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Epidemiology of Intestinal Parasitic Pathogens, *Plasmodium Falciparum* and their Contribution to Anaemia among School Children in Lagos State, Southwest, Nigeria

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Abstract: *Intestinal parasitic infections are among the neglected tropical diseases targeted for elimination by World Health Organization and are also among the most prevalent infections in school children residing in developing countries. This study was designed to determine the prevalence and intensity of intestinal parasites and its contribution to the development of anaemia among primary school children in Lagos State, Nigeria. Nine hundred (900) faecal and blood samples were collected among the school children. Direct saline preparation and formol-ether concentration techniques were used to determine the prevalence of intestinal parasites and Giemsa-stained blood smears were also used for the identification of Plasmodium falciparum. The overall prevalence of intestinal parasites and P. falciparum was 55.2% and 8.3% respectively. The prevalence of each intestinal parasite was 2.8% for Trichuris trichiura, 23.7% for Hookworm, 27.7% for Ascaris lumbricoide, and 1.1% for Strongyloides stercoralis. The only co-infection recorded was A. lumbricodes and P. falciparum (1.3%). Hookworm had the highest burden of 14,138.6 mean epg and Trichuris trichiura had the least burden of 84.6 mean epg. While no significant difference was observed between the mean serum ferritin for uninfected (73.70 ±10.41) and infected (73.20 ±8.72) (P = 0.4382), a significant difference was recorded between the mean PCV of uninfected (38.69 ±3.85) and infected school children (37.78 ±5.14) (P = 0.0047). Personal hygiene, public awareness and regular de-worming programme should be sustained as this would reduce the burden of intestinal worm infection among school children.*

Keywords*: Epidemiology, Intestinal Parasite, Plasmodium falciparum, Anaemia*

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I. Introduction

Intestinal parasitic pathogen infections have been recognized as an important public health concern, mainly in developing countries like Nigeria. Owing to this significance, there have been continuous efforts to determine the incidence and prevalence of intestinal helminthes in various places such as China, Southern Malawi, Uganda and Nigeria [1, 2, 3]. Most common among the intestinal helminthes are *Trichuris trichiura, Strongyloides stercoralis, Ascaris lumbricoides* and the anthropophilic hookworms (*Necator americanus* and *Ancylostoma duodenale*). About 1.2 billion people are estimated to be infected with *A. lumbricoides* globally while *T. trichiura* and hookworm infect up to 795 and 740 million people respectively [1]. Worm transmission is enhanced by poor socio - economic conditions, inadequate provision of sanitary facilities, and improper disposal of human waste, insufficient supplies of portable water, poor personal hygiene, substandard housing and lack of education [4].

Infection with helminthes such as hookworm, *Ascaris lumbricoides, Trichuris trichiura, Hymenolepis nana* are associated with conditions of poverty, unsafe water, sanitation and hygiene [5]. It is speculated that about two billion people are infected with helminthes mainly in the developing nations [6] Pre-school, school-aged children and pregnant women are at high risk of mobidity [7]. Helminthic infections are also deleterious to physical and mental growth during childhood, thwart educational advancements, and hinder economic development [8]. Adverse effects of helminthic infections are growth retardation, diminished physical fitness, delayed intellectual development and cognition [5, 7]. Malaria and intestinal helminthes are the most prevalent endemic parasitic diseases in sub-Saharan Africa due to amiable environmental conditions and their similarities in geographical distribution and co-infections [9]. In sub-Saharan Africa, the distribution of *P. falciparum* and helminthic infections is common and results in high rates of co-infections, which causes problems of poor nutrition and anaemia in the humans [9]. In Nigeria, reports have shown that falciparum malaria and helminthic infections are endemic and cause significant health problems among children [10]. Moreover, there is low level of awareness by the populace about the problem of helminthic infection and how to stem the tide of transmission. More importantly, the scale of the problem has long been neglected, partly because the diseases rarely kill. This study was therefore designed to determine the epidemiology of intestinal parasitic pathogens and their contribution to anaemia among school children in Lagos State, Nigeria.

II. Materials And Methods

Study Location: The study was carried out in five local Government Areas within Lagos State and comprises: Mushin Local Government Area (L.G.A.) with estimated population of 633,009, Surulere L.G.A. with estimated population of 503, 975, Ajeromi- Ifelodun L.G.A. with estimated population of 684,105, Badagry L.G.A. with estimated population of 426,735 and Ojo L.G.A. with estimated population of 598,071 according to the 2006 population census. These areas are located in the rain forest area with distinct rainy and dry seasons. These local government areas are predominantly with clustered homes, poor sanitary conditions and are densely populated with indiscriminate disposal of human wastes in drainages, streams and rivers. School children within the age bracket 2-13 years were recruited in this study. The children were selected across from the selected schools in all the five local government areas.

