, frequency of anti-malaria drug use and current use of anti-malarial drug predisposing to dengue virus infection.

The questionnaire was administered using the discussion method during which the researcher asks the patients questions contained in the questionnaire and thick as appropriate.The information gathered was recorded.

3.10 Determination of Malaria Parasites

This was done by microscopic examination of blood films stained with Giemsa, stain (Warhurst and Williams, 1996). Also, malaria rapid diagnostic kits were used (Appendix VII, VIII and IX).

3.10.1 Microscopic analysis

3.10.1.1 Thick blood film:

This was prepared by placing a drop of patient's blood on a clean grease free slide. Using the edge of a spreader (another clean microscope slide), a smear was prepared from the blood sample. The circular thick film was about 1cm in diameter. The smeared slide was allowed to air dry (Cheesbrough, 2010).The thick blood film provides enhanced sensitivity of the blood film technique and is much better than the thin film for the detection of low levels of parasitaemia and reappearance of circulating parasites during relapse or recrudescence.

3.10.1.2 Thin blood film:

This was prepared by placing a small drop of patient's blood on a clean grease free slide. Using a second clean as a spreader and with the slide with the drop of blood resting on a flat surface, the small drop of blood was touched with the spreader and the blood allowed to run along its edge. The spreader was gently but firmly pushed along the slide, keeping the spreader at an angle of 45^{o}. It was ensured that the spreader was in contact with the surface of the slide all the time that the blood was being spread. The smear was allowed to air dry. The smear was then fixed by dipping in a container of methanol for 3 seconds to prevent dehaemoglobinization. The fixed smear was then allowed to air-dry(Cheesbrough, 2010). The thin blood film emphasizes the parasites inclusion in red blood cells. Because of the fixed monolayer available in this procedure, the morphological identification of the parasite to species level is much easier and provides greater specificity than thick blood film examination.

3.10.1.3 Staining procedure

The prepared slides were placed in staining trough and flooded with 10% Giemsa solution for 30 minutes, out of sunlight. They were rinsed rapidly with clean water and placed film side downward in a slide rack to drain and dry making sure that the film does not touch the rack.

3.10.1.4 Microscopic examination of films

The stained slide was placed on the stage of the light microscope and the x100 oil immersion objective was positioned. A drop of immersion oil was added onto the slide and the objective was lowered until it touched the oil. The chromatin material of the parasite appeared dark red with blue cytoplasm. Schuffner's dots appeared pale red and *P. vivax* and *P. ovale* parastites malarial pigment appeared yellowish brown. Nuclei of small lymphocytes and neutrophils all appeared dark purple. Cytoplasm of mononuclear cells appeared blue-grey and red cells appeared grey to pale-mauve.

3.10.1.5 Estimation of parasitaemia

a. Number of parasites/µl of blood (thick film): This was accomplished by enumerating the number of parasites in relation to a standard number of leukocytes/µl (8000). Many microscopic fields (100) necessary to count 200 leukocytes were observed and the number of parasite/µl of blood determined (WHO, 1991).

$$\text{Parasite/µl of blood} = \frac{\text{Number of observed asexual parasites} \times \text{total WBC count/µl}}{200}$$

b. Number of parasites/µl of blood (thin film): The number of asexual parasites was counted in at least 25 microscopic fields against the standard values of 5000RBC/µl (males) and 4500RBC/µl (female) and the total parasite count/µl of blood was calculated as follows: (Shute, 1988).

$$\text{Parasite/µl of blood} = \frac{\text{Number of observed asexual parasites} \times \text{total RBC count/µl}}{\text{total number of RBC seen in 25 microscopic fields}}$$

3.10.2 Rapid diagnostic test (Immunochromatographic tests):

The Malaria Rapid Diagnostic Test kit that was used for this research is Malaria P.f/Pan (ACON Laboratories, Inc. Manufacturer San Diego, CA 92121, USA). The kit has a sensitivity of range of 97.6-100% for Pan and 93-100% for *P.f.* relative specificity is 99.1% and accuracy is 99.4% at 95% confidence interval.

3.10.2.1 Procedure for malaria rapid diagnostic test (MRDTs);

The test kit component was brought to room temperature (25^{o}C) and the pouch was opened to remove the device (cassette).Using disposable specimen pipette, whole blood sample was transferred into wells of test device. Two drops of the buffer were added to the buffer well and timer started.At the end of 15minutes, the control line and the test line were read and the result interpreted by comparing with the manufacturers guidelines (Appendix X).

3.11 Data Analysis

Datagenerated from the research were analysed using SPSS version 19 from SPSS Inc., USA. Chi square analysis was used to check the level of significance in the occurrence of dengue virus IgM and malaria parasite in relation to different

variables.These variables include risk factors such as use of mosquito bed nets, use of insecticides, presence of gutters and or bush close to or around residential area, sleeping in the afternoon and use of insecticide treated bed nets/insecticides when sleeping in the afternoon; demographic factors such as age, gender, highest educational level attained, marital status and occupation; symptoms such as fever, headache, muscle and joint pains, cold, abdominal discomfort, diarrhoea, vomiting and rash. Measurement of agreement (kappa) was used to measure the level of agreement between the two methods used for malaria diagnosis. Odds ratio analysis was also carried out to determine the extent to which exposure to risk factors could facilitate the occurrence of dengue virus IgM in the study population. Relationships with p-values less than 0.05 ($p<0.05$) were taken to be significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Prevalence of Dengue Virus in the Study Population

Figure 4.1 presents the prevalence of dengue virus IgM in the study population. Out of the 340 samples studied, 6 (1.8%) patients were sero-positive for dengue virus IgM and 2 of this 6 (1.3% of 158 positive malaria cases) were co infected with malaria and dengue virus (Table 4.1).

4.2 Prevalence of Malaria in the Study Population

The prevalence of malaria as obtained from this present study is 46.5% (158) using the microscopic technique as presented in figure 4.2.

4.3 Relationship between the Occurrence of Dengue Virus IgM and theOccurrence of Some Factors.

The relationship between potential risk factors and the occurrence of dengue virus IgM in the study population is presented in Table 4.2. The factors considered include use of mosquito bed net, use of insecticides, presence of ditch or bush around house, sleeping in the afternoon and use of insecticide treated bed net in the afternoon.

Therelationship between the presence of these factors and the occurrence of dengue virus IgM shows no association with p-values greater than 0.05. However odds ratio analysis shows that those who do not use mosquito bed nets at night are at 1.275 times more likely to be infected with dengue virus than those who use. Patients with ditches and

or bush close to or around their residential areas are 1.3 times likely to come down with dengue virus infection than those who do not (Table 4.2).

4.4 Relationship between Occurrence of Dengue Virus IgM and some Demographic Factors

The relationship between the percentage occurrence of dengue virus IgM and some demographic factors are presented in Tables 4.3 to 4.5. The demographic factors considered include age, gender, highest educational level attained, marital status, occupation.

There was no significant association between the occurrence of dengue virus IgM in the study population and these demographic factors. However, odds ratio analyses show that, according to age, age group 41-50 were 1.605 times more likely of having dengue virus IgM than those of other age groups (Table 4.3). Females were 2.877 times more likely to have dengue virus IgM than males. Considering highest educational level attained, patients with primary education were at 2.430 times more likely to have dengue virus IgM compared with those with other levels of education (Table 4.4). According to marital status, those who are married have the likelihood of 2.048 times of being dengue cases than those who are single and widowed (Table 4.4). The relationship between the percentage occurrence of dengue virus IgM and occupation is not significant (Table 4.5).

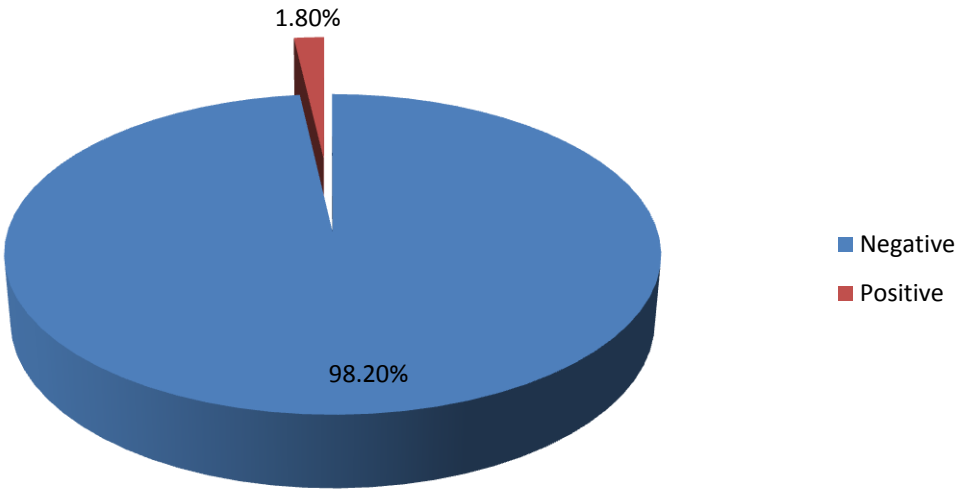


Figure 4.1: Incidence of dengue virus IgM in the study population

Table 4.1:Co-infection of dengue virus and malaria in the study population

	Dengue IgM			
Malaria status	**Total tested**	**Number positive**	**% occurrence**	**Number negative**
Positive	158	2	1.3	156
Negative	182	4	2.2	178
Total	340	6	1.8	334

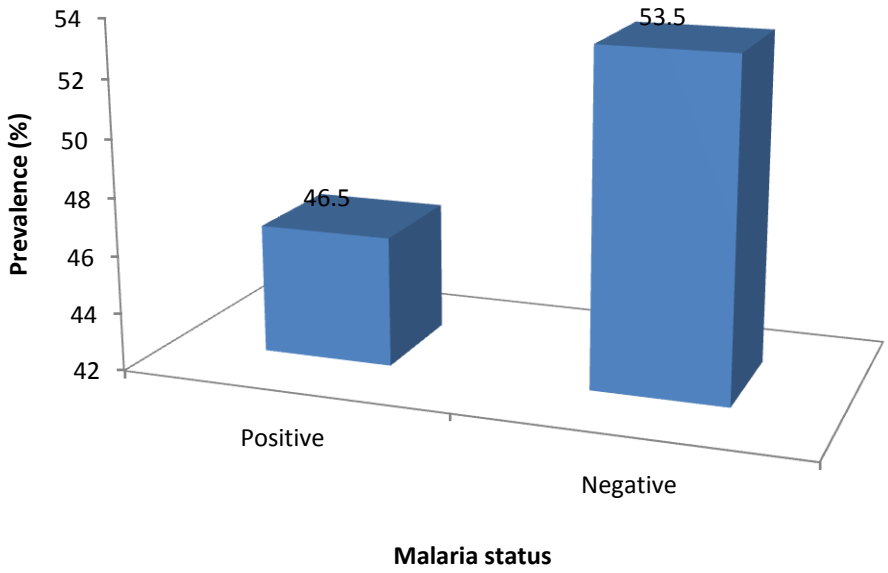


Figure 4.2: Prevalence of malaria in the study population

Table 4.2: Relationship between the occurrence of dengue virus IgM and the occurrence of potential risk factors.

