

**THE PREVALENCE OF MALARIA AND HELMINTH INFECTION IN PREGNANCY AT
BOOKING AND THEIR RELATIONSHIP TO ANAEMIA AT THE UNIVERSITY OF PORT
HARCOURT TEACHING HOSPITAL, SOUTHERN NIGERIA.**

Abstract:

Objectives: To determine the prevalence of malaria and helminth infection in pregnancy and their relationship with anaemia at booking at the University of Port Harcourt Teaching Hospital.

Materials and Methods: This was a cross-sectional descriptive study of 192 pregnant women who booked for antenatal care at the University of Port Harcourt Teaching Hospital between August 1, 2015, and March 31, 2016. Socio-demographic data were collected through a structured questionnaire. Blood samples were collected and thick and thin blood films made, stained and examined for malaria parasites under a light microscope using x100 objective lens with oil immersion. Wet mount was prepared from the stool specimen using direct smear method with normal saline and iodine preparation, and the concentration procedure using formol/ether for the identification of ova of helminths. Data obtained was analysed using Statistical Package for Social Sciences (SPSS) version 20.0. The results are presented in frequency tables and figures.

Results: The prevalence of malaria, helminth, and their co-infection at booking were 24.5%, 0.5% and 0.5% respectively while the prevalence of anaemia at booking was 16.7%. Malaria and helminth co-infection accounted for 3.1% of the study population with anaemia while 75% of those with anaemia had malaria infection alone. The helminth infection identified in this study was *Ascaris lumbricoides*. There was a statistically significant relationship between malaria and helminth co-infection, and the area of residence ($p= 0.036$).

Conclusion: The prevalence of malaria and helminth co-infection was very low and had no statistically significant relationship with anaemia. Malaria infection was mainly associated with anaemia at booking.

Keywords: Pregnancy, Malaria, Helminth, Co-Infection, Southern Nigeria.

1. INTRODUCTION

Parasitic infection during pregnancy is a major cause of anaemia in Africa [1]. Malaria and helminthiases are co-endemic especially in sub-Saharan Africa, and there is an extensive overlap between the area of malaria and helminth infection in Africa [1,2]. Their co-infection is not uncommon, and its additive effect may lead to worsening anaemia and other morbidities, this could have dire consequences on pregnancy [1,2].

According to the World Health Organization (WHO) estimate, there were 212 million new cases of malaria worldwide in 2015 (148-304million), with Africa

41 contributing 90% of the global cases of malaria. About 429,000 (235,000 –
42 639,000) deaths occurred from malaria worldwide, most of these deaths occurred
43 in Africa (92%) [3]. Nigeria is one of the most endemic countries for malaria, and
44 the susceptibility to this infection is increased by pregnancy [4]. Malaria infection
45 in pregnancy is a major health challenge as it poses a substantial risk to the
46 mother, her fetus and the neonate. Many studies have demonstrated the
47 relationship between malaria in pregnancy and maternal anaemia, miscarriage,
48 placental parasitaemia, low birth weight, congenital malaria, stillbirth, and
49 maternal death depending on the level of transmission [5-8].

50 Helminths are parasitic worms. Helminth infections are more common in the
51 developing countries and produce a global burden of disease greater than that of
52 other known infectious diseases such as malaria or tuberculosis [9]. It is estimated
53 that approximately one-third of the 3 billion people in the developing region of
54 Sub Saharan Africa, Asia and the Americas that live on less than 2 US dollar per
55 day are infected with one or more helminths [1,10]. Helminth infection during
56 pregnancy is associated with anaemia, prematurity, low birth weight, and
57 increased maternal morbidity and mortality [11]. Haemoglobin concentration of
58 <11 g/dl or PCV < 33% is considered as anaemia in pregnancy by WHO [12].

