

**Review Article** 

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# Burden of hookworm infestation and association with socio-demographic and clinical factors in a clinical setting in Nigeria

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# Abstract

Hookworm infestation is a major public health problem in developing countries and infestation in patients including pregnant women could result in anaemia and malnutrition. The study aimed to determine infection burden, socio-demographic risk factors, and impacts of hookworm infestation among pregnant women in Federal Medical Centre Keffi. The study was a cross-sectional survey of pregnant women presenting at the booking clinic of the hospital over 3 months. Participants were recruited using a systematic sampling technique. Their biodata and information on socio-demographic risk factors for hookworm infestation were obtained and recorded in a pre-designed proforma. Participants' clinical details were also assessed. Stool sample from each participant was analyzed for hookworm presence, egg burden, and participants' Packed Cell Volume was determined. The data was analysed using the Statistical Package for Social Sciences (SPSS) version 22. Categorical variables were compared using Fisher's exact test while t-test was used for the comparison of continuous variables and Spearman's Correlation was used to determine the relationship between non-parametric variables. P-value was significant if <0.05 at 95% Confidence Interval. The prevalence of hookworm infestation and anaemia (P-value =0.911). Hookworm infestation was associated with place of residence, sewage disposal system, and source of drinking water (P-values of 0.012, 0.001, and 0.002 respectively). Prevalence of hookworm infestation among the antenatal attendees was relatively high but had no association with anaemia.

Keywords Hookworm infestation, malnutrition, anaemia

# Introduction

Hookworm infestation has been recognized as an important public health problem. In 2012, there were 130 million people infected with hookworm (Brooker et al., 2010). This figure tripled to 428 million cases within the next 3 years (Wang et al., 1980–2015). Majority of the cases occur in tropical and subtropical countries, especially in areas of abject poverty and poor sanitation (Wang et al., 1980–2015; Jourdan et al., 2018). The two species of hookworm that commonly infect humans are *Ancylostoma duodenale* and *Necator americanus* 

(https://www.cdc.gov/parasites/hookworm/gen\_info/faqs.html) Although hookworm infestation is rarely fatal in pregnancy, its most common complication, anaemia, can be quite significant in heavily infested pregnant women. World Health Organization (WHO) estimate shows that anaemia affects over half of pregnant women in developing countries (Mpairwe et al., 2014). In Nigeria, 60% of women have anaemia in pregnancy with 7% having severe forms (Omigbodun et al., 2004; Komolafe et al., 2005; Agan et al., 2010). Studies have also shown a strong association between the degree of anaemia and pregnancy outcomes (postpartum haemorrhage, preterm births, low birth weight infants, and perinatal mortality and infant survival). Some studies done in Sub-Saharan Africa noted that about 50% of women with iron deficiency anaemia in pregnancy also have hookworm infestation (Haider et al., 2009). The combined effect of hookworm infestation, malnutrition and malaria in pregnancy have increased economic burden and adverse pregnancy outcomes in prevalent regions (Mpairwe et al., 2014).

In areas of high prevalence for various diseases, evidence-based interventions which improve pregnancy outcomes have been introduced in ante-natal care programme. These include the prevention and treatment of iron deficiency anaemia, malaria prophylaxis and prompt therapy, and the prevention of mother-to-child transmission of HIV. Due to the complications associated with hookworm infestation, there is a considerable potential associated with the administration of prophylactic anti-helminthic drugs alongside simultaneous iron and folic acid supplementation in disease prevalent regions<sup>10</sup>.

The World Health Organization recommends the deworming of pregnant women who live in areas with a greater than 20% prevalence of hookworm infestation or where anaemia is over 40% (http://www.who.int/reproductivehealth/news/antenatalcare/en/). Presently, third-world countries like Nepal, Sri-Lanka and Madagascar have added deworming to their antenatal care programme (Brooker et al., 2008). Despite available evidence supporting the introduction of presumptive treatment for hookworm infestation in pregnancy (Weatherhead et al., 2014), it is yet to be implemented in my centre. This is attributable to its categorization as non-mandatory in the list of interventions earmarked for focused antenatal care in Nigeria's Integrated Maternal New-born Strategy and Child Health (https://www.unicef.org/nigeria/ng publications IMNCHbroch ure.pdf). This study, therefore, aimed to ascertain the burden of hookworm infestation and its association with sociodemographic and clinical features in our clinical setting.