Study design: A total of nine hundred school children were examined for intestinal helminthes, and *Plasmodium falciparium*-helminthes co-infection. A well structured questionnaire was designed to collect information on the demographic, socio-economic and environmental background and personal hygiene practices that covers all the possible factors associated with intestinal parasites and its co-infection with malaria; was distributed among nine hunderd school children from the five local government areas under study. The parasitological survey was preceded by a pre-survey contact during which verbal permission was obtained from the Ethical review Committee and parents of the participating school children.

Sample Collection: School children were educated on how to collect fresh morning stool sample into sterile containers which were labeled and distributed to each of the participating school children a day preceding the program. Stool sample were collected as soon as they arrive the school premises and were packaged and transported to the laboratory without delay as stool specimen has to be examined within four hours of passage in order to ensure proper identification and presence of parasitic agents [11]. Three millilitres of venous blood sample was collected from each of the participants and transferred into EDTA containers and it was mixed gently [12].

Sample Analysis and Identification: Stool samples were visually examined to note the consistency, presence of abnormal features whether watery, bloody with mucus, formed or unformed [11]. Direct smear saline preparation of stool was examined for ova of parasites under the microscope within 24 h of collection using x10 and x40 objectives lenses as recommended by World Health Organisation [11]. Negative samples were subjected to concentration method using formol-ether sedimentation technique [12]. Kato- Katz thick smear technique was used for quantitative determination of helminthes in order to determine the intensity of infection and was expressed as the number of eggs per gram of faeces (epg) [11]. Thick and thin films were made from collected blood samples and stained with 10% Giemsa, in order to identify the species and quantify the malaria parasites in the blood and observed under microscope using oil immersion objective lenses. Malaria parasites were counted against 200 leukocytes, and counts were expressed as the number of parasites per microliter of blood, assuming an average leukocyte count of 8,000 cells/µl of blood [12].

Determination of Packed Cell Volume and Serum Ferritin

The packed cell volume (PCV, %) was determined by centrifuging microhematocrit tubes filled with blood in a microhematocrit rotor for 5 min at 10,000rpm and was read with the haematocrit reader [12] while Serum ferritin was estimated using commercially available rapid test kit produced by TECO DIAGNOSTICS, Lakeview Ave. Anaheim CA U. S.A. The procedure was according to the manufacturer's instruction.

Statistical analysis

Data analysis was carried out using SPSS version 16. Frequency tables and cross tabulations were produced for each of the study variables. Relationship between independent and dependent variables was assessed using chi-square test. Statistical significance was achieved if $P < 0.05$.

III. Results

A total of nine hundred (900) stool samples was examined from school children in the study areas from five L.G.A in Lagos state, Nigeria. The mean age and mean PCV were 7.5±2.78 and 33.28 ± 4.38 respectively. Five difference parasites were identified namely, *Ascaris lumbricoides*, hookworm, *Strongyloidies stercoralis, Trichuris trichiura and Plasmodium falciparum.* Majority (45.4%) of the participants are within the age range 6-9 years while the least was in age 10-13 years (22.9%). The male to female ratio was 461(51.2%):439 (48.8%). The mean packed cell volume was 33.28±4.38. *Ascaris lumbricoides* (27.7%) was the most common parasite while the least was *Strongyloides stercoralis* (1.1%). *Ascaris lumbricoides/ Plasmodium falciparum* co-infections has prevalence of (1.3%) (Table 1).

Prevalence of intestinal helminths among school children by age and sex is shown in Table 2. The overall prevalence of intestinal helminths was 55.2%. Age group 2-5years had the highest prevalence rate of 67.7% while the least age group was 10-13years (45.2%). There is significant difference between the age group and the intestinal helminthes (p=0.0001). Male (57.1%) were more infected than female countapact (53.3%) and there is also significant difference between the intestinal helminths and age (p = 0.0003).

Table 3 shows the prevalence and intensity of intestinal parasites and malaria parasite among school children by location. The intensity is calculated according to World Health Organisation guideline using arithmetic mean egg count per gram (AMEPG) of faeces. Hookworm had the highest intensity of 14,138.6 epg, followed by *A. lumbricoides* (12,695 epg) while the least was *T. trichiura* (86.4 epg).

