A STUDY ON ASYMPTOMATIC MALARIA IN APPARENTLY HEALTHY INDIVIDUALS IN AHMADU BELLO UNIVERSITY MAIN CAMPUS SAMARU, ZARIA

BY

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Msc/Sci/33483/02-03

AUGUST, 2006

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A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, AHMADU BELLO UNIVERSITY, ZARIA, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTERS OF SCIENCE (MICROBIOLOGY)

DEPARTMENT OF MICROBIOLOGY, FACULTY OF SCIENCE, AHMADU BELLO UNIVERSITY, ZARIA

AUGUST, 2006

DECLARATION

I hereby declare that the work presented in this thesis is original. It was carried out by me in the laboratory of the Department of Microbiology Ahmadu Bello University, under the close supervision of Prof. M. Galadima and Prof. W.N. Ogala. No part of this thesis has previously been submitted for any degree or qualification. References made to the work of other investigators are duly acknowledged.

_____

OJO O.T

CERTIFICATION

This thesis titled "A Study On Asymptomatic Malaria in Apparently Healthy Individuals in Ahmadu Bello University Main Campus Samaru, Zaria" by **OJO, OLUWOLE TEMITOPE** meets the regulations governing the award of the degree of Master of Science Ahmadu Bello University, Zaria and it is approved for its Scientific Contribution to knowledge and literary presentation.

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DEDICATION

This thesis is dedicated to my parents Deacon and Mrs. J.O. Ojo and all the victims of Malaria

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ABSTRACT

Four hundred apparently healthy individuals were sampled from Ahmadu Bello University Campus Samaru for Asymptomatic Parasitaemia using Giemsa stained thick and thin films as well as the ACON malaria parasite F kit. One hundred and five of the sampled individuals had Asymptomatic Parasitaemia with a parasite rate of 26.25% and parasite density of 3.43 per ul of blood. Plasmodium falciparum had the highest rate of occurrence (62%)while the lowest rate of occurrence was seen in P ovale (4.76%). P. malariae accounted for (21%) while 13.38% were mixed infections. Statistical analysis indicated that Asymptomatic Parasitaemia was associated with Age (P<0.05),with the highest occurrence (13.25%) in the 20-30 years age group. Asymptomatic Parasitaemia was also significantly associated with students (P<0.05), and the months of May, June and July. With this high parasite rates any form of stress on those asymptomatic individuals may lead to Clinical malaria. Compared with microscopy, the malaria parasite kit was 84% sensitive and 95% specific. The malaria kit will be more useful for epidemiological studies in P falciparum endemic areas rather than for diagnostic purposes. During raining season individuals, especially students should suspect malaria as soon as they develop any febrile illness and make appropriate consultation to allow early diagnosis and treatment.

CHAPTER ONE

1.0 INTRODUCTION:

Malaria or a disease resembling Malaria has been known for more than 4,000 years. Malaria is derived from the Italian word for "Bad Air". Malaria has probably influenced human populations and history to a great extent (C.D.C 2004a). Among the early victims of malaria, were Alexander the great, Oliver Crowell, Napoleon and his troops in his campaign against Palestine and eight German emperors (Ezedinachi 1991)

Asymptomatic parasitaemia is a condition in which an apparently healthy individual has a number of malaria parasites in the blood without showing any clinical malaria disease symptom (Rogers 1999). Malaria parasites are protozoans belonging to the subclass *Coccida* of the plylum *Apicomplexa*. The principal mode of transmission of malaria is by bites of the female anopheles mosquitoes.

Four major species namely *P. falciparum, P. malariae, P. ovale, and P. vivax* are the known etiological agents of malaria (Duguid *et al.,* 1987; Kakkilaya, 2005).

Though many control measures have been put in place, Malaria still kills both infants and adults. About 300-500 million individuals suffer the infection annually, resulting in about 1.5-2.7 million deaths World Wide (Rogers 1999, Communicable Diseases Fact Sheet 2004). In the year 2002, Malaria accounted

for 10.7% of children's death and ranked the 4th killer disease, after perinatal diseases (23.10%), lower respiratory tract infections (18.10%) and diarrhoeal diseases (15.20%) (WHO, 2003).

Routine methods used for malaria diagnosis includes microscopic examination of Giemsa stained thick and thin blood films, ELISA methods for antigen and antibody detection in the blood, Polymerase Chain Reaction (PCR) and Quantitative buffy coat method among others (Cheesbrough 1999).

Presently, over 500million people in endemic Africa, India, South East, Asia and South America are exposed to malaria disease with very high mortality rate potentials. After malaria attack, an individual may remain infective for 1-3years *P. falciparum*, 3-4years in *P. vivax* and 15-50years in *P. Malariae* (Kakkilaya 2005). Recently the Roll Back Malaria (RBM) program was represented at the Abuja O.A.U summit. Heads of states and International Agencies (UNICEF, WHO, NEPAD) committed themselves to intensify the fight against malaria in Africa (WHO, 2003; Malaria Foundation International, 2003). This is evidence that malaria still poses a threat to world's population and economy. Therefore it is important that a continuous survey of this important parasitic disease be carried out.

The aims and objectives of this study are to establish the prevalence of malaria parasitaemia in apparently healthy members of Ahmadu Bello University Community by:

(1) Determining asymptomatic malaria among students, staff, and children

(2) Determining the level of parasitaemia among the various social group

(3) Relating parasitaemia among the various age group and

(4) Comparing the sensitivity and specificity of the Malaria Parasite F kit with the conventional microscopic method of diagnosis.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Taxonomy of Malaria Parasite

The agents of Malaria are members of the *Phylum Apicomplexa*, because they are protozoans characterized by the presence of an apical complex consisting of polar rings, Conoid micronemes, rhoptries and subpellicular microtubules. All the members of the phylum are parasitic (Rogers 1999). Malaria parasites belong to the order Haemospororida and the genus *Plasmodium* (Kakkilaya, 2005)

2.2 HISTORY OF MALARIA

2.2.1 Ancient History

> The symptoms of malaria were described in ancient Chinese medical writings in 2700BC. Several characteristic symptoms of what would later be named malaria were described in the "Nei Ching" (The Canon of Medicine) which was edited by Emperor Huang T (CDC, 2004a).

In Greece, malaria became recognized by the 4th century. By the age of pericles (5th century) Hippocrates noted the principal symptoms of malaria in Asia (Ezedinachi, 1991; CDC, 2004a).

2.2.2 **Discovery of the Malaria Parasite**

Charles Louis Alphonse Laveran, a French surgeon stationed in Constantine, Algeria was the first to notice the parasite in the blood of a patient suffering from malaria. This occurred on the 6th of November 1880 (CDC 2004a).Camillo Golgo, an Italian Neurophysiologist, established that 2 forms of the disease occurred, one with tertian periodicity (fever every other day) and another with quartan periodicity (fever every 3rd day). This differentiation of malaria started in 1886 (CDC, 2004a). He also observed that fever coincided with the rupture of blood cells and release of merozoites into the blood stream.

In 1890, Italian investigators Giovani Batista and Raimonda Filetti introduced the names *Plasmodium vivax* and *P. malariae* for 2 of the species of the malaria parasite that affect humans. Laveran had believed earlier that there was only one species which he named *Oscillaria malariae*. In 1897, the naming of the parasite was reviewed by William H. Welch and he named the malignant tertian malaria parasite *P. falciparum*. The 4th human malaria parasite *P. ovale* was discovered by John W. Watson Stephen in 1922 (Bradley, 1996c; CDC, 2004a).

2.2.3 **Vectors of Malaria**

On August 20th, 1897 Ronald Ross a British officer in the Indian Medical service demonstrated that a mosquito (*Culex fatigans*) transmitted *Plasmodium relictum* from bird to bird. This discovery solved the problem of malaria

transmission. Within the year 1898-1899, a team of Italian investigators led by Giovani Batista Grassi, collected *Anopheles claviger* and fed them on malaria patients. The complete sporogonic cycle of *P falciparum*, *P. vivax* and *P malariae* was demonstrated during these studies. Therefore when *plasmodium* infected mosquitoes in Rome were sent to London in 1899, and were fed on two volunteers, both developed benign tertian Malaria. The transmission of the human Malaria parasites was thus established (CDC 2004 a).

2.3.0 **Biology of Malaria Parasites**

While it was recognized that the Anopheles Mosquito played a key role in the transmission of the disease, it was not until 1948 that all the stages in its Life cycle were identified. The parasite undergoes development in the female mosquito species that requires a blood meal for the maturation of her eggs (Bradley, 1996b; Gilles and Warell, 1993). Out of the 380 species of Anopheline Mosquitoes only 60 can effectively transmit Malaria (Bradley 1996b; Gilles and Warell, 1993). The Malaria parasites mainly (*P. falciparum, P malariae, P. ovale, and P. vivax*) have a life cycle which is split between a vertebrate host and their insect vector. All the *Plasmodium* species with the exception of *P. malariae* (which may affect the higher primates) are exclusively parasites of man (Bradley, 1996b).

