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STUDY OF A NEGLECTED TROPICAL DISEASE (NTD): *STRONGYLOIDES STERCORALIS* AND ITS ASSOCIATED RISK FACTORS AMONG SCHOOL CHILDREN IN AHODA-EAST LOCAL GOVERNMENT AREA, RIVERS STATE, NIGERIA

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ABSTRACT

Strongyloides stercoralis infection is very dangerous in immunocompromised individuals and those cases receiving corticosteroid therapy. Prevalence of *Strongyloides stercoralis* infection and associated risk factors among schoolchildren in Ahoada-East Local Government Area, Rivers State were investigated between May and July 2015. Three hundred and seventy stool samples from pupils in nine primary schools in the study area were examined for the presence of *Strongyloides stercoralis* larvae using direct smear and Baermann techniques on two stool samples collected over two consecutive days. A structured child questionnaire was used to assess the association of *Strongyloides stercoralis* infection with socio-demographic and socioeconomic variables. The data collected were analyzed using Chi-square test and logistic regression. Thirteen (3.5%) stool samples were found positive for *Strongyloides stercoralis* infection. Males had a higher rate of infection than females in all age groups examined. Pupils between 11 – 13 years had the highest infection (69.2%) among the age groups. Upata clan had the highest rate of infection (5.2%) among the clans investigated and had the highest parasite (11.6%) following soil analysis. Poor sanitary habit, non-use of footwear and lack of latrine at home were the key factors identified with this infection. This study stands as baseline information for further investigation on *Strongyloides stercoralis* infection in Rivers State. Awareness programs on sanitary and good hygiene among children, regular de-worming exercise mostly at the primary school level and legislation against indiscriminate faeces disposal are recommended.

KEYWORDS: Prevalence, *Strongyloides stercoralis*, Baermann techniques, Risk factors.

INTRODUCTION

Strongyloides stercoralis is an intestinal nematode that is highly neglected among the helminthes (Olsen *et al*., 2009), yet about 30 - 100 million people are affected by the disease in the world (Puthiyakunnon *et al*., 2014). S*trongyloides stercoralis* has auto-infection ability which may result in systemic infections with high worm burden, especially in immunosuppressed hosts (Basile *et al*., 2010; Khieu *et al*., 2013). Repeated internal auto-infection enables the parasite to exist within a host for decades even after leaving the contaminated environment. Strongyloidiasis is mostly prevalent in tropical and subtropical regions, however this disease is now prevalent in non-endemic countries as a result of migration and travel, being infection that is commonly found in migrants than in travelers (Buonfrate *et al*., 2012). The clinical syndromes of *Strongyloides stercoralis* vary greatly, depending on parasite densities and underlying immune response of the host. Great numbers of patients with *Strongyloides stercoralis* infection are asymptomatic or may show intermittent symptoms that mainly affect intestine, lungs and skin.

Systemic symptoms include weight loss and cachexia (Olsen *et al*., 2009). Warm moist temperatures, low socio-economic status as well as poor sanitary habits that lead to soil contamination with faeces, contribute immensely to high prevalence of *Strongyloides stercoralis* infection in tropical regions (Schär *et al*., 2013). The risk factors for *Strongyloides stercoralis* infection are male gender, alcoholism, low socio-economic status, poor sanitary standards and contact with contaminated soil (Chordia *et al*., 2011). Indiscriminate human waste disposal determines the level of *Strongyloides stercoralis* present in an area and promotes the development as well as transmission of geohelminths. Strongyloidiasis mostly occur in children due to their habit of playing outdoors barefooted in contaminated soil (Moon and Oberhelman, 2005) but advanced age is also a risk factor for severe strongyloidiasis, because of its association with immunosuppression. Immunocompromised individuals tend to develop hyperinfection syndrome and disseminate strongyloidiasis which are fatal if unrecognized (Marcos *et al*., 2011). The risk of *Strongyloides stercoralis* infection is increased by occupations that involve contact with faecal contaminated soil, which may include farming (Sanchez *et al*., 2001) and coal mining (Keiser and Nutman, 2004) depending on local practices.

