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Ascaris lumbricoides Infection Using Microscopy and IgG4 Detection Techniques in a School Children Population in Central Nigeria: An Epidemiological Study

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Abstract

Background: *Ascaris lumbricoides* infection is a major public health problem especially in developing countries. There is paucity of data on the prevalence of this parasite using an IgG4 detection method other than microscopy in Nigeria.

Aim: In a cross-sectional survey, the prevalence of *Ascaris lumbricoides* infection was carried out among school children using microscopy and IgG4 antibodies detection techniques in Central Nigeria.

Methods: After ethical clearance, stool and blood samples were collected from 400 children and examined for the parasite using microscopy and IgG4 *A. lumbricoides* ELISA kit in the selected schools in Nasarawa, Nigeria. The overall prevalence of the infection using microscopy and IgG4 detection was 28.0% and 30.5% respectively. The five classical microscopy techniques with the highest rates were $ZnSO_4$ solution and stoll egg counting techniques (28.5%). Tammah and Oversea primary school had the highest prevalence of 36.0% (P>0.05). Gender and age were not associated with the parasitic infection while occupation of the parents/guardians was a risk factor for the infection (p<0.05).

Conclusion: To the best of our knowledge, this is the first study that has reported the prevalence of *A. lumbricoides* infection using IgG4 detection technique in Central Nigeria. Intense awareness campaigns that will promote good hygiene and faecal deposition practices, creation of deworming programs in schools and provision of basic amenities that will help curb the parasite in the areas are urgently recommended.

Keywords: *Ascaris lumbricoides*; *Ascariasis*; Immunoglobulin G4; School children; Central Nigeria

Background

Ascaris lumbricoides is a roundworm of the large intestine that infects the gastrointestinal tract of man [1,2]. *A. lumbricoides* is a soil-transmitted helminth (STH), a group of human gastrointestinal nematodes transmitted through direct contact with eggs or larvae in soil environment [3,4]. The parasite is one of the commonest and most prevalent infection worldwide [5,6]. The infection has an estimated global prevalence of 25.0% [7]. The highest prevalence of *Ascariasis* occurs in the tropics where warm, wet climates provide favorable environment for year-round transmission of the infection [7]. The parasitic infection is usually asymptomatic and occurs mostly among children of school age in developing countries where there is contamination of soil by human feces and use of untreated feces as fertilizers [8]. The signs and symptoms of this parasitic infection include vomiting worms, bloody sputum, cough, low-grade fever, passing worms in stool, short breath, skin rash, abdominal pain and wheezing. It may also manifest in form of growth retardation, pneumonitis, hepatobiliary and pancreatic injuries and intestinal obstruction [9,10].

The parasitic infection is associated with elevated immunoglobulin G (IgG) and IgE responses in humans and animals [11,12]. Serological tests based on antibody detection may overestimate the prevalence of infection, due to the persistence of the antigen for a long time after deworming of patients. Although a novel, specific, and sensitive technique for the serodiagnosis of the parasite that involves the detection of Ascaris excretory-secretory (ES) antigen-specific IgG4 has been developed and used in research [13,14].

Transmission potential for *A. lumbricoides* in children is due to playing of pupils on the soil, malnutrition, unhygienic conditions, indiscriminate disposal of wastes and non-availability of portable water supplies in rural settings [15-17]. The prevention of the infection is assured by ensuring that soil is decontaminated, and this can be achieved by providing adequate toilet facilities in communities. The use of untreated human feces as fertilizers should be avoided and public health education should be advocated in rural settings [17].

There are data on *A. lumbricoides* infection among children in and outside Nigeria [2,4,14,18-20]. Relatively, there is little information on the use of IgG4 detection technique in estimating the prevalence of the parasitic infection in Nigeria. The aim of this study is to determine the prevalence of *A. lumbricoides* infection using microscopy and IgG4 techniques in a school children population in Central Nigeria.

Methods

Study area

This study was conducted in Nasarawa State, Nigeria. Nasarawa State has a central location in the Middle Belt Region of Nigeria. The state lies between latitude 7° and 9° 25'N of the equator and between longitude 7° and 9° 37'E of the Greenwich Meridian. It shares boundary with Kaduna state in the North, Plateau state in the East, Taraba and Benue states in the South while Kogi and the Federal Capital Territory flanks in the West [21].

Study population

The study population comprises of children aged 5-16 years old as adopted by FMOH [22] in the selected primary schools in Nasarawa who agreed to participate in the study. Structured questionnaire was administered to the parents/guardians of the four hundred (400) children recruited for the study. Participants who could not read or write in English Language were interviewed in Hausa by the researcher.

Four public primary schools were randomly selected in the study area. They are: Tammah Primary School (TPS), Oversea Primary School (OPS), Kofar-Kudo Primary School (KKPS) and Kwoto Primary School (KPS). The total population in the 4 primary schools is 1258 children.

