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Cyclospora cayetanensis and Intestinal Parasitic Profile in Stool Samples in Lagos, Nigeria

George E. ALAKPA and Adetayo F. FAGBENRO-BEYIOKU

Tropical Disease Unit, Department of Medical Microbiology and Parasitology, College of Medicine, University of Lagos, Lagos, Nigeria

Summary. Diarrhoea remains a leading cause of morbidity and mortality in the developing world and accounting for over 50 million deaths globally. The involvement of *Cyclospora cayetanensis* in diarrhoea cases globally has been documented, though with little from the Africa continent. This study was thus carried out to investigate the possible existence of this emerging pathogen and the parasitic profile in stool samples in the city of Lagos, Nigeria. A total of 1109 stool samples (216 of which came from diarrhoeal patients) were screened microscopically for intestinal parasites. Parasites were detected in just 16% (177). *Cyclospora cayetanensis* was detected from 11 stool samples: 10 diarrhoeal stools and one non-diarrhoeric stool. All stool samples positive for this emerging pathogen, were from patients aged 22 to 45½ years, seven of which were seropositive for HIV ($p > 0.05$). Result thus revealed for the first time in this state and country, the existence of *Cyclospora cayetanensis* and its possible involvement in diarrhoea cases. It also identified *Cryptosporidium parvum* as the most prevalent intestinal parasite in the state, a strong departure from previous reports. There is the need however for a more detailed study, in order to determine the exact extent of *C. cayetanensis* involvement in diarrhoea cases and the effect of seasons and cultural feeding practices on its epidemiology for effective management of diarrhoeal cases.

Key words: *Cyclospora cayetanensis*, diarrhoea, intestinal parasite, Lagos, Nigeria, stool.

INTRODUCTION

Diarrhoea remains one of the most important health problems globally and a leading cause of morbidity and mortality in children, especially in developing countries (WHO 1998). It accounts for over 50 million deaths (in all ages) worldwide and is ranked 3rd among diseases responsible for human mortality globally (WHO 1998). This disease condition remains a major problem in the developing countries due mainly to poverty, characterized by the absence of potable drinking water, proper sanitary habits, absence of good faecal disposal system, poor hygienic practices by the impoverished citizens and over crowding (WHO 1998).

Agents associated with diarrhoea cut across the major types of microorganisms: bacteria, viruses and protozoa. Bacterial agents mostly associated with diarrhoea are species of *Salmonella, Shigella, Campylobacter, Escherichia coli, Aeromonas, Plesiomonas* etc., while viral agents like Norwalk, and adenoviruses have been predominately associated with

Address for correspondence: George E. Alakpa, 43 F Bellows Lane, Towaco, NJ 07082, USA; Fax: 1-509-695-8377 or 1-718-518-0338; E-mail: edafealakpa66@hotmail.com

diarrhoea in infants and children (Alabi *et al*. 1998). The association of protozoa pathogens with diarrhoea has been on the increase (with the discovery of the *Cyclospora* in the mid and late 1990s), following the advent of the human immunodeficiency virus (HIV) infection and the acquired immunodeficiency syndrome (AIDS) pandemic. Traditional diarrhoeal protozoa agents are *Giardia lamblia*, *Entamoeba histolytica* and *Balantidium coli* and lately, coccidia agents like *Isospora belli*, *Cryptosporidium parvum*, microsporidia and the newest one; *Cyclospora cayetanensis*. (Clarke and McIntyre 1996a, Marshall *et al*. 1997, Terra *et al*. 1998, Allison *et al*. 1999, Fryauff *et al*. 1999, Wilson 1999).

The involvement of protozoan agents in humans can be traced back to the 19th century and these protozoa group constitute the highest group of parasites known to be associated with diarrhoea condition. Originally, the coccidia parasites are known to be pathogenic mainly to some species among the lower animals, insects, birds and non-human primates (Beneson 1995, Marshall *et al*. 1997, Fryauff *et al*. 1999).

Today, a newer coccidian *C. cayetanensis* has now been added to the list of protozoa agents that can induce diarrhoea. Prior to 1979, when the first human case of cyclosporiasis was reported (Ashford 1979), only four genera of the coccidia were known to infect humans and these are *Cryptosporidium, Isospora, Toxoplasma* and *Sarcocystis* (Clarke and McIntyre 1996b). *C. cayetanensis* is capable of causing prolonged gastrointestinal disorder (GIT). This is characterized by persistent and intermittent watery diarrhoea accompanied by weight loss and other symptoms in both immunocompetent and immunocompromised individuals irrespective of sex and age globally (Logar *et al*. 1997, Lepes 1998, Nassef *et al*. 1998, Terra *et al*. 1998, Fryauff *et al*. 1999, Mead *et al*. 1999). Prior to 1994 when its current name was given by Ortega *et al*. (1994), this pathogen was known by different names; *Cyanobacterium* (blue-green algae)-like bodies; *Cryptosporidium*-like bodies, coccidian-like bodies (CLBs) or even a species of *Isospora* or *Eimeria* genera (Ashford 1979; Long *et al*. 1990, 1991; CDCP 1991a,b; McIntyre and Lyons 1992, Ortega *et al*. 1993).