Co-infection of *P. falciparum* and hookworm has been reported to have an additive impact on haemoglobin by increasing the susceptibility to *P. falciparum*, which could have a great consequence on the pregnancy outcome because it results in intrauterine growth retardation, low birth weight, pre-term delivery and neonatal mortality [13]. The adverse perinatal outcomes may include premature, hypotrophic neonates and stillbirths in the malarial population, which may be linked to intervillous macrophages that decrease the maternal blood output and the perivillous excess of fibrin that reduces the materno-fetal exchanges [14].

Studies on the co-infection of *P. falciparum* and intestinal parasites among the pregnant women in Southern Nigeria are lacking. This study was conducted to determine the prevalence of malaria and helminth infection in pregnancy at booking and their relationship to anaemia.

2. MATERIALS AND METHODS

2.1 Study Site

The study was done at the ante-natal clinic of the University of Port Harcourt Teaching Hospital. The hospital is located in Alakahia town in Obio Akpor Local Government Area of Rivers State. It is the apex health institution in Port Harcourt and provides specialized healthcare services for Port Harcourt and the entire

77 Rivers State, as well as neighboring states. The antenatal clinic runs every day
78 from Monday to Friday headed by Consultant Obstetricians on each day. An
79 average of 5000 antenatal women is seen annually, which amounts to about 400
80 women monthly with about 3000 admissions.

81 The prevalence rates of malaria parasite infection among pregnant women in the
82 area were 27%, 27.3%, and 21% in the first, second and third trimesters
83 respectively [15]. Malaria control in pregnant women in the area is with the use of
84 insecticide-treated nets (ITNs) and Intermittent Preventive Treatment in
85 Pregnancy (IPTp) with sulphadoxie/pyrimethamine.

86 2.2 Sample size calculation

87 The sample size for this study was calculated using the formula for cross-sectional
88 studies

$$89 \quad n = (Z^2 P (1-P) / d^2) [16]$$

90 Where

91 n = sample size

92 Z = proportion of normal distribution corresponding to the required significance
93 level (5%) which is 1.96

94 P = prevalence of malaria hookworm co-infection which is 13% [17]

95 d = degree of accuracy or precision expected (0.05)

96 Thus

97 $n = 1.96^2 \times 0.13 (1-0.13) / 0.05^2 = 173.79 = 174$

98 The sample size was thus calculated to be 174. Giving an allowance for 10%

99 attrition rate, the minimum sample size for the study was therefore 191 women.

100 **2.3 Methods**

101 This cross-sectional descriptive study was conducted among pregnant women
102 booking for ante-natal care at the University of Port Harcourt Teaching Hospital
103 from August 1, 2015, to March 31, 2016. The sample size for this study was
104 calculated using the formula for cross-sectional studies, using a previous
105 prevalence of malaria hookworm co-infection which was 13% [17].

106 Pregnant women who had antimalarial drugs either as chemoprophylaxis or for
107 treatment prior to booking, those who had anti-helminthics prior to booking,
108 those with Human immunodeficiency virus infection (HIV), and pregnant women
109 with sickle cell disease and other haemoglobinopathies were all excluded from
110 the study.

All pregnant women who satisfied the criteria and gave consent were recruited for the study. Socio-demographic data were collected using a structured questionnaire and included age, level of education, occupation, marital status, area of residence, religion and parity. The questionnaires were administered by the principal investigator and some trained assistants.

2.4 Blood and Stool sample collection

Blood film was made on a clean slide by placing a drop of blood on the slide and spreading it to an area of approximately 1cm^2 . Safety procedure was followed in the collection of venous blood sample by swabbing the antecubital fossae with 70% alcohol and five milliliters of blood was drawn into an ethylene diamine tetra-acetic acid (EDTA) bottle with a sterile hypodermic needle. The thick film was air dried and the slide was dipped into Field's stain A for 3 seconds. The slide was then dipped into tap water for 3 seconds and then Field's stain B for another 3 seconds. It was washed gently with tap water for a few seconds until the excess stain was removed and allowed to dry vertically. The same procedure was used for the thin film except that Field's stain B was applied first. Thin film was used to identify parasite species which was *P. falciparum* while thick film was used for parasite density. Malaria parasite density was done using the number of parasites

129 per high power field (hpf) and classified as 1+ (1-9 parasites/100 hpf), 2+ (≥ 10
130 parasites/100 hpf), 3+ (1-9 parasites/hpf), 4+ (≥ 10 parasites/hpf) [18].