2.3.1 **Life Cycle of Plasmodium SPP**

The sporozoites from the mosquito salivary glands are injected into the human as the mosquito must inject anticoagulant saliva to ensure an ever flowing meal (Duguid *et al.*, 1987; Bradley, 1996b). The sporozoites are spindle –shaped organisms about 12-um in length (Duguid *et al.*, 1987). Once in the human blood stream, the sporozoites move to the liver and penetrate the hepatocytes (parenchyma cells) where they remain for 7-16 days multiplying to produce either merozoites or hypnozoites. The merozoites are contained in exo – erythrocytic schizonts (Bradley, 1996b; Rogers, 1999). Hypnozoites which are in the latent phase remain in the liver in the case of *P. ovale* and *P. vivax*. Consequently an individual may remain infective between 1-50 years (Duguid *et al.,* 1987; Wiser, 2004b; Kakkilaya, 2005). The Schizonts usually take 5-15days to develop. Through schizogony thousands of merozoites are released when the parenchymal cell ruptures. The free parasites then enter the red blood cell. The erythrocytic phase begins from this stage and the entrance initiate erythrocytic schizogony, the development of merozoites in this phase is fundamentally the same for all four species (Duguid *et al.*, 1987). The merozoites mature and become vacuolated to form the trophozoites so that in stained preparations it acquires a signet ring appearance. The trophozoites continue to enlarge to form the erythrocytic schizont. When this stage is reached, nuclear material divides in the schizont several times to form a corresponding number of merozoites, which are released into the plasma on

rupture of erythrocyte. These merozoites invade other erythrocyte, initiating further cycles of erythrocytic schizogony. Some merozoites develop into micro and macrogametocytes, the sexual form of the parasite (Rogers, 1999).

> Another mosquito arriving to feed on blood may suck up these gametocytes into its guts where exflagellation of microgametocytes occurs and the macrogametocytes are fertilized. The resulting Ookinete from this fertilization penetrates the wall of a cell in the mid-gut of the vector(peritrophic membrane), where it develops into an Oocyst. Sporogony within the Oocyst produces many sporozoites and when the Oocyst ruptures in the haemocoele, the sporozoites migrate to the salivary gland cell and lay in the vacuoles for up to 59 days becoming 1000 times more infective than Oocyst before injection into another host (Bradley, 1996b).

F.g 1 ***A diagramatic sketch of the life cycle of Malaria parasite***

2.3.2 Vectors of Plasmodium spp

This highly specialized life cycle of the malaria parasite requires specialized biology on the part of the plasmodium species. Not all mosquitoes are vectors of the parasite because refractory mosquitoes possess substances toxic to *Plasmodium* within their cells (Weathersby and mcCroddon, 1982). Trypsin – like activity in the mid gut of resistant Anopheles species have been reported by Frizzi (Frizzi 1974 cited by Bradley 1996b), possibly inhibiting Ookinete development. Although given sufficient time and contact the parasite seems to be capable of adapting to any suitable Anopheline mosquitoes (Bradley,1996b). Sporogony within the mosquito is governed by environmental temperature as Anopheline mosquitoes are poikilotherms (Bradley, 1996b; Gilles and warell, 1996).

2.3.3 Comparison of the Life Cycle of the Four Human Plasmodium spp

Once injected into the human host, all the 4 species will penetrate hepatocytes. However, *P. falciparum and P. malariae* sporozoites trigger immediate schizogony whereas *P. ovale and P. vivax* sporozoites may exibit either immediate or delayed schizogony. Delayed schizogony results in the development hypnozoites which may result into asymptomatic malaria

parasitaemia (Duguid *et al.,* 1987; Bradley 1996b). Some strains of Plasmodium like the North Korean strain of *P ovale* seem to form long lasting hypnozoites (Bradley,1996b).

P. vivax may have an incubation period of up to 10 months while other species incubation period varies. In *P. falciparum,* erythrocytic schizogony takes 48 hours and gametocytosis takes 10-12 days (Patrick *et al.,* 1998).

2.3.4 **Cellular and Molecular Biology of Plasmodium Invasion**

A biting mosquito transfers about 10% of its sprozoite load into the peripheral capillaries or perivascular tissue. The sporozoites begin their evasion of the host defencies, possibly by binding to some serum proteins such as heparin sulphate proteoglycans for camouflage (Schulman *et al.,* 1980 cited by Bradley,1996b). Some of these sporozoites are destroyed by macrophages or by antigen specific antibodies in immuned individuals, but in non-immuned individuals, they reach the hepatocytes and initiate schizogony or become hypnozoites depending on their delay trigger (Bradley, 1996b)

In plasmodium parasite 3 distinct invasive forms have been identified: sporozoites, merozoite, and ookinete (Wiser,2004a). Merozoites rapidly and specifically penetrate the erythrocytes due to their surface receptor-ligand interactions capacities. Four main steps occur in the invasion, namely; initial merozoites erythrocytes binding, reorientation and erythrocyte deformation, junction formation and parasite entry (Gratzer and Dluzewski*.,* 1993)

The initial contact between the merozoites and the erythrocytes result from random collision. Surface receptor proteins that have been implicated are MSP-1(Merozoites Surface Protein) and CSP (Circumsporozoite Protein) (Wiser, 2004a).

Plasmodium has an apical organelle. After binding to the erythrocyte, the parasite reorients itself so that the apical end is juxtaposed to the erythrocyte membrane and this coincides with transient erythrocyte deformation. Following re-orientation micronemes from the apical organelle are discharged into a junction formed between the parasite and host cell. Of note is the EBA-175, a n5KDa erythrocyte binding antigen from *P. falciparum* and DBP, Duffy binding protein from *P. vivax and P. knowlesi* (Reed *et al.*, 2000). After junction formation, the parasite moves into the erythrocyte by a force generated by myosin motors associated with the transmembrane parasite ligands moving along actin filaments within the parasite (Wiser, 2004a).

After invasion, the host erythrocyte is modified. The parasite undergoes a trophic phase followed by replicative phase in the erythrocyte. The parasite modifies the host erythrocytes to make it more permeable to small molecular weight metabolities needed for the growth of the parasite. Sequestration of the mature parasites in infected erythrocyte in capillaries and post-capillary venules leads to micro-circulatory alterations and metabolic dysfunctions. This modification could be responsible for many of the manifestation of severe falciparum malaria. This is because it causes cyctoadherence of infected

erythrocytes to endothelial cells which results in the subsequent sequestration (Wiser, 2004a). Sequestrations also help the infected erythrocytes to avoid the host spleen, and cytaodherance correlates with antigenic variation expressed on the erythrocyte surface (Hviid 1998; Smith,2001 cited by wiser, 2004a).

2.4.0 **The Four Human Plasmodium Species**

As earlier noted, there are 4 (four) human malaria parasites; these are *P. falciparum, P. malariae P. ovale and Plasmodium vivax*. These organisms have unique distinguishing features.

2.4.1 **Plasmodium Falciparum**

It is the most prevalent and virulent of all the malaria parasites (Duguid *et al.* 1987, Patrick *et al.,* 1998; Cheesbrough, 1999; W.H.O 2005). *P. falciparum* is found in mainly hotter and more humid regions of the world. It is the main species found in tropical and subtropical Africa. It is also endemic in parts of Central America, South America, Bangladesh, Pakistan, Afghanistan, Nepal, South East Asia, Indonesia, Philippines, etc (Cheesbrough, 1999). These human parasites does not demonstrate host selectively of erythrocytes. It invades any RBC at any stage in its existence. Many sporozoites can infect a single erythrocyte leading to 3or even 4 small rings seen in an infected cell. *P. falciparum* is often distinctively seen in host cell at the very edge or periphery of the cell membrane (appliqué or accole position) and is distinctive for this species. The gametocytes are crescent-shaped and infected RBC do not enlarge

and are not distorted. *P. falciparum* does not produce hypnozoites in the liver like *P. malariae* (Patrick *et al.*, 1998, Rogers, 1999; Cheesbrough, 1999).

P. falciparum causes the most severe disease of the 4 species. Hence, it is designated malignant tertian or falciparum malaria. The morbidity and mortality in falciparum malaria positively correlates with the higher parasitaemia during infections and the complications arising from sequestration. Factors contributing to these higher parasitaemia include large number of merozoites produced by each schizonts, lack of host erythrocyte preference, and its immune evasion characteristics (i.e. spleen avoidance) provided by sequestration (Wiser, 2004b).

The incubation period of *P. falciparum* is the shortest of all the plasmodia; it ranges from 7-10days. Daily quotidian chills and fever are rapidly produced after infections and the periodicity of attacks then become tertian (48hrs) resulting in the development of a fulminating disease. (Cheesbrough 1999). The term malignant tertian malaria is very appropriate for these infections because it has symptoms similar to those of intestinal infections namely (nausea, vomiting, diarrhea, among others) (Patrick *et al.*, 1998). Involvement of the brain (cerebral malaria) is most often seen in *P. falciparum* infections. Capillary plugging from accumulation of malarial pigment and masses of cells can result in coma and death. Also associated with the infection, is marked Haemoglobbuiria commonly called black water fever caused by kidney damage. Kidney damage due to intravascular hemolysis characterized by rapid destruction of RBC can

result in acute renal failure, tubular necrosis, nephrotic syndrome and death. (Wiser, 2004a; Cheesbrough 1999; Rogers 1999). Liver involvement is shown by abdominal discomfort, vomiting of bile, severe diarrhea and rapid dehydration (Hviid 1998; Patrick *et al.* 1998, Rogers 1999, Cheesbrough 1999).

2.4.2 **Plasmodium Malaria**

P. malariae has prevalence lower than *P. faciparum* and *P. vivax*. It is found in the tropics and subtropics. It accounts for 25% of plasmodium infection in the tropical Africa. In Guyana, India and Malaysia, it accounts for less than 10% of plasmodium infections. (Cheesbrough 1999).