Route for its transmission is still not very clear, but the oral-faecal route remains the most implicated, while its association with GIT infection or diarrhoea, remains proven with the continuous increase in the number of reported cases and outbreaks of cyclosporiasis globally (Jelinek *et al*. 1997, Marshall *et al*. 1997, Steiner *et al*. 1997, CDCP 1998, Ortega *et al*. 1998, Sterling and Ortega 1999).

In the developed countries like the USA and the UK, where a lot of documentation exists, there is a fair idea of the profile of parasitic pathogens. In USA, it is now a common practice for stool samples submitted to the laboratory, to be first screened for the presence of the commonest pathogenic parasites and when these are absent, the screening for newer agents like *C. cayetanensis* is done (Garcia-Lopez *et al.* 2000).

Three countries have so far been documented to report cases of *C. cayetanensis* infection from the continent of Africa. These are Morocco, South Africa and Egypt (Markus and Frean 1993, Nassef *et al*. 1998, Ortega *et al*. 1998). I am unaware of any reported case in Nigeria; neither has there been any attempt to investigate the possible presence of this emerging diarrhoeal pathogen in Lagos State. Reason can be attributed to the fact, as revealed from an earlier study (Alakpa *et al*. 1999), that physicians and health workers in the state and country were completely unaware of the existence of this pathogen. Also, in Nigeria, numerous studies into the prevalence of parasitic organisms have been conducted in recent years; however, there exists little or no published study specifically aimed at determining the parasitic profile of stool samples submitted to some laboratories in Lagos.

This study was thus carried out to determine the parasitic profile of stool samples in Lagos, with the main focus of investigating for the existence of "newer" parasitic agent(s) especially *C. cayetanensis* and its involvement in diarrhoeal cases.

MATERIALS AND METHODS

This study was conducted in Lagos metropolis, the most commercialized and cosmopolitan city in Nigeria and situated in the southern part of the country with over 5million individuals. The Yaba Central Laboratory; Nigerian Army Base Hospital, Yaba; General Hospital, Lagos; The Lagos University Teaching Hospital: Pediatric Unit and the Microbiology laboratory; Jimsan hospital, Ijora-Badiya and two private laboratories, constitute centers for sample collection.

This was a cross sectional laboratory based study commenced in March 1998 till April 2000 with the collection of all stool specimens submitted to the Microbiology and Parasitology department of the Health Institutions/laboratories mentioned above. Of the 1109 stool samples collected, only 216 (19.5%) were from confirmed diarrhoea cases.

Stool samples were collected from 55 normal healthy individuals with no prior illness (gastrointestinal/diarrhoeal), 3 weeks to the time the stool samples were collected, to serve as negative control, while

positive *Cyclospora cayetanensis* organism sent by Dr. Stuart Clarke of the Scottish Reference Laboratory, Glasgow, UK, served as positive control.

Collection of samples

All stool samples submitted to the Microbiology and Parasitology departments of the above mentioned health institutions within the period of the study were collected in waterproof screwed capped plastic containers and transferred to the laboratory for processing. Bio-data of the patients, whose stools were collected, were obtained from sample request forms and some by means of a questionnaire. Data like the age, sex and reason for visiting the hospital/stool being examined were obtained.

Stool processing

Stool samples were processed immediately for parasitology and bacteriology. Parasitology: Samples were first concentrated employing the formal-saline sedimentation method. Smears were stained by the modified Kinyoun carbol-fuchsin staining procedure (Visvesvara *et al*. 1997) and examined at x 400 magnification using a Nikon light microscope equipped with an eye-piece micrometer calibrated using a stage micrometer, to determine the size of the oocysts.

Loop size of stool samples were made into suspension with saline, a portion smeared for Gram staining, and other seeded onto 3 bacteriological agar media: MacConkey agar; Deoxycholate Citrate agar (DCA) and Thiosulphate Citrate Bile Salt agar (TCBS), so as to determine the presence of any pathogenic bacteria agent. Inoculated plates were incubated at 37°C for 24 h. Colonies morphology determined, followed by Gram staining and sugar fermentation tests. Culture of pure isolated on Kliglier Iron agar was also done.