Risk Factor	Dengue virus IgM					
	Total	Positive(%)	Negative(%)	P-value	OR	C.I.
Use of mosquito net						
No	208	4(1.9)	204(98.1)	0.781	1.275	0.230-7.058
Yes	132	2(1.5)	130(98.5)			
Use of insecticides						
No	120	2(1.7)	118(98.3)	0.919	0.951	0.165-5.071
Yes	220	4(1.8)	216(98.2)			
Ditches/bush around house						
Yes	205	4(2.0)	201(98.0)	0.748	1.323	0.239-7.328
No	135	2(1.5)	133(98.5)			
Sleep in the afternoon						
Yes	245	4(1.6)	241(98.4)	0.766	0.772	0.139-4.285
No	95	2(2.1)	93(97.9)			
Use of insecticides in the afternoon						
No	218	3(1.4)	215(98.6)	0.368	0.363	0.036-3.617
Yes	27	1(3.7)	26(96.3)			

Key**:**
OR = Odds Ratio
C.I. = Confidence Interval

Table 4.3: Relationship between the occurrence of dengue virus IgM and some demographic factors (age and gender).

Demographic Factors	Dengue virus IgM					
	Total	Positive(%)	Negative(%)	P-value	OR	C.I.
Age (Years)						
1 – 10	66	1(1.5)	65(98.5)	0.864	0.828	0.095-7.206
Others	274	5(1.8)	269(98.2)			
11 – 20	56	1(1.8)	55(98.2)	0.990	1.015	0.116-8.854
Others	284	5(1.8)	279(98.2)			
21 – 30	87	1(1.1)	86(98.9)	0.613	0.577	0.066-5.006
Others	253	5(2.0)	248(98.0)			
31 – 40	54	1(1.9)	53(98.1)	0.958	1.060	0.121-9.259
Others	286	5(1.7)	281(98.3)			
41 – 50	38	1(2.6)	37(97.4)	0.667	1.605	0.183-14.118
Others	302	5(1.7)	297(98.3)			
>50	39	1(2.6)	38(97.4)	0.687	1.558	0.177-13.691
Others	301	5(1.7)	296(98.3)			
Gender						
Female	217	5(2.3)	212(97.7)	0.316	2.877	0.332-24.914
Male	123	1(0.8)	122(99.2)			

Key**:**
OR = Odds Ratio
C.I. = Confidence Interval

Table 4.4: Relationship between the occurrence of dengue virus IgM and some demographic factors (Educational level and marital status).

Demographic Factors	Dengue virus IgM					
	Total	Positive(%)	Negative(%)	P-value	OR	C.I.
Educational level						
Tertiary	65	0(0.0)	65(100.0)	0.230		
Others	275	6(2.2)	269(97.8)			
Secondary	105	2(1.9)	103(98.1)	0.896	1.121	0.202-6.220
Others	235	4(1.7)	231(98.3)			
Primary	59	2(3.4)	57(96.6)	0.297	2.430	0.435-13.585
Others	281	4(1.4)	277(98.6)			
Quranic	55	1(1.8)	54(98.2)	0.974	1.037	0.119-9.053
Others	285	5(1.8)	280(98.2)			
Informal	56	1(1.8)	55(98.2)	0.990	1.015	0.116-8.854
Others	284	5(1.8)	279(98.2)			
MaritalStatus						
Single	163	2(1.2)	161(98.8)	0.470	0.537	0.097-2.973
Others	177	4(2.3)	173(97.7)			
Married	169	4(2.4)	165(97.6)	0.402	2.048	0.370-11.336
Others	171	2(1.2)	169(98.8)			
Widowed	8	0(0.0)	8(100.0)	0.701		
Others	332	6(1.8)	326(98.2)			

Key**:**
OR = Odds Ratio
C.I. = Confidence Interval

Table 4.5: Relationship between the occurrence of dengue virus IgM and a demographic factor (Occupation)

Demographic Factor	Dengue virus IgM				
	Total	**Positive(%)**	**Negative(%)**	**Chi-square**	**P-value**
Occupation					
Civilservant	29	0(0.0)	29(100.0)		
Selfemployed	32	0(0.0)	32(100.0)		
Military	53	0(0.0)	53(100.0)		
Others	226	6(2.7)	220(97.3)	3.081	0.379

4.5 Occurrence of Dengue IgM and Current use of Anti-malarial Drug.

The percentage occurrence of dengue virus IgM in patients currently on antimalarial drug is presented in Table 4.6. A higher percentage occurrence of 3.7% for dengue virus IgM was obtained in those who are currently on an anti-malaria drug. A percentage occurrence of 0.96% for dengue virus IgM was found in those who are not currently on any anti-malaria drug. The difference in the percentage occurrence of dengue virus IgM between those who are currently on anti-malaria drug and those who are not is not significant.

4.6 Occurrence of Dengue Virus in Relation to some Symptoms

The relationship between the occurrence of dengue virus IgM in relation to some symptoms was also studied, the symptoms include fever, headache, muscle and joint pains, cold, abdominal discomfort, diarrhoea, vomiting and rash. The association between the occurrence of dengue virus IgM and the presence of these symptoms in the study population is not significant. However, odds ration analysis show that the 253 patients with fever were at 1.743 odds of having dengue virus IgM than those without fever. One hundred and seventy seven patients with abdominal discomfort are at 1.861 odds of being dengue cases than those who do not have abdominal discomfort. The 55 patients with diarrhoea were 2.651 times more likely to be dengue cases than those who do not have diarrhoea (Table 4.7).

Table 4.6: Relationship between occurrence of dengue virus IgM and current use of anti-malarial drug.

Current use of anti-malarial drug	Dengue IgM			
	Total tested	Number positive	% occurrence	Number negative
Yes	108	4	3.7	104
No	232	2	0.9	230

$\chi^2 = 3.433$, df = 1, p = 0.064

Table 4.7: Occurrence of dengue virus IgM in relation to some symptoms

	Dengue virus IgM					
Symptoms	**Total**	**Positive(%)**	**Negative(%)**	**P-value**	**OR**	**C.I.**
Fever						
Yes	253	5(2.0)	248(98.0)	0.613	1.743	0.200-15.050
No	87	1(1.1)	86(98.9)			
Headache						
Yes	241	4(1.7)	237(98.3)	0.819	0.819	0.147-4.543
No	99	2(2.0)	97(98.0)			
Muscle and joint pains						
Yes	220	4(1.8)	216(98.2)	0.919	1.093	0.197-6.054
No	120	2(1.7)	118(98.3)			
Cold						
Yes	142	1(0.7)	141(99.3)	0.208	0.274	0.032-2.369
No	198	5(2.5)	193(97.5)			
Abdominaldiscomfort						
Yes	177	4(2.3)	173(97.7)	0.470	1.861	0.336-10.300
No	163	2(1.2)	161(98.8)			
Diarrhoea						
Yes	55	2(3.6)	53(3.6)	0.250	2.651	0.473-14.842
No	285	4(1.4)	281(98.6)			
Vomiting						
Yes	74	1(1.4)	73(98.6)	0.760	0.715	0.082-6.217
No	266	5(1.9)	261(98.1			
Rash						
Yes	7	0(0.0)	7(100.0)	0.720		
No	333	6(1.8)	327(98.2)			

Key**:**
OR = Odds Ratio
C.I. = Confidence Interval

4.7 Relationship between the Occurrence of Malaria and theOccurrence of Risk Factors.

The relationship between the frequency of use of mosquito nets and the percentage occurrence of malaria parasite is presented in Table 4.8. A lower percentage occurrence of malaria parasite of 32.3% occurred in those who always use mosquito nets. This is followed by those who do not use mosquito nets at all with percentage occurrence of malaria parasite of 49.5%. Those who occasionally use mosquito nets had a higher percentage occurrence of malaria parasite of 50.7%. Chi square value of 0.511 and p value of 0.039 shows that the difference in the percentage occurrence of malaria parasite according to frequency of use of mosquito net is significant.

The relationship between insecticide use and the occurrence of malaria is presented in Table 4.8. Those who always use insecticides had a higher percentage occurrence of malaria parasite of 51.7%. This is followed by those who occasionally use insecticides with percentage occurrence of 45.1% and those who do not use insecticides at all with percentage occurrence of 44.2% for malaria parasite. There was no significant difference in the percentage occurrence of malaria parasite in relation to frequency of insecticide use.