#### Materials and methods

#### **Study location**

The study was carried out in Federal Medical Centre, Keffi which is a tertiary health facility located in Nasarawa State, North-Central Nigeria. It serves Nasarawa and other neighboring states including Benue, Kaduna, and the Federal Capital Territory, Abuja.

The Obstetrics and Gynaecology Department of Federal Medical Centre, Keffi has 4 teams. Each team has weekly booking and follow-up ante-natal clinics between Monday and Thursday. The ante-natal clinic is run by doctors, who are assisted by nurses and midwives.

# **Ethical consideration**

Approval for this study was obtained from the Ethical Clearance

Committee of Federal Medical Centre, Keffi. All participants who accepted to participate in this were educated on the procedures. Written informed consent was then obtained.

Confidentiality was maintained. Only the initials and study identification numbers were used to identify each subject. The principle of beneficence was upheld as those who had hookworm infestation were treated and the outcome of the study would also help to improve the care of pregnant women.

#### Study design

The study was a prospective cross-sectional study that was carried out over a period of three months between 1<sup>st</sup> November 2019 and 31<sup>st</sup> January 2020.

# **Study population**

The study population comprised all pregnant women presenting for booking visits at the ante-natal clinic of Federal Medical Centre, Keffi who met the study criteria.

#### **Inclusion criteria**

Consenting pregnant women attending the booking clinic of FMC, Keffi who met the inclusion criteria were included in the study.

# **Exclusion criteria**

Those with the previous history of ingestion of anti-helminthics, failure to provide stool specimen, haemoglobinopathies, HIV positivity and those with 2 or more pluses of malaria parasites were excluded from the study.

#### Sample size

The sample size was calculated using the formula  $n = Z^2 \frac{pq}{d^2}$ 

However, the sample size was increased to 200 to ensure proper representation of the ante-natal clinic population as an average of 75 new clients were booked weekly at the clinic.

#### Methodology

The study was conducted between the 1<sup>st</sup> of November 2019 and 31<sup>st</sup> of January 2020. Eligible clients were recruited from the study location using a systematic sampling technique. The antenatal clinics of Teams A, B, C, and D formed the 4 sampling frames. The list of pregnant women presenting for booking at each clinic visit in their order of presentation was obtained from the ANC record office every week. The first client that booked was assigned as number 1 and every other third pregnant woman who met the inclusion criteria was selected to participate. The enrolled participants who met the inclusion criteria were counseled and written informed consent was obtained. A total of 720 new enrollees were seen at the booking clinic within the period and 220 were recruited based on the sample size and

inclusion criteria. Only 205 returned their stool specimen for analysis and 5 questionnaires were discarded due to discrepancies between questionnaire and laboratory samples.

The biodata, socio-demographic and clinical details of the participants were obtained by the researcher using closed-ended questions and recorded on the study proforma. The weight (in kg) of each patient was taken using an electronic weighing balance while their height (in m) was taken using a meter rule.

Stool and blood specimens were collected from each patient. Laboratory analysis was done by the researcher with the assistance of consultant microbiologist and haematologist in Federal Medical Centre, Keffi. Data on the presence, density of hookworm infestation as well as the packed cell volume of the patient was recorded on the proforma.

#### **Stool Specimen Collection and Examination**

# **Stool Specimen Collection**

#### Materials used

- 1. Fresh stool (stool sample collected within 3 hours)
- 2. Glazed tile
- 3. Microscope slide
- 4. Cellophane as cover slip
- 5. Gloves
- 6. Kato set (Template with hole, screen, nylon or plastics
- 7. Plastic spatula
- 8. Normal saline
- 9. Iodine
- 10. Microscope

A single fresh stool sample was collected into a wide-mouthed, clean, dry, leak proof bottle provided by the researcher.

Participants were counselled to ensure that no urine, water, soil, or other contaminants got into the container. The stool container was labelled with the participant's identification number. It was then transferred to the microbiology laboratory in a specimen transportation box for immediate examination.

# **Stool Specimen Examination**

The stool specimens were examined by wet-mount method for hookworm identification, Kato-Fatz technique for hookworm egg density estimation.

