Prevalence of malaria plasmodium in Abeokuta, Nigeria

Okonko, I. O.[1]*, Soleye, F. A.[2], Amusan, T. A.[3], Ogun, A. A.[4], Udeze, A. O.[5], Nkang, A. O.[1], Ejembi, J.[6] and Faleye, T. O. C.[1]

[1]Department of Virology, Faculty of Basic Medical Sciences, University of Ibadan College of Medicine, University College Hospital (UCH), Ibadan, University of Ibadan, Ibadan, Nigeria. WHO Regional Reference Polio Laboratory, WHO Collaborative Centre for Arbovirus Reference and Research, WHO National Reference Centre for Influenza, National HIV Reference Laboratory.
[2]Medical Laboratory Unit, Department of Health Services, University of Agriculture, Abeokuta, P.M.B. 2240, Abeokuta, Ogun State, Nigeria.
[3]Department of Veterinary Biochemistry, College of Veterinary Medicine, University of Agriculture, Abeokuta, Ogun State, Nigeria.
[4]Department of Epidemiology, Medical Statistics and Environmental Health, Faculty of Public Health, College of Medicine, University of Ibadan, Ibadan, Nigeria.
[5]Department of Microbiology, Faculty of Sciences, University of Ilorin, Ilorin, Nigeria.
[6]Department of Clinical Microbiology, Ahmadu Bello University Teaching Hospital (ABUTH), Zaria, Kaduna State, Nigeria.
E-mail: mac2finney@yahoo.com

_____

ABSTRACT

This study reports the prevalence of malaria caused by plasmodium between genders in Abeokuta, the capital city of Ogun State located in the forest zone of southwestern Nigeria between January 2002 and December 2004. Blood film examination for malaria parasites in 708 patients; 366 males and 342 females. Microscopic examination of thick films techniques was employed for this study. Of the 708 (100%) patients examined, 577 (81.5%) were *Plasmodium*-positive. A high malaria parasite prevalence rate of 81.5% was noted in this study. Female subjects were more infected (42.4%) than males (41.9%) however, there was no significant difference in the sex of the subjects studied (p=0.05). A high malaria parasite prevalence rate of 86.9% was noted in samples collected in year 2003 than in other years studied. There was significant difference in the years under study (p=0.05). This study shows that a good percentage of people were infested by malaria *Plasmodium*. This could be attributed to lack of adequate accommodation and poor sanitary conditions in the area under study. Although several efforts have been made to effectively control the high incidence of malaria in Nigeria, these have been largely unsuccessful due to a number of reasons such as irrigated urban agriculture which can be the malaria vector's breeding ground in the city, stagnant gutters and swamps in our environment where mosquitoes breed in millions, and lack of political will and commitment of the government in its disease management program, low awareness of the magnitude of malaria problem, poor health practices by individuals and communities and resistance to drugs. Therefore, future interventions in Nigeria should be directed toward controlling malaria in the context of a moderate transmission setting; thus, large-scale distribution of insecticide-treated nets or widespread use of indoor residual spraying may be less cost-effective than enhanced surveillance with effective case management or focused larval control.

Keywords: mosquito eradication, malaria, malaria interventions, *plasmodium*
_____

INTRODUCTION

Malaria is the world's most deadly parasitic disease and is caused by infection with single-celled parasites of the genus *Plasmodium* belonging to the apicomplexan phylum. *Anopheles* mosquitoes transmit these parasites from one person to another in their bites (Microsoft Encarta, 2009). Malaria is characterized by periodic bouts of severe chills and high fever. Serious cases of malaria can result in death if left untreated. More than a million people die of the disease each year, most of them in Africa, according to the World Health Organization (WHO) (Microsoft Encarta, 2009). It is one of the most prevalent and deadly widespread of all parasitic diseases in the world. Over 500 million people suffer clinical malaria episodes annually caused by *Plasmodium falciparum* infection alone resulting in a conservative estimate of 1 million deaths (Guinovart *et al*., 2006; Vaughan *et al*., 2008).

Malaria was once widespread in North America and other temperate regions. Today, the disease occurs mostly in tropical and subtropical regions, particularly in sub-Saharan Africa and Southeast Asia. The disease is also found in Central and South America, Oceania, and on some Caribbean islands. Public health officials had hoped to wipe out malaria during the 20th century. However, malaria parasites have developed defenses against many antimalarial drugs. This response, known as drug resistance, makes the drugs less effective. In addition, the

_____
*Corresponding author

Anopheles mosquitoes that transmit the disease have become resistant to many insecticides (Microsoft Encarta, 2009).