Table 1: Demographic characteristics and parasitic infection status of school children

Demographic variables	**Frequency (%) N= 900**
Age	
2-5	285 (31.7)
6-9	409 (45.4)
10-13	206 (22.9)
Sex	
Male	461 (51.2)
Female	461 (51.2)
Mean age ±SD	7.5 ±2.78
Mean age ±SD	33.28 ± 4.38
Parasites	
A . lumbricoides	249(27.7)
Hookworm	213 (23.7)
T. trichiura	25(2.8)
S. stercoralis	10 (1.1)
P. falciparum	75 (8.3)
A.lumbricoides+ P. falciparum	12 (1.3)

Table 2: Prevalence of Intestinal Parasitic Infection among school children by age and Sex

Parasites	**Age Group (Year)**					**Sex**			
	2-5 n=285	**6-9 n=409**	**10-13 n=206**	**Total N=900**	**P-value**	**Male n=461**	**Female n=439**	**Total N=900**	**P-value**
	NO. Infected (%)	**NO. Infected (%)**	**NO. Infected (%)**	**NO. Infected (%)**		**NO. Infected (%)**	**NO. Infected (%)**	**NO. Infected (%)**	
A. lumbricoides	96(33.7)	103 (25.2)	50 (24.3)	249 (27.7)	0.022 *	127(27.8)	122(27.6)	249(27.7)	0.000
Hookworm Spp	79 (27.7)	96 (23.5)	38 (18.5)	213(23.7)	0.058	119(25.8)	94(21.4)	213(23.7)	0.612
S. stercoralis	5(1.8)	3(0.7)	2(1.0)	10(1.1)	0.440	3(0.7)	7(1.6)	10(1.1)	1.000
T. trichiura	13 (4.6)	9 (2.2)	3 (1.5)	25 (2.8)	0.0746	14(3.0)	11(2.5)	25(2.8)	0.111
Total	193 (67.7)	211 (51.6)	93 (45.2)	497 (55.2)		263(57.1)	234(53.3)	497(55.2)	
p-value	0.0001					0.0003			

Table 3: Prevalence and Intensity of Intestinal Parasites and Malaria Parasite among School Children by location.

Parasites	**Rural n=450**		**Urban n=450**		**Total N=900**	
	No. Positive (%)	**Infection burden (AMEPG)**	**No. Positive (%)**	**Infection burden (AMEPG)**	**No. Positive (%)**	**Infection burden (AMEPG)**
A. lumbricoides	125(27.8)	8976	124(27.6)	3719	249(27.7)	12695
Hookworm	110(24.4)	12371.3	103(22.9)	1767.3	213(23.7)	14138.6
T.trichiura	16(3.6)	71.3	9(2.0)	15.1	25(2.8)	86.4
S. stercoralis	16(3.6)	*	4(0.9)	*	10(1.1)	*

Malaria	49(10.1	*	26(5.8)	*	75(8.3)	*
Malaria+*A. lumbricoides*	5(1.1)	**	7(1.6)	**	12(1.3)	**

*= Threshold value for calculation is not available in WHO criteria.
**= Could not be quantified because it is mixed infection.
AMEPG= Arithmetic mean egg per gram of faeces.

The association between intestinal parasites and malaria parasite in relation to packed cell volume and serum ferritin is shown in Table 4. Hook worm infection have a marked variation on the PCV level on infected school children (36.17±3.37) when compared with uninfected school children (38.61+3.34). This was closely followed by malaria with mean packed cell volume for infected school children (37.25±3.57) in relation to uninfected school children with Packed Cell Volume (38.76±3.19). In co-infection of Ascaris lumbricoides and malaria infection there is no marked difference in the mean packed cell volume of infected school children (38.02±4.21) when compared with uninfected counterparts (38.72±3.33). The total mean pack cell volume of infected school children was 37.78±5.14 while the uninfected mean pack cell volume was 38.69±3.85, therefore there is significant association between packed cell volume and parasitic infections (P= 0.0047).The total mean serum ferritin level of infected school children with parasitic infection was 73.20±8.72 while the total mean serum ferritin level of uninfected school children was 73.70±10.41. There is no significant association between serum ferritin level and parasitic infections (P= 0.4382).