Identification of *C. cayetanensis* oocysts was based on the size of the oocysts (8-10µm in diameter); the shape being oval/round with well-defined wall, with consistent staining colour variably from pink /red to colourless and comparing it with the positive control. Confirmation of positive stool samples was done at the Scottish Reference Laboratory in Glasgow, UK. Stools positive for *Cyclospora* oocysts, were crosschecked with results from bacteriology initial examinations.

Results were analyzed with the Epi-Info 6.4 version and "STAT" software. The chi-square and student-t tests used to determine the statistical significance.

RESULTS

General intestinal profile

From the 1109 total stool samples analyzed, parasite ova/cysts and oocysts were detected in 177 (16.0%). Frequency distribution showed that 111 (62.7%) of the 177 had protozoa cysts or oocysts, while 66 (37.3%) had helminthic parasites. *Cryptosporidium parvum* was the most detected pathogenic parasite (Table 1).

In terms of general prevalence, of the 1109 stools screened *C. parvum* was the most prevalent pathogenic intestinal parasite in Lagos (3.1%). This was closely followed by *Ascaris* sp. (2.6%), *Giardia* sp. (2.5%), *C. cayetanensis* (0.9%), *E. histolytica* (0.3%) and Hookworm (0.1%). Non-pathogenic *E. coli* had a prevalence of (3.1%) and *T. trichiura* (2.9%) (Table 2).

Few parasitic agents were detected in children less than 6 years, however among the age group 6-10years, the only pathogenic agent found was G. lamblia and non-pathogenic *E. coli*. In the age group 11-20 years, *A. lumbricoides*, *E. coli* and *T. trichiura* were detected. Majority of those aged 21 and above had *Cryptosporidium* oocysts in their stools. There was however no significant association between age and the detection of parasitic agents ($p > 0.05$). Females were found to have more parasitic organisms in their stools than the males; this was not statistically significant ($p>0.05$). A close observation revealed that most of the parasites were from diarrhoea patients with the exception of *A. lumbricoides*. Results also showed that 41 of the 177 (23.2%) had multiple infections, indicating the possibility that mono-infection could be predominating in our environment. There was a significant association between nature of infection with age group and sex,

Table 1. Prevalence of intestinal parasites, based on diarrhoea status. AS - *Ascaris lumbricoides*, CRYP - *Cryptosporidium* sp., CYC - *Cyclospora cayetanensis*, EC - *Entamoeba coli*, EH - *Entamoeba histolytica*, GL - *Giardia lamblia*, HK - Hookworm, ST - *Strongyloides stercoralis*, TT - *Trichuris trichiura*. $P < 0.05$

	Organisms								
Diarrhoeal status:	AS	CRYP	CYC	EC	EH	GL	HK	ST	TT
Positive (n=100)	7	29	10	22	2	19	0	1	9
%	7.0	29.0	10.0	22.0	2.0	19.0	0.0	1.0	9.0
Negative (n=77)	22	5	1	12	2	9	1	3	23
%	28.6	6.5	1.3	15.6	2.6	11.7	1.3	3.9	29.9

Table 2. Frequency distribution of parasitic agents detected in stool samples in Lagos

Organisms	No of stools (%)	Prevalence (%) (n=1109)
Cryptosporidium parvum	34 (19.2)	3.1
Entamoeba coli	34 (19.2)	3.1
Trichuris trichiura	32 (18.1)	2.9
Ascaris lumbricoides	29 (16.4)	2.6
Giardia lamblia	28 (15.8)	2.5
Cyclospora cayetanensis	11 (6.2)	0.9
Entamoeba histolytica	4 (2.3)	0.3
Strongyloides stercoralis	4 (2.3)	0.3
Hookworm	1 (0.6)	0.1
Total	177 (100.0)	

($p < 0.05$), but not with diarrhoeal status ($p > 0.05$) (Tables 3, 4).

Based on diarrhoeal status*; Cryptosporidium* sp. was also the most prevalent pathogenic organism in diarrhoea patients in Lagos, followed by *Giardia* sp. In comparison, the prevalence of *Cryptosporidium, Giardia and Cyclospora* in diarrhoeal cases were 29.0%, 19.0%, and 10.0% respectively, while in the non-diarrhoeal cases, it was 6.5%, 11.7% and 1.3% ($p < 0.05$) (Table 1).