131 MICROSCOPIC EXAMINATION

132 The thick film was used to determine the parasite densities. Stained slides were
133 examined under the light microscope using X100 objective lens in oil immersion.

134 Non-heparinized capillary tubes were filled with a blood sample from the EDTA
135 bottle. The tips of the capillary tubes were cleaned with cotton wool, sealed and
136 arranged inside a haematocrit centrifuge. They were centrifuged for 5 minutes at
137 12,000 revolutions per minute. The Packed Cell Volume was determined by using
138 a haematocrit reader. Anaemia was graded using WHO haemoglobin values for
139 anaemia in pregnancy as 10.0 - 10.9g/dl (mild), 7 – 9.9 g/dl (moderate), <7 g/dl
140 (severe) [19].

141 All patients were given clean labeled stool containers and instructed to bring
142 small quantities of their stool specimen on their next visit within an hour of
143 passing the stool to the laboratory. Wet mount was prepared from the stool
144 specimen using direct smear method with normal saline and iodine preparation,
145 and the concentration procedure using formol-ether for the identification of
146 helminth eggs as well as the protozoan cyst.

2.5 Statistical Analysis

Statistical package for Social Sciences SPSS version 20 was used for data analysis. The results are presented in means, standard deviation and frequency tables. Chi square was used to determine the correlation between variables. Statistical significance was reported at $P < 0.05$.

3. RESULTS

Of the 192 women recruited into the study, 160 (83.3%) were aged between 18-35 years, with a mean age at booking of 30.52 ± 4.75 years. The modal parity was para 1-4 with 53.1% of the study population in this group. Most (87%) of the women had tertiary education and majority (96.4%) of them resided in urban area while 3.6% resided in semi-urban area. Almost all (98.4%) of the women were Christians. These are shown in table 1.

The prevalence of malaria infection was 24.5%. Majority (89.4%) had mild parasite density and 10.6 % had moderate parasite density while that of helminth infection was 0.5%, and the infection was of moderate parasite density. The age, marital status, educational level and other variable had no significant association with the prevalence of helminth infection at booking. The prevalence of malaria and helminth co-infection in the study population was 0.5%. The mean Packed

Cell Volume was 32.67 ± 3.33 %. The prevalence of anaemia at booking was 16.7%. Majority, 28 (87.5%) of the study population with anaemia had mild anaemia while 12.5% (4) had moderate anaemia. These are all shown in table 2. Those who resided in semi-urban areas had the highest prevalence of malaria infection at 42.9%, however this was not statistically significant ($p>0.05$). The prevalence of malaria by parity was 29.3% in para 0, 21.6% in para 1-4, and 12.5% in $\geq$ para 5. The distribution of malaria parasitaemia among other co variates were as shown in figure 1.

Of the 192 women, only 1 had malaria and helminth co-infection and the helminth identified was *Ascaris lumbricoides*. The malaria parasite and helminth infection density were both moderate in this participant who resided in a semi-urban area. There was a statistically significant relationship between malaria and helminth co-infection, and the area of residence ($p= 0.036$). Other variables such as age, parity, marital status, level of education and religion had no statistically significant relationship with malaria and helminth co-infection ($p>0.05$). Figure 2 shows the results of malaria and helminth infection prevalence.

Most (71.4%) of the women with mild anaemia tested positive for malaria parasite while 28.6% (8) with mild anaemia were negative for the malaria

183 parasite. All the women with moderate anaemia tested positive for malaria
 184 parasite as shown in figure 3. There was a very low negative correlation ($r = -0.13$)
 185 between malaria and helminth co-infection, and anaemia in this study.

