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Evaluation of Hookworm Infections and Some Haematological Parameters amongst Primary and Secondary Schools Children in Aba Metropolis, Abia State, Nigeria

***[1]ABAH, AE; WOKEM, GN**

[1]Department of Animal and Environmental Biology, Faculty of Science, University of Port Harcourt PMB 5323 Port Harcourt 50001, Rivers State, Nigeria
[2]Department of Medical Laboratory Sciences, Rivers State University of Science and Technology, Nkpolu, Port Harcourt 50001, Rivers State, Nigeria
**Corresponding author: austin.abah@uniport.edu.ng or aeabah@yahoo.com*

ABSTRACT: Hookworm infections and some haematological parameter among children were assessed in five different schools in Aba metropolis, Abia State, Nigeria. Blood and Stool samples were collected and analysed, following ethical approval and consent from parents and teachers of the subjects. Stool samples were analyzed using standard parasitological technique, from the blood samples, Haemoglobin (Hb) levels, Esinophils and white blood cell counts (WBC) were determined. Out of 1500 samples examined, 120 (8.0%) were positive for Hookworm infection. There was variation in infection rate by school and there is significant difference in the prevalence of Hookworm infection within the schools ($P<0.05$). Sex related prevalence showed that infection was higher in females 8.17% than in males 7.89%. It was observed that hookworm infection is dependent on sex and age ($P<0.05$). There was a negative correlation between hookworm infection and Haemoglobin levels both in female and male subjects. Eosinophil count and hookworm infection by sex and age revealed that there is a correlation between hookworm infection and eosinophilia counts. There was an increase in eosinophilia with high rate of hookworm infection in the age group of 10 – 13 years. This result showed that there is higher value of eosinophilia in female children than in male children ($P < 0.05$). White blood cell counts (WBC) showed a proportional variation with hookworm infection in both male and female ($P<0.05$). In conclusion, Hookworm infection is relatively high among the children in Aba metropolis and the relationships between hookworm infection and Haemoglobin, Eosinophil and white blood cells were established. ©JASEM

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Keywords: Hookworm infections, Haemoglobin, Eosinophils, Leucocytes, School Children, Aba Metropolis.

Human hookworm infection is a common soil transmitted helminth infection that is caused by the nematode parasites, *Necator americanus* and *Ancylostoma deudenale* worldwide (Hotez et al., 2003). Hookworm belongs to the phylum Nematode and family Ancylostomatidae. An estimated 576-740 million people in the world are infected with hookworm (CDC, 2013). Hookworm infection thrives in areas where sanitary and environmental conditions favour the development of filariform larvae and infection of the host. Many of the affected population live in rural communities of developing countries where hookworm is endemic (Crompton, 1999 and Otesen *et al.,* 1997). The highest prevalence of hookworm infection being reported in sub-Saharan Africa, the Pacific Islands, India, East and South Asia, Latin America, and the Caribbean(De Silva *et al*.,2003 and Bethony *et al.,*2006).

Hookworm belong to group of infection generally referred to as intestinal parasitic infections which has been reported to have high prevalence in Nigeria among children because of their vulnerability (Ejezie and Igwe 1993; Awogun *et al.,*1995; Adeyeba and Akinlabi 2000,Leder and weller 2002; Barnabas 2005, Sam-Wobo *et al*., 2005). Specifically; prevalence of 25.0% was recorded by Abah and Arene (2015) among school children in Rivers State. Babamale *et* al., (2015) reported prevalence of 17.8% among school-aged children in Okuta community in Kwara State. Also Adeyeba and Tijani (2002) reported a prevalence of 3.2% in Igbo-ora in Oyo State and Odebunmi *et al*., (2007) reported 3.2% among school children in Vom, Plateau State. In Ethiopia prevalence of 25.5% was reported by Ibrahim *et al*., (1999).

The most serious effects of hookworm infection are blood loss leading to anaemia, in addition to protein loss (CDC, 2013). Most of the people infected are asymptomatic. Despite the lack of symptoms, hookworm substantially contributes to the incidence of anemia and malnutrition in developing nations. Hotez and Pritchard (1995) described hookworm as an intestinal parasite of human that usually causes mild diarrhoea or cramps. Heavy infection with hookworm can create serious health problems for new-borns, children, pregnant women and persons who are malnourished. Since hookworm infection occurs predominantly among the world's most impoverished people (De silva et al., 2003), It has assumed a notorious reputation of "hookworm disease" among others, for example, the reputation of pre 1949 china as the "sick man of Asia" was partly a result of the high prevalence and intensity of infection with hookworm (Hotez 2002).