Indeed, malaria is still remains one of the most significant public health problems in Nigeria and perhaps the commonest cause of ill health in Africa. Almost all the entire population of Africa is at risk of this parasitic disease that has continued to claim at least one million deaths. It is mosquito-borne and one of the killer diseases of the world, currently accounting for about 300 to 500 million clinical cases annually and over 1.2 to 2.7 million deaths worldwide each year (WHO, 1992). In Nigeria, statistics show that malaria accounts for 25% of under-five mortality, 30% of childhood mortality and 11% of maternal mortality. All Nigerians are at risk of malaria and the problem is compounded by the increasing resistance of malaria to hitherto cost-effective antimalaria drugs.

Previous studies have shown that *Anopheles* mosquito breeding decreases with increasing proximity to the center of urban areas (Robert *et al.*, 2003). Although the complex factors that contribute to malaria risk are not fully understood, availability of vector breeding sites is clearly essential (Robert *et al.*, 2003). Urban agriculture, promoted as a means of increasing food security, improving nutrition, and alleviating poverty, can, especially when irrigated, create breeding habitats that could increase malaria transmission in cities. This potential risk was indicated by other authors (Afrane *et al.*, 2004).

However, malaria remains a global health problem, and public health efforts today focus on controlling it. In addition, a worldwide effort is under way to develop a vaccine that protects people against the disease. In the meantime, research by the WHO has found that sleeping under bed nets treated with insecticide can greatly reduce deaths from malaria, especially among children (Microsoft Encarta, 2009). Over the years, a lot of efforts have gone into controlling malaria in Nigeria and other African countries, the most affected by the disease, but the problem has not shown any sign of abating. The reasons for the limited success in efforts to eradicate malaria-a disease of poverty in Nigeria include lack of political will and commitment, low awareness of the magnitude of malaria problem, poor health practices by individuals and communities and resistance to drugs (Yusuf, 2007). This present study reports the prevalence of malaria caused by *plasmodium* between genders in Abeokuta, the capital city of Ogun State located in the forest zone of southwestern Nigeria between January 2002 and December 2004.

MATERIALS

Microscopy is the main tool for laboratory diagnosis of malaria (WHO, 1992). The thick blood film and the thin blood film methods are employed. Field's stain A (A polychrome methylene blue, disodium hydrogen phosphate and potassium hydrogen phosphate) and Field's stain B (Eosin, disodium hydrogen phosphate and potassium hydrogen phosphate). Thin film is carried out following the examination of thick film to identify the particular species of the *Plasmodium* responsible for the

infection; it is stained with commercially prepared Leishman stain.

The materials employed in the study included a Leitz light microscope, EDTA (ethylene diamine tetra acetic acid) bottles, methylated spirit (methanol), cotton wool, tourniquet, syringes (5 mL) and needles (21 G) (Epidi *et al.*, 2008).

Study area

This study was carried out at the Department of Health Services, University of Agriculture, Abeokuta. Abeokuta is the capital city of Ogun State located in the forest zone of southwestern Nigeria between January 2002 and December 2004.

Study population

After informed consent was obtained, a total of 708 subjects; (366 males and 342 females) blood samples were collected from subjects attending the Department of Health Services, University of Agriculture, Abeokuta, South-Western, Nigeria. Thick blood films were prepared for each person.

METHODS

Sample collection

The method of sample collection employed was venepuncture technique (Carmel *et al.*, 1993; Ibhanesebhor *et al.*, 1996; Okocha *et al.*, 2005; Epidi *et al.*, 2008). Soft tubing tourniquet was fastened to the upper arm of the patient to enable the index finger feel a suitable vein. The puncture site was then cleansed with methylated spirit (methanol) and venepuncture made with the aid of a 21 G needle attached to a 5 mL syringe. When sufficient blood had been collected, the tourniquet was released and the needle removed immediately while the blood was transferred into an EDTA bottle (Epidi *et al.*, 2008).

