



Prevalence and Distribution of Hookworm Infection among Pupils of University of Calabar Staff School, Calabar, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author CIM designed the study and wrote the protocol. Authors DMMN and BEA performed the statistical analysis and wrote the first draft of the manuscript. Authors DMMN and BEA also managed the analyses of the study. Authors BEA, AVO and DMMN managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

For decades, hookworm infections have been known to be a major problem of public health, affecting mostly children and causing significant impact on their health. The aim of this study was to investigate the prevalence rates between age and sex of school children affected using University of Calabar Staff School, Calabar, Nigeria. Stool samples were collected from the pupils and examined using direct smear, formal-ether concentration and brine floatation techniques. The frequency of hookworm infection was more in males, 11(11.3%) than in females, 7(6.8%) consisting the 18 positive children sampled from the 200 pupils and the highest prevalence was found among the 13-15 years age group (33.3%). The statistical analysis showed no significant difference for both age and sex ($p>0.05$). Hookworm infections have decreased in recent years due to the

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increasing development of the country and other personal hygiene observed thus, continuous deworming program by Cross River State government and the Nigerian Government should be conducted in primary schools to lower the rate of the infection.

Keywords: Public health; prevalence; hookworm; smear and infection.

1. INTRODUCTION

Hookworm infection caused by *Ancylostoma duodenale* and *Necator americanus* affects about 740 million people worldwide with 80 million people severely infected. Developing countries are the most affected and within these, the major cases occur among school aged children [1]. The distribution of hookworm infection depends on many factors like socio-demographic variables associated with poverty such as reduced access to adequate sanitation, potable water, and healthcare, as well as the prevailing climatic and environmental conditions [2,3]. The economic burden caused by hookworm infection is high. Recently, it has been estimated to cost 1.8 million Disability-Adjusted-Life-Years (DALYs). Young children are reported to be disproportionately affected by hookworm infection compared to adults due to increased nutritional requirements and less developed immune system. Hookworm infection in this age group has been linked with significant reduced growth and increased risk for protein-energy malnutrition [4] including growth stunting, iron-deficiency, anemia intellectual retardation, cognitive and educational deficit.

The precise impact of hookworm infection on child nutrition, growth and development appears to depend on the species, burden and its impact on host nutrition tends to be long-termed. Despite recent advances, a number of important questions still remain unanswered regarding hookworm infection risk and its consequences in child population. For example, it is still not clear whether certain age, gender and even ethnic groups are most likely to become infected.

1.1 Life Cycle and Classification

Humans are infected with hookworm's third stage filarial-form larvae. The larva in soil penetrates through the skin particularly into area such as unprotected feet. Once infected, the filarial-form larva migrates into blood circulation. They break out of the pulmonary blood vessels into alveoli, then crawl up the trachea and are swallowed with saliva to re-enter the intestinal tract. They attach

themselves to the mucous membrane of the small intestine to mature into adults. The female adult releases eggs (*N. americanus* about 9000-10,000 eggs per day and *A. duodenale* 25000-34000 eggs per day) which are passed in the faeces of the human host. These eggs hatch in the environment within several days and cycle starts anew [5].

1.2 Epidemiology

No international surveillance mechanisms are in place to determine the prevalence and global distribution of hookworm infection. However, based on extensive research, hookworm is estimated to be in 740 million people [6], highest prevalence occurring in sub-Saharan Africa and Eastern Asia. Other area of rural poverty in the tropics including Southern China [7]. Indian subcontinent [8] and America [7] also have high transmission rates. In all regions, there is striking relationship between hookworm prevalence and low socioeconomic status.

Compared to other soil transmitted helminthes (STH) infections and schistosomiasis, hookworm infection exhibits a unique age-intensity profile. Whereas, the intensity of the former peaks in childhood and adolescence, hookworm intensity usually either steadily rises in intensity with age or plateau in adulthood [9]. According to [7], sub-Sahara and East Asia have about 198 and 149 million infected people respectively. Others are South Asia – 59 million, Latin America and Caribbean – 50 million, North Africa and Middle East – 10 million, India – 71 million, China – 39 million infected people. Other studies in Nigeria shows that in Niger-Delta 34.9% of 4990 people were infected [10] and in Vom, Plateau State, 3.2% of total 463 samples were positive [11].

Recent technological development are now used by researchers like Geographical Information System (GIS) and Remote Sensing (RS) to examine helminth ecology and epidemiology, other focus on the development of DNA-based tools that can be used for diagnosis of infection, specific identification and analysis of genetic variability in hookworm populations [12,13].