Information on prevalence, geographical variation of the infection and risk factors associated with them are fragmentary. Umar and Bassey (2011) documented on the incidence of *Strongyloides stercoralis* infection in four local government areas of Kano State Nigeria. Dada- Adegbola *et al*., (2010) reported on prevalence of strongyloidiasis among diarrhea patients in Ibadan Nigeria. In Rivers State a fast growing state in Niger Delta, information on *Strongyloides stercoralis* remained scanty and it became imperative to evaluate and assess the prevalence of strongyloidiasis infection and associated risk factors among school children which will serve as a baseline information for further investigation of the infection in Ahoada-East Local Government Area, Rivers State, Nigeria.

MATERIAL AND METHODS

Study area

Ahoada-East Local Government Area, Rivers state, South-South of Nigeria, lies between latitude 5^{o} and $5^{o}30^{1}$ North of equator to longitude 6^{o} 65^{1} East of Greenwich. It has a mean temperature of about 30°C, a relative humidity of between 80% and 100% as well as a mean annual rainfall of about 2,300m (Mmom, 2003) (Fig 1.1).

Sampling frame

This school-based survey was conducted from April to July 2015 among the school children in Ahoada-East Local Government Area of Rivers State. Nine schools were selected randomly from the list of all primary schools in the study area (Ahoada-East Local Government Education Authority). Three primary schools were selected from each of the clans in the study area (Upata, Akoh and Igbuahoada). Pupils between the ages of 8-16 years were selected for participation. They were randomly selected by drawing lots mostly from basic 3-6 in all the schools.

Data collection

Stool samples of three hundred and seventy (370) school children of different ages and sexes were collected from the population sampled and examined for the presence of *Strongyloides stercoralis* larvae. Every morning, after collecting the filled bottle and answered questionnaire, another pre-labeled empty container was given out for the next day. The risk factors associated with this infection was assessed using structured child questionnaire.

Also, one hundred and eighty (180) soil samples were collected from different parts of compound of the nine primary schools within the rainy season. About 20g of the top soil

(down to a depth of not more than 2cm) from the playground, front and behind classrooms and toilet areas were scooped into clean polythene bags using clean spoon and taken to the Laboratory for analysis (Chukwuma *et al*., 2009).

Parasitological examination

Direct smear technique was first used to analyze the stool samples. The Baermann technique was also performed on the stool samples. Stool sample of walnut size was placed on nylon mesh screen and two layers of laboratory tissue in a plastic funnel that was filled with warm water (43°C) and connected to clamp tubing. After few hours of incubation, the clamp was released and the water was collected into a beaker. It was distributed into centrifuge tubes and centrifuged at 1500rpm for two minutes. The supernatant was decanted and the sediment was left at the bottom of the tube. The sediment was mixed with last drop of fluid inside the tube by tapping the tube with finger. It was placed on glass slide, covered with cover slip and microscopically examined with x10 and x40 objectives for the presence of *Strongyloides stercoralis* larvae. The larvae were identified morphologically using the following parameters: short buccal cavity, prominent genital primordium (L1) and presence of forked tail (L3) (Cheesbrough, 2005).

For examination of soil samples, five (5) grams of each soil sample was placed in a test tube that contains formal saline. It was homogenized for one minute and the suspension was then strained through Whatman filter paper (pore size 2.5 µm) placed in a funnel to remove sand particles. Ether (3ml) was added to the filtrate (7ml) in a centrifuge tube and the mixture was shaken vigorously. It was centrifuged at 2,000 rpm for two minutes. The supernatant was decanted and the sediment was mixed with last drop of fluid inside the tube by tapping the tube with finger.

The sediment was placed on a clean glass slide, covered with a cover slip and microscopically examined .The larvae were identified with the aid of Atlas of Parasitology (Cheesbrough, 2005).

Data Analysis

SPSS version 21.0 software employing Chi-square analysis. The independent effect of the variables was determined using logistic regression analysis, by calculating the strength of association between risk factors and infection using odds ratio (OR) and 95% confidence interval (CI). P-value < 0.05 was considered statistically significant.

Ethical Consideration

The study was approved by University of Port Harcourt Research Ethics Committee. All relevant authorities (Rivers State Universal Basic Education Board, headmasters and school teachers) were notified of the purpose and procedures of the study. Informed written permit was obtained from Ahoada-East Local Government Education Authority, prior to commencement of study. Oral consent was obtained from head masters and class teachers.

