

CONCURRENT INTESTINAL PARASITOSEs WITH CRYPTOSPORIDIUM SPECIES AMONG PATIENTS WITH GASTROINTESTINAL DISORDER IN LAGOS, NIGERIA

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Abstract

Cryptosporidium, a protozoan parasite that causes gastrointestinal illness, is transmitted by ingestion of oocysts excreted in human or animal stools. Cryptosporidiosis presents with severe diarrhoea in HIV/AIDS patients and self-limiting in immunocompetent individuals. The parasite is yet to receive sufficient public health attention in Nigeria. This study was undertaken to investigate association of *Cryptosporidium* oocysts with other intestinal parasites in patients with gastrointestinal complaints. The collected stool samples were examined immediately with wet mount using saline solution and 5% lugol iodine. The aliquot was centrifuged in formal-ether using concentration technique, then stained with Kinyoun's Carbol-fuchsin. Two hundred and thirty-eight (238) stool samples were collected from patients with diarrhoea and other gastrointestinal symptoms. Of the 238 participants, 20(8.4%) were HIV-seropositive patients. *Cryptosporidium* oocyst was identified using Kinyoun carbol fuschin stain. The overall prevalence of *Cryptosporidium* was (6.3%). Of the 218 HIV-seronegative individuals, 4.6% were positive for *Cryptosporidium* and among the 20 HIV-seropositive patients, 15(75.0%) were positive. Apart from *Cryptosporidium*, 23.9% of the patients were positive for other intestinal parasites such as *Ascaris*, *Fasciola*, *Giardia*, *Trichuris*. *Entamoeba histolytica/dispar* ranked highest with (7.14%) positive, followed by *Ascaris* (6.3%). The highest infection rate (8%) was recorded in the age group 11-20 years and there was a statistical significant difference between infected and uninfected in relation to sex (Chi square =0.731, df=1, P<0.05). The occurrence of *Cryptosporidium* with other intestinal parasites such as *Entamoeba histolytica/dispar* and *Giardia* underscores the need for the inclusion of *Cryptosporidium* investigations when examining patients stool.

Keywords: *Ascaris*, *Cryptosporidium*, *Entamoeba*, *Giardia*, Intestinal parasitoses.

INTRODUCTION

Cryptosporidiosis is currently a public health concern world wide because it has been detected in different hosts, including

man, other mammals, birds, reptiles, amphibians, fish and water (Fayer and Ungar, 1986; Current and Garcia, 1991; Ferreira, 2000; Franco and Neto, 2002;

Karasawa *et al.*, 2002). Though, *Cryptosporidium* is highly reported in chronic and severe diarrhoea in immunodeficient individuals, especially those with HIV/AIDS, several studies have also reported the occurrence of diarrhoea caused by intestinal parasites in immunocompetent individuals (Crawford and Vermund, 1988; Dietz *et al.*, 2002). Prevalence studies showed that infections are higher in developing countries (Janoff and Reller, 1987; Tumwine *et al.*, 2003) and in children especially those less than 2 years old, than in adults (Fayer Ungar, 1986; Adegbola *et al.*, 1994). Data on the prevalence and incidence of *Cryptosporidium* and other intestinal parasites, in most rural and urban settings have been previously reported (Banwat *et al.*, 2004; Akujobi and Ogunsola, 2006; Molloy *et al.*, 2010, Maikai *et al.*, 2012). However, there are no current data on *Cryptosporidium*-associated diarrhoea in Nigeria. We therefore report the concurrent infection of *Cryptosporidium sp* with other intestinal parasites, among patients of different age groups from various clinics in Lagos, Nigeria.

MATERIALS AND METHODS

Study Population

The study was conducted among patients diagnosed with diarrhoea and other gastrointestinal symptoms from six (6) hospitals in Lagos, Nigeria. A total of 238 patients (1-80 years old) with diarrhoea and other gastrointestinal symptoms were parasitologically examined (128 males and 110 females). 20 of the patients had been confirmed as HIV sero-positive. The study population cuts across various age groups and sex. As one of the hospital sites is involved in the management of HIV/

AIDS patients, pre and post - counseling was done for the participants and voluntary consent was given for their stool samples to be examined. The HIV-seropositive individuals were immediately referred for proper management of their condition.

Parasitological Examination of Stools

A total of 238 stool samples obtained from the patients were examined for the presence of *Cryptosporidium* and other intestinal parasites. The consistency and presence of blood, or mucus were noted for each stool specimen. Fresh stool samples were collected and first examined immediately by light microscopy of direct saline and 5% lugol iodine preparations. Thereafter, microscopy was repeated after formol-ether concentration of approximately 2g of stool sample. *Cryptosporidium* oocysts were identified using a standard technique described by Garcia and Bruckner (1997). Briefly, smear made after centrifugation from the sediment were allowed to air dry and fixed in absolute methanol for 1 minute after drying and then stained with Kinyoun's Carbol-Fuchsin for 5 minutes. The slides were rinsed in tap water for 5 seconds, decolourized with 5% acid alcohol for 2 minutes, rinsed again in water, counterstained with methylene blue for 1 minute, rinsed with water and allowed to dry for 1 minute. The prepared slide was then examined with oil immersion objective. Bacterial and viral pathogens were not screened in this study. The nuclei of *Cryptosporidium* examined stained pinkish red, spherical and measured between 4-6 μm .