The relationship between socio-demographic factors and intestinal parasites is shown in Table 5. The prevalence of intestinal parasites was found to be higher in those with family size 3-4 (56.6%), and family size 1-2 had the least (13.3%). The highest prevalence was recorded in those whose parents had only primary education.There is low prevalence among those who drink well water and use filtration as a means of treating water with prevalence of 15.0% and 2.0% respectively. There was statistically significant association between the rate of intestinal helminthes infection and all the socio-demographic factors considered (p =0.0001)

Table 4: Association of Intestinal Parasites and Malaria Parasite in relation to Pack Cell Volume (PCV) and Serum Ferritin.

Parasites	**Mean Pack Cell Volume (%)**			**Mean Serum Ferritin /Ug**		
	Not infected ±SD N=345	**Infected±SD N= 555**	**p-value**	**Not Infected ±SD N=345**	**Infected ±SD N= 555**	**p-value**
A.lumbricoides	38.88 ±5.17	38.16 ±4.38	0.026*	73.62 ±1.70	74.50 ±8.77	0.0658
Hookworm	38.61 ±3.34	36.17 ±3.37	0.0001*	73.72 ±6.37	72.18 ±4.13	0.0001*
S. stercoralis	38.78 ±3.16	38.62±5.25	0.6092	73.68 ±9.31	73.42 ±8.75	0.6725
T. trichiura	38.92 ±3.22	38.19±3.49	0.0017*	73.81 ±7.93	73.62 ±5.69	0.6764
Malaria	38.76 ±3.19	37.25±3.57	0.0001*	73.72 ±8.24	73.70 ±8.24	0.9718
Malaria+ *A.lumbricoides*	38.72 ±3.33	38.02±4.21	0.0089*	73.72 ±11.89	69.00 ±8.98	0.9718
Total mean	38.69±3.85	37.78±5.14		69.00 ±8.98	73.20±8.72	0.0001*
P-value	0.0047*			0.4382		

Table 5: Socio-demographic of the school children infected with Intestinal Parasites.

Variables	**N= 555 Positive (%)**	**N=345 Negative (%)**	**N=900 Tot al (%)**	**P-value**
Family Size				
1-2	74 (13.3)	135(39.1)	209 (23.2)	
3-4	314 (56.6)	115 (33.3)	429 (47.7)	0.0001*
≥5	167 (30.1)	95(27.5)	262 (29.1	
Mother Education				
Primary	410 (73.9)	69 (20.0)	479 (53.2)	
Secondary	115 (20.7)	99 (28.7)	214 (23.8)	0.0001*
Tertiary	30 (5.4)	177 (51.3)	207 (23.0)	
Father Education				
Primary	421 (75.9)	86 (24.9)	507 (56.3)	
Secondary	96 (17.3)	104 (30.2)	200 (22.2)	
Tertiary	38 (6.9)	155(44.9)	193 (21.5)	
Source of drinking water				
Well water	83 (15.0)	82 (23.8)	165 (18.3)	
Bore hole	188 (33.9)	95 (27.5)	283 (31.4)	0.0001*
Stream water	195 (35.1)	57 (16.5)	252 (28.0)	
Tap and other sources	89 (16.0)	111 (32.2)	200 (22.3)	
Method of water treatment				
Boiling	35 (6.3)	114 (33.0)	149 (16.6)	
Filtration	11 (2.0)	46(13.3)	57 (6.3)	0.0001*
Alum	41 (7.4)	129 (37.4)	170 (18.9)	
None	468 (84.3)	56 (16.3)	524 (58.2)	

Bath room type				
Traditional	312 (56.2)	127 (36.8)	439 (48.8)	
Western	208 (37.5)	132(38.3)	340 (37.8)	0.0001*
Both	35 (6.3)	86 (24.9)	121 (13.4)	