1.3 Pathogenesis and Clinical Presentations

Hookworm larva get to the host either orally or by penetrating the skin to reach the small intestine. Hookworm's haematophagous habits cause pathogenesis of anemia and malnutrition. The worms attach using their mouth parts, each female worm is estimated to ingest a minimal of 0.1ml of blood per day. However, actual blood loss can be significantly greater, the worms change, their feeding sites several times a day, and the secretion of anticoagulants or proteins means that the vacated sites continues to bleed, contributing greatly to blood loss [14,15].

2. METHODS

2.1 Study Location

The study was carried out at the University of Calabar Staff School, Calabar, Cross River State, Nigeria, between the periods of October to December 2013. The age range of the pupils sampled was from 2-13 years.

2.2 Study Population

Stool samples were collected from 200 pupils aged 2-13 years. The faecal samples were collected in October – December, 2013. School was selected by convenience sampling. To augment the sample size due to a lower than expected number of children, a younger category was added.

2.3 Ethical and Consent Approval

Informed written consent was taken from parents and caretakers and the school authorities after explanation about the objectives and aims of the study.

2.4 Sample Collection

Pupils were provided with wide mouthed specimen bottles with screw caps and spoons with specific instructions to collect samples in the morning. The sample bottles were properly labeled with date, number, age and sex of the pupil. Total of 200 samples were collected, from 97 males and 103 females. They were given instructions to avoid contamination of samples. The samples were transported to the Microbiology laboratory, University of Calabar for analysis.

2.5 Questionnaire

Simple structured closed-ended questionnaires were given to pupils to gain information from the parents or guardians of subjects regarding child and household socio-demographic, housing, water and sanitation characteristics. These items included subject age, sex, ethnicity, family size, house construction, water, sanitation and garbage disposal, characteristics and the presence versus absence, type and density of domestic animals living in and around the home, shoe wearing habit, personal hygiene practices and related symptoms like headache, nausea, abdominal pains etc.

3. MACROSCOPY AND MICROSCOPY

3.1 Macroscopy

Before carrying out microscopic examination, macro-scopy was carried out to check the consistency of the stool, presence of blood or mucus, presence of proglottids, colour and smell of the stool, then recorded against the appropriate sample number [16].

3.2 Microscopy

Wet mounts (saline and iodine) [17].

3.2.1 Principle

It is a simple preliminary microscopic method that detects motility in organisms, eggs, cysts, larvae, trophozoites of the organism and gives clear images of fresh specimen under the microscope.

3.2.2 Procedure

Place a drop of saline on left half of the slide and one drop of iodine on the right half. With an applicator stick, pick up a small portion of the specimen and mix with saline drop, do same with iodine drop. Put the cover slip separately on both and examine under the microscope using 10X objectives to focus, the 40X objective to get a clearer view [16,17].

3.2.3 Results

Ova, cysts and trophozoites of organisms were found and identified mostly in the saline wet mount. Iodine wet mount revealed mostly amoebic and flagella cysts.

3.3 Concentration Techniques

3.3.1 Principle

This technique is used mostly when the number of parasites in the stool specimen is low. Eggs, cysts and larvae are covered but trophozoites get destroyed during this procedure.

Concentration procedures can be grouped under 2 categories:-

- i. Sedimentation procedure
- ii. Flotation procedure [17,18].

3.4 Formal-Ether Sedimentation Technique

3.4.1 Procedure

Transfer half teaspoon of faeces in 10 ml of water in a glass container and mix thoroughly. Place two layers of gauze in a funnel and strain the contents into a 15 ml centrifuge tube. Centrifuge for two minutes at about 500 g. Discard the supernatant and re-suspend the sediment in 10 ml of physiological saline. Centrifuge at 500 g and discard supernatant. Re-suspend the sediment in 7 ml of 10% formaldehyde (one part of 40% formalin in three parts of saline) and 3 ml of ether (or ethyl acetate). Close the tube with a stopper and shake vigorously or mix. Remove the stopper and centrifuge, rest the tube in a stand, four layers become visible. Pour off the liquid leaving a small amount of formalin for suspension of the sediment. With a pipette, remove the sediment and mix it with a drop of iodine, then examine under the microscope [17].

3.4.2 Results

Ova or cysts can be detected, since this method is very sensitive. Size and shape of the parasitic structure is maintained.

