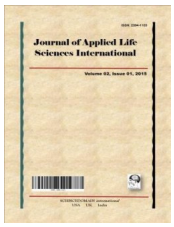

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Effects of Gravidity and Trimester on Malaria-geo-helminth Co-infections and Syndemics among Pregnant Women Attending Antenatal Care at Nandi Hills Sub-County Hospital, Nandi County in Kenya

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Authors' contributions

This work was carried out in collaboration between all authors. Author MRJ designed the study, wrote the protocol and wrote the first draft of the manuscript. Author NM managed the literature searches and analyses of the study. Author MJ managed the laboratory processes. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To investigate the effects of gravidity (primigravidae and multigravidae) and trimester on the co-infection with *P. falciparum* and geo-helminth parasites in pregnancy.
Study Design: The research was a cross sectional study of pregnant women attending antenatal care at Nandi Hills Sub County Hospital, Nandi County (Kenya) in the months of April to December 2015. Nandi Hills Sub-County lies within latitudes 0° and 0°34' North and Longitudes 34°45'' and 35°25'' East.
Methodology: Study population constituted consenting pregnant women attending antenatal care at Nandi Hills Sub County hospital in the year, 2015. The selection was an inclusive criteria and the

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respondents were stratified by gravidity and trimester. Pregnant women were selected by simple random Sampling to attain a sample size of 300.
Each participant was provided with a labelled screw caped stool container and informed on how to collect about 5 grams of stool sample. Stool was processed immediately at the hospital using formal- ether concentration technique. Consenting participants donated capillary blood sample by a finger prick. Thick and thin blood smears were prepared and stained using Field Stains A and B. Haemoglobin concentration was determined by drawing blood into the cuvettes and tested using diaspect haemoglobin test machine. Data was processed using statistical package for social sciences (SPSS Version 16). Chi square analysis were used in analysis.
Results: Difference in malaria and *A. lumbricoides* co-infection and malaria and hookworm co-infection in the two groups (primigravida and multigravida) was not significant (*P=.09* and *P=.10* respectively). Malaria parasites-*A. lumbricoides* and malaria parasites-hookworm co-infection were counter-syndemic and syndemic respectively by gravidity and trimester. Parasitic infections by gravidity and trimester did not significantly affect haemoglobin levels.
Conclusion: Gravidity and trimester did not alter the association of malaria – geo-helminth co-infection.

Keywords: Malaria; geo-helminth; co-infection; syndemics; counter-syndemics; gravidity; trimester; pregnancy.

1. INTRODUCTION

Plasmodium protozoa that cause malaria coexist alongside other pathogens and parasites many of which are also infectious to humans. Research and control of helminthiases is under-prioritized and under-funded and this has earned many parasitic worms a place among the WHO list of 'neglected tropical diseases' [1]. Adding to the global morbidity that results from human helminth infections are the observations that they have both direct and indirect effects on malaria and HIV/AIDS in developing countries [2].

Research is beginning to show some interesting and conflicting findings regarding the clinical and control implications of malaria co-infections with helminths [2]. Syndemics are an aggression of two or more diseases or afflictions in a population where there is a synergistic relationship which enhances and exacerbates the negative health effects of any or all of the diseases. Disease co-occurrence without any interaction is known as comorbidity or co-infection as opposed to syndemics whereby co-occurring diseases additively increases negative ill health [3].

Malaria and helminths have been classified as infectious syndemics of pregnancy in Africa and that vulnerability to syndemics involves factors that put groups in harm's way for cluster of diseases and those factors that contribute to the weakening of bodies or the degrading of the immune capacities [3]. It has increasingly been speculated that helminth infections may alter susceptibility to clinical malaria [4] and there is now increasing interest in investigating the consequences of this co-infection [5]. There is limited literature on helminth and malaria syndemics [3]. Gravidity and trimester have been evaluated in other studies only as risk factors of infection and co-infections and not the extent to which they can influence parasite association where malaria and geo-helminth parasites are comorbid in pregnancy.

Maternal health has been recalcitrant to progress in Sub-Saharan Africa and this has proved to be a formidable challenge in achieving millennium development goals (MDGs) [6]. This study therefore was important in testing the possibility that geohelminth infected pregnant women irrespective of gravidity or trimester could constitute a transmission hub for malaria parasites hence exacerbating their negative effects on the health of pregnant women.