Diarrhoea stools and *C. cayetanensis*

Results form this cross-sectional study revealed that of the 216 watery-diarrhoeal-stools in the study, only 10 (4.62%) were positive. Majority (73.2%) of the diarrhoeic stool samples came from individuals aged between 21 to 50 years and 20.4% from those age 0-20years. One hundred and thirteen (52.3%) of the stools were from men and the remaining 44.4% (96) were from females. All stools were watery in nature. Eighty-three (38.4%) were from patients whose stools were submitted for investigation following GIT complaints, 39.4% (85) from those that came for HIV test and the remaining 45 (20.8%) from routinely collected stool samples (Table 5).

Patients with stool positive for *Cyclospora*

All stool samples positive (eleven in total) for *Cyclospora* were from adults (age 22 to 45½years), however, 10 of these numbers were from diarrhoeic stools. Of these 10 three were males and the rest were females. All had histories of greater than 4 weeks of watery diarrhoea. Two of the 10 cases positive for *Cyclospora* were co-infected with *Cryptosporidium* sp. and both were also HIV positive patients with prolonged diarrhoea.

Generally, 7 of the 10 stools positive for *Cyclospora* oocysts, came from HIV seropositive diarrhoea patients

Table 3. Distribution of cysts/oocysts or ova in stool samples by patients' age, sex and diarrhoeal status. AS - *Ascaris lumbricoides*, CRYP - *Cryptosporidium parvum*, CYC - *Cyclospora cayetanensis*, EC - *Entamoeba coli*, EH - *Entamoeba histolytica*, GL - *Giardia lamblia*, HK - Hookworm, ST - *Strongyloides stercoralis*, TT - *Trichuris trichiura*

Organisms	AS	CRYP	CYC	EC	EH	GL	HK	ST	TT
Age group:									
0-5years	0	2	0	0	1	0	0	0	0
6-10 years	0	0	0	26	0	25	0	0	0
11-20years	18	0	0	21	1	0	0	0	11
21-50years	7	32	11	4	0	3	1	4	21
Not stated	4	0	0	2	2	0	0	0	0
Sex:									
Female	16	12	8	19	2	13	1	1	3
Male	13	20	3	15	1	9	0	3	12
Not stated	0	2	0	0	1	6	0	0	0
Diarrhoeal status:									
Positive	7	29	10	22	2	19	0	1	9
Negative	22	5	1	12	2	9	1	3	23

Table 4. Nature of infection by age, sex and diarrhoeal status

	Single	Multiple	P-value
Age group:			
0-5years	3	0	
6-10 years	1	25	$p < 0.05$
11-20years	18	10	
21-50years	67	5	
Not stated	6	1	
Sex:			
Female	44	24	
Male	48	11	$p < 0.05$
Not stated	3	6	
Diarrhoeal status:			
Positive	48	25	
Negative	47	16	$p > 0.05$

Table 5. Frequency distribution of diarrhoeal patients' stools

	Frequency (No)	%	*Cyclospora* oocysts positive No
Age group:			
Infants (0-5 years)	18	8.3	0
Children (6-10 years)	6	2.8	0
Teenager (11-20 years)	20	9.3	0
Adult (21-50 years)	158	73.2	10
Elderly (>51 years)	5	2.3	0
Not stated	9	4.2	0
Sex:			
Female	96	44.4	7
Male	113	52.3	3
Not stated	7	3.2	0
Cyclospora:			
Detected	10	4.6	
Not detected	206	95.4	
Reasons:			
Routine	45	20.8	0
GIT	83	38.4	3
HIV testing	85	39.4	7
Check-up	0	0	0
Control	0	0	0
Others	3	1.4	0
Total	216	100	10

(Table 6). Though there was a statistically significant association between cyclosporiasis and diarrhoea ($p < 0.05$), there was no significant association between the detection of *Cyclospora* oocysts in stools and age nor sex ($p > 0.05$). The study also revealed a statistical association between diarrhoea and consistency of stool, and reason for visiting the hospital ($p < 0.05$). The prevalence of *Cyclospora* and *Cryptosporidium* were found to be higher in stool from diarrhoeal patients, than in non-diarrhoeal patients (Table 2).

Table 6. Nature of infection by age, sex and diarrhoeal status. N. S. - not stated, HIV - human immunodeficiency virus positive, GIT - gastrointestinal tract disorder, Cryp - *Cryptosporidium* sp.