In contrast with *P. vivax, P. ovales and P. falciparum, P. malariae* can infect only mature erythrocytes with relatively rigid cell membrane (Patrick *et al.,* 1998).All stages of *P. malariae* are morphologically by a compact parasites and do not alter the morphology or the size of the host erythrocytes. Elongated trophozoites stretching across the erythrocyte, called band forms, are sometimes observed in *P. malariae* infection. Schizonts typically have 8-10 merozoites which are often arranged in a rosette pattern with a clump of pigment in the center (Wiser, 2004b). Occasionally, reddish granules called Ziemann's dots appear in the host cells(cheesbrough,1999). Unlike *P. vivax and P. ovale,* hypnozoites *of P. malariae* are not found in the liver and infection relapse does not occur. Recrudescence does occur and attacks may develop after apparent abatement of symptoms (Patrick *et al.*, 1998).

The incubation period of *P. malariae* is the longest of the plasmodia, usually ranging from or occasionally reaching 18 – 40 days several months to years. The early symptoms are influenza-like with fever patterns of 72 hours (quartan or malarial malaria). U

ntreated infections may last as long as 20-50years (Patrick *et al.* 1998; Wiser, 2004(b); Kakkilaya, 2005).

2.4.3 **Plasmodium Ovale**

P. ovale has a restricted distribution and low prevalence. It is found mainly in West Africa where it accounts for up to 10% of malaria infections. It has also been reported in the Philippines, Indonesia, China and parts of far East, South East and South America (Cheesbrough, 1999).

P. ovale is similar to *P. vivax* in many respects including its selectivity for young, pliable erythtocytes. As a consequence the host cell is enlarged and the shape is changed usually oval in form. The cell border is frequently fimbriated or ragged. The schizonts of *P. ovale* when matured has about half the number of merozoites 6-18 seen in *P. vivax* schizonts 14-24 (Patrick *et al.,* 1998;Cheesbrough,1999).Ovale tertian or Ovale malaria is the infection caused by *P. ovale* and untreated infections last only about a year instead of several years like those of *P. vivax*. It relapses and its recrudescence phase is similar to that of *P. vivax* (Wiser, 2004b).

2.4.4 **Plasmodium vivax**

P. vivax is capable of developing in mosquitoes at lower temperature than *P. falciparum*, and thus has a wide distribution in temperate and subtropical areas. It is the main specie in South America though it occurs in the Middle East, North Africa, India, Pakistan, Sri Lanka, Papua, New Guinea and Solomon Islands (Cheesbrough, 1999).

P. vivax is a very selective plasmodium species that invades only young immature erythrocytes. Infected Red Blood Cells (RBC) are usually enlarged and contains numerous pink granules or schuffners dots. The trophozoites are ring-shaped but amoeboid in appearance. Mature trophozoites and erythrocytes schizorists contains about 14-20 merozoites sometimes up to 24. (Patrick *et al.*, 1998, Wiser, 2004b). The gametocytes are round in shape. The numerous pink granules are the manifestation of Caveola-vesicle complexes that form on the erythrocyte membrane (Wiser, 2004b).

The infection caused by *P. vivax* is benign tertian or vivax malaria. After an incubation period of 10 to 17 days, the *P. vivax* infected patient experiences vague influenza-like symptoms which includes headache, muscle pains, photophobia, anorexia, nausea and vomiting (Wiser, 2004b). As the infection progresses there is an increased number of rupturing erythrocytes which liberates merozoites and toxic cellular debris and heamoglobins into circulation. This leads to the typical pattern of chills and fever that characterizes the malarial rigors (every 48 hours).. If left untreated chronic infection can lead to

brain , kidney and liver damage, as a result of malaria pigments and capillary plugging of these organs by masses of adherent erythrocytes. (Patrick *et al.*, 1998).

2.5.0 **Mechanical Mode of Malaria Parasite Transmission**

Malaria is spread mostly by bites of an infected Anopheles mosquito. Although with certain species, dormant forms can be produced which may cause relapses of malaria months to years later as mentioned earlier. Malaria may also be transmitted by Blood transfusion, use of contaminated needles or syringes and through mother to child during pregnancy.

2.5.1 **Transfusion Transmitted Malaria**

Malaria acquired by a blood transfusion recipient from a plasmodium infected donor is called transfusion malaria.

The risk of acquiring malaria via the transfusion of blood is extremely low in non-endemic countries such as Canada and the United States. A transfusion malaria risk of 0.25 cases/million donors unit has been estimated in the United States, with a fairly steady incidence of one to three cases per year reported by United States Centers for Disease Control and Prevention. In contrast the risk in endemic areas may be more than 50 cases/million donors (Slinger *et al.,* 1999).

In endemic areas, prospective recipients of blood are given anti-malaria Prophylactic treatment to help reduce the risk of transfusion malaria. Inadequate chemotherapy of clinical malaria infections, may facilitate the development of persistent sub clinical parasitaemia and increase the risk of such cases serving as reservoirs of malaria type resistant to chloroquine (Rogers 1999; Kakkilaya, 2005).

Most cases of blood transfusion acquired infections occur in cases of blood stored for less than 5 days in the blood bank and it is rare in blood stored for more than 2 weeks. Frozen plasma is not known to transmit malaria.(Kakkilaya,2005).

In transfusion malaria, Pre–erythrocytic schizogony does not occur and hence relapses due to dormant plasmodium forms does not occur. Transfusion malaria is particularly common in countries where commercial blood donation are common transactions involving blood donors from the less affluent social classes in endemic areas (Gilles and Warell, 1993). *Plasmodium vivax* infections are the most commonly incriminated in accidental infection following transfusion with plasmodium infected blood in temprate countries. This is followed by P.falciparum and P malariae infections involving donated blood by asymptomatic long-term malaria carriers (Gilles and Warell *et al.*, 1993; Kakkiliya, 2005).

2.5.2 **Congenital Malaria Transmission**:

Intra-uterine transmission of infection from mother to child malaria infection is very hazardous. When the placenta becomes heavily infected with the parasites, it can be easily passed to the growing foetus. This type is mostly common in 1st pregnancy among non immune populations (Kakkilaya, 2005). According to John Hopkins Bloomberg School of Public Health, Placental malaria infection during pregnancy significantly predisposes the risk of mother to child transmission of HIV. Their study of HIV-positive women in Rakia, Uganda found that the mother to child HIV transmission rates were 40 percent among women with placental malaria compared to 15.4 percent for women without malaria. These researchers believed that interventions to prevent malaria during pregnancy could potentially reduce mother to child transmission of HIV (Brahmbhatt *et al.,* 2003).

Mother to foetus transmission of malaria across the placental (Vertical transmission), accounts for high infant's mortality among pregnant women, abortion of foetus, infants deformities, among others(Gilles and warell,2003;Kakkilaya,2005).

2.5.3 **Needles Stick Malaria Transmission**

Transmission of malaria via needle stick injury though rare is mostly by occupational acquisition. It may also occur during laboratory preparation of

smear due to glass cuts. Accidental transmission by needle stick can also occur among drug addicts who share syringes (Kakkilaya, 2005).

2.6.0 **Epidemiology of Human Malaria:**

Epidemiology is the study of factors that influence the prevalence and distributions of diseases in the community (Duguid *et al*, 1987). Malaria is said to be endemic where there are constant incidences of cases over a period of many years. Epidemic malaria is a term, which indicates a periodic or occasional sharp increase in the level of malaria in a given indigenous community.

The epidemiology of human malaria is an example of an intricate biological system in which the parasite and its vertebrate and arthropod hosts interact in close dependence on environmental conditions.

2.6.1 **The Parasite Factors**

To survive the parasite must be present in human host long enough and be able to offer an adequate quantum of viable gametocytes of both sexes at a time when environmental conditions are suitable for transmission (Krier, 1980). The natural life span of human malaria infection varies widely according to the plasmodium species and strain (Kakkilaya, 2005). These periods may reflect the degree of the parasite's adaptation to man. Plasmodium-human tolerance apparently least in *P. falciparum,* a species which rapidly kills its host. On the other hand *P malariae* in adult man often exhibits almost commensalic features.

(Krier, 1980; Gilles and Warell, 1993; Kakkilaya 2005). In the cold temperate region, natural adaptation is shown by *P. vivax* species which possesses a long incubation period that allows it to remain in the vertebrate host until the next, usually is quite a short transmission period (Graham *et al.* ,1975).

The relatively late appearances of gametocyte in naturally acquired falciparum malaria which is about 15 days after patency is an important epidemiological feature. Moreover, studies indicated a period and duration of infections of *P. falciparum* which coincides with the period of highest biting activity of the vectors. Between 6p.m. and 6a.m. (Krier . 1980).

The development of Plasmodia in the Anopheles, depend on a minimum temperature below which it does not occur and above that which the amount of transmission is dependent on environmental factors (Gilles and Warell, 1993).

In areas where malaria eradication has been achieved and there is no reservoir of infection in local population, no transmission should occur unless it is imported from elsewhere.

2.6.2 Human Host Factors

Genetic, physical, behavioral, and economical factors, affects man's susceptibility to infection with malaria parasite. Sex and age are not important factors with regards to the malaria infection, but children have generally a higher degree of susceptibility than adults (Gilles and Warell, 1993).

Genetic differences in the susceptibility to particular parasites play important roles in the distribution of *P. vivax* which is virtually absent from west and central Africa and shows very distinct differences of incidence in Hamito-Semitic and Nilotic populations in Ethiopia (Krier, 1980). Distribution of *P. ovale* may suggest some relationship to ethnic factors. There are factors involved in the immune response; persons of African origin seem to have a greater innate immunity to some types of malaria than other races. Studies have shown that presences of haemoglobin S in population is due to evolutionary selection and these presence has a mitigating effect on the severity of *P. falciparum* infection. (Gilles andWarell, 1996).