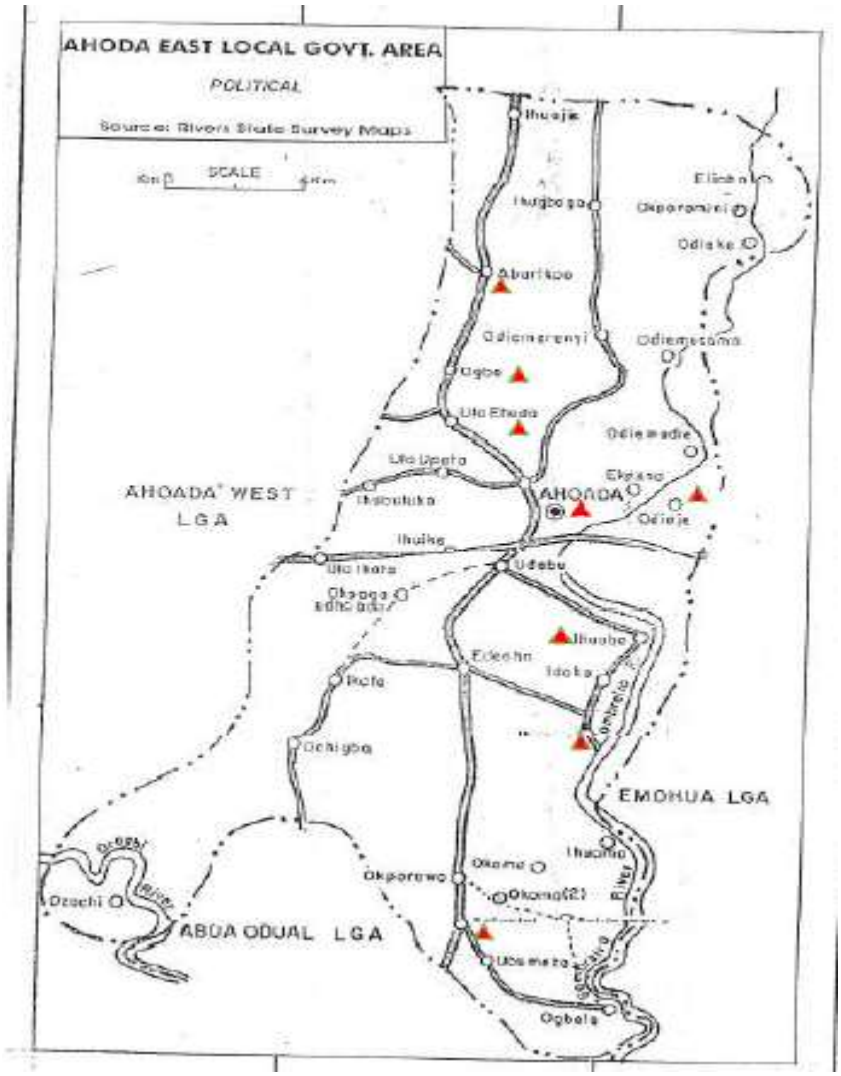

Fig 1: Sampled Communities in Ahoada-East Local Government Area, Rivers State.

RESULTS

Of the three hundred and seventy (370) stool samples examined, thirteen (3.5%) were positive for *Strongyloides stercoralis* infection. Eight out of one hundred and seventy three (173) males examined were infected, giving a prevalence rate of 4.6% while five (2.5%) of the one hundred and ninety seven (197) females were infected (Table1). Though there was difference in prevalence between the sexes, it was not statistically significant (X^2 = 1.183, p >

0.05). Regarding age groups, nine (4.4%) of two hundred and two (202) pupils between 11-13years were positive for this infection. Four (2.6%) out of one hundred and fifty five pupils between 8-10years were infected while none of the pupils between 14-16years was infected. Highest prevalence of infection was found within the age group 11-13years (Table 1).

Results reported in Table 2 showed that Ahoada central had the least prevalence rate of 2.4% infection, Akoh clan had 3.7% infection rate, while a higher prevalence of 5.2% was observed among the Upata clan. There was difference in the distribution of this infection among the three clans but the difference was not statistically significant ($X2 = 2.328$, $p = 0.312$). Sex-specific rates showed that male pupils had higher infection status across the communities. The difference between sexes was not statistically significant ($p > 0.05$).