The relationship between the presence of ditch/bush close to or around house and the occurrence of malaria parasite is presented in Table 4.8. Those who have gutters and or bush close to or around their houses had a higher percentage occurrence of malaria parasite of 48.5%. Those without bush or gutter around or close to their houses had a percentage occurrence of malaria parasite of 43.3%. Chi square analysis showed no significant difference in the occurrence of malaria parasite in relation to presence or absence of gutters/bushes close to or around residential areas.

The relationship between occurrence of malaria and the frequency of opening of doors in the early mornings and evenings is presented in Table 4.8. Those who occasionally open their doors at these times had a higher occurrence of malaria parasite of 48.7%. Those who always leave their doors open at these times had a percentage occurrence of malaria parasite of 36.5% while those who do not leave their doors open their doors at these time had a lower percentage occurrence of malaria parasite of 36.5%. There was no significant difference in the occurrence of malaria parasite in relation to frequency of opening of doors in the early mornings and evenings.

4.8 Occurrence of Malaria in Relation to some Demographic Factors

The percentage occurrence of malaria according to different age groups is presented in Table 4.9. A higher percentage occurrence of dengue virus IgM of 61.5% occurred in the age group >50. This is closely followed by 57.1% for the age group of 11 – 20. The least percentage occurrence of 35.6% occurred in the age group 21 – 30. No significant difference existed in the percentage occurrence of malaria according to age groups.

Table4.9 presents the percentage occurrence of malaria in the study population according to gender. From Table 4.9, females had a higher percentage occurrence of malaria of 44.70% while males had percentage occurrence of 45.5%. There was no significant difference in the occurrence of malaria according to gender.

The occurrence of malaria in the study population according to educational level is presented in Table 4.9. A higher percentage occurrence of 57.1% was found in patients with no education at all. This was followed by a percentage occurrence of 55.9% for patients with primary education as their highest educational level attained. A lower

percentage occurrence of 32.3% was found in patients with tertiary education. Chi square value of 10.131 and p value of 0.038 shows a significant difference in the percentage occurrence of malaria according to different educational level.

Table 4.9 presents the percentage occurrence of malaria in the study population according to marital status. A higher percentage occurrence of malaria of 50% was found in the widowed. Those who are single had percentage occurrence of 47.9%. The least percentage occurrence of malaria of 45.0% was found in the married population. Chi square analysis shows no significant difference in the percentage occurrence of malaria in the study population according to marital status.

The percentage occurrence of malaria according to occupation is presented in Table 4.9. The highest percentage occurrence of malaria according to occupation was found among civil servants with 51.7%. This is followed by others whose occupations were not particularly specified with percentage occurrence of 48.7%. The least percentage occurrence of malaria according to occupation was found in the military with 34.0%. There was no significant differences in the percentage occurrence of malaria according to occupation.

4.9 Occurrence of Malaria Parasite in Relation to some Symptoms

The percentage occurrence of malaria in patients with symptoms of fever is presented in Table 4.10. Patients with fever had percentage occurrence of malaria of 49.8% and those without fever had percentage occurrence of 36.8%. Chi square value of 4.412 and p value of 0.036 shows significant difference in the percentage occurrence of malaria in relation to symptoms of fever.

The occurrence of malaria in patients with headache is presented in Table 4.10. A higher percentage occurrence of 47.7% was found in patients with headache. A lower percentage occurrence of malaria parasite of 43.4% was found in patients without symptoms of headache. However, chi square analysis showed no significant difference in the percentage occurrence of malaria in relation to symptoms of headache.

The percentage occurrence of malaria parasite in patients with muscle and joint pain is presented in Table 4.10. Patients with muscle and joint pains have a percentage occurrence of malaria parasite of 49.5% and those without muscle and joint pain have a percentage occurrence of malaria parasite of 40.8%. There was no significant difference in the percentage occurrence of malaria parasite in relation to muscle and joint pain.

Table 4.10 presents the percentage occurrence of malaria in patients with symptoms of cold. A higher percentage occurrence of malaria parasite in this group was found in those with symptoms of cold (50.7%). Those without symptoms of cold had a lower percentage occurrence of 43.4%. No significant difference existed in the percentage occurrence of malaria parasite in relation to symptoms of cold.

The relationship between abdominal discomfort and the percentage occurrence of malaria shows that patients with abdominal discomfort had a higher percentage occurrence of malaria parasite of 47.5% (Table 4.10). Those without abdominal discomfort had a lower percentage occurrence of malaria parasite of 45.4%. There was no significant difference in the percentage occurrence of malaria parasite in relation to presence or absence of abdominal pain.

The percentage occurrence of malaria parasite in patients with diarrhoea is presented in Table 4.10. Patients with diarrhoea had a percentage occurrence of malaria parasite of 50.9%. Those without diarrhoea had a percentage occurrence of 45.6% for malaria parasite. There was no significant difference in the occurrence of malaria parasite in relation to symptoms of diarrhoea.

Patients with vomiting had a higher percentage occurrence of malaria parasite of 51.4% compared with those without vomiting with a percentage occurrence of 45.1% (Table 4.10). Chi square analysis showed no significant difference in the occurrence of malaria parasite in relation to symptoms of vomiting.

4.10 Occurrence of Malaria and Current use of Anti-malarial Drug

The relationship between the occurrence of malaria parasite and the current use of anti-malaria drug is presented in Table 4.11. Those who are currently using anti-malaria drug had a percentage occurrence of malaria parasite of 50.9% while those who are not currently on any anti-malarial drug had a percentage occurrence of malaria parasite of 44.4%. Chi square and p value of 1.263 and 0.261 respectively shows no significant difference in the percentage occurrence of malaria parasite in relation to current use of anti-malarial drug.

4.11 Comparison of Microscopy andRapid Diagnostic Test for the Diagnosis of Malaria

The performance of the two laboratory techniques employed in the diagnosis of malaria in the study population is presented in Figure 4.3. The microscopic method gave a

prevalence of 46.5% (153) while the rapid diagnostic test method gave a prevalence of 35.6% (121). Measurement of agreement Kappa value of 0.406 with p-value of 0.049 shows a significant level of agreement between the two methods, microscopy and malaria rapid diagnostic test in the determination of malaria status.

Table 4.8: Relationship between the occurrence of malaria parasite and risk factors

Risk factor	Malaria				
	Total	**Positive(%)**	**Negative(%)**	**Chi-square**	**P–value**
Use of mosquito net					
Always	65	21(32.3)	44(67.7)		
Occasionally	67	34(50.7)	33(49.3)		
Never	208	103(49.5)	105(50.5)	6.511	0.039*
Use of Insecticides					
Always	87	45(51.7)	42(48.3)		
Occasionally	133	60(45.1)	73(54.9)		
Never	120	53(44.5)	67(55.5)	1.320	0.517
Ditch/bush around house					
Yes	206	100(48.5)	106(51.5)		
No	134	58(43.3)	76(56.7)	1.973	0.373
Opening of doors in early morning and evenings					
Always	52	24(46.2)	28(53.8)		
Occasionally	236	115(48.7)	121(51.3)		
Never	52	19(36.5)	33(63.5)	2.548	0.280

*= Significant differences exist

Table 4.9: Relationship between occurrence of malaria parasite and some demographic factors

Demographic factors	Malaria				
	Total	**Positive(%)**	**Negative(%)**	**Chi-square**	**P–value**
Age (Years)					
1 – 10	66	32(48.5)	34(51.5)		
11 – 20	56	32(57.1)	24(42.9)		
21 – 30	87	31(35.6)	56(64.4)		
31 – 40	54	23(42.6)	31(57.4)		
41 – 50	38	16(42.1)	22(57.9)		
>50	39	24(61.5)	15(38.5)	10.957	0.052
Gender					
Male	123	56(45.5)	67(54.5)		
Female	217	102(47.0)	115(53.0)	0.069	0.793
Educational level					
Tertiary	65	21(32.3)	44(67.7)		
Secondary	105	48(45.7)	57(54.3)		
Primary	59	33(55.9)	26(44.1)		
Quranic	55	24(43.6)	31(56.4)		
None	56	32(57.1)	24(42.9)	10.131	0.038*
MaritalStatus					
Single	163	78(47.9)	85(52.1)		
Married	169	76(45.0)	93(55.0)		
Widowed	8	4(50.0)	4(50.0)	0.318	0.853
Occupation					
Civilservant	29	15(51.7)	14(48.3)		
Selfemployed	32	15(46.9)	17(53.1)		
Military	53	18(34.0)	35(66.0)		
Others	226	110(48.7)	116(51.3)	4.089	0.251

*= significant differences exist

Table 4.10: Relationship between Occurrence of Malaria Parasite and Some Symptoms

Symptom	Malaria				
	Total	Positive(%)	Negative(%)	Chi-square	P–value
Fever					
Yes	253	126(49.8)	127(50.2)		
No	87	32(36.8)	55(63.2)	4.412	0.036*
Headache					
Yes	241	115(47.7)	126(53.2)		
No	99	43(43.4)	56(56.6)	0.518	0.472
Muscle and joint pain					
Yes	220	109(49.5)	111(50.5)		
No	120	49(40.8)	71(59.2)	2.369	0.124
Cold					
Yes	142	72(50.7)	70(49.3)		
No	198	86(43.4)	112(56.6)	1.757	0.185
Abdominal discomfort					
Yes	177	84(47.5)	93(52.5)		
No	163	74(45.4)	89(54.6)	0.145	0.704
Diarrhoea					
Yes	55	28(50.9)	27(49.1)		
No	285	130(45.6)	155(54.4)	0.520	0.471
Vomiting					
Yes	74	38(51.4)	36(48.6)		
No	266	120(45.1)	146(54.9)	0.906	0.341

*= significant difference exists

Table 4.11: Relationship between occurrence of malaria parasite and current use of anti-malarial drug

Current use of anti-malarial drug	Malaria			
	Total tested	Number positive	% occurrence	Number negative
Yes	108	55	50.9	53
No	232	103	44.4	129