The major hookworm – related injury in humans occurs when the adult parasite causes intestinal blood loss, (Stoltzfus *et al*., 1997, Albonico *et al*.1998). Blood loss occurs when the worms use their cutting apparatus to attach themselves to the intestinal mucosa and sub-mucosa and contract their muscular oesophagi to create negative pressure, which sucks a

**Corresponding author: austin.abah@uniport.edu.ng*

plug of tissue into their bucal capsules. Capillaries and arteries are ruptured not only mechanically but also chemically, through the action of hydrolytic enzymes. Blood flow is ensured by the release of anti-clotting agents by the adult worm (Stanssens *et al*., 1996, Del Valle *et al*., 2003). The hookworm ingests a portion of the extravagated blood; some red cells undergo lysis, thereby releasing hemoglobin, which is digested by a cascade of hemoglobinases that line the gut of the parasite. Williamson *et al*., (2003) and Stoltzfus *et al*., (1997) observed that the major clinical manifestations of hookworm disease are the consequences of chronic intestinal blood loss. Iron deficiency anemia occurs and hypoalbuminemia develops when blood loss exceeds the intake and reserves of host iron and protein. Depending on the status of host iron, a hookworm burden of 40 to 160 worms is associated with hemoglobin levels below 11g per deciliter (Lwambo *et al*., 1992 and Bundy *et al*., 1995). However other studies have shown that anemia may occur with a lighter hookworm burden (Olsen *et al*., 1998). The degree of iron-deficiency anemia induced by hookworms depends on the species (Albonico *et al*., 1998). A. duodenale causes greater blood loss than infection with Necator americanus, for instance, in Zanzibar, among children who were infected only with N. americanus hookworms, the prevalence of hypoferitinemia (ferittin level, < 12ug per liter) was 33.1%, where as in children who were also injected with A. duodenale hookworms, the prevalence was 58.9% (Stoltzfus *et al*., 1997). When iron stores in the 11 host becomes depleted, there is a direct correlation between the intensity of hookworm infection and the reduction in hemoglobin, serum ferritin and protoporphyrin levels (Stoltzfus *et al*., 1997).

Most of the physical signs of chronic hookworm infection reflect the presence of iron – deficiency anemia in addition anasarea from extensive plasma hypoproteinemia is associated with edema of the face and lower limbs with potbelly. The skin becomes waxy and acquires a sickly yellowish color. Hookworm infection can cause hypothermia. That is severe enough to reduce fever caused by malaria (Maxwell *et al*., 1987). Other than hypochromic microcytic anemia, the most prominent laboratory finding is eosinophilia. Eosinophilia peaks at five to nine weeks after the outset of infection, a period that coincides with the appearance of adult hookworms in the intestine (Maxwell *et al*., 1987). A moderate or heavy hookworm burden results in recurrent epigastric pain and tenderness, nausea, exertional dyspnea, pains in the lower extremities, palpitations, joint and sternal pains, headache, fatigue and impotence (Anyaeze, 2003).

Other effects of hookworm infections include stunted growth, Impairment of intellectual and cognitive development in school age-children, immune impotence (Udonsi, 2002). Considering these effects of Hookworm and the fact that the environmental conditions which enhance the transmission of hookworm abound in Aba metropolis, this study was aimed at evaluating Hookworm infections and some haematological parameter amongst primary and secondary School children in Aba Metropolis, Abia state, Nigeria.

MATERIALS AND METHODS

Study Area: Aba is a city in the southeast of Nigeria and the main trading centre in Abia State. It lies along the west bank of the Aba River, and is at the intersection of roads leading to Port Harcourt, in Rivers State, Owerri in Imo State, Umuahia in Abia State, Ikot Ekpene, and Ikot Abasi in Akwa Ibom State. It has an area of 72 Km^2 and a humidity of 82%. There are two seasons in the year, namely: the rainy season and the dry season. The rainy season begins in March and ends in October with a break in August usually referred to as the "little dry season." The dry season which lasts for four months begins in November. Heavy thunderstorm is characteristic of the onset of the rainy season. The total rainfall decreases from 2200mm in the south to 1900mm in the north. The hottest months are January to March when the mean temperature is above 27°C. The relative humidity is usually high throughout the year, reaching a maximum during the rainy season when values above ninety per cent are recorded. The city became a collecting point for agricultural products following the British made railway running through it to Port Harcourt. Aba is a major urban settlement and commercial centre in a region that is surrounded by small villages and towns. The indigenous people of Aba are the Ngwa. Aba is well known for its craftsmen. As of 2006 census, Aba had a population of 534,265. Aba is surrounded by oil wells which separate it from the city of Port Harcourt. Its major economic contributions are textiles and palm oil along with pharmaceuticals, plastics, cement, and cosmetics which made the Ariaria International Market to become the largest market in West Africa seconded by the onitsha main market. There is also a brewery, a glass company and distillery within the city. Finally, it is famous for its handicrafts. Aba is the commercial hub of eastern Nigeria". The city has well over 90 primary schools.