2.6.3 **Parasite-Vector Relationship**

There is a condition known under the term "Anophelism without malaria". This generally means the presence of Anopheles spp which cannot transmit the infection either because they are non-vectors or because they do not normally feed on humans, preferring animal blood. Bradley, (1996c) stated that out of 380 species of anopheles mosquitoes only 60 can transmit malaria effectively. Kreier,(1980) reported that where there are more than 400 species of anopheles mosquitoes, 67 harbor sprorozoites. However, in a geographical area, there are usually not more than three or four anopheles species that can be regarded as important vectors (Gilles and Warell, 1993). To be an effective vector, a species must be present in adequate number in or near human

habitations, and those with preference for human blood rather than animal blood are better vectors (Krier, 1980;Gilles and Warell1993).

2.6.4 **Environmental Factors**

Variation in climatic conditions has a profound effect on the life of a mosquito and on the development of malaria parasite in it; hence, its capacity for transmission of the disease seasonally (Gilles and Warell,1993;CDC 2004b). The life expectancy of the vector, determines its survival through the time required to produce viable sporozoites. This longevity is largely dependent on environmental conditions such as relative humidity and temperature. (Kreier, 1980).Malaria parasites will not develop in a mosquito when the average temperatures are below 16^{O}C. The best condition for development of plasmodia in the Anopheles and the transmission of infection are when the mean temperature is within 20-30^{O}C range, while relative humidity is at least 60% (Kreier, 1980; CDC 2004b). At this temperature range,it takes 16 days for *P.vivax,* 30 for *P. malariae and* 22 for *P.falciparum* to complete their developmental cycle in the female Anopheline mosquito(Duguid *et a.,l* 1987)

Social and Economic factors of the environment such as sanitation, housing, occupation, poverty among others have an important effect especially in the underdeveloped endemic countries. (Gilles andWarell, 1993).

Wars and large-scale population movements are also factors in the epidemiology malaria as it can be spread through its vector and human host by ship or plane from one region to another. These are known as "imported malaria".

2.6.5 **Prevalence Of Malaria In Nigeria**

Malaria is the most widespread and most important single disease in the tropics with very high mobidity and mortality rate. Most malaria cases in the world (90%) occur in Africa (Okocha *et al.,* 2005). Nigeria located in West Africa has malaria prevalence variatons from place to place. In a recent study carried out by Okocha *et al* (2005), the malaria prevalence was 30.2% among blood donors in Nnewi Nigeria,with *P. falciparum* accounting for 76.6% parasitaemia. In a 6 month prevalence study carried out in Benin City south of Nigeria, the prevalence was observed to be 40% and *P. falciparum* was the dominant infecting species (Ibhanesebhor *et al*.,1996). In the year 2004 examination of 206 Children for neonatal malaria in Kaduna showed 8.25% *P. falciparum* prevalence (Orogade 2004). W.H.O reported a 21.03% prevalence of malaria parasitaemia in every 1000 Nigerians in the year 2003. This further confirms that malaria is very endemic in Nigeria.

In Nigeria, malaria caused by *Plasmodium falciparum* remains an important public health problem as it accounts for 25% of infant and 30% of childhood mortality (Olukosi *et al.,* 2005). In a National Malaria Survey in 2004,a

prevalence of 35.9% of malaria was recorded among children in Makarfi local government area (Ogala,2004). A World Health Organization Survey of Malaria burden in Nigeria (Global Data 2003) showed that in 2000: 2,476,608 were infected, in year 2001: 2,253,519, in year 2002: 2,605, 381, and in year 2003: 2,608, 479. The burden of infection kept increasing yearly and the average burden in individual less than 5 years was 43% while those above 5 years was 57% (W.M.R 2005).The coverage of the Roll Back Malaria (R.B.M) intervention remains unacceptably low. The limitations to this coverage among others includes:

(1)The inability of the program to get to rural areas due to poor road net works.

(2)The level of poverty in Nigeria does not allow for the majority to purchase insecticide treated mosquito net.

(3)The literacy level of the majority of Nigerians who live in rural areas is very low, therefore most members of the population have no knowledge of the dangers of malaria and how it could be prevented.

2.7.0 **Malaria Survey And Diagnostic Methods:**

Diagnosis is important as malaria infection may be confused with any other fever especially during the low transmission season of the parasite. A careful examination of blood specimen is thus important to effectively diagnose parasitaemia (Gilles and Warell, 1993; Cheesbrough, 1999).The following are

some of the methods that are currently in use for the detection of malaria parasitaemia from suspected cases.

The traditional methods of diagnosis of malaria still depends on the microscopic detection of the causative Plasmodium in the blood smear.

2.7.1 **Blood Microscopy For Plasmodium**

Blood examination for malaria is best done by the microscopic examination of the thick and thin blood films. Blood films are best prepared on clean glass slides free from grease and scratches (Cheesbrough, 1999). The miscroscopy of thick and thin film is the gold standard in diagnosis of malaria and requires an expert microscopist(Gilles and Warell, 1993; Cheesbrough, 1999). The thick or thin blood films are stained with Giemsa , field , or Leishman stains. These stains all give excellent demonstration of the malaria parasite. The parasite are seen as red chromatin dots in the Red Blood Cells(RBC) in films which shows their forum clearly thus it can be used for species differentiation (Rogers, 1999; Cheesbrough, 1999).

2.7.2 **Indirect Fluorescent Antibody Technique(IFAT)**

Serological diagnosis of malaria and its complications has become of practical value following the introduction of Indirect Fluorescent Antibody Test (IFAT) in 1962(Gilles and warell, 1993). It has been used in the screening of blood in blood banks (Gilles and Warell, 1993; Rogers 1999). In the IFAT procedure, for the parasite to be detected, the test blood sample is spread as a

film of blood on a microscope slide. The slide is covered first with a diluted test serum. A solution of antihuman globulin labeled with fluorescent isothiocynate is then added. After washing and drying the slides are examined with a fluorescence microscope. Antibodies in the test serum combines with antigens of the malaria parasites contained in the whole blood smear and these reactions, are indicated by the fluorescence of the parasite. The indirect haemagglutination (IHA) test is also used but more as field method since it does not require the special fluorescent microscope (Gilles and Warell, 1993).

2.7.3 **Enzyme Linked Immunosorbent Assay (ELISA)**

The enzymes linked immunosorbent assay (ELISA) can also be used in malaria diagnosis. The test is similar to IFAT. A base of a plastic tube or plate is coated with a soluble antigen. The serum containing the antibody is incubated in the coated tube and the excess of antibody is removed by washing buffers. The antibody specific globulin labeled with the appropriate enzyme is then added to the tube and the excess of it removed. The enzyme substrate is then added and its change of colour is proportional to the antibody concentration in the test serum. The disadvantages of this method is the difficulty in standardizing the antigen and detection of low levels of antibodies. It is less accurate than with the IFAT (Gilles and Warell, 1993).

2.7.4 **Malaria Rapid Diagnostic Technique (RDTS)**

These Kits are developed to diagnose malaria rapidly without the need of a microscope. The test are based on the immuno-chromographic detection of antigen HRP2 (Histidine Rich Protein 2) or specific PLDH (Parasite Lactate Dehydrogenase). Both HRP 2 and PLDH are produced by the falciparum parasites during their growth and multiplication in the red cells. This test is still being developed as of recent, and it is only specific for detection of *P. falciparum* and *P. vivax* infections. Example of these kits among others are ParaSight F, ICT malaria pf and OptiMal test kits (Cheesbrough, 1999).

2.7.5 **Quantitative Buffy Coat (QBC) Microhematocrit Centrifugation Method**

Microhematocrit centrifugation with use of the QBC malaria tube has been used for the detection of blood parasites (Cheesbrough, 1999). When venous or capillary blood is centrifuged for 5 min in a QBC centrifuge, (14, 387 x g,) parasites or erythrocytes containing parasites are concentrated into a small 1 to 2mm region near the top of the erythrocyte column and are held closed to the wall of the tube by a plastic float, thereby making them readily visible by microscopy. Tubes pre-coated with acridine orange provides a stain which induces fluorescence in the parasites. This method automatically prepares a concentrated smear which represents the distance between the float and wall of the tube. Once the tube is placed in a plastic holder (Paraviewer) and immersion

oil is applied onto the tip of the hematocint tube (no covership is necessary) the tube is examined with a x40 to x60 oil immersion objective (it must have a working distance of 0.3mm or greater) (Lynne *et al.,* 1999;cheesbrough,1999).

Other methods that could be used to detect malaria parasites include the use of DNA and RNA hybridization technique or the polymerase chain reaction methods (PCR) (Rogers 1999, W.H.O 2005). Among these approaches discussed above none of these methods has yet replaced routine microscopic diagnosis, although a malaria infection could be detected by these methods PCR is very sensitive and specific but the expensive nature of the equipments limits this method (CDC 2004c).

2.8.0 **Chemotherapy And Drug Resistance In Malaria.**

Antimalaria drugs are classified into several chemical groups. Some of these are no longer in use today because drug resistance strains of malaria have emerged and have become a major problem. Antimalaria drugs have a selective action on the different phases of the Plasmodium parasite life cycle in human host. This may be divided into "causal prophylactic" which prevents the establishment of the parasite in the liver and "blood schizontocidal" drugs

which attack the parasite in the red blood cell, preventing and terminating clinical attack. (Gilles and Warell, 1993).

Tissue schizontocides are compounds acting on pre-erythrocytic forms in the liver. The gametocytocidal drugs destroy sexual form of the parasite in the blood. Some of the anti-malaria drugs are hypnozotocidal, while others are sporontocidal drugs which will kill dormant hypnozoites and inhibit the development of oocyst on the stomach wall of mosquitoes, that have fed on the gametocytes carrier respectively (Krier, 1980, Gilles and Warell, 1993; Bradley 1996). Failure to take recommended chemoprophylaxis and refusal or delay in seeking medical care, is a risk factor for fatal malaria (CDC, 2004a).