Laboratory Analysis

The collected blood samples were analyzed within 1 to 2 h of collection. Thick and thin blood films were prepared according to the technique outlined by Cheesebrough (2004) and described by Epidi *et al.* (2008). A drop of each blood sample was placed in the center of a grease-free clean glass slide. Thereafter, the reverse side of the slide was cleaned with cotton wool and kept for air-drying and staining with field's stain. The slide was held with the dried thick film side facing downward and dipped in field's stain A (eosin) for 5 s. It was washed off gently in clean water and then dipped in field's stain B (methyl azure) for 5 s and washed again in clean water. The back of the slide was cleaned with cotton wool and kept in the draining rack to air-dry for eventual examination under the microscope, using oil immersion at 100X magnification to observe for *Plasmodium* parasites. Presence of ring forms

of *Plasmodium* and Trophozoites of *Plasmodium* indicate positive results. A blood smear was considered negative if no parasite seen after 10 min of search or examination under 100 high power fields of microscope.

Identification

Positive specimens were identified on the basis of microscopy. Using standard methods (CDC, 2007), a trained laboratory technician at UNAAB Health Services Department interpreted the malaria blood slides. Prevalence of *Plasmodium* was calculated as the proportion of sampled persons with a positive result divided by the number of persons who provided blood samples. All point estimates were weighted, with empirically estimated standard errors used to account for prevalence.

Data analysis

The data generated from this study were presented using descriptive statistics. With SPSS version 15.0 for Windows (SPSS, 2006), was used to evaluate whether or not there were associations between the malaria *Plasmodium* prevalence and the subjects' variables. SPSS computer software was used for data analysis. Confidence level was set at p= 0.05

RESULTS

A total of 708 persons from different households in different communities and locations were enrolled in the study. Microscopy at UNAAB Health Services Department identified 577 (81.5%) malaria infections among the 708 (100.0%) persons who had provided a blood sample. Presence of ring forms of *Plasmodium* and Trophozoites of *Plasmodium* indicate positive results. The malaria *plasmodium* of these 708 blood samples collected from apparently healthy persons in this study with *Plasmodium*-positive and -negative slides are shown in Table 1. Table 1 shows the *plasmodium* of the apparently healthy persons in Abeokuta, Nigeria from 2002 to 2004. This study shows 267 (80.7%) cases of malaria *Plasmodium* among the subjects in 2002; 133 (86.9%) cases in 2003 and 177 (79.0%) in 2004 (Table 1).

Table 2 shows the frequency and distribution of malaria *Plasmodium* between genders of apparently healthy subjects from 2002 to 2004. The distribution of the *Plasmodium* species found in the 577 (81.5%) positive subjects is shown in Table 2; of which 297 (41.9%) of the male subjects tested positive for *Plasmodium* and 300 (42.4%) of the female subjects had *Plasmodium* (Table 2).

Table 3 shows the frequency and distribution of malaria *Plasmodium* between genders of apparently healthy subjects by year (2002). The distribution of the malaria *Plasmodium* found in 331 (100.0%) subjects is shown in Table 3; of which 267 (80.7%) of them were positive for *Plasmodium*, 134 (85.9%) of the positive subjects were males and 133 (76.0%) were females as shown in Table 3.

Table 4 shows the frequency and distribution of malaria *Plasmodium* between genders of apparently healthy subjects by year (2003). The distribution of the *Plasmodium* species found in 153 (100.0%) subjects for the year 2003 is shown in Table 5; of which 133 (86.9%) of them were positive for *Plasmodium*; 55 (83.3%) of the positive subjects were males and 78 (89.7%) were females as shown in Table 4.

Table 1: Malaria *Plasmodium* among apparently healthy subjects from 2002 to 2004

Year	No. tested (%)	No. positive (%)	No. negative (%)
2002	331 (46.8)	267 (80.7)	64 (19.3)
2003	153 (21.6)	133 (86.9)	20 (13.1)
2004	224 (31.6)	177 (79.0)	47 (20.9)
Total	708 (100.0)	577 (81.5)	131 (18.5)

Table 2: Distribution of malaria *Plasmodium* between genders of apparently healthy subjects from 2002 to 2004

Year	No. tested	No. positive (%)		Total (%)
		No. males (%)	No. females (%)	
2002	331	134 (40.5)	133 (40.2)	267 (80.7)
2003	153	55 (35.9)	78 (50.9)	133 (86.8)
2004	224	97 (43.3)	80 (35.7)	177 (79.0)
Total	708	297 (41.9)	300 (42.4)	577 (81.5)