Based on soil sample analysis, seventeen (9.4%) out of one hundred and eighty soil samples collected from the nine primary schools were found to be positive for S*trongyloides stercoralis*.

The distribution of *Strongyloides stercoralis* in soil according to clans showed that Ahoada central had a prevalence of four (6.7%), Akoh Clan had six (10%) and Upata Clan had seven (11.6%) (Figure 1). Upata clan had the highest prevalence of *strongyloides stercoralis* larvae in soil while Ahoada central had the least prevalence. In terms of distribution of *Strongyloides stercoralis* in soil with respect to the sample sites (different parts of school compound), back of classrooms had six positive samples (15.5%), toilet area had seven (13.3%), playground had three (6.7%) and front of classrooms had one (2.2%) (Figure 2).

In assessing the risk factors associated with *Strongyloides stercoralis* infection observed across the communities, Table 3 revealed that age group, gender, place of residence, family size and mother's occupation were not statistically different between infected and non-infected pupils ($P > 0.05$). Three key factors identified with this infection were defecation habit, non-use of footwear and lack of latrine at home. These three key factors were significantly associated with this infection. Poor sanitary habit was the potential risk factor for this infection. Pupils who defecate at open field were 20.9 times more likely to contract *Strongyloides stercoralis* infection than those that use water cistern (OR: 20.9, 95% CI: 3.24 – 134.84, $P = 0.001$ and OR: 11.358 for dug hole versus water cistern, 95% CI: 2.137 – 60.377, $P = 0.004$). Pupils that defecate in dug holes were 11.358 times more likely to be infected by this parasite than those who use water cistern (Table 4). Similarly, study subjects

who do not put on footwear at all were 18.58 times at the risk of having *Strongyloides stercoralis* infection than those who wear shoe always (OR: 18.58, CI: 2.119 – 163.052, P = 0.008). Pupils who sometimes put on footwear at home were more likely to be infected than those who wear always (OR: 8.55, CI: 1.039- 10.326, P = 0.046).

Lack of latrine at home was also identified as a risk factor for *Strongyloides stercoralis* infection. Pupils who had no latrine at home were more likely exposed to this infection than those that had latrine (OR: 7.764, CI: 2.456 – 24.541, P = 0.005) (Table 4).

Table 1: Prevalence of *Strongyloides stercoralis* infection among school children in Ahoada-East Local Government Area, Rivers State, Nigeria.

Age group	No. of females examined	No. of females positive (%)	No. of males examined	No. of males positive (%)	Total number examined	Total number positive (%)
8-10yrs	84	2 (2.4)	71	2 (2.8)	155	4 (2.6)
11-13yrs	108	3 (2.7)	94	6 (6.3)	202	9 (4.4)
14-16yrs	5	0	8	0	13	0
Total	197	5 (2.5)	173	8 (4.6)	370	13 (3.5)

Table 2: Distribution of *Strongyloides stercoralis* infection in three clans of Ahoada-East Local Government Area, Rivers State, Nigeria.

Clan	No. of males examined	No. of males positive (%)	No. of females examined	No. of females positive (%)	Total number examined	Total number positive (%)
Ahoada central	75 2	(2.6)	91	2 (2.2)	166	4 (2.4)
Akoh	52 3	(5.7)	56	1 (1.7)	108	4 (3.7)
Upata	46 3	(6.5)	50	2 (4)	96	5 (5.2)
Total	173	8 (4.6)	197	5 (2.5)	370	13 (3.5)