*Study Population***:** The five schools randomly selected and sampled for Hookworm ova in this study were:

1. Christ the king primary and secondary school 400
2. School Road Primary School 260
3. St. Joseph Primary and secondary school, 300
4. Cameroun Road Primary School 220

5. Okigwe Road Secondary School 320

The subjects were all residents in Aba urban and were grouped into five zones according to the location of the schools.

Ethical Clearance: Permission was sought and obtained from chairman of the Local Government Area, School heads, teachers and consents of Parents were also obtained.

Sample collection: A total of 1500 faecal samples were collected from subjects. Each subject was provided with a wide mouthed 20ml universal container, directing them to use the spatula attached to the cover of the bottle to collect a pea sized stool sample the next morning. They were advised to provide early morning stool samples as this has been proved to contain more parasites than those collected during the day (Bundy and Michael, 2000).

Stool analysis: Samples were analysed following the standard procedure described by WHO (1991) and Cheesbrough (2005).

All the stool samples were concentrated using formol ether concentration technique. One milliliter of a well-mixed stool sample was put in a tube containing 4 mL of 10% formalin. Three milliliters of the 10% formalin was again added and mixed by shaking. The suspension was sieved using a coffee strainer into a centrifuge tube. Three milliliters of diethyl ether was added and stoppered. It was then shaken vigorously for 1 min. The stopper was removed and the suspension centrifuged for 1 min at 400 rpm. The entire column of the fluid below the faecal debris and ether was carefully removed using a Pasteur pipette and transferred into another centrifuge tube. Ten per cent formalin was added to the transferred suspension to make up to 10 mL. It was then centrifuged at 1000 rpm for 10 mins. The supernatant was decanted and the bottom of the tube tapped to re-suspend the deposit. The deposit was examined by light microscopy at 100x and 400x magnifications for the presence of ova of hookworm parasite.

Blood sample analysis: The blood samples were analyzed to determine Haemoglobin (Hb) levels, Esinophils and white blood cell counts (WBC)

Haemoglobin estimation by cyanmethaemoglobin method (Baker *et al*., 1998) This was done by dispensing 0.02ml (20u/l) of well-mixed blood samples of subjects into 5ml of 'Drabkins' solution in a test tube using micropipepette and tube stoppered. The diluted blood was left at room temperature for 4 – 5 minutes protected from sun light. A yellow – green filter (11Ford 608) was placed in the calorimeter and calorimeter zeroed using 'Drabkins' fluid 25 after which absorbance of samples of subjects were obtained and recorded. The actual Hb values of subjects were then obtained by reading of the absorbance against corresponding Hb concentration on the already prepared calibration graph use in the laboratory where this work was carried out.

Eosinophil count by counting-chamber method (John and Ronald, 1991) Using a micropipette,0.02ml (20u/l) of EDTA blood was dispensed to 0.38ml of diluting fluid to give a dilution of 1:20 in a test tube. The suspension was mixed and allowed to stand for not longer than 30 seconds and the counting chamber was filled using a Pasteur pipette. The Eosinophils were counted as soon as they were settled. A chamber with the fuchs –Rosenthal ruling was used. This has an area of 16mm and volume 3.2u/l. The Eosinophil count was calculated as follows: Count/L = No. of cells counted x Dilution x 106 Volume counted (u/l) 1

Total leucocyte counts (white cell counts) using micropipette, 0.02ml (20u/l) of EDTA blood was dispensed to 0.38ml of diluting fluid (2% glacial acetic acid) to make a dilution of 1:20 in a test tube and tube was corked tightly. The dilution was mixed by rotating the cell suspension mixer for 1 minute. The neubeur counting chamber was filled with the dilution by means of Pasteur pipette and allowed to stand for 5 minutes. The number of cells was then counted using x10 objective and x40 objective; the cells are calculated as follows. 26 Count/L = No. of cells counted x Dilution factor Volume counted (u/l) 1.

Statistical Analysis: Proportions obtained in the study were analysed using percentage and descriptive statistics (Tables and Charts) and Chi square (X^2) analysis at 5% significant level. SPSS package was used.