Table 3: Frequency and distribution of malaria *Plasmodium* between genders of apparently healthy subjects by year 2002

Sex	No. tested (%)	No. positive (%)	No. negative (%)
Males	156 (47.1)	134 (85.9)	22 (14.1)
Females	175 (58.9)	133 (76.0)	42 (24.0)
Total	331 (100.0)	267 (80.7)	64 (19.3)

Table 4: Frequency and distribution of malaria *Plasmodium* between genders of apparently healthy subjects by year 2003

Sex	No. Tested (%)	No. Positive (%)	No. Negative (%)
Males	66 (43.1)	55 (83.3)	11 (16.7)
Females	87 (56.9)	78 (89.7)	9 (10.3)
Total	153 (100.0)	133 (86.9)	20 (13.1)

115

Table 5: Frequency and distribution of malaria *Plasmodium* between genders of apparently healthy subjects by year 2004

Sex	No. tested (%)	No. positive (%)	No. negative (%)
Males	120 (53.6)	97 (80.8)	23 (19.2)
Females	104 (46.4)	80 (76.9)	24 (23.1)
Total	224 (100.0)	177 (79.0)	47 (21.0)

Table 5 shows the frequency and distribution of malaria *Plasmodium* between genders of apparently healthy subjects by year (2004). The distribution of the *Plasmodium species* found in 224 (100.0%) subjects for the year 2004 is shown in Table 5; of which 177 (79.0%) of them were positive for *Plasmodium*; 97 (80.8%) of the positive subjects were males and 80 (76.9%) were females as shown in Table 5.

DISCUSSION

Malaria occurs almost exclusively in the tropics and sub-tropics (WHO, 2006) and approximately, 40% of the world's population, mostly those living in the world's poorest countries, are at risk of malaria (Atif *et al.*, 2009). Every year, more than 500 million people become severely ill with malaria-most cases and deaths occur within sub-Saharan Africa (WHO, 2007).

In this study, the presence of ring forms of *Plasmodium* and Trophozoites of *Plasmodium* indicate positive results. Results from the survey showed the overall prevalence of *Plasmodium* infection from 2002 to 2004 to be 85.1% in this area of Abeokuta, Southwestern, Nigeria. This differ from the overall prevalence of 59.9% reported in a study by Ojo and Mafiana (2005) among children <15 years in Abeokuta, Southwestern Nigeria and 51.5% reported by Epidi *et al.* (2008) among blood donors in Abakaliki, Southeastern Nigeria. Our results are substantially higher than previous estimates from passive surveillance of suspected malaria case-patients (Beatty *et al.*, 2007). Atif *et al.* (2009) reported an incidence rate of 10.5% malaria infection in a similar study among 1000 patients in Hyderabad, Sind, Pakistan.

Prevalence rate of 81.5% in this study represents a substantial level of illness, especially when one considers that the severity of the disease is likely high given the low level of acquired immunity among this population (Klinkenberg *et al.*, 2005). This high prevalence underscores the fact that, malaria is still a heavy burden on the continent, despite all that has been done.

Malaria can affect all the age humans groups and both male and female sexes. Studies have also shown seasonal variations in the rate of infections and differences in the types of malarial parasite depending upon the geographical conditions (Ghulam *et al.,* 2004). This study also showed that malaria parasite (*Plasmodium*) was higher in females (42.4%) than in male (41.9%) subjects. This is in agreement with the findings of Ibekwe

(2004) and Ibekwe *et al.* (2009) in similar studies in South-eastern, Nigeria but differs from Atif *et al.* (2009) who reported infection rate to be higher among young adult males in Pakistan.

This study shows that a good percentage of the apparently healthy persons were infested by malaria with *Plasmodium*. Majority of the subjects belonged to urban areas of Abeokuta and they were educated with higher socio-economic class. This is also contrary to Atif *et al.* (2009) who reported malaria infections to be prevalent among majority of uneducated patients with lower socio-economic class belonging to the rural areas of Hyderabad, in Pakistan.

The prevalence of *Plasmodium* is attributed to its ability to resist attack of most drugs that are commonly in use in the study area. The stagnant drainage systems in the University, Abeokuta Metropolis and its environs created favourable environmental conditions for the breeding of mosquitoes that act as vectors of malaria parasites and so this enhances the proliferation of the *Plasmodium* species. The prevalence of the *plasmodium* among these subjects could also be attributed to the effect of climatic features on vector breeding and transmission as wet season usually promote mosquito breeding.