A randomized clinical trial to look at the effect of helminthic treatment on malaria infections. Showed that treatment of *A. lumbricoides* was associated with two-fold increase in malaria parasitaemia in adults [7], suggesting a protective effect of *A. lumbricoides* co-infection.

It is well documented that a woman's immune response during pregnancy and chronic helminth infection shift towards type 2 immunity and anti-inflammatory cytokines. During pregnancy activities of CD4+ T cells, T cell type 2 (Th2) and their inflammatory cytokines interleukines 4, 5 and 10 increase. Inversely, the activities of

T helper 1 (Th1) cells and their inflammatory cytokines as well as inflammatory macrophages and natural killer (NK) cells decrease. Protective immune response therefore against a majority of intracellular pathogens during pregnancy is weakened. Infectious diseases including malaria during pregnancy are very common because Th1 immune response and their cytokines are down-regulated [8]. Apart from either the synergistic or antagonistic effects of the parasite on each other, a key symptom of both malaria and helminth infections and resultantly co-infections is anaemia in which pregnant women, children and immunocompromised people are at risk [1].

a cautious conclusion that can be drawn from the human studies conducted so far, is that helminth infections seem to increase the susceptibility to malaria infection [9]. There are no studies that have been done to evaluate the effect of pregnant women obstetrics on parasite co-infection and syndemics in Nandi Hills sub county. The area around town is inhabited by mainly the tea plantation and factory casual labourers whom the majority are those who have moved in from the neighbouring high parasite transmission areas of either the former Nyanza or western provinces.

Gravid levels and trimester stages could be among the factors that make pregnant women vulnerable to malaria-geo-helminth co-infection and syndemics. There are no available studied currently for the entire Nandi county (within former Rift Valley province) on parasitic infections and pregnancy. The county which is an epidemic zone has been neglected by researchers whose focus is mainly on the endemic area of Western and Nyanza regions of the country.

2. METHODOLOGY

2.1 Study Area

The study was carried out in Nandi Hills Sub-County Hospital in Nandi County (Fig. 1). It lies within latitudes 0° and 0°34' North and Longitudes 34°45'' and 35°25'' East [10]. Access to economic resources is low for women owing to the traditional division of labour that places women at the household level for domestic chores. Cases of early marriages are high in the county and are the major cause of school dropouts among the girls. Common diseases within Nandi County are malaria, upper respiratory tract infection, skin diseases and diarrhea. Period with highest outpatient cases is March and October when the area experiences heavy rains [11].

2.2 Study Population and Selection Criteria

Study population constituted consenting pregnant women attending antenatal care at Nandi Hills Sub County hospital in the year, 2015 from the month of April to December. There was no criteria for excluding pregnant women who were selected to participate. Pregnant women were randomly selected by lottery method whereby specific numbers were written on small pieces of paper, folded into small sizes, placed in a hat then mixed thoroughly. Individuals who picked a number similar to that picked by the researcher formed the subjects of the study.

2.3 Study Design and Sample Size

The current study was a cross sectional survey study of pregnant women attending antenatal care at Nandi Hills Sub-County hospital. The data was collected from April to December 2015 to obtain enough information that enabled drawing of inferences. Sample size was 300 and it was calculated basing on 95% confidence interval and 5% marginal error. A formula used to estimate the sample size (n) was as follows [12]:

$$n = \frac{Z^2 P(1-P)}{D^2}$$

where n is the sample size, D is the marginal error (0.05), P is the estimated prevalence (20%) and the Z is the normal standard deviation that corresponds to 95% confidence interval (1.96). Sample size was thus $1.962 \times 0.2(1-0.2)/0.05^2$. Sample size was found to be 245 individuals but it was expanded to 300 individuals because a larger sample is used, when research is to evaluate other parameters other than prevalence and intensity [13].

2.4 Stool Sample Collection and Laboratory Processing

Each consenting participant was provided with a labeled screw caped stool container and informed on how to collect about 5 grams of stool sample. Stool was processed immediately at the hospital using formal- ether concentration technique [14]. Microscopic examination was done using X10 objective magnification after staining with 1% lugol's iodine and results recorded in terms of presence/absence of ova in stool.