Ethical permission and administrative clearance

In line with the Helsinki Declaration which specifies the code of ethics for biomedical research involving human subjects, clearance for this study was obtained from the Health Research Ethics Committee of Nasarawa State Ministry of Health, Nigeria. Further consent for the work was sorted and obtained from the Heads of the schools. Head Masters, Teachers, Parents/Guardians of the children and children were informed properly on the objectives of the study.

Sample collection

About 20 g of stool sample was collected from each of the 400 children. A clean dry sample container was given to each pupil for their stool specimen. The samples collected were then transported in ice packs to where they were examined for the parasites. Subsequently, 2 ml of blood sample was collected from each participant by venipuncture into a clean labeled plain tube. This was allowed to clot at room temperature and spun for 5 minutes at 3000 rpm. The resultant sera were harvested into well labeled cryovials and stored at -20°C until ready for ELISA assay.

Laboratory Investigations

Microscopy

The stool samples were checked macroscopically to note the color, odor, presence of mucus and/or blood. The stool samples were examined microscopically within 24 hours after collection. Multiple approaches were used for the examination of eggs and larvae of the parasite. The stool samples were concentrated using the formol-ether concentration technique and examined for the presence of *Ascaris* eggs by direct smears using normal saline and iodine solutions. Furthermore, sodium nitrate and zinc sulphate floatation techniques, Baermann and stoll egg counting techniques were adopted to investigate and count worm eggs and larvae [23-25].

Enzyme linked immunosorbent assay (ELISA)

The IgG *Ascaris lumbricoides* ELISA kit (Abcam Scientist Inc, USA) was used to detect anti-Ascaris IgG antibody levels in subjects' sera according to the manufacturer's specifications as described by Funk et al. [24]. Test procedure and result interpretations were performed according to the manufacturer's instructions.

Statistical analysis

The data obtained were subjected to descriptive statistical analysis using Smith's Statistical Package (SSP version 2.80, Claremont, California-USA). Chi square statistical test was used to determine the association of the prevalence of *A. lumbricoides* infection among school children with the studied risk factors. Values obtained were considered statistically significant at $P \leq 0.05$.

Results

A total of 400 school children in four public primary schools in Nasarawa participated in this study. The distribution of *A. lumbricoides* infection among the subjects based on different microscopic techniques and their respective prevalence rates is shown in **Table 1**. The overall prevalence of the parasitic infection by microscopy and IgG4 antibodies was 112 (28.0%) and 122 (30.5%) respectively (**Table 2**). **Tables 3 and 4** shows the prevalence of *A. lumbricoides* infection based on the schools studied using the microscopy and ELISA methods

(p>0.05). The prevalence of the infection in relation to sociodemographic characteristics using IgG4 antibodies detection technique is shown in **Table 5**.

Table 1 Distribution of *A. lumbricoides* infection among school children in relation to classical microscopic techniques used in the study area.

Techniques Used	Number Examined	Number Positive	Prevalence (%)	Chi square value	P value
Direct Smears	400	112	28		
$NaNO_3$ Solution	400	112	28		
$ZnSO_4$ Solution	400	114	28.5	0.0332	0.9999
Baermann Technique	400	112	28		
Stoll Egg Counting Technique	400	114	28.5		

Table 2 Comparison of Microscopy and ELISA methods for the Detection of *A. lumbricoides* in the study area.

Methods	No. Examined	No. Positive	Prevalence (%)	Chi square Value	P value
Microscopy	400	112	28		
IgG4 ELISA	400	122	30.5	0.3307	0.5652

Table 3 Prevalence of *A. lumbricoides* infection among school children in relation to schools studied using Microscopy in Nasarawa, Nigeria.

School No.	No. Examined	No. Positive	Prevalence (%)	Chi square Value	P value
TPS	100	36	36		
OPS	100	22	22		
KKPS	100	24	24	3.3053	0.3468
KPS	100	30	30		
Total	400	112	28		

Legend: TPS (Tammah Primary school), OPS (Oversea Primary school), KKPS (Kofar-Kudu Primary school), KPS (Kwoto Primary school)

Table 4 Prevalence of *A. lumbricoides* infection among school children in relation to schools studied using ELISA method in Nasarawa, Nigeria.

School No.	No. Examined	No. Positive	Prevalence (%)	Chi square Value	P value
TPS	100	32	32		
OPS	100	36	36		
KKPS	100	26	26	1.4722	0.6887
KPS	100	28	28		
Total	400	122	30.5		

Table 5 Prevalence of *A. lumbricoides* infection among school children in relation to sociodemographic characteristics in Nasarawa, Nigeria using ELISA method.