RESULTS AND DISCUSSIONS

From a total of 1500 samples examined for Hookworm infection, 120 (8.0%) were positive. Infection rate by school showed that Okigwe Road School had higher infection rate of 30(9.4%) followed by St. Joseph's School 25(8.3%) and 20(7.7%), 30 (7.5%) for School road and CKC respectively while Cameroun road school had the least infection rate of 15(6.8%) as shown in Table 1. The statistical analysis showed that there is significant difference in the prevalence of Hookworm infection within schools. (P<0.05)

Table1: Prevalence of Hookworm infection within schools in Aba metropolis

School	No.Examined	No.+ve	% +ve	Pi Values	Nipi
CKC	400	30	7.5	0.075	2.25
St.Joseph's	300	25	8.3	0.083	2.08
School Rd	260	20	7.7	0.077	1.54
Cameroun rd	220	15	6.8	0.068	1.02
Okigwe Rd	320	30	9.4	0.094	2.82
Total	1500	120	8.0		∑nipi=9.71

Sex related prevalence showed that infection was higher in females 8.17% than in males 7.89%. More females within the age range of 14-17years were more infected (10.3%), followed by those within age range 10-13years(9.47%) while those within the age range of 6-9years had the least infection rate of 6.02%. More males within the ages 10-13years had higher infection (9.09%), followed by those within the ages 6-9years (7.94%) while the least rate of infection of 6.74% was recorded among those within ages 18-22years (Table 2). It was observed that hookworm infection is dependent on sex and age ($P<0.05$).

Table 2: Prevalence of Hookworm infection among Age group by sex in Aba

Age group	No.Examined		No.Infected		% infected	
in years	m	f	m	f	m	f
6-9 yrs	151	133	12	8	7.94	6.02
10-13yr	286	190	26	18	9.09	9.47
14-17yr	181	97	14	10	7.73	10.30
18-22yr	282	180	19	13	6.74	7.22
Total	900	600	71	49	7.89	8.17

Table 3. Distribution of mean Haemoglobin (HB) % by sex and age in Aba metropolis

Age	Sex	No, Examined	Mean HB (%)	No. Infected	% infected
6-9yr	M	151	54	12	7.9
	F	133	49	8	6.02
10-13yr	M	286	60	26	9.09
	F	190	56	18	9.47
14-17yr	M	181	72	14	7.73
	F	97	64	10	10.2
18-21yr	M	282	70	19	6.74
	F	180	68	13	7.22
Total		1500		120	8.0

Table 3 showed that there is a negative correlation between hookworms infection and Haemoglobin levels both in female and male subjects; this work recorded the highest mean haemoglobin rate of 72% in male subjects among the age group of 14-17yrs with percentage infection rate of 7.7% in the female also the highest mean haemoglobin rate is 68% of age group 18-21years and the percentage infection rate of 7.2%. This works is in agreement with the findings of Cheesbrough (1998) who also suggested a negative correlation between hookworm infection and Haemaglobin levels. Stoltfus et al., (1997), Williamson et al., (2003), Hotez and Pritchard (1995) all suggested a negative correlation between hookworm burden and blood percentage but this normally 43 occurs when there is “hookworm disease, i.e. iron deficiency anemia that results from moderate to heavy infection and there is usually heavy blood loss. This work also agrees with the work of Stolztfus et al., (1997), which showed the status when iron stores in the host becomes depleted; there is a direct correlation between the intensity of hookworm infection and the reduction in haemoglobin; serum ferritin and protophorphyrin levels (Stoltzfus et al., 1997).

Table 4. Distribution of mean Eosinophil count by sex and age in Aba metropolis

Age	Sex	No. Examined	Mean Eosinophil	No. Infected	% infected
6-9yr	M	151	$1.0x10^9/L$	12	7.94
	F	133	$1.5x10^9/L$	8	6.02
10-13yr	M	286	$1.8x10^9/L$	26	9.09
	F	190	$2.0x10^9/L$	18	9.47
14-17yr	M	181	$0.8x10^9/L$	14	7.73
	F	97	$0.9x10^9/L$	10	10.2
18-21yr	M	282	$0.9x10^9/L$	19	6.74
	F	180	$1.0x10^9/L$	13	7.22
Total		1500		120	8.0

Table 4 revealed that there is a correlation between hookworm infection and eosinophilia counts. It showed an increase in eosinophilial with heavy burden of hookworm infection in the age group of 10 – 13 years but a decrease in eosinophilia count in the age 14 – 17 years. This work also showed that there is higher value of eosinophilia in female children than in male children ($P < 0.05$) this is also in agreement with the work of Maxwell et al., (1987) which observed eosinophilia instead of the usual hypochromic microcytic anemia. Eosinophilia peaks at about 5 to 9 weeks of the onset infection. Carroll and Grove (1986), Wolfe (1999), also shared the same view of eosinophilia as a result of hookworm infection and reinfection