The high prevalence of malaria *Plasmodium* obtained in this study is worrisome because high-density urban African populations are not often considered particularly vulnerable to malaria infection. In other West African urban areas, malaria prevalence rates from 2% to 16% have been reported with large variation between communities (Sabatinelli *et al.*, 1986). According to Klinkenberg *et al.* (2005), the observed low level of sensitivity of microscopy for identifying *Plasmodium* infections is similar to findings observed elsewhere (Kasehagen *et al.*, 2006). This could be attributable to 2 factors: 1) many of the infections likely occurred at low parasite densities, and 2) the laboratory technician was responsible for reading a large number of slides with low parasite prevalence over a relatively short period.

Malaria transmission was highly localized; all 577 (81.5%) infections were in persons from different areas of Abeokuta, which suggest that transmission is potentially based on a set of discrete ecologic determinants. Such clustering is consistent with the observed tendency for *Anopheles* mosquitoes to over disperse (Klinkenberg *et al.*, 2005). Recently, several authors focused attention on urban malaria (Robert *et al.*, 2003) and stressed the need to investigate risk factors for urban malaria. Although levels of transmission in urban areas may be lower than in contiguous rural areas, high population densities and possible lower immunity (Trape and Zoulani, 1987) may result in more disease impact in urban settings. Furthermore, although not the sole cause, irrigated urban agriculture which is practiced at the university may further increase the risk for malaria by providing suitable breeding sites.

The human behavioural pattern is a major epidemiological factor that impacts on disease transmission and progression in Africa and there is growing evidence that with appropriate awareness,

education, attitude, attention to and chemotherapy of, the key symptoms of malaria, the incidence of severe malaria can be drastically reduced especially in the rural and urban areas where most of the estimated 2 to 3 million deaths per year from malaria occur (Miller *et al.*, 1994; WHO, 2000). Prompt and accurate diagnosis of malaria is the key to effective disease management and therefore it is one of the main interventions of the global malaria control strategy (WHO, 1993). Identification of the species of malarial parasite is very important for its effective and curative treatment as resistance to chloroquine and other anti-malarial drugs has been reported previously (Rahim and Younas, 2003; Muhammad *et al.*, 2004; Atif *et al.*, 2009). Malaria in pregnancy is significantly associated with higher mortality and morbidity including cerebral malaria, maternal malaria, intrauterine growth retardation, abortions, still birth and premature labour (Kochar *et al.*, 1998; Sullivan *et al.*, 1999; Atif *et al.*, 2009).

Although several efforts have been made to effectively control the high incidence of malaria in Nigeria, these have been largely unsuccessful due to a number of reasons such as lack of political will and commitment, low awareness of the magnitude of malaria problem, poor health practices by individuals and communities and resistance to drugs (Sambo, 2007). Consequently, as long as there are stagnant gutters and swamps in our environment where mosquitoes breed in millions, there shall be no respite to the malaria scourge and its attendant effect on the health and socio-economic life of Nigerians and by extension Africans (Yusuf, 2007). Reducing poverty and improving sanitation and access to health care in malaria endemic regions would go a long way to reduce the malaria burden in Africa (Atif *et al.*, 2009). For those living in malaria endemic countries, limited resources frequently makes malaria prevention very difficult to implement. Vector control (reducing the breeding grounds by spraying or destruction of habitat) has only had very limited success. More successful strategies could include (WHO, 2005): Use of insecticide-treated bed nets (ITNs), indoor residual spraying, targeted chemoprophylaxis for those most at risk for pregnant women and travelers (Atif *et al.*, 2009)

In light of the current progress of malaria control efforts in Nigeria, where most states are not malaria free and the total number of cases has been steadily increasing, Nigeria is not yet on its way to achieving those original eradication goals. A key aspect of future research in Nigeria should therefore focus on understanding treatment-seeking behavior, barriers to accessing health services among febrile persons, and quantifying patterns of malaria transmission (Klinkenberg *et al.*, 2005). Future malaria intervention and preventive measures for the future hopes in the development of fatal malaria should include reducing poverty and improving access to health care in malaria endemic regions in Africa (Suh *et al.*, 2004). According to Suh *et al.* (2004) and Atif *et al.* (2009), malaria prevention for effective result should include measures taken both against mosquitoes' vectors and against the malarial parasite. Such interventions include vector control programs managed by government health authorities, personal protection measures to avoid mosquito bites and the use of chemoprophylaxis. However, due to the development of drug resistant parasites, drug side-effects and contraindications, the control of vector mosquitoes and avoidance of their bites have become increasingly important (Suh *et al.*, 2004; Atif *et al.*, 2009). Furthermore, the development of new anti-malarial drugs for prophylaxis and treatment as well as vaccines against malaria is also one important area under grand challenge exploration and the Malaria Genome project researches which involve molecular manipulation of the mosquito genome to produce transgenic mosquitoes that cannot infect humans will hopefully to provide new targets for both drugs and vaccines against malaria infection.