3.5 Brine Flotation Technique

3.5.1 Procedure

Place about one milliliter of faeces in a flat bottomed container with capacity of 15-20 ml, add few drops of brine and stir it to make a fine emulsion, then more brine so that the container is nearly full, stirring the solution thoroughly. Remove floating coarse materials, fill the

container with a dropper till a meniscus is formed. Place a glass slide carefully on top, allow for 20 minutes, lift slide quickly and overturn smoothly, after putting a coverslip, examine under the microscope [17].

3.5.2 Results

Ova or cysts can be detected, but unfertilized eggs of *A. lumbricoides* cannot be seen because they do not float. If suspension is left for more than 20 minutes, protozoan cysts and thin walled nematodes eggs will collapse and become distorted in appearance.

3.6 Statistical Analysis

Frequency distribution tables, percentage prevalence and intensity of infection are estimated using standard formulae. Simple percentage was used to compare differences in prevalence between age groups and sex, Fisher's Exact (SISA) was used to find odd ratios (O.R), confidence interval (C.I) and probability (P)-values. Significant level was set at confidence limit 95% and $p < 0.05$.

4. RESULTS

Two hundred (200) samples were collected from the pupils consisting of 97 males and 103 females. The mean age group fell under 7-9 years, the age range of the pupil was from 1-13 years. Hookworm was recovered from a total of 18 samples revealing a prevalence rate of 9%. The highest prevalence for hookworm infection was found in age group 13-15 years (33.3%) and lowest in <4 age group, which had no positive sample. Frequency distribution was highest in males than in females (11.3% and 6.8% respectively). Statistical analysis of the data showed no significant difference for both age and sex ($p > 0.05$).

Table 1. Prevalence of hookworm infection in sample population (n=200)

Category	No. of subject	Percentage frequency (%)
Hookworm +	18	9
Hookworm -	182	91
Total	200	100

This table shows that 9% of the samples examined were hookworm positive, 18 samples out of the 200 samples had hookworm

Table 2. Distribution of hookworm infection by age of study subjects

Age (Years)	No. involved	No. +	% +	C.I	O.R	p-value
<4	2	-	-	-	-	-
4 – 6	42	5	11.90	0.62-3.04	1.50	0.17
7 – 9	103	9	8.70	0.60-1.57	0.94	0.19
10 – 12	50	3	6.00	0.22-1.87	0.57	0.17
13 – 15	3	1	33.30	0.48-53.07	5.29	0.23

This table reveals that the highest prevalence is found among age group 13 – 15 years (33.3%), followed by 4 – 6 years (11.9%). This does not show a significant difference (p>0.05)

Table 3. Distribution of hookworm infection by sex of study subjects

Sex	No. examination	Positive	% age	C.I	O.R	p-value
Male	97	11	11.3	0.87-1.93	1.75	0.11
Female	103	7	6.8			

This table shows distribution of hookworm infection by sex, males had highest prevalence (11.3%) compared to females (6.8%), but this shows no significant difference (p>0.05)

Table 4. Prevalence of hookworm infection according to demographic characteristics of study subjects

Residential Area	No. (%)	No. + (%)
Unical Staff Quarters	30 (15.00)	2 (6.67)
Calabar South	97 (48.50)	11 (11.34)
Calabar Municipality	73 (36.50)	5 (6.85)

This table shows the prevalence of hookworm infection based on the residential area, it reveals that those who stay in Calabar South had highest frequency of infection (11.34%) and University of Calabar Staff Quarters had the lowest frequency (6.67%).