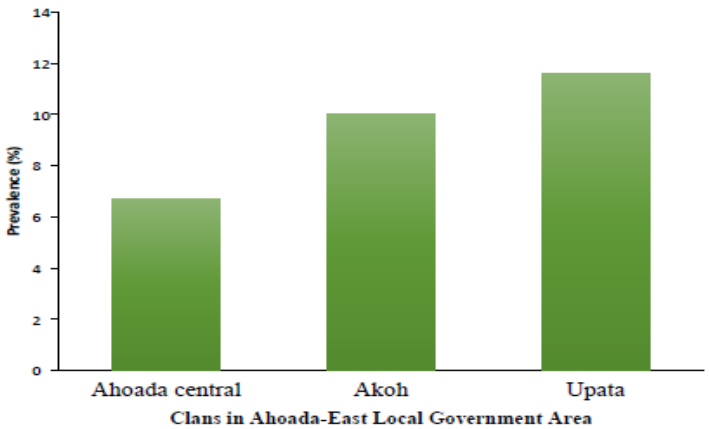

Figure 4.1: Distribution of *Strongyloides stercoralis* as observed from soil samples in three clans of Ahoada-East Local Government Area, Rivers State.

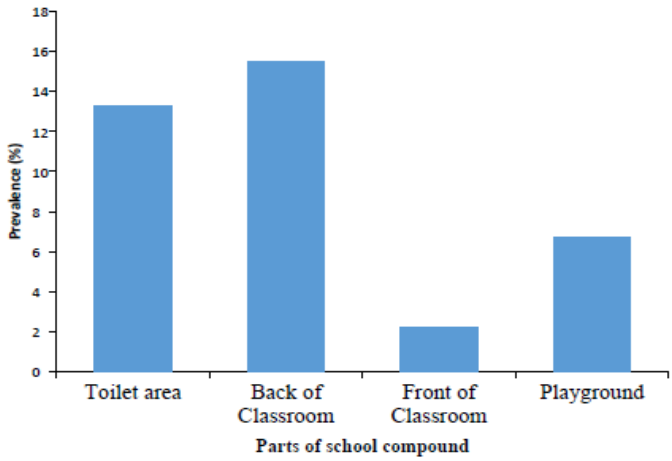

Figure 2: Prevalence of *Strongyloides stercoralis* in soil samples with respect to sample sites (parts of school compound).

Table 3: Association of Demographic and Socio-economic Variables with *Strongyloides stercoralis* Infection.

Variables	Infection		X^2	P-value
	Positive (%)	Negative (%)		
Gender				
Male	8 (4.6)	165 (95.4)	1.183	0.277
F Female	5 (2.5)	192 (97.5)		
Age group				
8 – 10yrs	4 (2.6)	151 (97.4)	1.40	0.497
11 – 13yrs	9 (4.4)	193 (95.6)		
14 – 16yrs	Nil (0)	13 (100)		
Defecation habit				
Open field	3 (17)	15 (83)	19.244	0.000
Dug hole	5 (10)	46 (90)		
Latrine	3 (3.3)	87 (96.7)		
Water cistern	2 (0.9)	209 (99.1)		
Use of footwear				
Always	1 (0.6)	171 (99.4)	10.991	0.004
Sometimes	7 (4.8)	140 (95.2)		
Not at all	5 (9.8)	46 (90.2)		
Latrine availability				
No	8 (11.6)	61 (88.4)	16.337	0.000
Yes	5 (1.7)	296 (98.3)		
Place of residence				
Semi-urban	3 (2)	152 (98)	1.959	0.162
Rural	10 (4.6)	205 (95.4)		
Family size				
1-3 persons	2 (2.7)	70 (97.3)	0.232	0.890
4-6 persons	4 (3.3)	117 (96.7)		
>6 persons	7 (4)	170 (96)		

Table 4: Strength of Association between Selected Risk Factors and *Strongyloides stercoralis* Infection.

Risk factor	Infection		OR	95% CI	P-value
	Negative	Positive			
Defecation habit					
Open field	153		20.9	3.240 – 134.84	0.001
Dug hole	46	5	11.358	2.137 – 60.377	0.004
Latrine	87	3	3.603	0.592 – 21.944	0.164
Water cistern	209	2	1	-	-
Use of footwear					
Always	171	1	1	-	-
Sometimes	140	7	8.550	1.039 – 70.326	0.046
Not at all	46	5	18.58	2.118 – 163.052	0.008
Latrine availability					
No	8	61	7.764	2.456 – 24.541	0.005
Yes	296	5	1	-	-