$\chi^2 = 1.263$, df = 1, p = 0.261

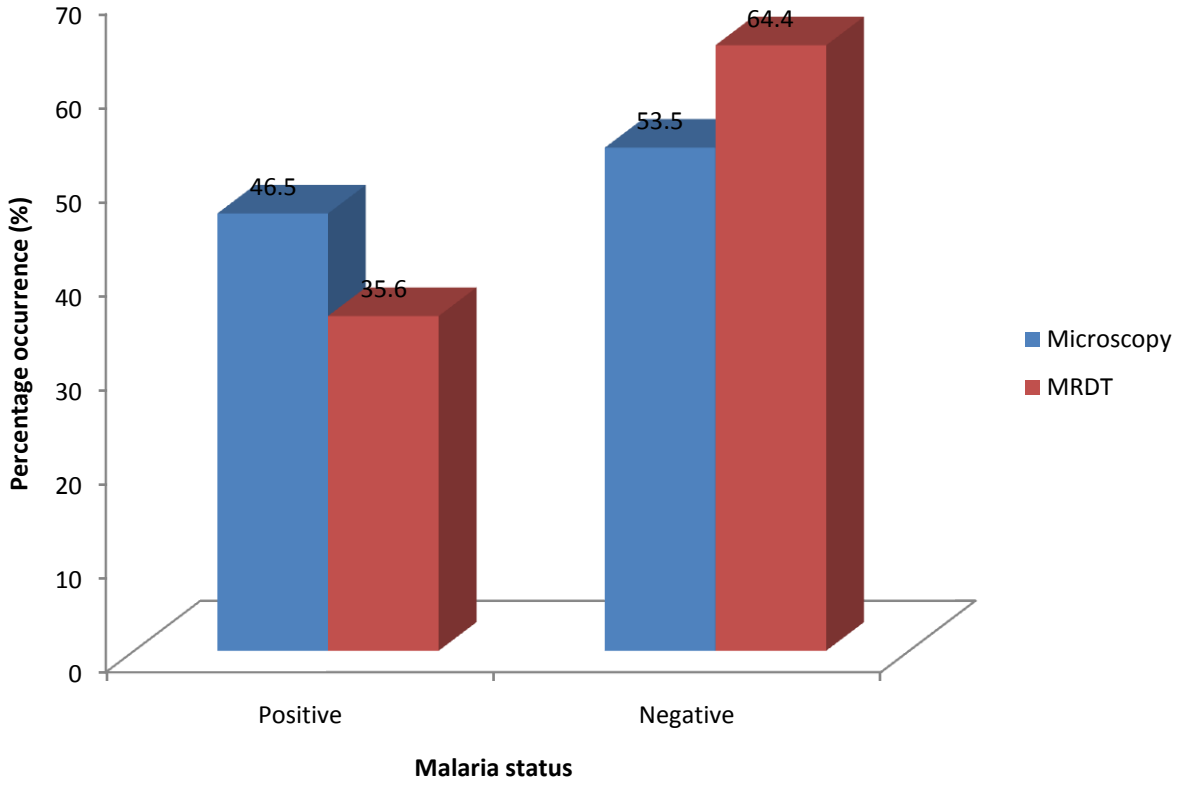


Figure 4.3: Comparison of microscopy and malaria rapid diagnostic test for diagnosis of malaria.

Measurement of agreement Kappa = 0.406, p = 0.049
Key: MRDT = Malaria rapid diagnostic test

CHAPTER FIVE

5.0 DISCUSSION

A sero-prevalence of 1.8% for dengue virus IgM was obtained from the study. Although, the prevalence of dengue virus IgM obtained in this study is quite low, it has brought to lime light the occurrence of the virus within Kaduna metropolis, Nigeria. A positive case of dengue virus in a community is of epidemiologic importance as mosquitoes can transmit the virus from an infected person to a high proportion of susceptible individuals within the same environment. This is in line with previous studies by Dawurung *et al.* (2010) with a prevalence rate of 2.2% and Baba *et al.* (2009) with dengue IgM prevalence of 0.67%.

Out of the 158 positive malaria cases, 2 (1.3%) were sero-positive for dengue virus IgM. This result is very important because Nigeria is one of the few African countries that limit investigation of febrile illnesses to malaria and perhaps typhoid with complete neglect to viral infections. Generally, viral infections suppress the natural immunity of the host and this often allows opportunistic infections to set in (Dawurung *et al*., 2010). Therefore, a co-infection of dengue and malaria as observed in this study could be very devastating to the host.

The prevalence of malaria obtained in this study using microscopy method is 46.5%. This prevalence rate is higher than that obtained by Anumudu *et al*. (2006) and Abdulahi *et al*. (2009) with prevalence of 17% and 27% respectively. This is because the population employed in this study were suspected of malaria and sent to the laboratory to carry out confirmatory malaria tests.

The relationship between the percentage occurrence of the dengue virus IgM and risk factors is not significant going by their p-values which were greater than 0.05. This could be due to the fact that the total number of the study population infected with dengue virus was quite small at the time of this research. However, primary protection against infection with dengue virus depends on the control of mosquitoes (Mahyoub *et al*., 2013). Such control measures include the use of insecticide treated bed nets, spraying of insecticides and other measures to reduce the reproduction and multiplication of mosquito vectors of disease and reduce their number in the environment to the extent that they do not spread disease (WHO, 1990). In Africa, *Aedesaegypti* breeds primarily in natural habitats such as trees, holes and leaf axils and artificial containers (Rozendaal, 1997). Thus the need for effective environmental management cannot be overemphasized. Insecticide treated bed nets can be effectively used to protect infants and night workers while sleeping in day time since the vector species bite during the day time, early morning or late afternoon.

The relationship between the percentage occurrence of dengue virus IgM and some demographic factors such as age, gender, highest educational level attained, marital status and occupation was not significant going by their p-values which were greater than 0.05. Dengue infection is not peculiar to any age group or sex (Dawurung *et al*., 2010) indicating that everyone is at risk of contracting the disease. However, with regards to gender distribution, the prevalence of dengue virus IgM in females (2.3%) was higher than that of males with percentage occurrence (0.8%) and odds ratio analysis show that females are 2.877 times more likely to come down with dengue virus infection than males. This could be due to the fact that *Aedes* sp breeds in human dwelling and surroundings and women are

more likely to remain in and around the home, carrying out domestic activities during the day when the mosquitoes are most active, feeding indoors and outdoors. This agrees with previous research by Shu-yuan, (2007); Dawurung *et al*.(2010); Idris *et al*.(2010). With regards to highest educational level attained, dengue virus IgM was not detected in those with tertiary education. Dengue transmission by *Aedes aegypti* has been associated with poor hygienic conditions, which in turn may be associated with low socio economic levels and to low schooling of the house dwellers (Rodriguez-Figuerod *et al*., 1995). In Kenya, the use of insecticide-impregnated bed nets was significantly related to mother's educational level (Marsh *et al*., 1996).

The difference in the percentage occurrence of dengue virus IgM in relation to current use of anti-malarial drug is not significant ($p = 0.064$), although, 3.7% of those that were on anti-malarial drug during the time of the research were sero-positive for dengue virus IgM. This is of immense public health importance as dengue can be misdiagnosed (by under-diagnosis or over-diagnosis) despite available clinical guidelines (Jose *et al*., 2007). Given the lack of specificity of the symptoms of dengue fever, clinicians can confuse dengue with symptoms of other diseases such as influenza, 'chikungunya', leptospirosis, malaria or typhoid fever (Halstead, 1997). Misdiagnosis can be influenced by treatment guidelines, for example, although guideline for the treatment of febrile children aged 2 months to 5 years are useful to ensure that children with fever and no alternative explanation are empirically treated for malaria, this guideline may contribute to the misdiagnosis of dengue, particularly in area of low malaria transmission or where physicians are not routinely using malaria smears to confirm diagnosis (Jose *et al*., 2007).

The percentage occurrence of dengue virus IgM in relation to symptoms showed no significant association ($p>0.05$). However, people with fever,diarrhoea and abdominal discomfort had higher percentage occurrences of dengue virus IgM and were more likely to have dengue virus IgM than those who do not display these symptoms. Similar clinical manifestations have been reported in different studies (Ayyub *et al*., 2006; Shahin *et al*., 2009). This is of immense public health importance because people who present with these symptoms at clinics and hospitals are presumably treated for malaria especially in malaria endemic country like Nigeria (FMH, 2001).

The relationship between the occurrence of malaria and some risk factors from this study showed that a significant difference exist in the percentage occurrence of malaria parasite according to frequency of use of mosquito bed net ($p=0.039$). The relationship between the occurrence of malaria parasite and frequency of use of insecticides, presence of ditches and or bush close to residential areas and opening of doors in the early mornings and evenings was not significant going by their p-values which were greater than 0.05. The significant difference that existed in the percentage occurrence of malaria parasite according to frequency of mosquito bed net use is in consonance with the work of Gahutu *et al.* (2011). Those who live in residential areas where sanitation is very good are more likely to belong to higher socio economic class and thus better endowed to acquire mosquito bed nets (Ogunlesi *et al*., 2005). People are therefore admonished to use mosquito bed nets and repellents on their windows and doors irrespective of their abode as this is relevant in the determination of who develops malaria or not.

The relationship between the percentage occurrence of malaria and some demographic factors showed that the occurrence of malaria in relation to different age

groups and gender is not significant. (p=0.052 and 0.793 respectively). However, the occurrence of malaria in relation to highest educational level attained is significant (p=0.038) in this study. Marital status and occupation showed no significant association with malaria (p=0.835 and 0.251 respectively). Some researchers however, reported that pregnant females of less than 20 are more at risk of coming down with malaria due to their decreased level of immunity (Agomo *et al*., 2009; Tyndall *et al*., 2012). The present research did not look at the pregnant status of females used in the study population.