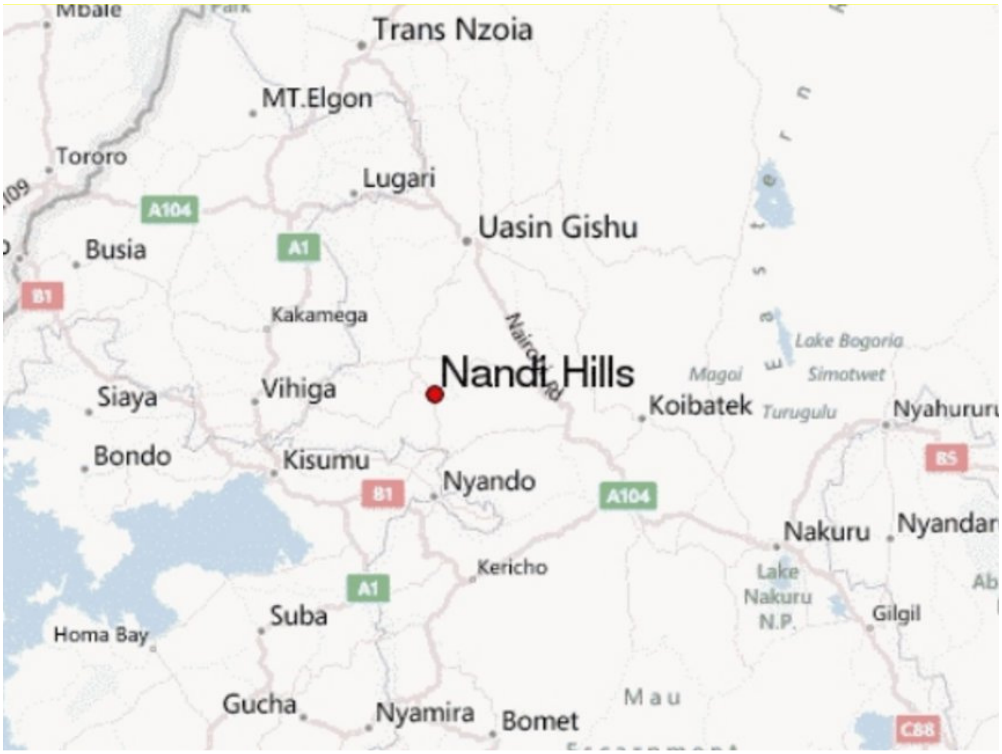

Fig. 1. A map showing Nandi-Hills sub County of Nandi County and neighbouring counties in the western part of Kenya; area shown in red is the study area
Source: Google Maps

2.5 Blood Sample Collection and Laboratory Processing

Consenting participants donated capillary blood sample by a finger prick. The finger was cleansed with alcohol moistened swab then dried with a piece of cotton. It was then punctured with a disposable blood lancet. Thick and thin blood smears were prepared and stained using Field Stains A and B. Microscopic examination was done under oil immersion at x100 objective to determine the level of parasitaemia and identify parasite species [15]. Estimation of *P. falciparum* densities was done by counting the number of *P. falciparum* parasites in relation to standard number of leucocytes in the thick film. Parasite density was thus calculated using the formula;

Parasite /µl of blood= number of parasites counted x 8000 leucocytes/ 500 leucocytes

Where 8000 leucocytes represents total number of white blood cells (WBCs) in an individual and 500 leucocytes represents the number of WBCs against which the parasites are counted [16]. Haemoglobin levels were determined by drawing blood into the cuvettes and tested using diaspect haemoglobin test machine then recorded as either hb≥11 (non-anaemic) or hb<11 (anaemic). Experienced laboratory technicians did microscopic examination and one with more experience did quality control.

2.6 Obstetric Information Collection

Obstetric information (gravidity and trimester) was obtained from the ANC booklet of the respondents. Each variable was coded and responses were accorded numerical values when recording the data in SPSS work sheet. Gravidity was categorized into (1) primigravidae and (2) multigravidae, whereas trimester was accorded values as (1) 1st trimester, (2) 2nd trimester and (3) 3rd trimester.

2.7 Analysis of Data

Data was recorded in a laboratory notebook and later transferred to excel spreadsheet protected by a pass word only accessible to the investigator. Data was processed using statistical package for social sciences (SPSS Version 16).