Nonetheless, future malaria interventions in Nigeria should also be directed toward controlling malaria in the context of a moderate transmission setting; thus, large-scale distribution of insecticide-treated nets or widespread use of indoor residual spraying may be less cost-effective than enhanced surveillance with effective case management or focused larval control.

REFERENCES

Afrane, Y. A., Klinkenberg, E., Drechsel, P., Owusu-Daaku, K., Garms, R., and Kruppa, T. (2004). Does irrigated urban agriculture influence the transmission of malaria in the city of Kumasi, Ghana? *Acta Tropica* **89, 125-134.**

Atif, S. H., Farzana, M., Naila, S., and Abdul, F. D. (2009). Incidence and pattern of malarial infection at a tertiary care Hospital of Hyderabad. *World Journal of Medical Sciences* **4, 9-12.**

Beatty, M. E., Hunsperger, E., Long, E., Schurch, J., Jain, S., and Colindres, R. (2007). Mosquitoborne infections after Hurricane Jeanne, Haiti, 2004. *Emerging Infectious Diseases* **13, 308-310.**

Carmel, B., Kenmogne, D., Copin, N., and Mbitsi, A. (1993). Plasmodium prevalence and parasite burden in blood donors of Brazzaville, Congo. *Annal of Society for Belgium Medicine Tropical* **73, 179-187.**

Centers for Disease Control and Prevention (2007). Laboratory identification of parasites of public health concern. 27th May, 2003. [cited 19th April, 2009.] http://www.dpd.cdc.gov/dpdx/html/diagnosticprocedures.htm

Cheesebrough, M. (2004). District laboratory practice in tropical countries. Part 2. Cambridge University Press. **pp. 357.**

Epidi, T. T., Nwani, C. D., and Ugorji, N. P. (2008). Prevalence of malaria in blood donors in Abakaliki Metropolis, Nigeria. *Scientific Research and Essay* **3, 162-164.**

Ghulam, M., Memon, I. A., Noorani, I.A., and Mehmood, A. K. (2004). Malaria prevalence in Sindh. *Medical Channel* **10, 41-42.**

Guinovart, C., Navia, M. M., Tanner, M., and Alonso, P. L. (2006). Malaria: burden of disease. *Current Molecular Medicine* **6, 137-140.**

Ibekwe, A. C. (2004). Baseline *Salmonella* agglutinin titres and *Plasmodium* load investigation in apparently healthy freshmen in Nnamdi Azikwe University, Akwa. B.Sc. Project in the Department of Applied Microbiology and Brewing, Faculty of Natural Sciences, Nnamdi Azikwe University, Akwa. **pp. 41.**

Ibekwe, A. C., Okonko, I. O., Onunkwo, A. I., Ogun, A. A., Udeze, A. O., and Ejembi, J. (2009). Comparative prevalence level of *Plasmodium* in freshmen (first year students) of Nnamdi Azikwe University in Awka, South-Eastern, Nigeria. *Malaysian Journal of Microbiology* **5, 51-54.**

Ibhanesebhor, S. E., Otobo, C. S., and Ladipo, O. A. (1996). Prevalence of malaria parasitaemia in transfused blood donor in Benin-City, Nigeria. *Annals of Tropical Paediatrics* **16, 93-95.**

Kasehagen, L. J., Mueller, I., McNamara, D. T., Bockarie, M. J., Kiniboro, B., and Rare, L. (2006). Changing patterns of *Plasmodium* blood-stage infections in the Wosera region of Papua New Guinea monitored by light microscopy and high throughput PCR diagnosis. *American Journal of Tropical of Medicine and Hygiene* **75, 588-596.**

Klinkenberg, E., McCall, P. J., Hastings, I. M., Wilson, M. D., Amerasinghe, F. P., and Donnelly, M. J. (2005). Malaria and irrigated crops, Accra, Ghana. *Emerging Infectious Diseases* 11, 1095. August 2005 [cited 18th January, 2009]. http://www.cdc.gov/ncidod/EID/vol11no08/04-1095.htm

Kochar, D. K., Thanvi, I., Joshi, A., Subhakaran, S. A., and Kumawat, B. L. (1998). Falciparum malaria and pregnancy. *Indian Journal of Malariology* **35, 123-130.**

Microsoft Encarta (2009) [DVD]. "Malaria." Redmond, WA: Microsoft Corporation, 2008.