2.8.1 **Quinine**

Following their arrival in the new world, the Spanish learnt of a medicine used for fever treatment by the Indians which was the bark of Chinchona Tree or "Peruvian bark". This medicine became known as quinine (CDC, 2004a). Quinine has been used for more than 3 centuries and until the 1930's it was the only effective agent for malaria treatment. It is now only used for treatment of severe falciparum malaria (CDC 2000), partly because of undesirable side effects such as massive intravascular haemolysis and haemoglobinuria i.e. black

water fever. Quinine and Mefloquine cause blebbing of the parasite membrane and causes aggregation of haemozoin to form. Parasites resistance occurs by uncertain mechanisms, but it is stable and transmissible (Bradley, 1996a; Nosten 1999, Richard and Graham, 2004).

2.8.2 **Chloroquine**

It is a very effective 4-aminoquinolone for both treatment and prophylaxis. It was first used in the 1940's shortly after the second world war. It was found to be effective in curing all forms of malaria. This drug that was discovered by a German Hans Andesag in 1934 (CDC 2004a) is now no longer as effective as it used to be due to increasing strains of malaria parasites resistant to it (Gilles *et al.,* 1996). Chloroquine works by preventing haemoglobin metabolism of malaria parasite, but resistant parasite though are unable to produce haemozoin, they are still able to digest haemoglobin (Richard and Graham, 2004).

2.8.3 **Mepacrine**

These drugs are blood schizontocides similar to chloroquine. They are derivatives of 9 amino-acridine developed in the early 1930's (Gilles *et al.,* 1996). Some parasites have become resistant to it just like chloroquine and are no longer in routine use (Richard and Graham, 2004).

2.8.4 **Sulfonamides**

Sulfonamide drugs work by inhibiting para-amino benzoic acid which is needed to synthesize the dihydropteroate which is an intermediate compound in the synthesis of tetrahydrofolate. Thus it is a drug that can be used for treatment and prophylaxis due to its ability to inhibit metabolism of malaria parasites. The mechanism of resistance of malaria parasite strains to these drugs are not known but resistant parasites strains seem to be available in all stages of the metabolism. But the mechanism of gene amplification is a possibility (Bradley 1996a, Richard and Graham, 2004).

2.8.5 **Primaquine**

The 8-aminoquinolones are believed to be converted in the liver to quinone active metabolites (Gilles and Warell, 1993). These drugs have been used against gametocytes and hypnozoites. The drug works by inhibiting electron transport chain of the parasite. The resistant strains to these drugs have increased numbers of mitochondria suggesting that the resistance mechanism involves the production of extra number of these organelles to compensate for the damage caused by drugs (Bradley 1996. Richard and Graham, 2004, Net Doctors 2005).

2.8.6 **Antibiotics**

Antibiotics such as tetracycline, doxycycline, clindamycin among others have little action on pre erythrocytic stages and have slow but marked actions

on blood stages (Gilles and Warell, 1993). The drugs are inhibitors of ribosomal protein synthesis. They can be used in cocktail with other drugs like chloroquine to combat resistant forms of falciparum malaria (Bradley 1996a; Richard and Graham, 2004).

2.8.7 **Atovaquone**

These are slow acting but potentially valuable experimental drugs which act upon electron transport chain in the malarial mitochondria. They are potentially active on growing stages of parasites and have prophylactic potentials (Richard and Graham , 2004, Attaran *et al.,* 2004).

2.8.8 **Malarone**

This is a combination of proguanil and atovaquone. These new combination was released in Australia. When atovaquone is combined with proquanil a synergistic effect is achieved against the malaria parasite. These synergistic effects have been found effective against the resistant strains of malaria. (Richard and Graham, 2004, Attaran *et al.,* 2004).

2.8.9 **Artemisinins**

These are among the newest and most effective of all antimalarial drugs and seem to affect protein synthesis (Bradley,1996a). These drugs must be protected and used rationally to prevent the emergence of resistant Plasmodium strains to it(Attaran *et al.,* 2004)

This peroxide antimalarial drug (artemisinin) is derived from the Chinese medicinal plant *Artemisia annua*, and semi-synthetic analogues such as artemether and artecther. Artesunate and artelinic acid are blood schizontocides (Gilles and Warell, 1993). The prophylactic use of these drugs will be unwise because of their embryotic effects and because of the likely development of resistance by Plasmodium to these promising drugs (Gilles and Warell, 1993, W.H.O. 2005).

2.8.10 **Proguanil**

This drug falls into the biguanide class of antimalarials and was first synthesized in 1946. This drug is known t inhibit specifically dihydrofolate

reductase which is used by the parasite to make folinic acid cofactors. Because they inhibit all growing stages of malaria parasite, they are used as prophylactics and sporontocides (Gilles and Warell, 1993, Kreier 1980). The parasite resistant to these drugs have dihydrofolate reductase enzymes that binds to the biguanides 400-800 times less readily thaan the enzymes of drug sensitive strains. A cocktails of biguanides and sulfonamides have been used effectively for treatment of biguanide resistant strains (Bradley,1996a).

2.9.0 **Social - Economic Impact Of Malaria:**

Malaria imposes substantial cost to both individuals and governments. These includes maintenance of health facilities, purchase of drugs and supplies of drugs. Public health intervention against malaria is also another area that has indebted government in areas such as provision of insecticide, treated nets and spraying of insecticides.

Other importance of malaria includes lost of work- days with resulting in loss of income and loss of opportunities for job and economic ventures. Malaria also affects tourism such that it reduces income gain from tourists because malaria endemic countries will be an impediment to the availability of tourist visiting from a malaria free country.

Malaria is a killer of infants mostly because they have not yet developed sufficient degree of immunity to it (CDC,2004a). Because these persons are

most vulnerable the world stand a chance of losing children who would be tomorrows leaders, children who are the hope of future generations.

Pregnant women whose immunity is decreased by pregnancy especially during the first and second pregnancies are vulnerable to the infection as well. This could thus lead to fetus incapacitation and deformities such as blindness, neurological damages and even fetus death, which may cause psychological trauma to the mother (Branmbhatt *et al.,*2003).

People subjected to frequent malaria infection can develop anemia (CDC 2004a). Subsequently they may need blood transfusion, which in developing countries can expose them to HIV infection. This may lead them to being abandoned and rejected by their families and spending of money extra for the treatment of the accidentally acquired HIV infection.

It is important that control and preventive measures are put in place to this disease that has put 41% of the world's population at risk, caused, more than 300million episodes of acute illness yearly and at least one million deaths each year (W.H.O,2003).

2.10.0 **Problems Mitigating the Effective Control and Prevention of Malaria**.

The malaria parasite is vector borne; therefore any climatic change in habitat of mosquito will have an impact on the prevalence of the disease. Any change in

the environment (e.g. Construction of drink water reservoirs or deforestations) will either increase or decrease the prevalence of the vectors respectively (Chris and Paul, 2000; Arjen, 2006). As with many infectious diseases, the sudden increase in mobility will increase the probability of the emergence of malaria in regions were the disease have been eradicated (Arjen, 2006).

Indiscriminate use of both antimalarials and pesticides has resulted in increased resistance of both malaria parasite and it vectors (Malaria foundation International, 2003; Arjen 2006). Although effective antimalarials are in existence, the price of these drugs are too high for people in malaria endemic countries and therefore unsuitable for wide spread use (Arjen, 2006). Antimalarials may be prophylactic or curative. An effective vaccine for malaria has not yet been discovered (Malaria Foundation International 2003). Natural resistance against malaria only reduces the severity of the disease, but is incapable of rendering an individual completely immune.(Malaria Foundation International, 2003). An effective malaria vaccine will have to overcome the problem of antigenic variations associated with the parasite. (Elizabeth, 2006).

Lack of coordination in the implementation of malaria control programmes has militated against the control of malaria adversely. The last global effort to eradicate malaria was attempted in the 1960s (Malaria Foundation 2003), with the discovery of new effective antimalarias and the use of powerful insecticides such as DDT. This attempt failed due to lack of coordination (Malaria Foundation International, 2003; Arjen 2006).

Modern technologies, such as Geographical Information System (GIS) are highly effective tools in mapping malaria risk. But these are also expensive and hard to access for many developing countries (Malaria Foundation International, 2003).

Perhaps the major problem in the control of malaria in the developing world is corruption and grafts. These are often problems as people within administrative structures fight over available resources which affect malaria control programs.

2.11.0 **Control And Prevention Of Malaria**

The control of malaria is rather dynamic and not static. The measures that may work in one region may thus not necessarily apply in others due to socio-economic status and the environment conditions of the ecotype. However, the following processes could be used in control and prevention of malaria worldwide. According to the classification proposed by Russell in 1952 (Gilles and, 1993), which will still be very useful and effective today are:-

(1) Measures designed to prevent mosquitoes from feeding on humans.

(2) Measures designed to prevent or reduce breeding of mosquitoes.

(3) Measures designed to destroy larvae

(4) Measures designed to eliminate malaria parasite in human host

(5) Measure designed to destroy adult mosquitoes

But these cannot be entirely relied upon to give complete control in practice but it does speak of much in theory because of the human and

economic factors involved. In summary, the followings may be helpful in prevention and control of malaria.