Education can have a positive impact on malaria burden (Dike *et al*., 2006). The action taken by mothers when the child has malaria differed with their level of education (Kakai *et al*., 2009). Mothers with post-secondary education were more likely to take their sick children to hospital than take any other action, while those with no formal education were more likely to take other action than hospital (Kakai *et al*., 2009). There is a direct relationship between formal education and occupation. Those with the highest level of education were more likely to be better employed and thus better able to afford better medicare (Dike *et al*., 2006). Marital status had no significant effect of the percentage occurrence of malaria in the present study though, the single were more infected than the married, this agrees with Okwa and Ibidapo(2010).

The relationship between the percentage occurrence of malaria according to symptoms of fever was significant in the resent study (P=0.036). This is very important in the present study as a higher percentage of people who had fever were sero-positive for dengue virus IgM. Therefore, patients presenting in the clinic with suspected fever should be subjected to laboratory diagnosis for conformation. Diagnosis on the basis of clinical evidence alone is unreliable, misleading and could lead to development of drug resistance,

increase in adverse drug reactions, accumulation of toxic malaria drug and increase in drug budgets (Jonkman *et al*., 1995; Igbeneghu *et al*., 2009). The relationship between occurrence of malaria and symptoms of headache, muscle and joint pains, cold, abdominal discomfort, diarrhoea and vomiting was not significant going by their p-values which were greater than 0.05. Although, WHO(2011) report these as the symptoms of malaria, these signs and symptoms are not discriminatory for malaria (Marc *et al*., 2013) as observed in the present study.

The association between occurrence of malaria and current use of anti-malaria drug was not significant ($p = 0.261$). This may be due to worldwide reported case of multidrug resistance by malaria parasite (Olurishe *et al*., 2007). Despite the wide range resistance that has been reported in various parts of the country, and indeed the West African sub region, several health care professionals still prescribe chloroquine as the drug of choice for uncomplicated malaria (Ndube, 2004). This may not be unconnected with the apparently low cost of obtaining chloroquine in any of its pharmaceutical dosage forms (Olurishe *et al*., 2007). This has led the World Health Organization to recommend combination therapy as first line treatment, with regimens containing artemisinine based compounds as gold standard (Olurishe *et al*., 2007). However, the cost of artemisinine combination therapies compared to available anti-malaria drug is high, for example, artemether-lumefantrine combination has no substitute and is beyond the reach of average citizen (Olurishe *et al*., 2007),

The comparison of microscopy and rapid diagnostic tests as used for the determination of malaria status in this research shows no significant difference between the two methods in the determination of malaria status. However, microscopy using Giemsa

stain performed better than the rapid diagnostic test kit. Giemsa microscopy is regarded as the most suitable diagnostic instrument for malaria control because it is inexpensive to perform, able to differentiate malaria species and quantify parasites. In the age of high quality light emitting diodes (LED) illumination and solar battery chargers, microscopy has become more feasible in remote areas (Wongsrichanalari *et al*., 2007). However, microscopy requires well-trained, competent microscopist and rigorous maintenance of functional infrastructures plus effective quality control.

CHAPTER SIX

6.0 SUMMARY AND CONCLUSION

6.1 Summary and Conclusion

1. In this research, the presence of dengue virus in the study population was determined giving a prevalence of 1.8% using the ELISA technique.
2. The presence of malaria parasites in the study population was detected. The prevalence was 46.5% using the microscopy method.
3. The risk factors identified did not have any significant association with the occurrence of dengue virus in the study population. However, use of insecticide treated bed nets and highest educational level attained were significantly associated with the occurrence of malaria.
4. A total of 2(1.3%) of the malaria positive cases were also infected with dengue virus.

6.2Recommendations

1. Long lasting physical transformation of vector habitats, for example, improved water supply, mosquito proofing of overhead tanks, cisterns or underground water reservoir should be enhanced.
2. Effort should be made to reduce man-virus contact by mosquito proofing of houses with screens on doors/windows.
3. Protective clothing and repellents which are common means of protection against mosquitoes and other biting insects should be enhanced.

4. Biological control of mosquitoes should be stepped up for example, larvivorous fish are recommended for the control of *Aedes aegypti* in large water bodies or large water containers and endotoxin-producing bacteria, *Bacillus thuringensis* serotype H-14 (Bt H-14) has been found to be an effective mosquito control agent.
5. Chemical control measures are recommended in permanent/big water containers where water has to be conserved or stored because of scarcity of water or irregular and unreliable water supply.
6. Involvement of household, community for mosquito control is of paramount importance as the problem revolves mainly around man and his environment. There should be a continuous dialogue between health personnel and the community so that people may accept *Aedes* control programmes as their own programme. Community should be involved in the task of elimination of *Aedes* breeding in and around their houses for keeping houses free of larval breeding and reduction/elimination of adult mosquitoes. Towards these objectives, massive, repetitive, intense and persistent Behaviour Change Communication campaign is crucial. The community must be assured that these are preventable disease and empowered with the knowledge about mode of transmission, vector control options, availability of services in addition to correct treatment, so that timely and appropriate action is taken. Special campaigns may be carried out through mass media including local vernacular newspapers/magazines, radio and TV, especially using local cable networks as well as outdoor publicity like drum beating, rallies, etc. Health education materials should be developed and widely disseminated in the

form of posters, pamphlets, handbills. Inter-personal communication through group meetings, traditional / folk media particularly must be optimally utilized.

7. In order to achieve sustainability of successful DF/DHF vector control programme, it is essential to focus on involvement of hospitals, non-health sector departments including schools/colleges, civil society organizations (NGOs, Faith Based Organizations and Community Based Organizations like Residents. Welfare Organizations, Self-Help Groups).

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APPENDICES

Appendix I: Ethical Consent obtained from the Kaduna State Ministry of Health



MINISTRY OF HEALTH, KADUNA STATE

All Communications to be Addressed to:
THE HON. COMMISSIONER
Quoting Reference and Data
Tel: (062) 248084
(062) 248252

Independence Way,
P.M.B. 2014,
Kaduna,
Kaduna State, Nigeria.

MOH/ADM/744/Vol.1/195

7th September, 2012.

.....
.....
.....

ETHICAL APPROVAL

I am directed to convey the Honorable Commissioner's wish to grant approval to Idoko, Matthew Owoicho, currently on his MSc. program from Department of Microbiology, Faculty of Science, ABU – Zaria. Topic: **SERO – EPIDEMIOLOGICAL SURVEY OF DENGUE VIRUS INFECTION IN MALARIA PATIENTS** in parts of Kaduna State.

It is expected that necessary assistance be accorded to him in the process of his research, please.

However, it is mandatory for the researcher to submit a copy of his research finding(s) to the office of the Honorable Commissioner, Health as soon as it is concluded as you have the assurances of my highest regards.

2/10/12.

JACOB KAYIT MUSA
SECRETARY, ETHICAL COMMITTEE

Appendix II: Informed Consent Form 1.

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 men, women and children attending some selected Hospitals in Kaduna State. We are inviting you to participate in this research work titled "Sero-epidemiological survey of dengue virus infection in malaria patients in some parts of Kaduna State, Nigeria. Dengue virus is a small tiny organism (a virus) that causes diseases such as dengue fever, dengue hemorrhagic fever and dengue shock syndrome. The research will involve the collection of blood samples of participants. The results obtained thereby may be used in any way to improve the understanding and management of the disease in our community. 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 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: Informed Consent Form 2

DEPARTMENT OF MICROBIOLOGY
SCHOOL OF POSTGRADUATE STUDIES
AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA.
Head of Department: **Prof. S.A. Ado;** B.Sc, M.Sc, PhD E-mail: microbiology@abu.edu.ng

INFORMED CONSENT FORM (ICF)

This Informed Consent Form is for Malaria patients attending clinics in some hospitals in Kaduna State. We are inviting you to participate in this research work titled "Sero-epidemiological Survey of Dengue Virus Infection in Patients suspected of Malaria in some parts of Kaduna State, Nigeria.

Principal Investigator: Idoko, Matthew Owoicho

Collaborating Investigators: Prof. S.A. Ado

Prof. V.J. Umoh

Name of Organization: Department of Microbiology

Ahmadu Bello University, Zaria - Nigeria

Name of Sponsor: Self

Name of Proposal: Postgraduate Research Proposal

This Informed Consent Form has two parts:

- Information Sheet (to share information about the research with you)
- Certificate of Consent (for signatures if you agree to take part)
  You will be given a copy of the full Informed Consent Form

PART I: INFORMATION SHEET

Introduction

I am Idoko, Matthew Owoicho,a postgraduate student of the Department of Microbiology, Ahmadu Bello University, Zaria, carrying out a research work under the supervision of Prof. S.A. Ado and Prof. V.J. Umoh.

We are doing a research work on Sero-epidemiological Survey of Dengue Virus Infection in Patients suspected of Malaria in some parts of Kaduna State, Nigeria. I am going to give you information and invite you to be part of this research. You do not have to decide today whether or not you will participate in the research. Before you decide, you can talk to anyone you feel comfortable with about the research.

There may be some words that you do not understand. Please ask me to stop as we go through the information and I will take time to explain. If you have questions later, you can ask them of me, the study doctor or the staff.

Purpose of the research
The purpose of this research is to survey the sero-epidemiology of dengue virus infection in malaria patients in parts of Kaduna State, Nigeria.

Participant selection

We are inviting all men, women and children with febrile illness who are attending hospitals in some parts of Kaduna State to participate in the research work on the Sero-epidemiological survey of Dengue virus infection in malaria patients.

- *Do you know why we are asking you to take part in this study? YES.....NO……..*
- *Do you know what the study is about? YES..... NO. .....*

Voluntary Participation

Your participation in this research is entirely voluntary. It is your choice whether to participate or not. Whether you choose to participate or not, all the services you receive at this clinic will continue and nothing will change. If you choose not to participate in this research project, you will be offered the treatment that is routinely offered in this clinic/hospital for malaria patients and we will tell you more about it later. You may change your mind later and stop participating even if you agreed earlier.