186 TABLES

VARIABLE	NUMBER	PERCENTAGES
AGE (years)		
<18	0	0%
18-35	160	83.3%
>36	32	16.7%
MARRITAL STATUS		
Married	192	100%
Single	0	0%
Divorced	0	0%
Separated	0	0%
LEVEL OF EDUCATION		
Tertiary	167	87.0%
Secondary	24	12.5%
Primary	1	0.5%
PARITY		
0	82	42.7%
1-4	102	53.1%
>4	8	4.2%
AREA OF RESIDENCE		
Urban	185	96.4%
Semi-urban	7	3.6%
Rural	0	0%
RELIGION		

Christianity	189	98.4%
Islam	3	1.6%
Others	0	0%

187 **TABLE 1: Socio-demographic characteristics**

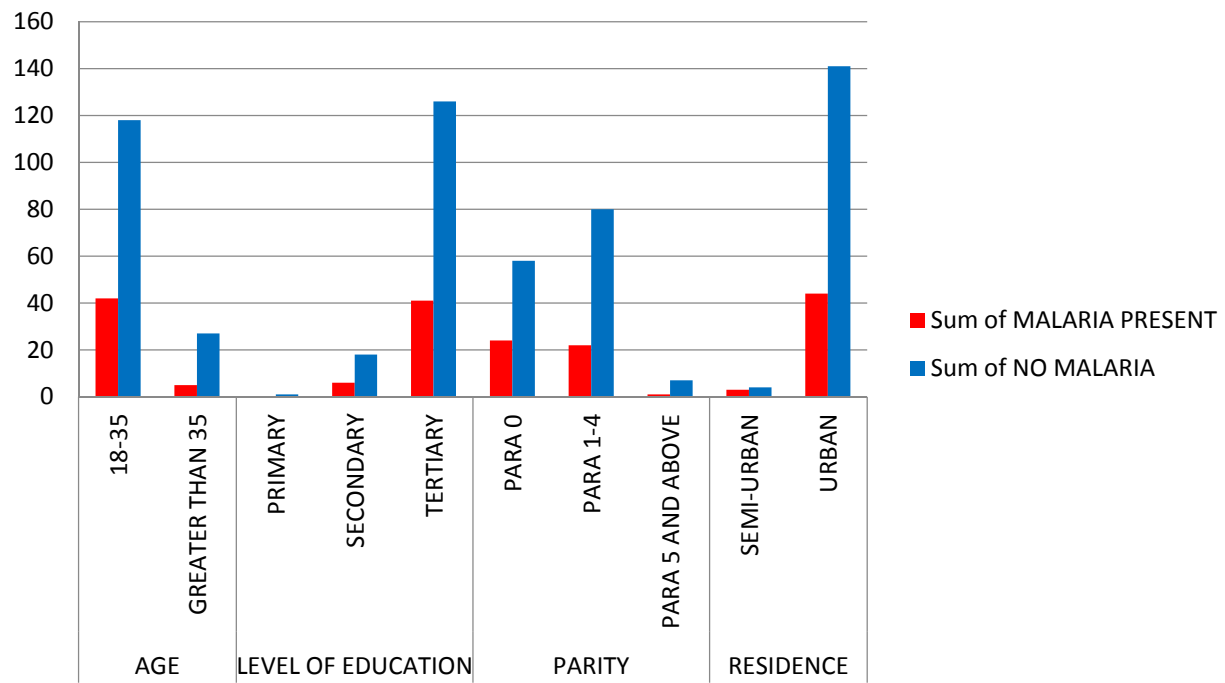
188 **TABLE 2: Laboratory results**

VARIABLE	FREQUENCY	PERCENTAGE
RESULTS FOR MALARIA INFECTION		
Positive for malaria parasite		
No	145	75.5%
Yes	47	24.5%
Parasite density		
+	42	89.4%
++	5	10.6%
+++	0	0%
RESULTS FOR HELMINTH INFECTION		
Stool microscopy positive		
No	191	99.5%
Yes	1	0.5%
Density of infection		
Mild	0	0%
Moderate	1	100%
Severe	0	0%
MALARIA HELMINTH CO-INFECTION		
No	191	99.5%
Yes	1	0.5%
HAEMATOLOGICAL RESULT		
Normal PCV	160	83.3%
Anaemia	32	16.7%
DEGREE OF ANAEMIA		
Mild	28	87.5%
Moderate	4	12.5%
Severe	0	0%