Plate 1: Rhabditiform larva of *Strongyloides stercoralis.*

DISCUSSION

Present study records a prevalence rate of 3.5% for *Strongyloides stercoralis* infection among three hundred and seventy school children in nine primary schools of Ahoada-East Local Government Area, Rivers state, Nigeria. This is the first report in Ahoada-East Local Government Area on the importance of *Strongyloides stercoralis* infection using different diagnostic techniques (Direct smear and Baermann technique) on two stool samples collected over two consecutive days. The observed prevalence in this study was low compared to other studies that used similar methods of diagnosis in other parts of the country. In Kano state, Umar and colleague diagnosed *Strongyloides stercoralis* in 37.14% of school children (Umar

and Bassey, 2011). The low prevalence could be connected with recent effort by Rivers state government to reduce infant mortality and improve environmental sanitation through construction of modern classrooms in all communities of the state and schools de-wormingprogramme. However, a prevalence rate of 3% similar to the one observed in this study was recorded in 2010, among diarrhea patients in Ibadan, Nigeria (Dada-Adegbola *et al*., 2010).

In the present study, prevalence of *Strongyloides stercoralis* infection was higher in males than in females. Males had higher rate of infection than females in all the age groups examined. This coincides with the reports of Umar and Bassey, (2011) and Khieu *et al*., (2014). Yelifari *et al*., (2005) reported a prevalence rate of 12.7% in men and 10.6% in women. The high prevalence of this infection in male pupils could be due to their habit of going to school, hunting and fishing without foot wear (Chukwuma *et al*., 2009). Pupils within 11 – 13years age group had the highest rate of infection (69.2%) among the age groups examined. This observation is similar to the one made by Umar and Bassey, (2011) in their study. Barefooted movement is commonly seen among males, mostly those within the age group 11 – 13years which were found to have high rate of this infection.

The study revealed that Upata clan had the highest occurrence of this parasite in both stool (5.2%) and soil (11.6%). Primary schools sampled at Upata clan did not have toilet facilities. The pupils normally defecate in the nearby bushes surrounding the school. This resulted in larvae being washed into the school compound when it rained, resulting in the school compound and surrounding area being highly contaminated with larvae of *Strongyloides stercoralis*. This results in poor sanitary and hygienic conditions of the clan. Moreover, most of the school children in Upata clan go to school barefooted leading to exposure of the children to *Strongyloides stercoralis* larvae in this area. Ahoada central which had the least occurrence of this parasite in stool (2.4%) and in soil (6.7%) was more of semi-urban than rural settlement. Environmental sanitation and personal hygiene tend to be higher there. Most of the pupils in Ahoada central claimed to use water cistern for defecation. In all the clans of the study area, back of classrooms (15.5%) and back of toilets (13.3%) were found to harbour highest number of *Strongyloides stercoralis* from soil analysis. This coincided with observation of Chukwuma *et al*., (2009). This is most likely due to the hidden nature of back of classrooms and toilets areas, as pupils prefer to defecate in easy and fast way.

Three significant risk factors were recorded including poor sanitary habit, non-use of footwear and lack of latrine at home. This findings also agree with that of Dada-Adegbola *et al*., (2010) and Umar and Bassey (2011). The presence of eggs of helminth and larvae of *Strongyloides stercoralis* in soil is indicative of faecal pollution. *Strongyloides stercoralis* is mostly transmitted through penetration of exposed skin by the infective (filariform) larvae found in soil contaminated with faeces. Improvement in basic personal hygiene such as wearing of shoes when having contact with soil and defecating in a toilet could interrupt the transmission of *Strongyloides stercoralis* infection.

This study examined two stool samples per pupil. The prevalence rate reported may be underestimated, as shedding of *Strongyloides stercoralis* larvae in faecal samples considerably varies on daily basis (Knopp *et al*., 2008; Schär *et al*., 2014). A recent study which observed the excretion of *Strongyloides* larvae in stool samples over seven consecutive days has shown that it ranged from 0.003 larvae to 151.2 larvae per gram (Schär *et al*., 2014). Nevertheless, no single method of diagnosis (Baermann technique or Koga Agar Plate culture) can detect all *Strongyloides stercoralis* infection even with multiple stool samples examination (Khieu *et al*., 2013). The combined use of diagnostic methods on several stool specimens was encouraged to increase sensitivity since a true "gold standard" diagnostic method is not available.

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