#### Wet Mount Method

A drop of physiologic saline (sodium chloride 0.9w/v) and iodine was placed on either end of a glass slide. With the use of an applicator stick, a small amount of sample (about 2grams) was mixed with drops of physiological saline and iodine on different glass slides. The emulsified faeces were covered with a covered slip while avoiding air bubbles. Each slide was examined using ×10 and × 40 power on the microscope. The presence of ova was then recorded.

#### **IiKato-Fatz Technique**

A glass slide was labeled with a sample number and a glazed tile was placed on it. A small amount of faecal material was placed on the tile and a piece of nylon screen was pressed on top of it so that some of the faeces would sieve through the screen and accumulate on top of it. A flat-sided spatula was used to scrape the upper side of the screen to collect the sieved faeces. A template was placed on the slide and the sieved faeces was added with the spatula so that the hole in the template was filled. A spatula was passed over the filled template to remove excess faeces from the edge of the hole. The template was removed carefully so that a cylinder of faeces will be left on the slide. The faecal material was covered with a pre-soaked cellophane strip. The slide was subsequently inverted and the faecal sample pressed firmly against the hydrophilic cellophane strip to spread evenly. The slide was then examined systematically and the egg was multiplied by the appropriate factor specified on the Kato kits to give the density of infection in number of eggs/gram of faeces (Bärenbold et al., 2017).

#### The egg density was classified as follows:

Nil:	0 egg/gram
Light density:	1 to 1999 egg/gram +
Moderate density:	$\geq$ 2000 to 3999 egg/gram ++
Severe density:	$\geq$ 4000 egg/gram

#### **Blood Sample Collection and Examination**

#### **Blood Sample Collection**

A capillary blood sample was obtained for packed cell volume estimation the same day the stool sample was collected. The tip of the pulp of the middle finger of the participant's left hand would be swiped clean with alcohol swab and would then pricked with a lancet. Subsequently, the ensuing drop of blood was allowed to flow by capillary action into the micro-haematocrit capillary tube. After this, the tube was labeled with the study number of the participant before it was transferred to the laboratory for processing.

#### **Blood Sample Analysis**

The packed cell volume (PCV) of the participant was determined using blood collected into a heparinised capillary tube. One end of each capillary tube was occluded using plasticine. Thereafter, the capillary tube was mounted on a micro-haematocrit centrifuge and was spun for 5 minutes. Each capillary tube was then mounted on a haematocrit reader for the reading of the red cell column, measured in height (millimetres as a percentage of height in millimetres) of the whole capillary blood column. The haematocrit was taken as the volume of erythrocytes expressed as a percentage of whole blood in the sample (Bull et al., 2000).

# Data analysis

The data collected was entered into the proforma sheet designed

for the study and subsequently analysed using the Statistical Package for Social Sciences (SPSS) software version 22. It was summarized as frequency and percentages for categorical variables, mean and standard deviation for normally distributed continuous variables, and median and range for non-parametric data. Independent samples t-test was used for the comparison of continuous variables. The p-value was significant if <0.05.

#### Results

A total of 220 consenting pregnant women were recruited and 205samples were returned, five questionnaires were discarded due to irreconcilable discrepancies between questionnaires and laboratory samples.

During the study period, a cumulative total of 1808 pregnant women were seen in the four antenatal booking clinics targeted by the study and 720 coming for the first time. The ages of the participants ranged between 19 and 45 years with a mean of 30.28 (Table 1). Most of the subjects were in the age group of 30-39 years (56%) while the age groups of less than 20 were the least accounting for 1%.

Almost all the subjects were married (99.5%) with only one participant being single. The level of education attained by the subjects was tertiary (52%) while 3.5% had no form of education. Majority of the participants were civil servants 32.5%, this was closely followed by those not gainfully employed i.e. housewives 29.5% while farmers accounted for just 1%. Majority of the subjects were rural dwellers (64%).

With regards to household characteristics (Table 2), 56% of the participants had separate toilet facilities and 43% shared their toilets with other households, only 1% of the subjects practice open defecation. The most common type of toilets used by the participants was the water closet which accounted for 97%. With reference to refuse disposal majority of the respondents, 88% disposed their refuse via the incinerator. About 97.5% of the subjects use footwear frequently at home, 63% routinely wash their hands following defecation and 75.5% accessed the borehole for drinking water.