Comparison of prevalence of geo-helminthes and malaria co-infection was done using chi-square test. Synergistic and antagonistic relationships of parasites were determined using Phi and Cramer's V test of strength of association by mainly considering Phi ($r\varphi$) value. The test was used to determine the interaction of geo-helminth and malaria parasites by gravidity and trimester to show whether they were syndemic or counter syndemic.

3. RESULTS

3.1 Malaria and Geo-helminth Co-Infection and Syndemics

Out of the 300 pregnant women, only 2.7% were co-infected with malaria and either with *A. lumbricoides* or hookworm parasites. 13% of the pregnant women who were positive for *Plasmodium* were co-infected with *A. lumbricoides,* while 30% of the malaria parasites infected pregnant women were co-infected with hookworm parasites and none of them had *Plasmodium* and *T. trichiura* parasites. Differences in malaria parasites and *A. lumbricoides* co-infection was not significant (*P=.07*) compared to malaria parasites and hookworm co-infection which was significant (*P=.04*).

There was however a negative association between malaria parasites and *A. lumbricoides* co-infection and the strength of association ($r\varphi$) was -.105. Association between malaria parasites and hookworm was positive, ($r\varphi$=.121). Malaria parasites and *A. lumbricoides* were antagonistic while malaria and hookworm parasites were synergistic.

3.2 Parasite Infection by Gravidity

3.2.1 Single parasite infections by gravid groups

Primigravidae constituted 75(25%) of the study population while multigravidae made up 225(75%) of the study population. More of the primigravidae group had *Plasmodium falciparum* parasites. Malaria parasites infection within primigravidae group was 11(15%) whereas within multigravida group it was 12(5%) (Table 1). The difference in malaria parasites infections between these gravid groups was significant (*P=.01*). In regard to helminth infections, 22(29%) of the primigravidae were infected with *A. lumbricoides*, while 67(30%) of the multigravida were infected with *A. lumbricoides* and the difference in infection proportions in these groups was insignificant *(P=.94)*, individuals among primigravidae who were infected with hookworm were 14(19%) while 32(14%) infected among the multigravidae group. The difference in infection proportions by hookworm was not significant (*P=.36*). All the three pregnant women infected with *T. trichiura* were multigravidae (*P=1.19*) (Table 1).

3.2.2 Malaria and geo-helminth co-infection and syndemics among different gravid groups

Out of the total 11 primigravidae women who were positive for malaria parasites, 2 of them (18%) were co-infected with *A. lumbricoides* and 4 (36%) were co-infected with hookworm. Out of the total 12 multigravidae women positive for malaria parasites, 1 (8%) was co-infected with *A. lumbricoides* and 3 (25%) were co-infected with hookworm parasites (Table 2).

The difference in *P. falciparum* and *A. lumbricoides* co-infection and *P. falciparum* and hookworm co-infection in the two groups (primigravida and multigravida) was not significant (*P=.09*). There was no co-infection between malaria and *T. trichiura* parasites in any gravidity. There was an insignificant negative association (counter-syndemic relationship) between malaria- *A. lumbricoides* and insignificant positive association (syndemic relationship) in malaria-hookworm co-infection by gravidity. Strength of association between malaria parasites and *A. lumbricoides* and that between malaria parasites and hookworm in the primigravid group was weak (*$r\varphi$=-.102* and *$r\varphi$=.19* respectively). The strength of association of malaria and geo-helminth parasites in the multigravida group was also weak (*$r\varphi$ =-.111* and $r\varphi$=.07) for malaria parasites-*A. lumbricoides* and malaria-hookworm respectively (Table 2).

3.3 Parasite Infection by Trimester

3.3.1 Single parasite infection by trimester groups

Pregnant women in their 1st trimester were 19.3%, 57% were in 2nd trimester and 23.7% were in 3rd trimester. Infection with *Plasmodium falciparum* was high among the 1st trimester group. 12(21%) of the 1st trimester individuals had malaria parasites, while 8(5%) and 3(4%) in

the 2nd and 3rd trimester respectively were positive for malaria parasites. There were significant differences in malaria infection proportions by trimesters (*P=.00*).

For *A. lumbricoides* infection, 16(28%), 55(32%) and 18(25%) of those in 1st, 2nd and 3rd trimesters respectively were infected. 14(24%), 17(10%) and 15(21%) of those in their 1st, 2nd, and 3rd trimesters respectively had hookworms, while the three infections (2%) of *T. trichiura* were found among the 2nd trimester group. The difference in infection proportions in *A. lumbricoides* and *T. trichiura* by trimester was not significant (*P=.53* and *P=.08* respectively). The difference in hookworm infection proportion by trimester was significant (*P=.01*).