Key: Unical = University of Calabar; No. = Number; % = Percentage; + = Positive

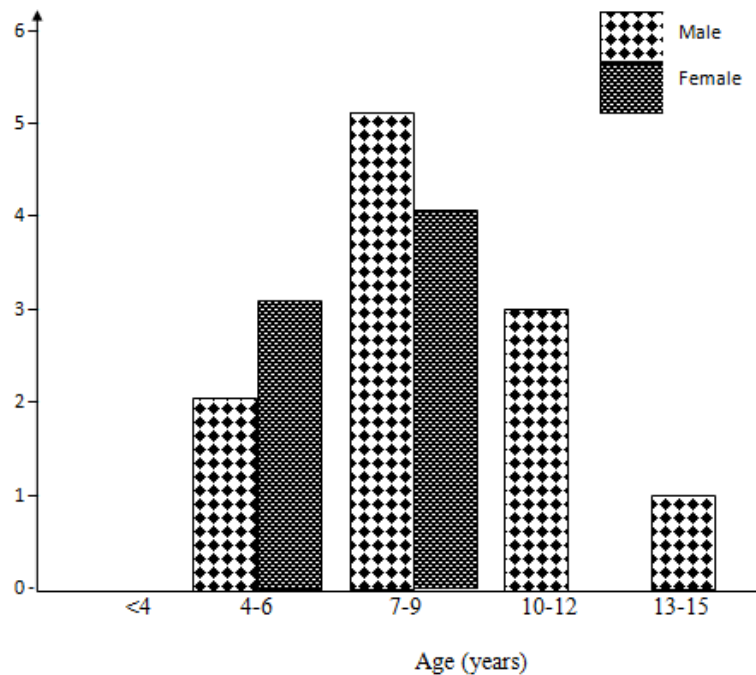


Fig. 1. Distribution of hookworm infection by age and sex

Table 5. Distribution of hookworm infection by age and sex

Age (years)	Sex	No. Examined	No. Infected	% Infected
>4	M	-	-	-
	F	2	-	-
4 -6	M	19	2	11.9
	F	23	3	
7 – 9	M	47	5	8.7
	F	56	4	
10 – 12	M	29	3	6
	F	21	-	
13 – 15	M	2	1	33.3
	F	1	-	

5. DISCUSSION

This study has shown that hookworm infection has an overall prevalence rate of 9%. The overall prevalence of hookworm in this study is however high when compared with findings of Odebunmi et al. [19] which showed 3.2%. This could be attributed to the poor personal hygiene observed among subjects in the study area and unsanitary conditions such as inadequate sanitation facilities like latrines. The results in the contamination of the soil with eggs and larva of the parasite to which the children are exposed. However, the common habit of working and walking barefoot results in higher exposure of these children to the infective larvae.

It was observed that the prevalence of hookworm infection was higher in males 11(11.3%) than in females 7(6.8%). The observation confirms an earlier report of a pronounced sex bias in hookworm infections in Nigeria [10] and consistent with reports from African [20] and Asian [12,21] countries where males have been shown to carry heavier infections of hookworms than females. The possible mechanisms underlying such differences in pattern of parasitic infections carried by sexes have been review in the past [22] and although innate and immunological factors may contribute, the most important factor is believed to be behavioural and sex dependent differences in exposure to infection. Males are often engaged in predisposing activities such as football playing, playing in streams or ponds and they also help their parents in farming, where it is observed that human faeces is occasionally combined with animal faeces in fertilizing agricultural plots, a practice which has been shown to be associated with higher abundance of hookworm infection in those who use fresh, as distinct from treated human faeces or non-faecal fertilizer [23].

Age group 13-15 years recorded higher prevalence, this agrees with findings of Luka et al. [24] and Nxasana et al. [25]. This might be due to their workload such as cleaning of surroundings, water fetching, farming activities increase risk occurrence when they work barefooted. Age group 4-6 had the next high prevalence, this may be due to their indiscriminate defecation practices as suggested by Odebunmi et al. [19], hand-washing practices and eating discarded food remains from the street.

Prevalence of this infection was higher in those living in Calabar South Local Government Area, Nigeria, 11(11.34%). This may be due to the fact that Calabar Municipality and University of Calabar staff quarters are more developed and the type of occupation engaged by parents/guardians are mostly white-collar jobs, while in Calabar South, parents/guardians are engaged mostly in farming, fishing and trading. These activities predispose the children to hookworm infection while assisting their parents. The houses in this area are mostly built with mud and human faecal material is often incorporated into material used to strengthen dwellings.

6. CONCLUSION

Hookworm infection is more common in children and the infection deteriorates psychological and physical development and symptoms like poor growth, mental retardation, emaciation and pains can be observed. From this study, prevalence of hookworm was more in males than females and highest in age group 13-15 years. Frequency of the infection showed 9%, serious complications may occur if the infection is not treated. Special attention should be given to treatment of children and regular deworming programmes should be carried out in schools by the Cross River State

Ministry of Health or the Federal Ministry of Health, Nigeria.

7. RECOMMENDATIONS

- Environmental measures have to be involved and government support such as provision of adequate and safe water and introduction of acceptable sanitation system.
- Improvement of personal and community hygiene practices.
- Local health officers should visit the schools regularly for routine deworming and health education to improve conditions.
- People should also be informed about the signs, symptoms and prevention methods of this infection.
- Cases should be reported to the hospital on time for proper medical management.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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