Out of the 200 stool samples examined, hookworm was seen in 20 of the samples giving a prevalence of 10.0%. Other organisms seen were entamoeba histolytica [14(7%)], Escherichiacoli [4(2%)] and ascarislumbricoides [2(1%)]. Both stool samples that had Ascaris lumbricoides also had hook worm, while 4 out of the 14 stool samples with Entamoeba histolytica also had hookworm, only 1 of the 4 samples that had Escherichia coli also had hookworm in addition.

Twelve (6%) of the participants had a packed cell volume below 30 which was the lower limit used for anaemia in the study, however none of the women who had packed cell volume below the lower limit had hookworm or other helminths in their stools.

The association between hookworm infestation and subjects' socio-demographic variables were sought and shown on (Table

5). Education and occupation did not have any statistically significant association (p-value of 0.252 and 0.588 respectively), however place of residence was found to be statistically associated with hookworm infestation (p value of 0.012).

With regards to the hygiene habits, type of toilet usage and source of drinking water were statistically associated with hookworm infestation with p-values of 0.001 and 0.002 respectively. In contrast, frequent use of footwear at home, routine hand washing after defaecation, and refuse disposal system were not statistically significant (p-values of 0.450, 0.097 and 0.503 respectively).

The prevalence of anemia among the study population was 6.0%. There was no statistically significant association between packed cell volume and body weight with hookworm infestation (P-values of 0.911 and 0.172 respectively).

#### Discussion

In this study, the prevalence of hookworm infestation in pregnant women attending the antenatal clinic services of FMC Keffi was 10% which is also similar to that found in a study in Uganda in 2016 which was9.9%. This is higher than the prevalence rate of 8.2% (Green et al., 2015) and 6.9% (Alli et al., 2011), in Niger delta and Ibadan respectively, on the other hand, a higher prevalence rate was reported in Ghana (17.6%), (Baidoo et al., 2010). The probable reason for this marked difference in prevalence in relation to Ghana may be because this study was done in the hospital setting and the women attending antenatal care are better informed. It may therefore be said that the prevalence could be higher if the same study is carried out in the rural setting outside the hospital where the literacy level is low.

The association between place of residence and hookworm showed a statistical significance. it was found that people who reside in the rural areas had the highest prevalence of hookworm 90% this is not surprising because people that live in the rural areas predominantly feed on fresh vegetables and less processed food which has a high risk of transmission a high prevalence was also found in people who don't routinely wash their hands after defaecation 80%.

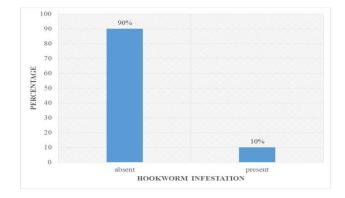
There was no statistical significance between hookworm infestation and anaemia with a p-value of 0.911. This is in contrast to a study in Europe which revealed a direct relationship between hookworm infestation and anaemia (Feldmeier et al., 2012) and that in Sub-Saharan Africa which also revealed that 50% of women with iron deficiency anaemia in pregnancy also had hookworm infestation (Haider et al., 2009). Another study done in Nepal also revealed a high prevalence of hookworm infestation with associated morbidities like anaemia and reduced immune resistance from other nutritional disorders made the condition worse (Bondevik et al., 2000). Malaria, sickle cell disease and aids in pregnancy are possible co-morbidities that may contribute to low packed cell volume in the other studies, and these were excluded in my study.

Safe disposal of human faeces is essential for the control of

hookworm infestation (De Silva et al., 2003). Sanitary measures include the provision of toilets along with training on their use as well as appropriate treatment of human waste before it is used for agriculture as manure. There was a statistical association of hookworm infestation in this study with sanitary practice. It was found that those who shared toilet facilities accounted for 65% of those infested with hookworm (Hotez et al., 2003).