3.3.2 Malaria and geo-helminth parasites co-infection and syndemics by trimester

Out of the 12 *P. falciparum* infected individuals, 2(17%) were co-infected with *A. lumbricoides,* and 4(33%) of them were co-infected with hookworms in their 1st trimester. Among those in the 2nd trimester, there was only one case (12%) of malaria parasites and *A. lumbricoides* co-infection and 2(25%) cases of malaria parasites and hookworm co-infections out of 8 *P. falciparum* infected individuals. There was no *P. falciparum-A. lumbricoides* in the 3rd trimester but for *P. falciparum*-hookworm, co-infection was insignificant at 33% (Table 2). There were no cases of malaria- *T. trichiura* co-infections in all the three trimesters. Differences in co-infection proportions in all the trimester groups was not statistically significant for *P. falciparum-A. lumbricoides* (*P=.34, P=.22* and *P=.30)* for 1st, 2nd and 3rd trimester respectively. Similarly, differences in *P. falciparum*-hookworm co-infection proportions were not significant (*P=.40, P=.15* and *P=.60*) for 1st, 2nd and 3rd trimesters respectively (Table 2).

Co-infection with *P. falciparum* and *A. lumbricoides* had a negative association in all the three trimester groups and the strength of association was r^{φ} =-.125, r^{φ}=-.093 and r^{φ}=-.122 for 1st, 2nd and 3rd trimesters respectively. Co-infection with *P. falciparum* and hookworm had a positive association in all the trimester groups (r^{φ}=.110, r^{φ}=.111 and r^{φ}=.063 for 1st, 2nd and 3rd trimesters respectively). With trimester, *P. falciparum- A. lumbricoides* remained counter syndemic whereas *P. falciparum*-hookworm remained syndemic.

Table 1. Parasite infection rates by gravidity

Parasites		Primigravidae n=75	Multigravidae n=225	Total	P value
P. falciparum	Positive	11 (15%)	12(5%)	23(8%)	0.01
A. lumbricoides	Present	22(29%)	67(30%)	89(30%)	0.94
Hookworm	Present	14(19%)	32(14%)	46(15%)	0.36
T. trichiura	Present	0(0%)	3(1%)	3(1%)	1.19

P. falciparum and hookworm infections were significant at .05 infections while A. lumbricoides and T. trichiura were not significant; (%)= percentage within gravid group, 75 for primigravidae and 225 for multigravidae

Table 2. *P. falciparum*-geohelminth co-infections in relation to gravidity and trimester

Characteristics	n	*P. falciparum-A. lumbricoides*				*P. falciparum*-hookworm			
		Present	Absent	P value	r^{φ}	Present	Absent	P value	r^{φ}
Gravidity									
Primigravidae	11	2(18%)	9(82%)	.38	-.102	4(36%)	7(64%)	.10	.188
Multigravidae	12	1(8.3%)	11(91.7%)	.10	-.111	3(25%)	9(75%)	.27	.073
Trimester									
1st	12	2(17%)	10(83%)	.34	-.125	4(33%)	8(67%)	.40	.110
2nd	8	1(12%)	7(88%)	.22	-.093	2(25%)	6(75%)	.15	.111
3rd	3	0(0%)	3(100%)	.30	-.122	1(33%)	2(67%)	.60	.063

n= total P. falciparum in each category; (%) = co-infection percentage within P. falciparum; r^{φ}= Phi value for strength of association

3.4 Effects of Parasite Infection, Gravidity and Trimester on Haemoglobin Levels of Pregnant Women Attending Antenatal Care at Nandi-Hills Sub County Hospital

3.4.1 Effects of parasite infection on haemoglobin levels of pregnant women

A larger proportion of pregnant women (71.7%) had normal haemoglobin levels (Hb≥11mg/dl of blood) while only 28.3% were anaemic (Hb<11 mg/dl of blood). Only 4(2%) of those who had normal haemoglobin levels (Hb≥11 mg/dl of blood) were positive for malaria while 19(22%) of those who were anaemic tested positive for malaria. Among those infected with hookworm 11(5%) had normal haemoglobin levels, while 35(41%) of the hookworm infected pregnant women had haemoglobin levels of less than 11 (Hb<11; Table 3)

Infection proportions with either *P. falciparum* or hookworm parasites in relation to haemoglobin levels of the pregnant women was significantly different (*P=.00*). Furthermore, there was significant negative association between either *P. falciparum* or hookworm infection and haemoglobin levels of the pregnant women (r^{φ}=-.347 and r^{φ}=-.451 respectively).