Socio-demographics	No. Examined	No. Positive	Prevalence (%)	Chi square Value	P value
Gender					
Male	196	50	25.5		
Female	204	72	35.3	2.411	0.1204
Age (Years)					
<6	108	32	29.6		
06-Oct	176	52	29.5	0.2074	0.9015
>10	116	38	32.8		
Occupations of Parents/Guardians					
Farmers					
Traders	110	46	41.8		
Civil servants	92	20	21.7		
Artisans	90	16	17.8	10.3188	0.0159
	108	40	37		

Discussion

Ascaris lumbricoides infection is a common occurrence in developing countries such as Nigeria with school children carrying the hardest hit of the associated morbidity. An overall prevalence rate of 28.0% and 30.5% using microscopy and IgG4 detection techniques was recorded among school children in Central Nigeria which is in consonance with the reports of 28.1% among school pupils in Imo [4], 22.2% among school children in Ondo [26], 45.8% among volunteers in Delta [3], 67.0% in Ijebu [2], 21.42% in Katsina [27], 1.0% in Jos [18] and 19.1% among Almajiris in North Eastern Nigeria [28]. Higher rates compared to findings in the present study have been reported in other countries like 42-74% in Kenya [29], 37.8% in Sri Lanka [20], 29.0% in South Africa [19] and 23.6% in Ethiopia [1]. The relatively high prevalence of the infection reported in this study might be due to lack of good sanitary facility and poor personal and environmental hygiene habits observed among the school children in the area.

To our knowledge, this is the first study that has reported the prevalence of *A. lumbricoides* infection using IgG4

detection technique in Central Nigeria. Few studies have reported *A. lumbricoides* IgG4 antibodies [14,24,25].

The major strength of this study lies on the unprecedentedly large panel of arrays of diagnostic techniques used. The diagnostic rigor resulted in high quality data regarding the prevalence of the parasite in the area. Prevalences observed here are thus likely to approach the true prevalence of *A. lumbricoides* infection among children, in contrast to other prevalence studies, where generally only one microscopic technique is used. This approach led to several important observations concerning the presence of the parasite in the target population. In practice, the microscopic technique especially the direct smears are widely used in endemic areas, in both clinical and research settings including the healthcare facilities in Nasarawa State which could impede the true prevalence of the infection in the area.

The highest prevalence of the infection (36.0%) was reported in children attending Tammah Primary school and Oversea Primary school using the microscopy and ELISA methods ($p>0.05$). This might be unconnected to the fact that the schools are surrounded by overgrown grasses which could predispose the children to defecating in the bushes. The children also play around in school fields with their hands which could potentiate them infecting their selves with the parasite.

In a related development, there was no statistically significant association between the prevalence of *A. lumbricoides* infection and gender of the school children ($p<0.05$). The prevalence of the parasite was higher among female (35.3%) than their male counterparts (25.5%). This is in consonance with the reports within Nigeria and beyond [2,20,30]. This may be related to female exposure levels of play groups on sand, hawking, sharing of food with unwashed hands and other domestic chores. It can also be pin pointed to the fact that females have large surface area of genital opening which allows easy entry of the parasite ova during defecation.

This study further revealed that the infection was highest among children aged >10 years (32.8%). The high infection prevalence reported in this group is attributed to age-related changes in diet, hygiene, and daily activities with regard to the exposure to parasite infective stages. Comparable findings have been reported elsewhere [1,29,30]. In Nigeria, at this age both genders are actively involving in outdoor activities in swampy and muddy contaminated soils and collects water from unprotected wells. These endangered them to parasitic infections than the other age groups.

There was a statistically significant association between occupation of parents/guardians of the children and the infection ($P<0.05$). The highest prevalence was recorded among parents/guardians that are farmers (41.8%) and least among parents/guardians that are civil servants (17.8%). This means that, the prevalence rate of the infection cut across the socio-economic background of the parents/guardians which is in consonance with an epidemiological report [31].

Conclusion

This study demonstrated a relatively high prevalence of *A. lumbricoides* infection among school children in Nasarawa, Nigeria with potential health consequences. The specific IgG4 ELISA assay was used to detect antibodies specific for *A. lumbricoides* antigens in blood samples. To the best of our knowledge, this is the first study that has reported the prevalence of *A. lumbricoides* infection using IgG4 detection technique in Central Nigeria. The schools studied, gender and age of children were not associated with the parasitic infection while occupation of parents/guardians of the children was associated with the infection. IgG4 detection technique can detect *A. lumbricoides* more accurately but is generally not feasible in resource-poor setting. Hence, there is need for a more field-friendly and sensitive diagnostic tools for evaluating parasitic infection other than the gold standard microscopy. Health Education taught in schools should emphasize more on preventive measures against those infections whose control revolves around personal and environmental hygiene and effective primary health care system.

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Competing Interests

Authors have declared that no competing interests exist.

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