189

190

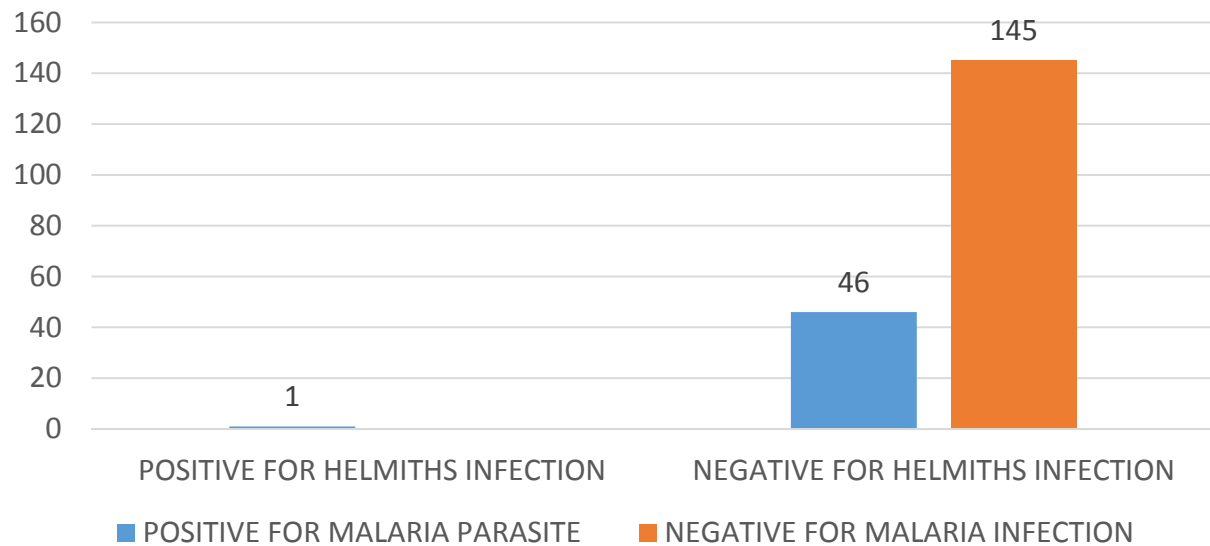
FIGURE 1: DISTRIBUTION OF MALARIA PARASITE INFECTION AMONG VARIABLES



191

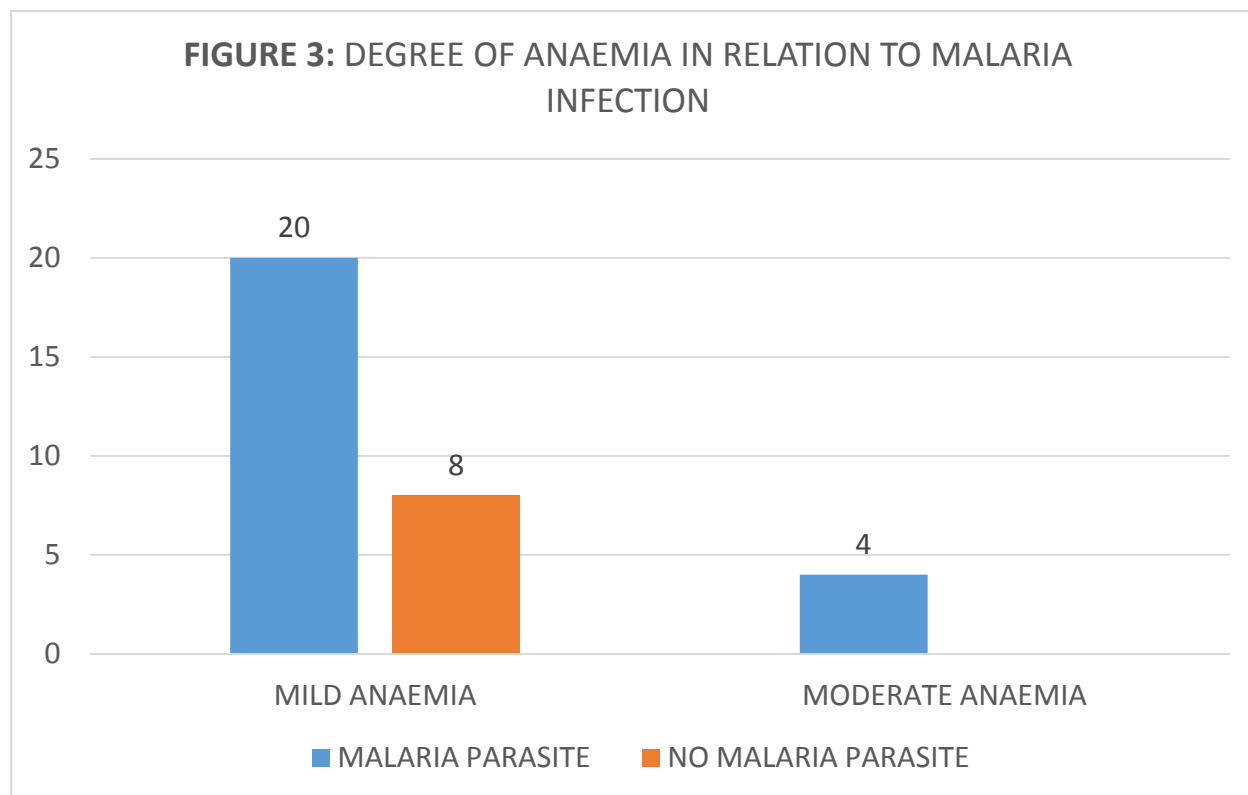
192

FIGURE 2: MALARIA AND HELMINTH INFECTION PREVALENCE



193

194



195

196

197 4. DISCUSSION

198 The findings from this study suggest that pregnant women were more susceptible
199 to malaria than helminth infection at booking at the University of Port Harcourt
200 Teaching Hospital. This was similar to reports from previous studies [20,21].
201 The prevalence of malaria was similar to that obtained in Gabon [21]. This may be
202 because the study population was similar, although the sample size of this study
203 was approximately half that of the one conducted in Gabon. It was however lower

204 than that reported in some other studies [17,20,22]. The higher prevalence in
205 these studies could be as a result of the study location, since all the study
206 participants resided in semi-urban areas with a lot of bushy surroundings, poor
207 sanitation and waste disposal facility while the majority of the participants in our
208 study resided in urban areas. In this study, those residing in semi-urban areas had
209 the highest prevalence of malaria compared to those who resided in urban areas.
210 It can therefore be deduced that residence in a semi-urban area was associated
211 with a higher prevalence of malaria. The study further reaffirms a higher
212 prevalence of malaria infection among primigravidae when compared to
213 multigravidae which were also reported in earlier studies [17,21,22].

214 The prevalence of helminth infection in this study was 0.5%, which was very low
215 when compared to reports from other studies [17,21,23]. This very high
216 prevalence can be attributed to the methodology used in those studies, the
217 sample population and their area of residence. In the study done in Gabon, stool
218 samples have tested a minimum of three times for helminth infection at monthly
219 interval if the initial stool sample tested was negative unlike in this study where
220 the stool samples of participants were tested only once. The high prevalence of
221 helminth infection as reported in Enugu and Anambra when compared to this
222 study could be attributed to the fact that the studies were done in semi-urban

223 and rural areas respectively, which were surrounded by thick forest with no
224 modern toilet and water drainage facilities. In contrast, the majority of
225 participants in this study were well educated and resided in an urban area with
226 modern toilet and water drainage facilities, which may have accounted for the
227 low prevalence of helminth infection in the study.