There was only one individual (25%) out of the 4 malaria parasites infected pregnant women in the non-anaemic category (Hb≥11) who was co-infected with *A. lumbricoides* but this was not statistically significant (*P=.83*). Among those in the anaemic category (Hb<11), 2(10.5%) out of 19 malaria parasites infected individuals were co-infected with *A. lumbricoides* and the difference in co-infection proportion in this category was significant (*P=.04*; Table 4).

Similarly, an insignificant proportion 1(25%) out of 4 malaria parasites infected individual in the non-anaemic category was co-infected with hookworm (*P=.07*) and 6(31.6%) out of 19 malaria parasites infected individuals in the anaemic category were co-infected with hookworm and the difference in co-infection proportion was insignificant (*P=.33*; Table 4). *P. falciparum- A. lumbricoides and P. falciparum*-hookworm co-infection in relation to haemoglobin level of <11, that is, the anaemic category had a negative association (r^{φ}=-22 and r^{φ}=-0.105 respectively).

3.4.2 Effects of parasite infection and gravidity on haemoglobin levels of pregnant women

In the non-anaemic category (Hb≥11), the proportion of pregnant women infected with *P. falciparum* was insignificant 2(0.9%) for both primigravidae and multigravidae (*P=.19*). Similarly, there was an insignificant difference in the proportions of those infected with *P. falciparum* in the anaemic (Hb<11) category (*P=.10*). All the three geohelminths did not significantly affect haemoglobin levels of the pregnant women in both primigravidae and multigravidae (Table 5).

Table 3. *P. falciparum*, geohelminth infections and haemoglobin levels of pregnant women

		Hb≥11 n=215	(%)	Hb<11 n=85	(%)	Total	(%)
Plasmodium	Positive	4	2%	19	22%	23	8%
Ascaris	Present	64	30%	25	29%	89	30%
hookworm	Present	11	5%	35	41%	46	15%
Trichuris	Present	2	1%	1	1%	3	1%

Table 4. *P. falciparum* –geohelminth co-infections in relation to haemoglobin levels

	Haemoglobin levels	
	Hb≥11 n=4	Hb n=19
P. falciparum- A. lumbricoides	1(25%)	2(10.5%)
p value	*P=.83*	*P=.04*
r^{φ}	r^{φ}=-.01	r^{φ}=-.22
P. falciparum-hookworm	1(25%)	6(31.6%)
P value	*P=.07*	*P=.33*
r^{φ}	r^{φ}=.12	r^{φ}=-.11

n= number of P. falciparum infections in the anaemic and non-anaemic categories; p=level of significance at .05; r^{φ}= strength of association

3.4.3 Effects of parasite infection and trimester on haemoglobin levels of pregnant women

Pregnant women in the non-anaemic category (Hb≥11) and were infected with *P. falciparum* in their 1st, 2nd and 3rd trimesters were 1(0.5%), 1(0.5%) and 2(0.9%) respectively while those in the anaemic category infected with *P. falciparum* were 11(12.9%), 7(8.2%) and 1(1.2%) respectively. The difference in infection proportions by *P. falciparum* in the non-anaemic and anaemic categories was insignificant (*P=.10* and *P=.07*) respectively (Table 6).

There was no significant difference in infection proportions of those infected with *A. lumbricoides* in the non-anaemic category, 10(4.7%), 42(19.5%) and 12(5.6%) in the 1st, 2nd and 3rd trimesters respectively (*P=.25*). similarly, those in the anaemic category were insignificantly affected by *A. lumbricoides,* 6(7.1%), 13(15.3%) and 6(7.1%) respectively in the 1st, 2nd and 3rd trimesters respectively (*P=.24*; Table 6).