(A) Education of the Public:

Training of health workers that will help in diagnosis and treatment of malaria is a very essential aspect of malaria control. These workers can give awareness lectures on the dangers of malaria and it will also be a way of improving epidemic preparedness and response to the out break of malaria therefore availability of trained workers is very essential to malaria control(Malaria Control Programme 2004)

(B) Provision of Nets

Mosquitoes nets both the non treated or the insecticide treated nets when used will help prevent mosquitoes bites. The insecticide treated net serves as a method of vector prevention when the mosquitoes come in contact with the nets and die. Mosquito nets when fitted at doors and windows of rooms and homes also help control and prevent the spread of malaria infections as these vectors are carriers of the malaria parasite. Recently the Nigerian government has promoted the use of insecticide treated nets.It is a good and less costly method of malaria prevention. This method will also prevent asyptomatic carriers of *Plasmodium spp* from being bitten by mosquitoes (Kathleen,2002;Malaria Foundation International,2003).

(C) Development of vaccines

Despite long and intensive research efforts, no vaccines against malaria in humans that is effective and suitable for mass production has been developed (Malaria Foundation International,2003;Elizabeth,2006). It is thus important that money and effort be put in this aspect of control. The development of vaccines will be cheaper than continuous production of new drugs that malaria parasite easily becomes resistant to. When and if vaccine is discovered, it will be the best thing that happens to man in the study of malaria.

(D) Prophylaxis

Medicines to prevent malaria should be prescribed to individual traveling from non-malaria endemic areas to endemic areas. And this prescription should be strictly followed as it will help prevent importation of malaria from a malaria infested area to a malaria free zone.

For efficient control of malaria, development of new drugs of choice is important as the parasites have developed resistance to the common drugs formerly used. Attention should be paid to these aspects of control until vaccines are eventually discovered. Drug prescribed should also be used according to prescriptions to prevent drug resistant strain development.

(E) Early Detection:

Another good control measure is the early detection of malaria parasite and treatment of the infection in individuals. Individuals may suffer

asymptomatic parasitaemia, but if regular check up is performed especially in endemic areas, this could be detected and treated before it results to an actual infection.

(F) Spraying of Insecticides:

This control method was used in some parts of Europe and North America between 1947 and 1951 (CDC,2004a). This method was used effectively and it led to the eradication of malaria from the regions. Spraying of insecticides will help to kill the mosquitoes vectors that carry these parasites and thus reduce or eradicate the malaria parasite. Some mosquitoes have become resistant to some insecticides, therefore alternative insecticides should be made available when this control measure is being used (Malaria Foundation International, 2003).

(G)Location of Dams:

Building of dams have been associated with increase in malaria infection (Gilles and Warell,1993; CDC,2004b). Thus if dams can be build far from human communities this might help reduce the spread of the diseases. Because mosquitoes use dams and stagnant waters as breeding grounds.

The control of stagnant water and bushes around homes and communities will also help in reducing and preventing the spread of the malaria infection.

(H) Biological Control:

The use of Laviparous fishes such as *Nothobranchius guentheri* and *Cynolebius whitec* are a good control measure. These fishes will eat up the anopheles larva and thus prevent the development of the larvae to adult mosquitoes that are vectors of malaria parasite. Other biological agents that have been successfully used to control mosquitoes includes fungi of the genera *Coelomomyces, Culicinomyles and Lagenidium;* Bacteria (*Bacillus sphaericus* that produces toxins harmless to humans and animals but potent to mosquitoes) and Nematodes of the family *Mermithidae* most especially the *Romanomermis culicivorax* which can greatly reduce larval populations and survives in a variety of habitats. (Balaraman and Hoti ,1987;Gilles andWarell, 1993; Kathleen,2002).

However, the disadvantage of the biological control method is that occasionally the biological agent becomes predators of other ecologically useful aquatic animals.

(I) Improving Health Facilities:

Availability of health facilities in rural and urban areas will help control and prevent malaria to a good level, as good treatment and case management can be obtained from good health care centers. The more the number of available good health facilities the better the chances of preventing death caused by malaria infection.

(J) Malarial Control Program:

The most important aspect of malarial control is to establish a control programme with trained professional available to run the program. This will help put other control methods discussed earlier in place and monitor the various control methods effectively. (W.H.O 1998). Sanwo in the year 2001 suggested the treatment of the people by the people, for the people. This is an appropriate step in control of malaria in Nigeria. It is important to note that for an effective malaria program to be achieved, a multiple strategy is needed. This is because there are numerous species of vectors and four species of plasmodium affecting human being. Drug resistance exist among parasites strains and thus alternative drug should be made available. The areas and type of home people live in will require different type of control strategies.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study area sampled was the Ahmadu Bello University Community Main Campus Samaru, located in Zaria, Kaduna State, Northern Nigeria.

3.2 Study Type

The cross sectional study method was the type used in these studies. Apparently healthy individuals were tested for the presence of malaria parasite in their blood.

3.3 Sampling Method

Volunteers were sampled between the study period(May-October2005) from the various faculties (Science, Engineering, Medicine, Pharmacy, Education, Social Science, Arts, Vet Medicine, and Agricultural Science) and the community health center on the campus. This was done by seeking consent of individuals, enlightening them on the work being done, and asking them questions to ensure that the inclusion and exclusion criteria were met. Samples were collected using sterile disposable lancets or sterile disposable needles and syringes. Either of these sterile disposable objects was used in pricking a finger from which 3-5 drops or 1 ml of blood were collected for processing on a clean slide and in EDTA tubes respectively.

3.3.1 **Study Population**

4 groups of A.B.U community main campus were sampled

(a) Academic Staff

(b) Non Academic Staff

(c) Students

(d) Children (6 months – 5 years)

3.3.2 **Inclusion Criteria**

It was ensured that:-

(a) The subject has been apparently healthy for 2 weeks without fever symptoms.

(b) The subjects did not have any forms of malaria symptoms

(c) The subjects were members of A.B.U community main campus Samaru.

3.3.3 **Exclusion Criteria**

It was ensured that any subject that showed any of the following were exempted from the study:

(a) Individuals that had been treated of malaria not more than 2 weeks prior to sampling.

(b) Individuals that had malaria symptoms or that were on anti-malaria prophylactic treatment.

(c) Individuals who were not members of A.B.U community Samaru.

(d) Pregnant women

3.3.4 **Sample Size**

The sample size was determined according to Ipsen *et al* 1970 as follows:

$$n = \frac{Z^2 (Pq)}{\alpha^2}$$

Where n = sample size, Z = standard normal deviation at 95% confidence interval 1.96

P = prevalence of malaria 35.9%(Ogala ,2004).

α = allowable error 5%

q = 1-P

With attrition addition of 10%, 400 samples were collected. One hundred samples were collected from each group.

3.3.5 **Sample Processing**

The samples collected were processed using the methods described by (Cheesbrough 1999)

(a) Blood Films Preparation

Thick films are the "gold standard" for detection of malaria parasite because of the relatively large volume of blood that can be examined directly. Thin films are gold standard for species identification.

(i) Thick films: from the samples, two-three drops of blood was touched with a grease free clean slide in an inverted position. The slide was then reserved and the blood was spread evenly with the edge or corner of another slide to make a circular patch of moderate thickness which allowed the easy reading of the slide through. The slide was kept horizontally and allowed to dry and protected from dust and flies.

(ii) Thin film:- a drop of blood was placed on a slide, and the smooth edge of another clean slide was then applied at an angle of 45^{O} to the drops of blood. The drop of blood was touched with the inclined slide till the blood was spread along the edge. The spreader was pushed forward maintaining the same angle until the blood was evenly spread with a fish tail appearance. The films were then air dried similar to the thick films.

3.4.0 Parasitological Screening Of Sampled Population

(A) Preparation Of Geimsa Stain And Buffered Water

(a) Geimsa Stain:- the stock solution was prepared from a commercially available Giemsa powder by AVONNALE LABORATORIES ENGLAND. The following reagents were used in the preparation.

Giemsa powder 3.8g

Glycerol (pure) 250 mls

Absolute methyl alcohol 250 ml

3.8 grams of Giemsa was weighed and transferred to a brown dry bottle of 2 litres capacity which contains few glass beads. To this was added 250mls absolute methanol and the solution was mixed well. Also 250mls of glycerol was added to the stain and the resulting solution mixed The bottle containing the stain was placed in a water bath at 55^{Oc} for 2 hours to dissolve the stain. The bottle was labeled and the stain was stored in the dark.

(b) Buffered Water. The water used for dilution of the Giemsa stain was prepared using KH_2PO_4 (0.7g), Na_2HPO_4 (1.0g) and Distilled water 1 litre to obtain a solution of PH7-7.2. This solution was used to make a 10% dilution when ever a diluted Giemsa stain was needed for the staining of the slides.

(B) **Staining Procedures**

For the thick film, the slides containing the blood dried smears were placed horizontally on the staining rack. A 10% dilution of Giemsa stain was used to flood the blood smear and allowed to stay for 10-15 minutes. The stain was then washed off gently using normal tap water until the exess stain was completely removed. The slides were air dried and made ready for microscopy. For the thin films containing the blood smears, the slides were first fixed with methyl alcohol for 2 minutes before the same staining process used in the thick film were then applied.