228 Malaria and helminth co-infection in this study were rather low when compared
229 to previous reports [17,20-22]. The high prevalence of malaria and helminth co-
230 infection in these studies could be attributed to the methodology used, socio-
231 demographic variables such as residence in a rural area or semi-urban area, parity
232 and low socio-economic status. Participants in this study resided mainly in an
233 urban area and were highly educated, which could account for the low prevalence
234 of malaria and helminth co-infection. However, there was a statistically significant
235 relationship between malaria and helminth co-infection and area of residence,
236 which showed an association between those residing in semi-urban area and
237 malaria and helminth co-infection.

238 The prevalence of anaemia in this study was lower than the findings from other
239 reports [17,23,24]. However, there was no statistically significant association
240 between malaria and helminth co-infection with anaemia. Majority of the women

with anaemia had malaria, this suggested that malaria infection was a more common cause of anaemia than helminth infection or their co-infection in our center. A similar finding was also reported in other studies [24,25].

5. LIMITATION

This study was carried out at a tertiary center located in an urban area, therefore it may not reflect the true picture of malaria and helminth co-infection and their effect on anaemia in the rural communities of Rivers State.

6. CONCLUSION

There was a very low prevalence of malaria and helminth co-infection at booking at the University of Port Harcourt Teaching Hospital. Larger studies and rural community-based studies are warranted.

CONSENT

A written informed consent was given by the patients to participate in the study and a verbal informed consent was obtained from them for the publication of this work.

ETHICAL APPROVAL

257 The authors hereby declare that the research was approved by the hospital ethics
258 committee. The ethical approval protocol number is
259 UPTH/ADM/90/S.II/VOL.X/500.

260 **COMPETING INTERESTS**

261 The authors have declared that no competing interests exist.

262 **REFERENCES**

- 263 1. Hotez PJ, Molyneux DH, Fenwick A, Ottesen E, Ehrlich Sachs S, Sachs JD.
264 Incorporating a rapid-impact package for neglected tropical diseases with
265 programs for HIV/AIDS, tuberculosis and malaria. Plos Med 2006; 3: 102.
- 266 2. Broker S, Akhwale W, Pullan R, Estambale B, Clarke SE, Snow RW, Hotez PJ.
267 Epidemiology of plasmodium-helminth co-infection in Africa: Population risk,
268 potential impact on anaemia and prospects for combining control. Am J Trop
269 Med Hyg 2007; 77: 88-98.
- 270 3. WHO Factsheet: World malaria report 2016.
- 271 4. WHO. A strategic framework for malaria prevention and control during
272 pregnancy in the African region. Brazzaville 2004, WHO Regional Office for
273 Africa; <http://www.afro.who.int/.../147-a-strategic-framework-for-malaria->

- 274 [prevention-and-control-during-pregnancy-in-the-african-region.html](http://www.who.int/prevention-and-control-during-pregnancy-in-the-african-region.html). Accessed
275 March 17 2014.
- 276 5. Steketee RW, Warima JJ, Campbel CC. Developing effective strategies for
277 malaria prevention programme for pregnant African women. Am J Trop Med
278 Hyg 1996; 55:95-100.
- 279 6. Luxemburger C, Ricci F, Nosten F, Raimond D, Bathet S, White NJ . The
280 epidemiology of severe malaria in area of low transmission in Thailand. Trans R
281 Soc Trop Med Hyg 1997; 91: 256-262.
- 282 7. Ezechukwu CC, Ekejindu IM, Ugochukwu EF, Egbuonu I, Oguata M, Chukwuka
283 JO. Congenitally acquired malaria in hyperendemic area: A cohort study. Trop J
284 Med Res 2004; 8: 44-48.
- 285 8. Ekejindu IM, Udigwe GO, Chijioke IRC. Malaria and Anaemia in pregnancy in
286 Enugu Southeastern Nigeria. Afr J Med Sci 2006; 351-353.
- 287 9. Hotez PJ, Brindley PJ, Bethony JM, King CH, Pearce EJ, Jacobson J. Helminth
288 infections: The great neglected tropical diseases. J Clin Invest 2008; 118: 1311-
289 1321.
- 290 10. Hotez PJ, Molyneux DH, Fenwick A, Kumaresan J, Ehrlich Sachs S, Sachs JD,
291 Savioli L. Control of neglected tropical diseases. N Engl J Med 2007; 357: 1018-
292 1027.