Patients bio-data					
Age (years)	Sex	Diarrhoeal status	Nature of stool	Reason	Other organism
45.½	M	positive	watery	HIV	N. S.
25	F	positive	watery	HIV	*E. coli*
43	F	positive	watery	HIV	N. S.
23	F	positive	watery	HIV	*Giardia*
N. S.	F	negative	soft	GIT	Ascaris
32	F	positive	watery	GIT	N. S.
41	M	positive	watery	HIV	N. S.
32	F	positive	watery	GIT	*E. coli*
26	F	positive	watery	GIT	N. S.
45	M	positive	watery	HIV	Cryp
22	F	positive	watery	HIV	Cryp

DISCUSSION

The World Health Organization (WHO) reported in 1998 that 33% of global deaths are as a result of infectious and parasitic diseases, while the effect of mortality and morbidity are as a result of some parasitic infections (WHO 1998). The place of intestinal parasites in the over all health of man is also very important.

In terms of pathogenic importance, parasites such as *Cryptosporidium, Cyclospora, E. histolytica* and *Giardia*, have been shown to be responsible for severe diarrhoeal episodes especially in immunosuppressed individuals globally. Depending on the degree of infection especially in children, *Ascaris* and *Trichuris* have been known to cause complications. While *Ascaris* may result in pneumonitis characterized by cough, dyspnoea, fever, eosinophilia, intestinal obstruction, malnutrition and malabsorption following heavy infection. *Trichuris trichiura*, causes inflammatory eosinophilic reactions in the caecum or rectum, which causes chronic diarrhoea and sometimes rectal prolapse (Ihezuoh 1998, Shanson 1999).

The results from this study have shown that *Cryptosporidium* is the most prevalent intestinal pathogenic parasite in Lagos, especially among diarrhoeal cases. While this organism was found mostly in adults, *Giardia* was found mostly among those aged 6-10 years. This result is similar to those reported by Marshall *et al*. (1997), Gilbert *et al*. (1998), and Okono (1999). The steady increase in the prevalence of *Cryptosporidium* in Nigeria may be attributed to increase in awareness about this pathogen among clinicians and laboratory scientists. Another factor could be the pandemic HIV/AIDS disease conditions, which is believed to have otherwise converted asymptomatic parasite-induced infection into life threatening diseases (Oyerinde 1999).

One of the "newer" emerging diarrhoeal pathogen detected in this study was *Cyclospora cayetanensis*, with a general prevalence of 0.9% (Table 2). Aderounmu (1999) reported *E. histolytica* as the most prevalent protozoan pathogen in Lagos, as against *Cryptosporidium*, as observed from this study. This difference may be attributed to the increase in awareness of *Cryptosporidium*, since this study was more extensive and distributed among more laboratories than that of Aderounmu.

In a study conducted in West Java - Indonesia, *C. cayetanensis* was found to be the dominant pathogenic intestinal parasite in that area, with 11.5% (29) of the 253 cases of GIT illness and diarrhoea being positive for the pathogen (Fryauff *et al*. 1999). From another study conducted, this time in Egypt where the stool samples of 130 immunocompetent diarrhoeal patients (80 children and 50 adults) were screened for cayetanensis, 9% (7) of the 80 children and 10% (5) of the adults were found to be positive, thus giving an incidence of 9.2% (11/130) (Nassef *et al*. 1998).

Results of this study show for the first time, the detection of *C. cayetanensis* oocysts in diarrhoeal stool in Lagos State. It has also shown the association between this pathogen and diarrhoea. Several studies have documented the fact that *C. cayetanensis* is a diarrhoea causing agent (Ortega *et al*. 1993, 1994; Clarke and McIntyre 1996a,b; Nassef *et al*. 1998; Fryauff *et al*. 1999). So far only very few countries in Africa have been documented to report case(s) of cyclosporiasis: South Africa (Markus and Frean 1993), Morocco (Ortega *et al*. 1998) and Egypt (Nassef *et al*. 1998). The low rate or report from Africa can well be attributed to lack of awareness, inadequate technology and limited studies. It is my belief, that the results of this study will stimulate interest among medical professionals in this state, country and sub-region in cyclosporiasis. A much more detailed and specific hospital base study will however, be necessary to provide more information about this agent of diarrhoea in this country.

In summary, these results thus provide an important finding with the documentation for the first time, the detection and presence of *C. cayetanensis* in stool samples in the state and country. Also, it showed that *Cryptosporidium parvum* is the most prevalent intestinal parasite in the state. We do believe, this findings will generate some scientific interest among clinicians and scientist, so that the much needed more studies that will focus more on the epidemiology of this emerging pathogen, especially in relation to seasonal affect and its distribution will be investigated. We also therefore recommended that, there should be more intensive education to health practitioners about this emerging diarrhoeal pathogens, since *C. cayetanensis* can be easily mistaken for *Cryptosporidium* sp. by an inexperienced microbiologist as they both share some similar staining characteristics.

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