Non-anaemic category infection proportion with hookworm was not significantly different (*P=.14*) where infection proportions were 3(1.4%), 4(1.9%) and 4(1.9%) in 1st, 2nd and 3rd trimesters respectively but infection proportions of those in the anaemic category was significant (*P=.05*) whereby 11(12.9%), 13(15.3%) and 11(12.9%) respectively were infected with hookworm in 1st, 2nd and 3rd trimesters (Table 6).

Table 5. Parasitic infection of primigravidae and mutigravidae in relation to haemoglobin levels of pregnant women

	Gravidity	Haemoglobin level	
		hb≥11 n=215	**hb<11 n=85**
P. falciparum	Primigravidae	2(0.9%)	9(10.6%)
	Multigravidae	2(0.9%)	10(11.8%)
p value		*P=.19*	*P=.10*
A. lumbricoides	Primigravidae	16(7.4%)	6(7.1%)
	Multigravidae	48(22.3%)	19(22.1%)
P value		*P=.54*	*P=.32*
Hookworm	Primigravidae	3(1.4%)	11(12.9%)
	Multirgavidae	8(3.7%)	24(28.2%)
P value		*P=.67*	*P=.96*
T. trichiura	Primigarvidae	0(0)	0(0)
	Multigravidae	2(0.9%)	1(1.2%)
P value		*P=.45*	*P=.50*

(%) = percentage within haemoglobin level category; p= level of significance at .05

Table 6. Parasitic infections of different trimester groups in relation to haemoglobin levels of pregnant women

	Trimesters	Haemoglobin levels	
		Hb≥11; n=215	**Hhb<11;n=85**
P. falciparum	1st trimester	1(0.5%)	11(12.9%)
	2nd trimester	1(0.5%)	7(8.2%)
	3rd trimester	2(0.9%)	1(1.2%)
P value		*P=.29*	*P=.07*
A. lumbricoides	1st trimester	10(4.7%)	6(7.1%)
	2nd trimester	42(19.5%)	13(15.3%)
	3rd trimester	12(5.6%)	6(7.1%)
P value		*P=.25*	*P=.24*
Hookworm	1st trimester	3(1.4%)	11(12.9%)
	2nd trimester	4(1.9%)	13(15.3%)
	3rd trimester	4(1.9%)	11(12.9%)
P value		*P=.14*	*P=.05*

(%) = percentage within haemoglobin level categories; p value= level of significance at .05

4. DISCUSSION

The study was done in Nandi-Hills sub county hospital in Nandi County where no other study had been conducted before. Nandi-Hills is part of the western highlands of Kenya which is an epidemic area with seasonal transmission, hence a low transmission region for parasitic infections [17]. Available studies in western part of Kenya have been concentrated in the former Nyanza province which is a high transmission area and have shown a high parasitic infections for both malaria and geohelminth parasites in pregnant women [18,19] and school children [20]

Malaria parasites and *A. lumbricoides* were counter-syndemic among the pregnant women attending antenatal care at Nandi Hills Sub County hospital, Nandi County, while malaria parasites and hookworm co-infections were syndemic. The outcome of the current study had similarities with a meta-analysis study [21] which showed that pregnant women with hookworm infections had 1.36 times higher risk of malaria infection than those mothers without hookworm and a randomized clinical trial [7] to look at the effect of helminthic treatment on malaria infections. They noted that treatment of *A. lumbricoides* was associated with two-fold increase in malaria parasitaemia in adults, suggesting a protective effect of *A. lumbricoides* co-infection.

The proportion of malaria infected primigravidae was higher than the multigravidae. This outcome suggested that multigravida women may have been exposed to different *P. falciparum* strains consequently developing better immunity [22] as opposed to primigravids seen in the current study.

Geo-helminth infection did not show preference for any gravid group. This suggested that irrespective of the number of pregnancies one had had, they were equally exposed to the infective stages of geo-helminth infection. Those co-infected with *P. falciparum* and any of the three geo-helminths by gravidity did not show any significant difference. Negative association between *P. falciparum* and *A. lumbricoides* in the primigravidae and multigravidae groups and positive association between *P. falciparum* and hookworm for primigravidae and multigravidae implied that gravidity cannot alter association of malaria parasites and geohelminths in pregnancy.