- *If you decide not to take part in this research study, do you know what your options are?YES…… NO……*
- *Do you know that you do not have to take part in this research study, if you do not wish to? YES…… NO …….*
- *Do you have any questions? YES……. NO. ……*

Procedures and Protocol

When you have agreed to participate in this research, we will take blood from your arm using a syringe and needle and also prick your finger using a sterile needle to collect blood. Vial or other small container containing small amount of EDTA in it, will be used to show you the amount of blood that we will collect from you).At the end of the research, any leftover blood will be destroyed.

Description of the Process

During the research you will make just one visit to the clinic.

In the visit, a small amount of blood will be taken from your arm with a syringe. This blood will be tested for the presence of malaria parasite and Dengue virus and rapid analysis will be carried out. We will also ask you a few questions about your general health and other measures so as to investigate the epidemiologic risk factors associated with the occurrence of Dengue virus.

Duration

The research will take place over two months. During that time, it will be necessary for you to come to the clinic just once. After that, the research will finish.

- *How many times are weasking you to come to the hospital to participate in the research?.....*
- *Can you tell me the number of time we will take your blood sample?.....*
- *Do you have any questions?.....*
- *Do you want me to go through the procedures again?.....*

Risks

There is a risk that you may share some personal or confidential information by chance, or that you may feel uncomfortable talking about some of the topics. However, we do not wish for this to happen. You do not

have to answer any question or take part in the research if you feel the question(s) are too personal or if talking about them makes you uncomfortable.

Benefits

If you participate in this research, you will have the following benefits:

- ✓ You will have the opportunity of having tested for malaria.
- ✓ Your blood will be screened for Dengue virus.
- ✓ There may not be any benefit to the society at this stage of the research, but future generations are likely to benefit.
- ✓ Apart from these few benefits listed above, your participation is likely to help us find answer to the research questions.

Reimbursements

You will not be provided with any special incentive or travel allowance for you to take part in the research. But we advise that your visit to the clinic will have to be on your normal schedule clinic days. However, you will have the opportunity of doing free malaria test.

Can you tell me if you have understood correctly the benefits that you will have if you take part in the study?.....

- ➢ *Do you know that the study will not pay for your travel costs?.....*
- ➢ *Do you know the kind of benefit you can get by participating in this research?.....*
- ➢ *Do you have any other questions?.....*

Confidentiality

With this research, it is possible that if others in the community are aware that you are participating, they may ask you questions. We will not be sharing the identity of those participating in the research.

The information that we collect from this research project will be kept confidential. Information about you that will be collected during the research will be put away and no-one but the researchers will be able to see it. Any information about you will have a number on it instead of your name. Only the principal investigator will know what your number is and we will lock that information up with a lock and key. It will not be shared with or given toanyone except the principal investigator.

- ➢ *Did you understand the procedures that we will be using to make sure that any information that we as researchers collect about you will remain confidential?.....*
- ➢ *Do you have any questions about them?.....*

Sharing the Results

The knowledge that we get from doing this research will be shared with you through community meetings before it is made widely available to the public. Confidential information will not be shared. There will be small meetings in the community and these will be announced. After these meetings, we will publish the results in order that other interested people may learn from our research.

Right to Refuse or Withdraw

You do not have to take part in this research if you do not wish to do so, refusing to participate will not affect your treatment at this clinic in any way. You will still have all the benefits that you would otherwise have at this clinic. You may stop participating in the research at any time that you wish without losing any of your rights as a patient here. Your treatment at this clinic will not be affected in any way.

Who to Contact

If you have any questions you may ask them now or later, even after the study has started. If you wish to ask questions later, you may contact any of the following:

IDOKO, Matthew O. 08038178086, 08059534953

This proposal has been reviewed and approved by Scientific Ethical Committee (SEC) of the Ministry of Health and other hospitals, which is a committee whose task is to make sure that research participants are protected from harm. If you wish to find out more about the SEC, contact the office of the Assistant Dean, School of postgraduate studies, ABU, Zaria.

- *Do you know that you do not have to take part in this study if you do not wish to? ……*
- *Do you know that you can ask me questions later, if you wish to? ……*
- *Do you know that I have given the contact details of the person who can give you more information about the study?.....*
- *You can ask me any more questions about any part of the research study, if you wish to. Do you have any questions?.....*

PART II: CERTIFICATE OF CONSENT

I have read the foregoing information, or it has been read and translated to me in language that I understand. I have also talked it over with the doctor to my satisfaction. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I understand that my participation is voluntary. I know enough about the purpose, methods, risks and benefits of the research study to judge that I want to take part in it. I understand that I may freely stop being part of this study at any time. I have received a copy of this consent form and additional sheet to keep for myself. I therefore consent voluntarily to participate as a participant in this research.

Name of Participant:

.....

Signature of Participant: .....

Date: .....

Day/month/year

Statement by Witness

I have witnessed the accurate reading of the consent form 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: ..... AND Thumb print of participant

Signature of witness: _____

Date _____

Day/month/year

Statement by the Researcher/Person Taking Consent

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands that the following will be done:

1. *Blood sample will be taken*

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.

A copy of this ICF has been provided to the participant.

Name of Researcher/person taking the consent:.....

Signature of Researcher /person taking the consent: _____

Date _____

Day/month/year

Appendix IV: ELISA procedure

The pack containing the kits and reagents were removed from the freezer where they were stored at -2^{o}C before usage. The reagents were removed and brought to room temperature (25^{o}C). Using separate plain sterile microtitre plates, the number of wells needed were broken off (first well for blank, next two for positive control, another two for positive control plus number of samples). The positive and negative controls were not diluted further as they were supplied pre-diluted. Forty micro litres of RF absorbent (goat anti-human IgG) was added to four microtubes and 100 µl of positive and negative controls were added to two tubes each and mixed well. All 140 µl mixtures were transferred to the reaction wells after ten minutes of incubation. Patient sera were diluted 1:40 using the dilution buffer provided. One hundred micro litres of the 1:40 diluted sera were transferred to separate microtubes and 40 µl of RF absorbent was added and mixed well. These were incubated for 10 minutes at room temperature and all 140 µl of test samples were transferred to appropriate wells. These were incubated at room temperature (25ºC) for 10 minutes.

The contents were shaken out and washed three times with diluted wash buffer (concentrated buffer and surfactant). Two drops of enzyme conjugate (anti-human IgM conjugated to peroxidase) were added to each well and incubated at room temperature for 10 minutes. The contents were shaken out and washed three times with wash buffer and thereafter wells were slapped against paper towels to remove all liquid. Two drops of the chromogen (tetramethylbenzidine) was added to each well and incubated at room temperature for 10 minutes. Two drops of the stop solution (1M phosphoric acid) were added to each well and mixed by tapping the strip holder. Reading was done in less than

thirty minutes of adding the stop solution using the ELISA reader which was set for biochromatic readings at 450/630 nm (Appendix X and XI).

Appendix V: Questionnaire administered for the assessment of risk factors, demographic data and symptoms.

QUESTIONNAIRE

A research on **Sero-epidemiological Survey of Dengue Virus Infection in Malaria Patients in parts of Kaduna State, Nigeria.** Please do honestly fill this questionnaire as this will go a long way in affecting the result of the research.

Thank you very much in anticipation of your cooperation.

SECTIONA:BIODATA

1. Reference number: …….
2. Age (years): 1 – 10 ( ), 11 – 20 ( ), 21 – 30 ( ), 31 – 40 ( ), 40 – 50 ( ), 50 and above ( ).
3. Gender: Male ( ), Female ( ).
4. Educational level: Tertiary ( ), Secondary ( ), Primary ( ), Quranic ( ), None ( ).
5. Marital status: Single ( ), Married ( ), Widowed ( ), Separated ( ).
6. Occupation: Civil servant ( ), Self-employed ( ), Military ( ), Others ( ).

SECTIONB: **CLINICALDATA**

Presenting symptoms of ailment

1. Fever, Yes ( ) No ( )
2. Headache, Yes ( ) No ( )
3. Muscle and joint pains, Yes ( ) No ( )
4. Cold, Yes ( ) No ( )
5. Abdominal discomfort, Yes ( ) No ( )
6. Diarrhoea, Yes ( ) No ( )
7. Vomiting, Yes ( ) No ( )
8. Rash, Yes ( ) No ( )

