AGE AND SEX RELATED PREVALENCE AND DISTRIBUTION OF HOOKWORM INFECTION AMONG PUPILS OF UNIVERSITY OF CALABAR STAFF SCHOOL, CALABAR, NIGERIA

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 increasing development of the country and other personal hygiene observed thus, continuous deworming program should be conducted in primary schools to lower the rate of the infection.

KEYWORDS: Public health, prevalence, hookworm, smear and infection

INTRODUCTION

Hookworm infection caused by *Ancyclostoma duodenale* and *Neatorsmericanus* 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 (Bethony *et al.,* 2006). 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 (Monstressor *et al.,* 1998).

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 (Stephenson *et al.,* 2000) 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.

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 stars anew (Hawdow and Hotez, 1996).

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 (de Silva *et al.,* 2003), highest prevalence occurring in sub-Saharan Africa and Eastern Asia. Other area of rural poverty in the tropics including Southern China (Hotez, 2003). Indian subcontinent (Yadla *et al.,* 2003) and America (HJotez, 2003) 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. (Bethony *et al.,* 2002). According to Hotez (2005), 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 (Agi and Awi-Waadu, 2008) and in Vom, Plateau State, 3.2% of total 463 samples were positive (Odebunmi *et al.,* 2007).

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 (Brooker *et al.,* 2007, Gassen *et al.,* 2009).

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 (Bungiro and Capello, 2004, Devaney, 2005)

METHODS

Study Location

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

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.

Ethical Approval

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

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.

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.

MACROSCOPY AND MICROSCOPY

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.

Microscopy

Wet mounts (saline and iodine)

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.

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.

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.

Concentration techniques

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 cab be grouped under 2 categories:-

i. sedimentation procedure

ii. Flotation procedure

Formal-ether sedimentation technique

Procedure: - Transfer half teaspoon of faeces in 10ml of water in a glass container and mix thoroughly. Place two layers of gauge in a funnel and strain the contents into a 15ml centrifuge tube. Centrifuge for two minutes at about 500g. Discard the supernatant and re-suspend the sediment in 10ml of physiological saline. Centrifuge at 500g and discard supernatant. Re-suspend the sediment in 7ml of 10% formaldehyde (one part of 40% formalin in three parts of saline) and 3ml 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. (Cheessbrough, 2002).

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

Brine flotation technique

Procedure:- Place about one milliliter of faeces in a flat bottomed container with capacity of 15-20ml, 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. (Koneman, 1997).

Result: - 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.

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 $p < 0.05$.

RESULTS

Two hundred (200) samples were 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)

KEY: Unical = University of Calabar

No. = Number

% = Percentage

\+ = Positive

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%).

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	-	

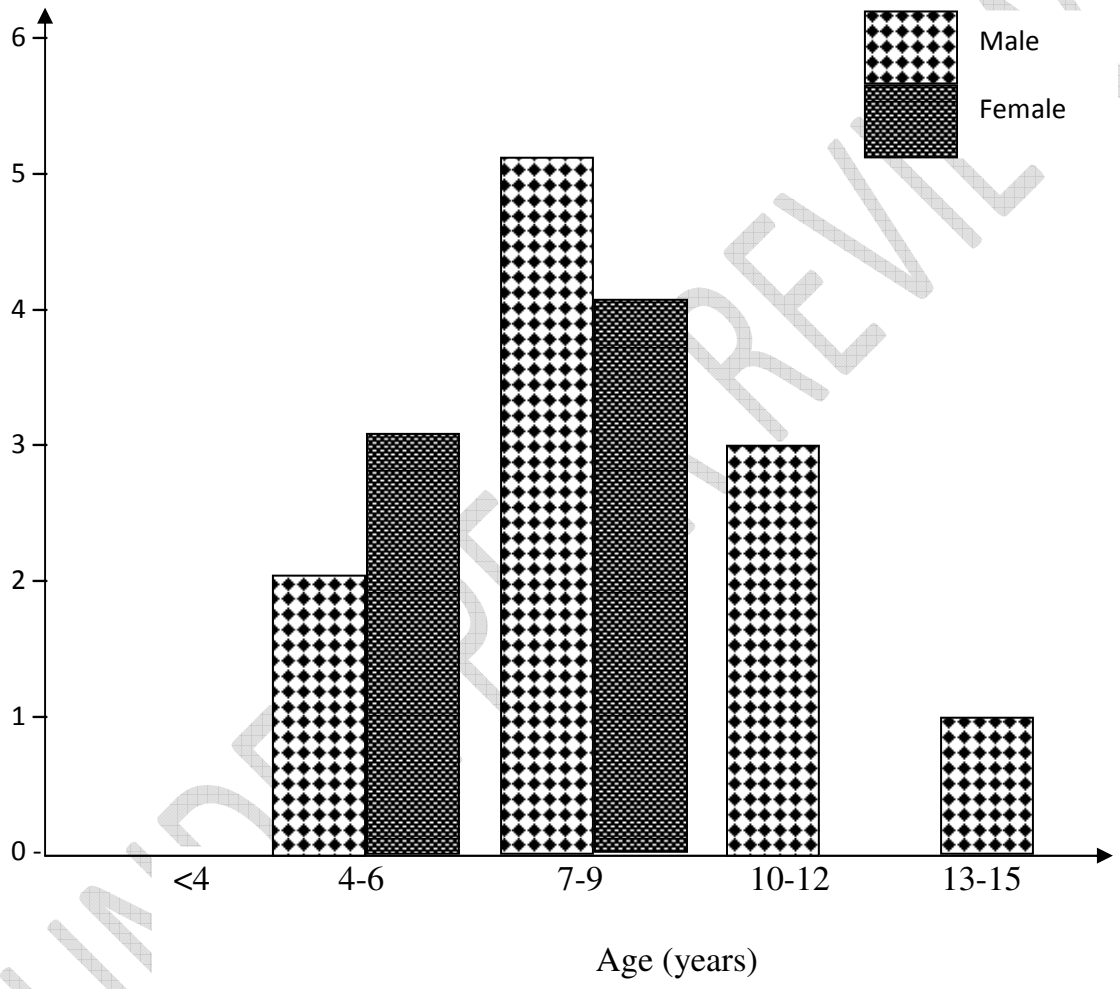

Figure 1: Distribution of hookworm infection by age and sex.

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