(C) **Detection Of Parasites And Identification**

Using oil immersion objectives, fields of each slide were examined for the characteristics of the parasites before conclusions were made either as positive or negative for parasitaemia. The cytoplasm of the parasite stained blue and the nuclear material stained red to purple-red. Shuffner's dot and other inclusion in the erythrocyte be stained red

(D)**Estimation Of Parasitaemia**

The number of parasites were counted against one hundred white blood cells as described by Cheesbrough(1999) A white cell count of 8000/ul was used in this survey. The formular used to get the number of parasites was:-

$$\text{Number of parasite per ul of blood} = \frac{\text{WBC count(8000) x Parasites counted against 100 WBC}}{100}$$

3.5.0 **ACON Malaria Kit**

The malaria parasite kits used were obtained from GILERCY INTERNATIONAL CO. LTD. Warri street Kaduna. The kit type was ACON ONE STEP TEST DEVICE, manufactured by ACON laboratories, Inc. 1408 Sorren to Valley Blud. San Diego, CA92121, USA

The tests were carried out according to the Kits manufacturer's instructions. Fingers to be used were cleaned with methanol (methylated spirit) after which the prick was made using sterile disposable lancets. From the prick site drops of blood were placed in the kits reaction chamber. Some blood

samples collected via the syringe and placed in EDTA tubes were processed using the kits pipette to withdraw the specified volume of blood, and placing specified volume into the chamber. The reaction chamber contains antibody specific to the malaria parasite antigen joined to a colored dye. The buffer solution lyses the blood, the antigen joins with the antibody and a color change is observed on the test pad. Three drops of the reaction buffer was added into the kits chamber. The kits were placed on a horizontal surface and after 15 minutes the results were recorded.

When the reaction showed a reddish brown band at the test and control region it indicated a positive reaction for parasitaemia. A band in the control not in the test region indicates a negative reaction. Likewise a band in the test and not in the control is a null reaction.

3.6.0 **Ethical Consideration**

Permission was obtained from the ethical committee of ABUTH Zaria and the University Authority.

3.7.0 **Data Analysis**

Data were analyzed using the χ^2 to check for association of parasitaemia to age, sex and status. ANOVA and students t test were used to compare the mean differences between the various study groups. The level of significance used was 0.05 (5%). For detail analysis see appendix iv.

CHAPTER FOUR

4.0 **RESULTS**

Four hundred individuals were sampled during the study in four groups of a hundred each (Academic staff, Non Academic Staff, Students, and children aged 6 months – 5 years).

Out of the four hundred samples, one hundred and five (26.25%) showed positive Parasitaemia. This consisted of 19(19%) Children, 49(49%) Students, 14(14%) Academic Staff, and 23(23%) Non-Academic Staff.

Statistically, significant association was established when the parasite rate was compared with age, status and period of sampling. The highest parasitaemia was seen in students with 49% of them having asymptomatic parasitaemia with specific parasite rate of 12.25%. The lowest parasitaemia was seen in Academic staff (14%) with this group contributing a specific parasitaemia rate of 3.5% to the total parasite rate of 26.25%.

There was a significant association between Parasitaemia and the social groups, with student having the highest prevalence. This is shown in table 1.

Table 1 **ASYMPTOMATIC PARASITAEMIA RATE PER STATUS**

Group	No of Samples Tested	No of Positive Samples	Parasite rate %	Specific Parasite Rate %
Children	100	19	19	4.75
Students	100	49	49	12.26
Non Academic Staff	100	23	23	5.75
Academic Staff.	100	14	14	3.5
Total	400	105		26.25

$\chi^2 = 37.74657 \quad P < 0.05$

4.1 **Distribution Of The Parasite Rate Per Study Group**

The parasite rate in the males is 12% while the female had 26% with each of them contributing 1.5% and 3.25% respectively to the total 26.25% parasite rate of the study samples. There was no significant association between parasitaemia and sex of the children (table 2). As seen in table 3, 49% of the students had asymptomatic parasitaemia; 53.13% in males and 41.67% in females. They

contributed 8.5% and 3.75% specifically to the total 26.25% parasite rate obtained in the study. The prevalence of parasitaemia was not significantly associated to the males or females. About 23% of the non-academic staff had asymptomatic parasitaemia; 18.03% parasite rate and 2.75% specific parasite rate was seen in males while 30.75% parasite rate was seen in females. There was no statistically significant association between parasitaemia and sex (table 4). Table 5 shows that14 % of the academic staff had parasitaemia; the males contributed 2.1% while the females 1.5% to the total parasite rate 26.25% observed in the study. No statistically significant association between parasitaemia and sex.

Table 2 **PARASITE RATE AMONG CHILDREN**

	Males	Female	Total
No of positive samples		**6**	**13**
19			
No of negative samples	44	37	81
Total no tested	50	50	100
Parasite rate (%)	12	26	38
Specific Parasite Rate (%).	1.5	3.25	4.75

$\chi^2 = 2.33918$ $P > 0.05$

Table 3 **PARASITE RATE AMONG STUDENTS**

	Males	**Female**	**Total**
No of positive samples	34	15	49
No of negative samples	30	21	51
Total no tested	64	36	100
Parasite rate (%)	53.13	41.67	94.8
Specific Parasite Rate (%).	8.5	3.75	12.25

$\chi^2 = 0.79539$ $P > 0.05$

Table 4 **PARASITE RATE AMONG NON ACADEMIC STAFF**

	Males	**Female**	**Total**
No of positive samples	11	12	23
No of negative samples	50	27	77
Total no tested	61	39	100
Parasite rate (%)	18.03	30.76	47.79
Specific Parasite Rate (%).	2.75	3.0	5.75

$\chi^2 = 1.51925$ $P > 0.05$

Table 5 **PARASITE RATE AMONG ACADEMIC STAFF**

	Males	**Female**	**Total**
No of positive samples	8	6	14
No of negative samples	58	28	86
Total no tested	66	34	100
Parasite rate (%)	12.12	17.64	29.76
Specific Parasite Rate (%).	2.1	1.5	3.5

$\chi^2 = 0.20268$ $P > 0.05$

A total of 110 samples were tested among the age group 21-30, of which 53(48.18%) had asymptomatic parasitaemia. This age group contributed the highest rate of parasitaemia 13.25% to the total 26.25% parasite rate obtained in the study. A statistically significant association was established between this age group and parasitaemia observed from the smears. Ages 11-20 had 11 samples tested out of which 6 (54.54%) were positive. They contributed a specific parasite rate of 1.5% to the total rate of 26.25% observed in this study (table 6). 24.48% of males and 28.93% of the females had asymptomatic parasitaemia. Each contributed 14.75% and 11.50% specific parasite rates respectively to the total 26.25% parasite rate obtained in this study. When parasitaemia was compared with sex no statistically significant association was seen (table 7). The month of May, June, July had a statistically significant association with the prevalence of asymptomatic parasitaemia. The parasitaemia was highest in May (61.53%) and June (44.89%); both contributing 6% and 5.5% specific parasite rate respectively. The prevalence of 39.53% and specific parasite rate of 4.3% were recorded in the month of July (table8).

Table 6 **PARASITAEMIA RATE PER AGE GROUP**

Age(Years)	**No of Samples Tested**	**No of positive samples**	**Parasite rate (%)**	**Specific Parasite Rate (%)**
0-5	100	19	19.00	4.75
11-20	11	6	54.54	1.50
21-30	110	53	48.18	13.25
31-40	100	17	17.00	4.25
41-50	53	8	15.10	2.00
51-60	24	2	8.33	0.50
61-70	2	0	0	0.00
Total	400	105		26.25

$\chi^2 = 47.22947$ $P < 0.05$

Table 7 **PARASITE RATE PER SEX**

Sex	No of Samples Tested	No of positive samples	Parasite rate (%)	Specific Parasite Rate (%)
Males	241	59	24.48	14.75
Females	159	46	28.93	11.50
Total	400	105		26.25

$\chi^2 = 0.76332$ $P > 0.05$

Table 8 **PARASITAEMIA RATE PER MONTH**

Period (Months)	No of Samples Tested	No of positive samples	Parasite rate (%)	Specific Parasite Rate (%)
May	39	24	61.53	6.0
June	49	22	44.89	5.5
July	43	17	39.53	4.3
August	23	3	14.29	0.8
September	118	22	18.64	5.5
October	131	17	12.98	4.3
Total	400	105		26.25

$\chi^2 = 56.45972$ $P < 0.05$

4.2 **Parasite Densities**

The degree of parasitaemia per ul of blood is the parasite density. This was obtained from the parasite count. Parasite density index is given by ^{total} $^{\mathbf{xy}}/_{\text{total }\mathbf{y}}$ = 3.43. i.e. an average of 3.43 parasite of malaria per ul of blood. There was no significant association between parasite count and sex (table 9). In table 10 individuals in the age group 21-30years had a parasite density index of 3.87 in the 53 positive samples of the 110 individuals of the group. A parasite density index of 4.00 was seen in ages 51-60 years of which 2 samples were positive of the 24 samples tested. The lowest parasite density index (2.84) was in children. However, parasite count was not significantly associated with age. The students had the highest density index of 3.89. The lowest density index of 2.84 was seen in children. Parasite count had no statistically significant association to group status (table 11).

4.3 **Parasite Density Index Per Sex**

Out of the 400 samples tested, 241 were males, 59 had parasitaemia with a density index of 3.45 while among 159 females, 46 were positive for parasitaemia with parasite density index of 3.37.