SECTION C: RISKFACTORS

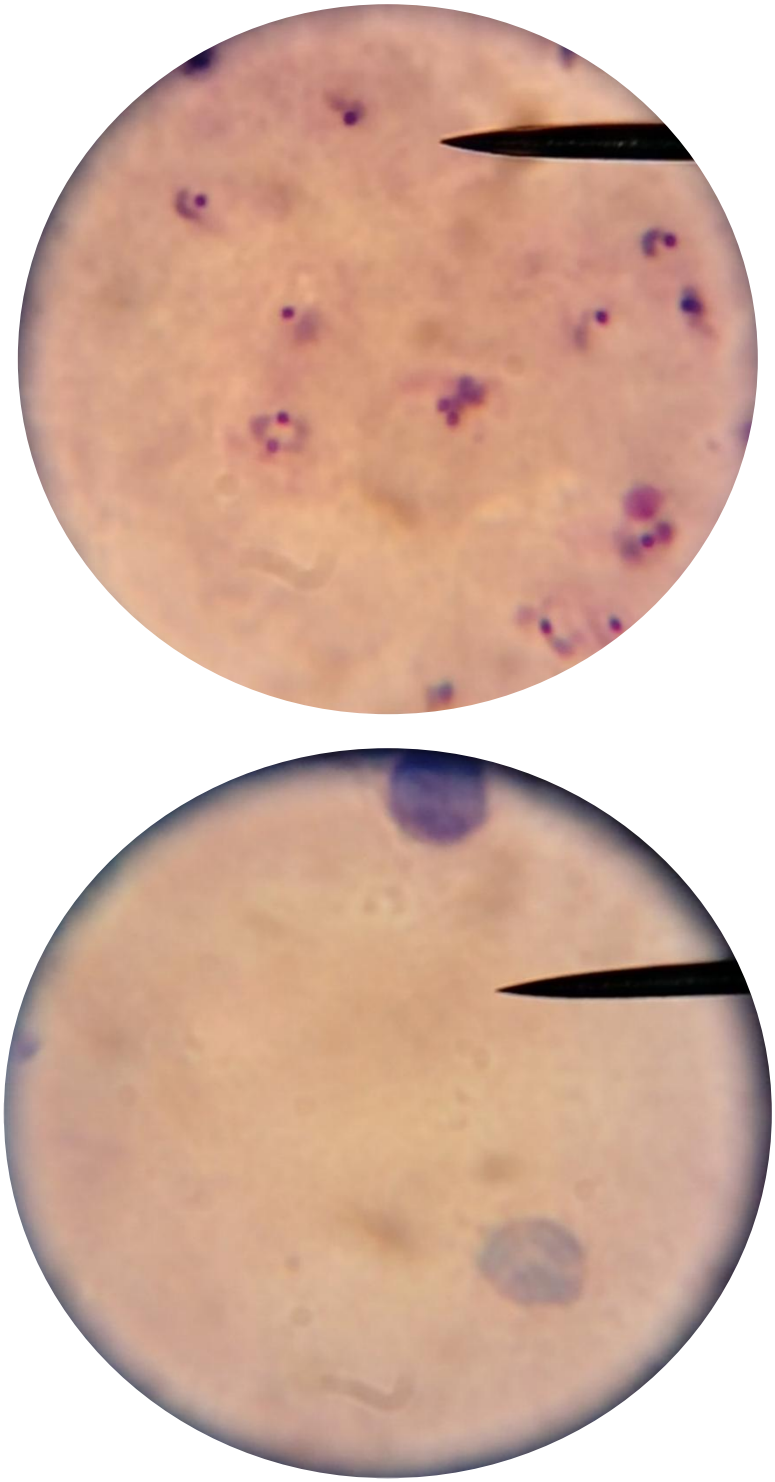
1. Do you use mosquito net? Always ( ), Occasionally ( ), Never ( ).
2. Do you use insecticides? Always ( ), Occasionally ( ), Never ( ).
3. Do you have gutters, bushes etc., around or near your house? Yes ( ), No ( ).
4. Do you sleep in the afternoon? Always ( ), Occasionally ( ), Never ( ).

5. How often do you use mosquito net and insecticides when you sleep in the afternoons? Always ( ), Occasionally ( ), Never ( ).
6. Do you leave your doors open in the evenings? Always ( ), Occasionally ( ), Never ( ).
7. How often do you use anti-malaria drugs? Always ( ), Occasionally ( ). Never ( ).
8. Are you currently on any anti-malaria drug? Yes ( ), No( ).

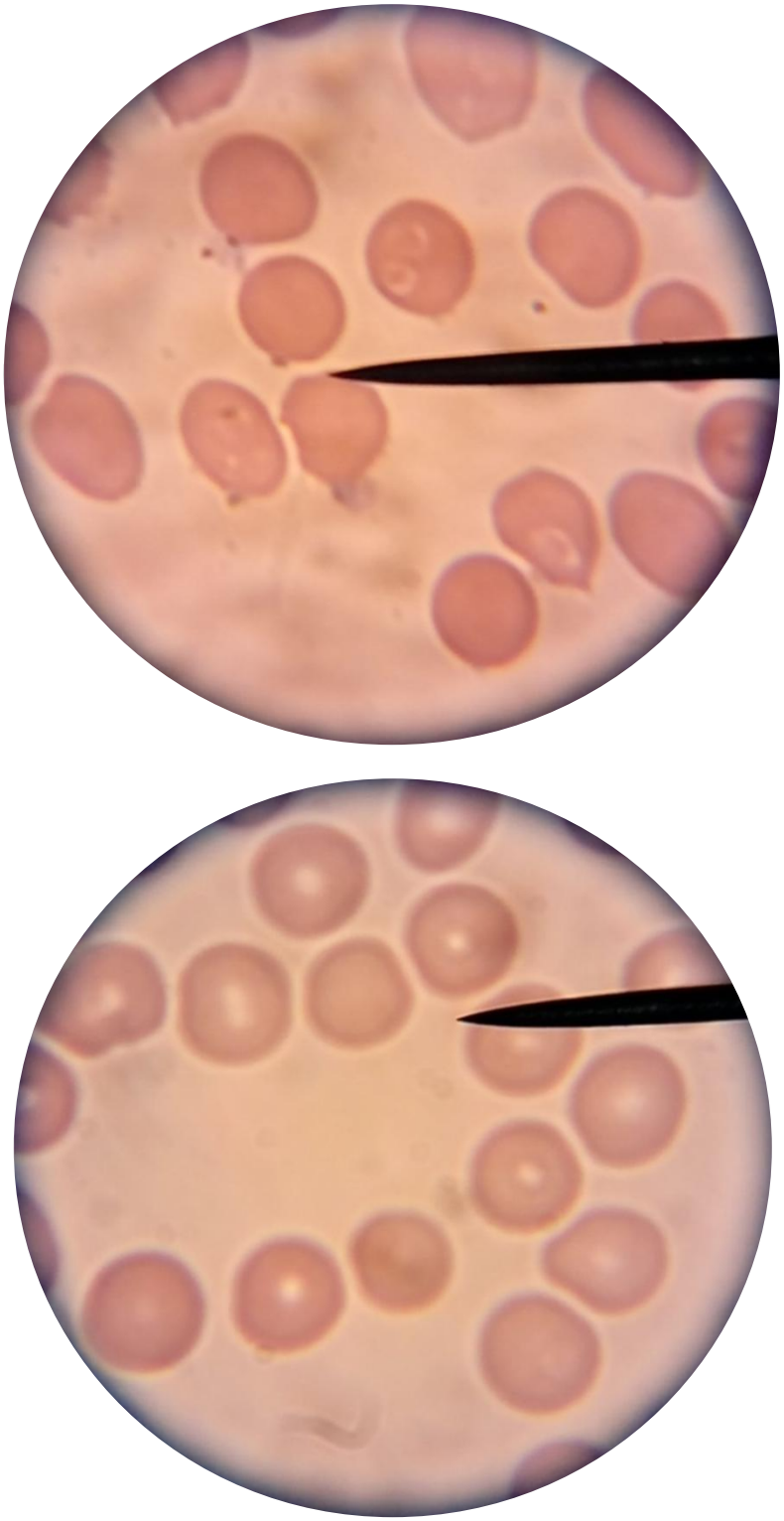
Appendix VI: Prepared thick and thin blood smears ready for staining and malaria rapid diagnostic test kit



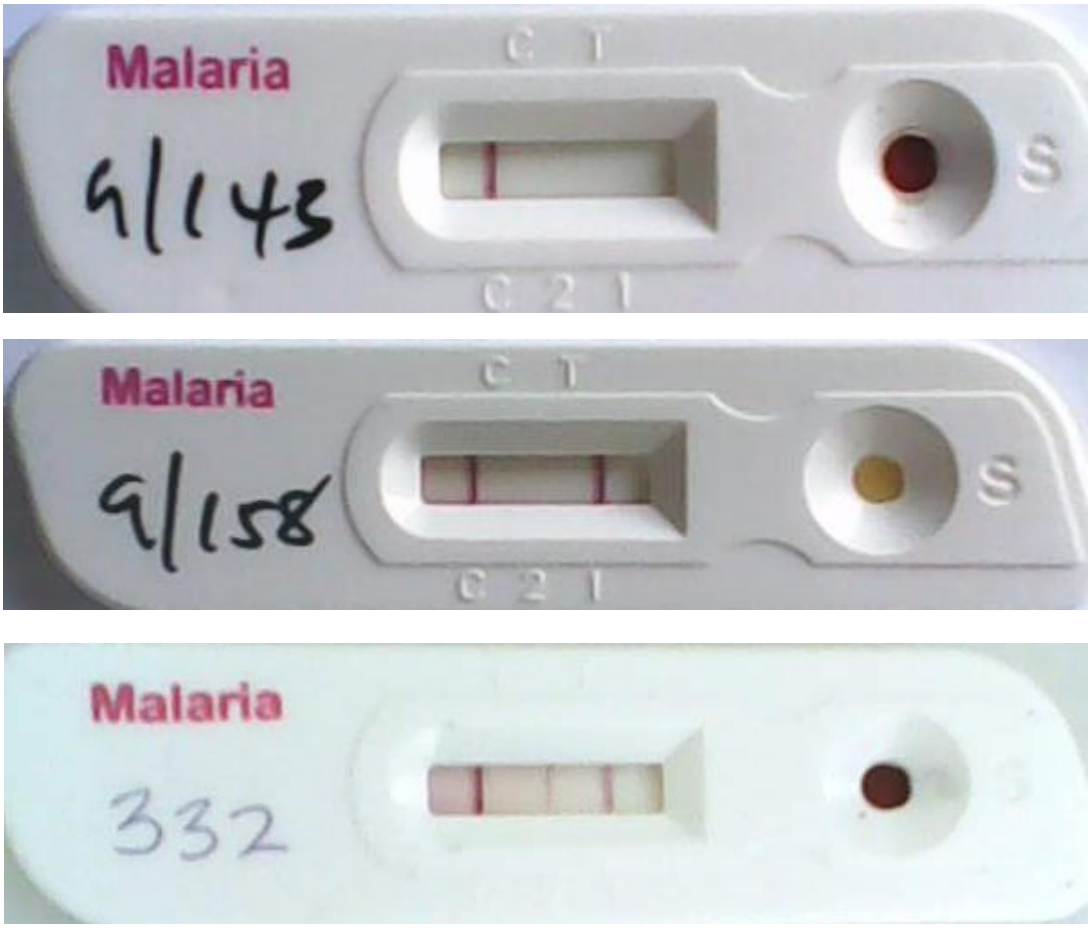
Appendix VII: Malaria thick film micrograph (Positive and negative slides respectively)