Table 1. Socio-demographic	variables o	of the	participants
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Age group (yrs.)	Frequency	Percentage
<20	2	1.0
20 - 29	79	39.5
30 - 39	112	56.0
40 - 49	7	3.5
Marital status		
Single	1	0.5
Married	199	99.5
Education		
attainment		
None	7	3.5
Primary	12	6.0
Secondary	75	37.5
Tertiary	106	52.0
Occupation		
Housewife	59	29.5
Farming	2	1.0
Trading	55	27.5
Civil servant	65	32.5
Student	11	5.5
Artisan	8	4
Residence		
Rural	129	64.5
Urban	71	35.5



# Fig. 1 Prevalence of hookworm infestation among the participants

Estimate for difference: 0.8; 95% CI for difference: (0.741, 0.859) Z = 26.67, P-Value < 0.001

Table 2.	Household	characteristics of	f the	study	participants

Toilet facility	Frequency	Percentage
Separate	112	56.0
Shared	88	44.0
Sewage disposal system		
Open	1	0.5
Pit latrine	5	2.5
Water closet	194	97.0
Refuse disposal system		
Open dump	18	9.0
Burying	4	2.0
Incinerator	178	88.9
Source of drinking water		
Surface water	1	0.5
Well	4	2.0
Pipe borne water	5	2.5
Borehole	151	75.5
Treated water	39	19.5
Use of footwear to toilet		
Yes	195	97.5
No	5	2.5
Hand washing after defecation		
Yes	74	37.0
No	126	63.0

Table 3. Clinical complications among the studyparticipants

	Frequency	Percentage
Pica		
Absent	199	99.5
Present	1	0.5
Dizziness		
Absent	199	99.5
Present	1	0.5
Easy fatigability		
Absent	199	99.5
Present	1	0.5

	Hookworm infestation			
	Present (20) n (%)	Absent (180) n (%)	$\chi^2$	p-value
Educational attainment			4.086	0.252
None	1 (5.0)	6 (3.3)		
Primary	3 (15.0)	9 (5.0)		
Secondary	5 (25.0)	70 (38.9)		
Tertiary	11 (55.0)	95 (52.8)		
Occupational status			1.062	0.588
Unemployed	5 (25.0)	65 (36.1)		
Unskilled workers	8 (40.0)	57 (31.7)		
Skilled workers	7 (35.0)	58 (32.2)		
Residence			6.311	0.012
Rural	18 (90.0)	111 (61.7)		
Urban	2 (10.0)	69 (38.3)		
Toilet facility		· · · /	3.977	0.046
Separated toilet	7 (35.0)	105 (58.3)		
Shared toilet	13 (65.0)	75 (41.7)		
Type of toilet system		· · · ·	14.332	0.001
Open	0 (0.0)	1 (0.6)		
Pit latrine	3 (15.0)	2 (1.1)		
Water closet	17 (85.0)	177 (98.3)		
Refuse disposal system			1.373	0.503
Open dump	3 (15.0)	15 (8.3)		
Burying	0 (0.0)	4 (2.2)		
Incinerator	17 (85.0)	161 (89.5)		
Hand washing after defecation			2.755	0.097
Yes	4 (20.0)	70 (38.9)		
No	16 (80.0)	110 (61.1)		
Source of drinking water	, , ,		16.990	0.002
Surface water	1 (5.0)	0 (0.0)		
Well	2 (10.0)	2 (1.1)		
Pipe borne water	0 (0.0)	5 (2.8)		
Borehole	13 (65.0)	138 (76.7)		
Treated water	4 (20.0)	35 (19.4)		
Use of footwear			0.570	0.450
Yes	19 (95.0)	176 (97.8)		
No	1 (5.0)	4 (2.2)		

# Table 4. Relationship between hookworm infestation and selected factors

# Table 5. Relationship between hookworm infestation, pcv and weight

	Hookworm infestation					
Factors	Present (20)	Absent (180)	Mean diff.	95% CI	t	p-value
	Mean (SD)	Mean (SD)				
PCV	35.39 (4.42)	34.84 (4.43)	0.55	-2.66 - 1.55	0.52	0.911
Weight	74.71 (12.58)	70.44 (12.94)	4.27	1.87 – 10.41	1.37	0.172

#### Conclusion

The prevalence of hookworm infestation among obstetric population was considerable but no significant association with anaemia. However, its preponderance among rural dwellers calls for screening among this set of pregnant women.

# **Conflict of Interest**

The author hereby declares no conflict of interest.

#### **Consent for publication**

The author declares that the work has consent for publication

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