Table 9 **DISTRIBUTION OF TOTAL PARASITE COUNT**

X	Parasite count per ul of blood	Frequency		Total Y	Xy
		Male	Female		
1	Less than 100	5	3	8	8
2	101-200	5	9	14	28
3	201-400	20	14	34	102
4	401-800	17	11	28	112
5	801-1600	10	6	16	80
6	1601-3200	2	3	5	30
Total		59	46	105	360

$\chi^2 = 1.71456$ $P > 0.05$

Table 10 **PARASITE COUNT AND DENSITIES PER AGE GROUP**

Count per ul of blood	**Frequencies per Age Group (Years)**						
	0-5	**11-20**	**21-30**	**31-40**	**41-50**	**51-60**	**61-70**
Less than 100	3	-	2	2	1	-	-
101-200	5	1	3	3	2	-	-
201-400	5	3	13	9	3	1	-
401-800	4	-	24	-	-	-	-
801-1600	2	2	7	3	1	1	-
1601-3200	-	-	4	-	1	-	-
Total	19	6	53	17	8	2	0
No of samples tested	100	11	110	100	53	24	2
Parasite density **Index**	2.84	3.50	3.87	2.94	3.13	4.00	0.00

$\chi^2 = 5.74130$ $P > 0.05$

Table 11 **PARASITE DENSITY INDEX PER STATUS**

Group Status	Parasite density	No Positive	No sampled
Children	2.84	19	100
Students	3.89	49	100
Non Academic Staff	3.0	23	100
Academic Staff	3.24	14	100
Total		105	400

$\chi^2 = 3.67722$ $P > 0.05$

4.4 **Plasmodium Species prevalence and group distribution**

The *Plasmodium spp* rate was obtained for the four species of which *Plasmodium falciparum* had the highest species rate. *P. falciparum* had the highest prevalence of approximately 62% while *P. malariae* had a high rate of approximately 21%. Mixed infection was found in 13.38% of the samples examined. This is shown on table 12.

Table 12 **PREVALENCE DISTRIBUTION OF THE PLASMODIUM SPP**

Malaria Parasite	**No of Positive samples n = 105**	**Species Rate (%)**	**Species Infection (Specific Rate %)**
P. falciparum	65	61.90	16.25
P. malariae	22	20.95	5.5
P. ovale	5	4.76	1.25
P. vivax	0	0.00	0.00
Mixed infection	13	13.38	3.25
Total	105	100	26.25

4.5 Validity And Accuracy Of The Malaria Test Kit (Sensitivity And Specificity)

A total of 105 samples were tested using the malaria test kit. Compared to the gold standard (Microscopy 100%), of the total of 105 samples used in this study, the ability of the test Kit to give correctly a positive result when individual smears are truly positive with parasitaemia (Sensitivity) was 84%. The proportion of individuals negative to the malaria smear and test kit (Specificity) was 95%. Using Kappa Statistics, the malaria kit was found to be almost perfectly reliable with a Kappa statistic of 0.93 (table 13).

Table 13 **COMPARISM OF RESULT USING MALARIA RDT KIT AND MICROSCOPY**

	Microscopy	**Malaria Kit**
True Positive	86	72
False Positive	0	1
False Negative	0	14
True Negative	19	18
Total	105	105

Sensitivity = 84% Specificity = 95%

CHAPTER FIVE

5.0 **DISCUSSION**

Malaria is still a major public health problem in Nigeria (WMR 2005). The results obtained from this research is a proof of high prevalence of asymptomatic malaria in the population studied. The high rate of positivity for malaria parasite in apparently healthy individuals in Ahmadu Bello University Campus Samaru, Zaria (A.B.U) is quite worrisome. This is a reflection of high rate of asymptomatic parasitaemia in the area. The implication of this is enormous. Any form of stress to the body system of malaria parasite carriers such as cold, accidental cuts or other infections could lead to blood stage schizogony and its sequelae. Further more, with regards to blood donation for transfusion purposes, it poses a risk especially to pregnant women, children and immune deficient patients who are vulnerable to malaria. Apart from transfusion malaria risk, it increases the cost of treatment since prophylactic treatment will be needed either to accompany or precede the transfusion process (CDC 2004a, Okocha *et al.,* 2005).

The highest specific parasite rate of 12.26% was obtained within the age group of 21-30 years, majority being students. This age group was statistically associated with asymptomatic parasitaemia. Most of the individuals in this group are those who are likely to have developed some immunity level due to previous exposures to the parasite in this malaria endemic community. The bulk of malaria related morbidity and mortality in an endemic setting is concentrated

in children below ages of five years (WMR 2005), and the increasing resistance to infection and disease with age is conventionally thought to reflect a slow and gradual acquisition of protective immunity (Hviid 1998). Furthermore, these students are likely to be more exposed to malaria infections because they read at night in mosquito infested rooms and sleep in hostel rooms where mosquitoes abound carrying malaria parasite ready to be passed unto the human host during their blood meals. The conditions in which these students live (are often characterized by stagnant waters from blocked drainages, busted pipes, refuse dumps) are favorable for malaria transmission. If better reading environments (mosquito nets on windows and doors, insecticide treated nets in hostels and good care of refuses) are made available, the parasite rate in the students would be reduced drastically. These carrier groups are a risk to the public; they could be a reservoir of infection readily available for the feeding mosquitoes to acquire and transmit the parasite to the less immune children of the community.

The low parasite rate of 19% seen in children with specific parasite rate of 4.75% is not an unusual finding. Children of these ages (0-5years) have not yet developed pre-immunity to malaria; therefore on contact with the parasite, infections set in and are treated quickly in the community.

The prevalence rate of the asymptomatic parasitaemia was seen to be higher in non-academic staff (23%) compared to the academic staff (14%) of the university community. This could probably be due to their socio-economic differences and educational status, as a great proportion of them are Junior Staff

who live in areas with poor drainages suitable from malaria transmission. Association between socio-economic status, education, and malaria has been well documented (Gilles and WArell, 1993, CDC 2004a, Maduka 2004). The academics on the average are better paid and have better housing conditions, thus they eat well and live well as compared to the majority of the non-academic staff that live on low wages. It is also expected that greater awareness for malaria will be higher among academic than non-academic staff. There is therefore a need to revisit the living condition made available for the students and staff of the university community.

For the total 400 samples examined microscopically, an average parasite density index of 3.43 was observed per ul of blood. Though raw data showed that most individuals had parasite counts of 201-400 malaria parasites per ul of blood, no statistically significant association was established between different individuals and the parasite count ($\chi^2 = 5.74130$ $P > 0.05$). The highest density index of 4.0 was seen in ages 51-60 years. This means that individuals in this age group have the tendency of having the highest number of malaria parasite in their blood without coming down with the infection. These individuals have probably been exposed to the malaria infection over a long period of time, therefore have a higher level of some immunity. As such it will take a heavy load of the parasite in their body or stress and other diseases for them to come down with malaria.

During the period of this study (May-Oct. 2005) the prevalence of malaria parasitaemia among apparently healthy A.B.U community members Samaru, showed the highest peak in May, which was the on set of raining season that year. Apparently this period provided the right environmental condition for the proliferation of the malaria parasite vectors. This finding was statistically significant ($P < 0.05$) and similar to those found in southeast Nigeria at the onset of raining season in that region (Okocha *et al.*, 2005). Parasitaemia was particularly low in August because at this period, the frequency of the rain is high and the rate of flow of water does not support mosquito breeding (Krier, 1980;Gilles and Warell, 1993; Rogers, 1999; CDC, 2004 b).

The sensitivity and specificity of the malaria kit used in this study was seen to be 84% and 95% respectively when compared to microscopy. This is an indication that microscopy still remains the method of choice in clinical diagnosis. Although for research purposes for falciparum malaria study alone, it is very useful. This was confirmed by Kappa statistics value of 0.93, which showed it to be almost perfectly reliable. However, for clinical diagnosis, malaria kit should be used in conjunction with microscopy. Microscopy helps to detect and identify any of the parasite types while the malaria parasite kit are specific only for *P. falciparum* and *P. vivax*.

In this study, 62% of the 105 positive smear samples were *P. falciparum* with an infection rate of 16.25%. This species has already been determined to be

prevalent near the equator in West African regions and their prevalence could go as high as 70-80% (CDC 2004b, Okocha *et al.,* 2005). Other species identified in this study included: *P. malariae and P. ovale*. Only *P. vivax* was not seen, as these species are associated with temperate or cold regions of the world. (Macleans *et al.,* 2004, CDC 2004b). *P. malariae* accounted for 21% of the parasitaemia obtained in this study with an infection rate of 5.5%. This high rate of *P. malariae* is similar to the 23.4% found among blood donors in south-East Nigeria (Okocha *et al.*, 2005). Although in 1995, a study conducted in southwest of Nigeria showed a low rate of 7.4% *P. malariae* in the rural subjects, it was not seen in the urban subjects in the same study (Ademowo *et al.*, 1995).

P. ovale accounted for 4.76% of the 105 positive samples with an infection rate of 1.25%. Previous research has indicated that *P. vivax and P. ovale* preferentially invade reticulocytes and therefore rarely cause parasitaemia of high rate (Rogers, 1999). The low parasite rate of *P. ovale* is therefore not unusual. About 13.38% of the parasitaemia were mixed infections occurring either as *P. ovale* with *P. falciparum or P. malariae* with *P. falciparum*. Results similar to these had also been obtained in the southwest of Nigeria (Adewomo *et al.* 1995, Onyenekwe *et al.,* 2004) and southeast Nigeria (Okocha *et al.,* 2005).

The high prevalence of asymptomatic parasitaemia on Ahmadu Bello University campus Samaru, Zaria, with the highest rate among students necessitate the following recommendation:

(1) Effort should be intensified in educating students on the ills of malaria, self medication and incomplete treatment which may predispose to asymptomatic malaria.

(2) Prophylactic drugs for malaria control and prevention be made readily available to the community health center of the institution.

(3) Malaria parasite F Kit should be used in the University laboratories for emergency screening of Patients suspected with malaria, so that treatment can begin before the confirmation of the result with microscopy.

(4) Malaria surveillance system should be strengthened on A.B.U Campus Samaru to help identify asymptomatic carriers who should be adequately treated to prevent transmission to other people.

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APPENDIX I

Source CDC 2004

APPEDIX II

Ethical Certification
APPENDIX III

Permission obtained from A.B.U

APPENDIX IV
Statistical analysis using SPSS