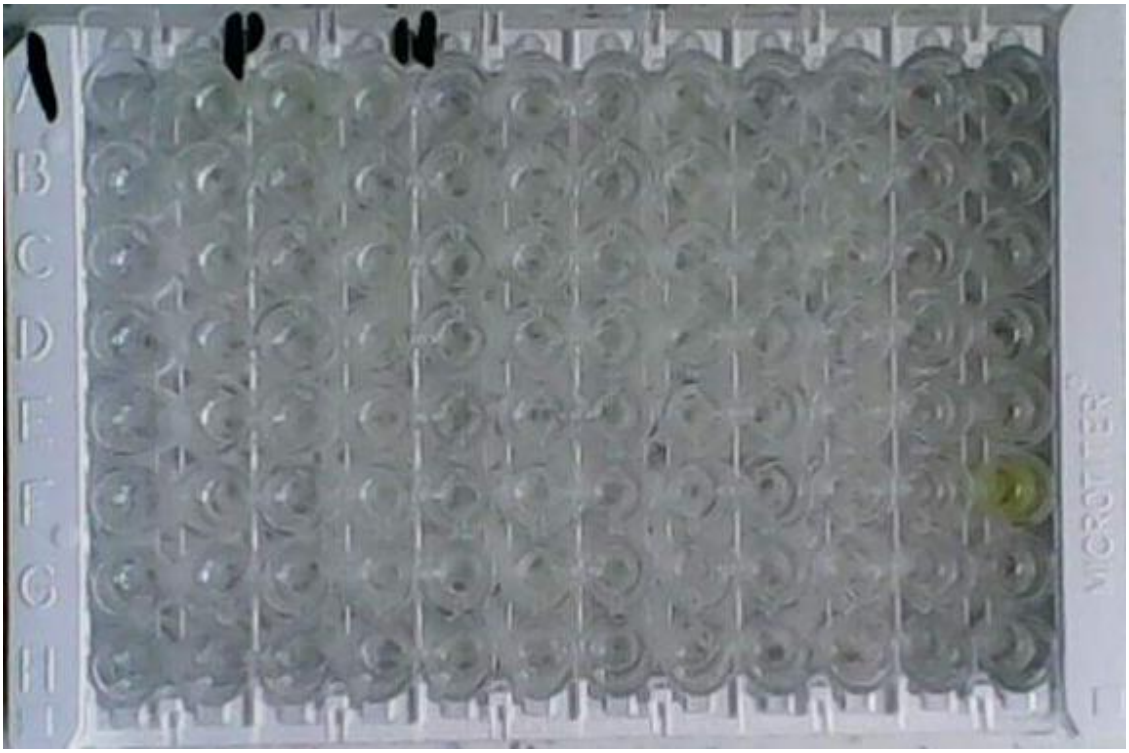
Appendix VIII: Malaria thin film micrograph (Positive and Negative slides respectively)



Appendix IX: Malaria Rapid Diagnostic Test kit (Negative test, Positive test for *Plasmodium falciparum* and Positive test for *Plasmodium falciparum* and Pan)



Appendix X: ELISA Microtitre Plate used for Determination of Dengue virus IgM



Appendix XI: Result of ELISA Optical Density for Dengue virus IgM, Plate 1.

	1	2	3	4	5	6	7	8	9	10	11	12
Hole	B	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
OD	0.005	0.074	0.079	0.000	0.004	0.001	0.000	0.003	0.003	0.000	0.001	0.002
Hole	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23
OD	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Hole	S24	S25	S26	S27	S28	S29	S30	S31	S32	S33	S34	S35
OD	0.011	0.009	0.012	0.010	0.013	0.012	0.011	0.011	0.008	0.009	0.010	0.008
Hole	S36	S37	S38	S39	S40	S41	S42	S43	S44	S45	S46	S47
OD	0.005	0.006	0.007	0.004	0.006	0.006	0.004	0.009	0.006	0.005	0.006	0.006
Hole	S48	S49	S50	S51	S52	S53	S54	S55	S56	S57	S58	S59
OD	0.003	0.004	0.002	0.003	0.001	0.004	0.001	0.005	0.000	0.003	0.001	0.007
Hole	S60	S61	S62	S63	S64	S65	S66	S67	S68	S69	S70	S71
OD	0.001	0.002	0.004	0.000	0.002	0.005	0.001	0.006	0.001	0.000	0.022	0.382
Hole	S72	S73	S74	S75	S76	S77	S78	S79	S80	S81	S82	S83
OD	0.009	0.007	0.009	0.007	0.012	0.013	0.007	0.009	0.009	0.004	0.005	0.008
Hole	S84	S85	S86	S87	S88	S89	S90	S91	S92	S93	S94	S95
OD	0.009	0.007	0.011	0.007	0.010	0.013	0.004	0.003	0.004	0.004	0.004	0.008

Appendix V Continued Plate 2:

	1	2	3	4	5	6	7	8	9	10	11	12
Hole	B	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
OD	0.004	0.011	0.013	0.002	0.002	0.001	0.003	0.000	0.000	0.000	0.000	0.000
Hole	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23
OD	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.022	0.000	0.002	0.000	0.000
Hole	S24	S25	S26	S27	S28	S29	S30	S31	S32	S33	S34	S35
OD	0.006	0.015	0.011	0.010	0.009	0.013	0.017	0.014	0.015	0.016	0.013	0.012
Hole	S36	S37	S38	S39	S40	S41	S42	S43	S44	S45	S46	S47
OD	0.003	0.008	0.006	0.005	0.006	0.007	0.009	0.008	0.008	0.007	0.005	0.006
Hole	S48	S49	S50	S51	S52	S53	S54	S55	S56	S57	S58	S59
OD	0.002	0.003	0.004	0.002	0.005	0.002	0.004	0.001	0.004	0.000	0.002	0.000
Hole	S60	S61	S62	S63	S64	S65	S66	S67	S68	S69	S70	S71
OD	0.000	0.006	0.011	0.003	0.001	0.007	0.003	0.003	0.003	0.000	0.004	0.002
Hole	S72	S73	S74	S75	S76	S77	S78	S79	S80	S81	S82	S83
OD	0.009	0.012	0.010	0.009	0.008	0.033	0.009	0.006	0.008	0.009	0.010	0.010
Hole	S84	S85	S86	S87	S88	S89	S90	S91	S92	S93	S94	S95
OD	0.008	0.007	0.009	0.007	0.007	0.010	0.006	0.006	0.009	0.007	0.008	0.007

Appendix V Continued Plate 3

	1	2	3	4	5	6	7	8	9	10	11	12
Hole	B	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
OD	0.001	0.006	0.009	0.000	0.002	0.004	0.001	0.002	0.000	0.005	0.003	0.003
Hole	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23
OD	0.000	0.000	0.000	0.000	0.000	0.002	0.004	0.000	0.002	0.002	0.000	0.000
Hole	S24	S25	S26	S27	S28	S29	S30	S31	S32	S33	S34	S35
OD	0.013	0.013	0.017	0.013	0.015	0.015	0.013	0.016	0.014	0.018	0.015	0.016
Hole	S36	S37	S38	S39	S40	S41	S42	S43	S44	S45	S46	S47
OD	0.009	0.010	0.009	0.009	0.008	0.009	0.011	0.011	0.015	0.012	0.010	0.010
Hole	S48	S49	S50	S51	S52	S53	S54	S55	S56	S57	S58	S59
OD	0.007	0.005	0.006	0.004	0.005	0.006	0.010	0.005	0.006	0.004	0.005	0.004
Hole	S60	S61	S62	S63	S64	S65	S66	S67	S68	S69	S70	S71
OD	0.002	0.009	0.010	0.011	0.000	0.011	0.011	0.010	0.008	0.006	0.008	0.009
Hole	S72	S73	S74	S75	S76	S77	S78	S79	S80	S81	S82	S83
OD	0.016	0.015	0.015	0.014	0.015	0.017	0.013	0.013	0.013	0.016	0.016	0.012
Hole	S84	S85	S86	S87	S88	S89	S90	S91	S92	S93	S94	S95
OD	0.012	0.011	0.011	0.011	0.010	0.012	0.011	0.011	0.010	0.011	0.011	0.011

Appendix V Continued Plate 4

	1	2	3	4	5	6	7	8	9	10	11	12
Hole	B	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
OD	0.004	0.005	0.004	0.005	0.024	0.000	0.009	0.100	0.003	0.000	0.001	0.003
Hole	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23
OD	0.010	0.010	0.009	0.009	0.005	0.009	0.015	0.011	0.012	0.013	0.013	0.011
Hole	S24	S25	S26	S27	S28	S29	S30	S31	S32	S33	S34	S35
OD	0.005	0.003	0.008	0.005	0.017	0.013	0.007	0.005	0.005	0.008	0.005	0.005
Hole	S36	S37	S38	S39	S40	S41	S42	S43	S44	S45	S46	S47
OD	0.005	0.008	0.001	0.005	0.000	0.004	0.003	0.004	0.003	0.003	0.002	0.009
Hole	S48	S49	S50	S51	S52	S53	S54	S55	S56	S57	S58	S59
OD	0.007	0.004	0.002	0.002	0.000	0.007	0.002	0.003	0.002	0.005	0.002	0.004
Hole	S60	S61	S62	S63	S64	S65	S66	S67	S68	S69	S70	S71
OD												
Hole	S72	S73	S74	S75	S76	S77	S78	S79	S80	S81	S82	S83
OD												
Hole	S84	S85	S86	S87	S88	S89	S90	S91	S92	S93	S94	S95
OD												