Miller, L. H., Good, M. F., and Milon, G. (1994). Malaria pathogenesis. *Science* **264, 1878-1883.**

Muhammad, A. K., Smego, R. A., Razi, S. T., and Muhammad, A. B. (2004). Emerging drug resistance and guidelines for treatment of malaria. *Journal of College of Physicians and Surgeons Pakistan* **14, 319-324.**

Ojo, D. A., and Mafiana, C. F. (2005). Epidemiological studies of malaria parasitaemia in Abeokuta, Ogun State, Nigeria. *In:* the Book of Abstract of the 29th Annual Conference & General Meeting (Abeokuta 2005) on Microbes As Agents of Sustainable Development, organized by Nigerian Society for Microbiology (NSM), University of Agriculture, Abeokuta, 6-10th November, 2005. **pp. 50.**

Okocha, C. E. C., Ibeh, C. C., Ele, P. U., and Ibeh, N. C. (2005). The prevalence of malaria parasitaeia in blood donors in a Nigerian Teaching Hospital. *Journal of Vector Borne Diseases* **142, 21-24.**

Rahim, F., and Younas, M. (2003). Malaria in children: Clinical profile and response to treatment. A district level experience. *Journal of Medical Sciences* **11, 21-25.**

Robert, V., Macintyre, K., Keating, J., Trape, J.F., Duchemin, J. B., and Warren, M. (2003). Malaria transmission in urban sub-Saharan Africa. *American Journal of Tropical of Medicine and Hygiene* **68, 169-176.**

Sambo, A. (2007). Why efforts to eradicate malaria fail. *Pharmanews* **29, 1-64.**

Sabatinelli, G., Bosman, A., Lamizana, L., and Rossi, P. (1986). Prevalence of malaria in Ouagadougou and the surrounding rural environment during the period of maximal transmission. *Parassitologia* **28, 17-31.**

SPSS (2006). Statistical Package for Social Sciences 15.0 for Windows. http://www.spss.com

Sullivan, A. D., Nyirenda, T., Cullinan, T., Taylor, T., Harlow, S. D., and James, S. A. (1999). Malaria infection during pregnancy; intrauterine growth retardation and Preterm delivery in Malawi. *Journal of Infectious Diseases* **179, 1580-1583.**

Suh, K. N., Kain, K. C., and Keystone, J. S. (2004). Malaria. *Canadian Medical Association Journal* **170, 1693-1702.**

Trape, J. F., and Zoulani, A. (1987). Malaria and urbanization in central Africa: the example of Brazzaville. Part III: Relationships between urbanization and the intensity of malaria transmission. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **81, 19-25.**

Vaughan, A. M., Aly, A. S. I., and Kappe, S. H. I. (2008). Malaria parasite Pre-Erythrocytic Stage Infection: Gliding and Hiding. *Cell Host and Microbe* **4, 209-218.**

World Health Organization (WHO) (1992). Expert Committee on Malaria. 19th report. WHO technical report series No. 735. Geneva.

World Health Organization (WHO) (1993). A global strategy for malaria control. World Health Organization (WHO); Geneva.

World Health Organization (WHO) (2000). Severe Falciparum Malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **94 Suppl. 1.**

World Health Organization (WHO). 2005. Roll Back Malaria partnership: World Malaria Report, 2005; World Health Organization (WHO); Geneva.

World Health Organization (WHO). 2006. WHO Map of malaria endemic countries, World Health Organization (WHO); Geneva.

World Health Organization (WHO) (2007). WHO Fact sheet 94: Malaria, World Health Organization (WHO); updated May 2007.

Yusuf, M. (2007). Africa Malaria Day should focus on ridding Africa of mosquitoes. *Pharma News* **29, 1-64.**