IV. Discussion

Globally, intestinal parasitic infections, including helminths and protozoa, remain endemic. About one-third of the world's populations, more than 2 billion people, are infected with the largest majority being children [13, 14]. In this study, the prevalence of intestinal parasites was 55.2%. This high prevalence is an indication of the presence of conducive climate for parasite growth, rapid and unplanned urbanization, and social practices of open defecation and lack of community education and sanitation, which impedes control of infection in Lagos, Nigeria. A similar study in south-west Nigeria reported lower prevalence of 48.4% [15] and 50.4% [16] respectively. In contrast, some other studies reported higher prevalence of 86.2% in Lagos state [17], 95.7% in Osun state [18], 94% in Ondo state [19] and 30.2% in North central Nigeria [20]. This high prevalence reported in these different locations within the country alludes to the fact that helminth infection is still endemic in the country. The predisposing factors that facilitate the continuous spread of these parasites are widespread in the country. In other African countries, a prevalence of 55.2% was reported in Cote'd'Ivoire [21], which are in agreement with this study. Differences in prevalence might be explained by the timing or seasonal differences of conducted surveys, environmental conditions, or other factors like mass deworming programs in the study areas. This high prevalence recorded can be attributed to ignorance, poverty, poor environmental and personal hygiene, shortages of clean potable water and indiscriminate defecation as most vegetable farmers use excreta as manure which is a veritable source of infection since children and their mothers often go to the farm to tender to the vegetables. *Ascaris lumbricoides* was the most prevalent parasite (27.7%) followed by *P. falciparum* (8.3%) in this study. This is consistent with results of previous studies in other parts of Nigeria reporting the predominance of *A. lumbricoides* among other intestinal parasites in children [18, 20].

In this study, the highest prevalance of parasites (67.7%) were recorded among school children aged 2-5years which was in agreement with a study in North central Nigeria which reported the highest prevalence of 66.7% among closely related age group [20]. Another study in Central Turkey also reported a prevalence of 51.0% in primary school children [22]. The least prevalence was observed in the older group 10-13yrs (45.2%). Besides, there was significant association between the age groups and *A. lumbricoides* infection (p = 0.022). The reduction in prevalence as the age increases could be associated with a higher level of awareness of personal hygiene as they grow older.

It was equally observed that male (57.1%) school children have higher prevelance than their female (53.3%) counterparts and the difference was statistically significant (P = 0.0003). Even, though gender is not a significant risk factor for prevalence of intestinal helminth infection [23]. It is however important to affirm that more males were enlisted in the study than females. Nevertheless, this result are in agreement with those of Damen *et al.,* [20] and Simon *et al.,* [24] but contrary Luka *et* al., [25] in Kaduna. This difference in gender prevalence could be attributed to gender bias in sending children to school.

In this study, the prevalence of *P. falciparum* is 8.3% and their co-infection with *A. lumbricoides* among school children is 1.3%. A similar study reported a prevalence of malaria (52.3%) and its co-infection with *A. lumbricoides* (57.1%) [26] in Nigeria, while another study in Cameroun reported a prevalence of 98.5% for malaria and co-infection with intestinal helminthes as 11.9% [27]. These differences in prevalence *P. falciparum* and intestinal helminthes co-infection could be due to the presence of different environmental, socio-economic and behavioural factors that can increase the risk of concurrent infection with both intestinal helminthes and *P. falciparum*. Moreso, the increased density of *P. falciparum* among children co-infected with intestinal helminthes could be due to down regulation of the immune system.

The packed cell volume (PCV) recorded in this study varies between children with one intestinal parasites to another when compared with un-infected school children as expected. In this study, school children habouring hookworm infection have a pronounced low level of PCV (anaemia) closely followed by school children with *P. falciparum* infection. A similar study in Nigeria reported anaemia to be associated with children infected with hookworm and *P. falciparum* co-infection compared to *P. falciparum* infection only [28]. The result of the statistical analyses shows that the occurrence of anaemia among children in this study was strongly influenced by Hookworm and *P. falciparum* co-infection.

This study found that the risk factors that significantly influence the spread of intestinal helminthes and malaria infection among school children include family size, parent education, source of drinking water, method of water treatment, and bathroom type. Sources of water in this study were mostly from the streams, while lack of functional toilet facilities makes majority of the children to defecate in bushes near rivers which could be responsible for some of the spread of the intestinal parasites infections. School children who came from a family of $\geq$ 5 members are at higher risk of *A. lumbricoides* infection compared to children from smaller families as ascariasis is associated with overcrowding. Based on this study, Lagos State falls within the category II (medium risk community) which calls for urgent intervention considering the fact that these infections always leads to anaemia, malnutrition and stunted growth [29], poor intellectual development and school performance [30], high rate of school absenteeism [31] and poor productivity [32].

In view of the high prevalence of intestinal helminth recorded in this study, there is need for urgent intervention such as regular yearly mass administration of anti-helmenthic among school children. Other measures that will enhance the eradication of intestinal helminthes infection include health education and improved personal and public hygiene. There is urgent need to start an integrated and effective intestinal parasites control programme in school-age children such as implementing periodic school-based de-worming programmes, providing proper health education pertinent to good personal hygiene and good sanitary practice which will help in reducing the prevalence and intensity of intestinal parasites and malaria in these communities.

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