- 293 11. Christian P, Khatry SK, West JP. Antenatal anthelmintic treatment,
294 Birthweight and infant survival in rural Nepal. Lancet 2004; 364:981-983.
- 295 12. World Health Organization. Prevention and Management of Severe Anaemia
296 in Pregnancy: report of a technical working group. Geneva: 1994.
297 WHL/FHE/MSM/93.3.
- 298 13. Steketee RW, Nahlen BL, Parise ME, Menendez C. The burden of malaria in
299 pregnancy in malaria-endemic areas. Am J Trop Med Hyg 2001; 64:28-35
- 300 14. Philippe E, Walter P. Placental lesions in malaria. Arch Fr Pediatr 1985; 42(2):
301 921-923.
- 302 15. Wogu MN, Florence O. Nduka FO, Wogu M. Prevalence of Malaria Parasite
303 Infection among Pregnant Women Attending Antenatal Clinics in Port
304 Harcourt, Rivers State, Nigeria. Int J Trop Dis Health 2013; 3(2):126-132.
- 305 16. Jekel JF, Katz DL, Elmore JF. Sample size, randomization and probability
306 theory. Epidemiology biostatistics and preventive medicine. 2nd edition.
307 Philadelphia. WB Saunders 2001: 196.
- 308 17. Ekejindu IM, Okeke EK, Akah B, Okpala E, Ezeagwuna DA, Onwurah O. Malaria
309 and Hookworm co-infection among pregnant and non-pregnant women in a semi-
310 urban area in Anambra State, Nigeria. World J of Med Sci 2011; 6: 33-35.

- 311 18. World Health Organization. Basic Malaria Microscopy. Part 1. Learner's Guide.
312 2nd ed. Geneva: WHO; 2010.
- 313 19. World Health Organization. Preventing and Controlling Iron Deficiency
314 Anaemia through Primary Health Care. WHO Publications; 1989. Aug, (1989).
- 315 20. Egwunyenga AO, Ajayi JA, Nmorsi OPG, Duhlinska-Popova DD. Malaria/
316 intestinal helminth co-infection among pregnant Nigerian women. Mem Inst
317 Oswaldo Cruz 2001; 96: 1055-1059.
- 318 21. Adegnika AA, Ramharther M, Agnandji ST, Ngoa UA, Issifou S, Yazdanbakhsh M,
319 Kremsner PG. Epidemiology of parasitic co-infections during pregnancy in
320 Lambarene Gabon. Trop Med Int Health 2010; 15: 1204-1209.
- 321 22. Yatich NJ, Yi J, Agbenyega T, Turpin A, Rayner JC, Stiles JK, Ellis WO,
322 Funkhouser E, Ehiri JE, Williams JH, Jolly PE. Malaria and intestinal
323 helminth co-infection among pregnant women in Ghana: Prevalence and Risk
324 factors. Am J Trop Med Hyg 2009; 80: 896-901.
- 325 23. Agu P, Ogbosi JS, Akpoigbe K, Okeke T, Ezugwu E. Impact of plasmodium

falciparum and hookworm infections on the frequency of anaemia in pregnant women of rural communities in Enugu, South East Nigeria. Pan Afr Med J 2013; 14: 27.

24. Muhangi L, Woodburn P, Omara M, Omoding N, Kizito D, Mpairwe H, Nabulime J, Ameke C, Morison LA, Elliott AM. Association between mild to moderate anaemia in pregnancy and helminth, malaria and HIV infection in Entebbe, Uganda. Trans R Soc Trop Med Hyg 2007; 101: 899-927.

25. Mwangi TW, Bethony J, Brookers S. Malaria and helminth interactions in human: an epidemiological viewpoint. Ann Trop Med Parasitol 2006; 100: 551-570.

343

344

345