Those in their 1st trimester had a higher proportion of infection of malaria infection as opposed to those in 2nd and 3rd trimesters. Malaria parasites are frequent during the second half of pregnancy, probably because of immunosuppression associated with pregnancy [15]. It is plausible to note that most women do not take their pregnant status seriously to even consider protecting themselves from malaria infections before the pregnancies begin to advance into second trimester. This is probably due to their inability to access protective measures such as prophylaxis because in Nandi County generally women are lower resource owners [11] and men/husbands cannot invest resources in preventing infection in a pregnancy that they are not able to see.

Any of the three geo-helminthes (*A. lumbricoides*, hookworm and *T. trichiura*) could infect the pregnant women irrespective of their trimesters. Co-infection with *P. falciparum* and any of the three geo-helminths by trimester was not significant. Negative association between *P. falciparum* and *A. lumbricoides* by trimester and a positive association between *P. falciparum* and hookworm by trimester was probably due to independent effects of the malaria and geo-helminth parasites on each other. Lack of *P. falciparum- A. lumbricoides* co-infection in the 3rd trimester could have resulted from low *P. falciparum* infection rate in the 3rd trimester.

Proportion of anaemic versus non-anaemic women in relation to either *P. falciparum* or *A. duodenale* infections was statistically significant. There were no cases of severe anaemia recorded in the current study. Proportion of anaemic versus non-anaemic women in relation to either *P. falciparum* or hookworm infections was statistically significant. In Tanzania, women who had parasitic infections were more susceptible to anaemia as compared to their counter parts who were not infected [23].

An important consequence of both malaria and helminth infection is anaemia, [24] an important public health problem in the tropics. Anaemia though develops only if the dietary intake of the affected individual is inadequate in iron [25]. Due to different mechanisms of by which malaria parasites and helminths cause anaemia, their impact on haemoglobin levels could be additive [26]. Malaria can potentiate the expected anaemia of pregnancy [15].

Outcome of the current study showed that malaria parasite-hookworm co-infection had a negative impact on the haemoglobin levels of the pregnant women, that is, co-infection with the two parasites decreased haemoglobin levels. As it has been noted, anaemia is a key symptom of malaria and helminth infections in pregnancy [6,1]. However, haemoglobin levels of pregnant women were not measured against hookworm intensity. It was likely that low haemoglobin levels recorded was as a result of low nutritional status of the subjects due to consumption of food which are poor sources of iron and folate [27]. The county experiences food poverty of 46.7% because most food crops are grown on small scale farms once a year [11]. There was interaction between malaria parasites-helminth co-infections and haemoglobin level of pregnant women in western Kenya [18]. Other parasites that were found infecting pregnant women at Nandi-Hills sub county hospital were *Taenia* parasites (1.7%) and *Entamoeba histolytica* (2.3%).

5. CONCLUSION

There existed a counter-syndemic relationship between malaria parasites and *A. lumbricoides* and a syndemic relationship between malaria by *P. falciparum* and hookworm. Gravidity and trimester did not alter the relationship between malaria causing parasites and geo-helminth parasites. Parasitic infections did not reduce haemoglobin levels of the pregnant women in both gravid groups and all trimester groups, that is, syndemic effect of parasite infection on Hb in relation to gravidity or trimester was not significant. There is need however to diagnose pregnant women for both *P. falciparum* and geo-helminth parasites as soon as they present themselves for ante-natal care and be advised on cost effective measures of caring for themselves during the course of pregnancy. Further research should be done on intensity of geohelminth infections and malaria parasites and evaluate their syndemic effects on haemoglobin levels of pregnant women in Nandi-Hills sub county. Those who were found to be infected with *P. falciparum* and geohelminth parasites were recommended for treatment with suitable drugs in the hospital [28] preferably IPT-SP for malaria parasites and albendazole for geohelminths. A follow up was made with the nurse in charge of MCH to ensure that treatment was effected. All pregnant women were recommended to take iron supplements. It is important to carry out a study on intestinal parasite infections among pregnant women to prevent any ill effects on the mother and unborn child in Nandi-Hills Sub County.

CONSENT TO PARTICIPATE

Pregnant mothers gave consent to participate by signing an informed consent form which was read to them and translated in mother tongue when it was necessary.

ETHICAL APPROVAL

The research protocol was approved by Ethics and Research Committee (ERC) of Jaramogi Oginga Teaching and Referral Hospital (JOOTRH) accreditation number 01713. The study was assigned reference number: ERC.1B/VOL1/178.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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