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Table 5. Distribution of mean WBC count by sex and age in Aba metropolis

Age	Sex	No. Examined	Mean WBC	No. Infected	%infected
6-9yr	M	151	$17x10^9$/L	12	7.94
	F	133	$16x10^9$/L	8	6.02
10-13yr	M	286	$18x10^9$/L	26	9.09
	F	190	$20x10^9$/L	18	9.47
14-17yr	M	181	$12x10^9$/L	14	7.73
	F	97	$24x10^9$/L	10	10.2
18-21yr	M	282	$9x10^9$/L	19	6.74
	F	180	$10x10^9$/L	13	7.22
Total		**1500**		**120**	**8.0**

Table 5 showed that WBC varies proportionally with hookworm infection rate. The peak of hookworm infection was recorded in this work at the age group 14 – 17years to be 10.3% for the female age group and WBC count of $24x10^9$ /L whereas the male of the same age group has 7.7% with WBC count of $12x10^9$ /L. This showed that females have higher WBC count than the male subjects. The proportionality of WBC count increases with hookworm infection. This agrees with the early work of Carroll and Grove (1986). He recorded that the Total White Blood Cell (TWBC) before hookworm infection increases to about 2.5 times in 42 days after hookworm infection.

The overall prevalence of 8.0% of hookworm infection recorded in this study is relatively high when compared with some reports by earlier researchers from other part of Nigeria. Adeyeba and Tijani (2002) reported a prevalence of 3.2% in Igbo-ora in Oyo State. Odebunmi *et al*., (2007) also reported 3.2% among school children in Vom, Plateau State. The high prevalence had been attributed to poor sanitary standard which Aba is notorious known for (Wokem *et al*., 2016), as there are no proper drainages and waste disposal facilities across the town. Consequently, when there is rainfall everywhere is flooded with faecal materials and other waste matters moving all over. Also personal hygiene may contribute to this high prevalence but generally the relative humidity, temperature and other environmental factors in Aba are very favourable for the transmission of hookworm in the area. However, the 8.0% recorded is lower than 17.8% reported by Babamale *et* al., (2015) among school-aged children in Okuta community in Kwara State ;25.0% recorded by Abah and Arene (2015) among school children in Rivers State and 25.5% reported in Ethiopia by Ibrahim *et al*.,(1999). Elsewhere, Odebunmi *et al*., (2007) had attributed the variation in the prevalence of hookworm to some habits such as the use and abuse of drugs accounting for the low shedding of eggs by infected people thereby giving a false impression of low infection rate. That explanation is still apt here because Aba is a commercial hub of pharmaceutical activities. Although there was no significant difference in prevalence of infection between the schools, subjects from Okigwe Road School had higher infection rate of 30(9.4%) followed by St. Joseph's School 25(8.3%) this might be as a result of the level of emphasis placed on good sanitary habit and personal hygiene by the schools' managements.

Infection was higher in females 8.17% than in males 7.89%. This finding is consistent with Odebunmi *et al*., (2007) who observed that the prevalence of hookworm infection was higher in females than in males in vom, Plateau State. Abah and Arene(2016) that observed higher prevalence of intestinal parasitic infections in females than males in the urban area of Rivers State; and Wokem and Wokem (2014) who reported higher prevalence of hookworm in females (10.0%) than males (8%) but statistically not significant in their work on intestinal helminhtiasis among school children in Port Harcourt. The observation may be due to the female chores such as food preparation in an open kitchen in the compound, cleaning and sweeping of surroundings and water fetching which are usually done relaxed with barefoot. However, this finding is at variance with Babamale *et al*.,(2015) and some other researchers that reported otherwise. Their explanation suggested that immunological and hormonal factors play a role in reduction of infection in females. We conclude that if other factors such as environmental and level of exposure are favourable, transmission and hence prevalence will be high.

Hookworm infection was found to be dependent on age as more females within the age range of 14-17years were more infected (10.3%), followed by those within age range 10-13years(9.47%). These groups belong to the teenage/ adolescent girl and corroborate Crompton (2000) who suggested that teenage girls and pregnant women were more at risk of hookworm and hookworm diseases such as iron deficiency anaemia than any other age group. We also observed that more males within the ages 10-13years had higher infection (9.09%). This agrees with the finding of Holland *et al*.,(1989) who observed that the bulk of helminth infections were within the age group 10-15 years and Odebunmi *et al*., (2007) who recorded the highest prevalence in the age group 13-15 years.

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