RESULTS

From the two hundred and thirty-eight (238) stool samples collected, twenty (20) patients examined had been confirmed as HIV-seropositive. One hundred and twenty-eight

128 (53.8%) of the sampled population were males (Mean age, 32years) while 110 (46.2%) were females (Mean age, 25 years). Soft-formed stool was 173(72.7%); hard-formed stool 8(3.4%), loose stools, 30(12.6%) and watery stools, 27(11.3%). Of the 128 males, 35(27.3%) and 24(21.8%) of the 110 females were also positive for other parasitic infection. *Ascaris* had the highest prevalence of intestinal parasites among the different age groups, especially amongst the age group 11-20years (Table1). *Cryptosporidium* was detected more in HIV-seropositive females 9(60.0%) than in males 6(40.0%), and in the age group 31-40years 6(40.0%) and this was statistically significant ($P<0.05$) though the HIV-seropositive patients sample size were small. The lowest rate (13.3%) was recorded in the age group (41-50) years. There was more concurrent association of

Cryptosporidium with *Entamoeba histolytica/dispar* and *Giardia* infection. The overall prevalence rate of *Cryptosporidium* was 6.3%. Within the age groups 1-10 and 11-20 years, 2(11.1%) and 6(33.3%) were positive for *E. histolytica/dispar* respectively while, in age group 1-10 years, one (1) was positive for Hookworm ova. *Entamoeba histolytica/dispar* ranked highest among the various parasitic pathogens, closely followed by *Cryptosporidium species* and *Ascaris lumbricoides*. Mixed infection was common between *E. histolytica/dispar* and *Giardia lamblia*, 9(0.44%) and this was more prevalent in males than in females. Apart from *Cryptosporidium*, 57(23.9%) of the 238 stools were positive for other intestinal parasites.

TABLE 1: PREVALENCE OF INTESTINAL PARASITES IN DIFFERENT AGE GROUPS OF PATIENTS

Age (Years)	<i>E.histolytica /dispar</i> No. (%)	Hookworm No. (%)	<i>Giardia lamblia</i> No. (%)	<i>Ascaris</i> No. (%)	<i>E.coli</i> No. (%)	<i>Trichuris trichuiria</i> No. (%)	<i>Fasciola hepatica</i> No. (%)	<i>Cryptosporidium</i> spNo.(%)	TOTAL
1-10	2(11.1)	1(50.0)	2(33.3)	2(13.3)	1(11.1)	2(25.0)	—	—	10
11-20	6(33.3)	—	1(16.7)	7(46.6)	1(11.1)	—	1(50.0)	4(26.7)	20
21-30	4(22.2)	—	—	3(20.0)	2(22.2)	—	1(50.0)	3(20.0)	13
31-40	1(5.5)	—	1(16.7)	1(6.7)	—	3(50.0)	—	6(40.0)	12
41-50	3(16.7)	1(50.0)	1(16.7)	2(13.3)	3(33.3)	1(12.5)	—	2(13.3)	13
51-60	1(5.5)	—	—	—	1(11.1)	—	—	—	2
61.70	1(5.5)	—	1(16.7)	—	—	—	—	—	2
TOTAL	18	2	6	15	8	6	2	15	72

DISCUSSION

Human parasitic diseases are caused by numerous widely disparate infectious organisms. Many require transmission by complex vectors and result in chronic infection that often cause severe morbidity in those infected. *Cryptosporidiosis* and other intestinal parasitic infections are more likely to be prevalent in most developing countries of the world, especially in subtropical and tropical countries as a result of poor hygiene and sanitation (Tumwine *et al.*, 2005).

In this study, *Cryptosporidium* oocysts and other intestinal parasites were observed more in males than females. This was diagnosed in various age groups and the highest prevalence of 40.0% was recorded in age group 31-40 years, in our view, it may be due to nature of their work or as a result of poor personal hygiene and possibly consumption of contaminated water and food, while the least percent positive 13.3% was within 41-50 years, who were confirmed HIV-seropositive patients. This result is consistent with those of other workers (Bhatticharge *et al.*, 1997; Agbakwuru *et al.*, 1998; Menon *et al.*, 1999; Banwat *et al.*, 2004), where they all reported various prevalence level of gastrointestinal infection from patients in countries from Asia and Africa.

However, Adegbola *et al.*, (1994) reported a high prevalence of *Cryptosporidium* oocysts in children less than 5 years of age in Gambia. They attributed this to closeness of the people to domesticated animals such as chickens, goats and sheep. In this study such epidemiological information were not collected because it was not part of the study design. Moreover, Banwat *et al.*, (2004) reported prevalence of 0% in HIV/AIDS

patients and 3.8% in HIV-seronegative children using the Ziehl-Neelsen stain in Jos, Nigeria. Interestingly, Tumwine *et al.*, (2005) reported the concurrent occurrence of *Cryptosporidium* (31.3%) and microsporidia (32.9%) in Uganda children with persistent diarrhoea with or without concurrent infection with HIV. The prevalence of 6.3% for *Cryptosporidium* recorded in this study was found to be lower than that of Tumwine *et al.*, (2005) as a result of the advanced technique used to differentiate the various species of *Cryptosporidium* and Microsporidia in their study.

The overall rate of infection of 23.9% in this study correlates slightly with other reports. Fashuyi (1983) and Adedoyin *et al.*, (1990), gave similar prevalence rates in their studies, though *Ascaris* ranked next to *E. histolytica/dispar* in this report. Another major limitation in the study was the inability to differentiate between *E. histolytica/dispar*. The high prevalence rate of *Ascaris lumbricoides* among the parasitic nematodes is supported by the fact that it is one of the commonly reported parasitic infections in developing countries (Fasuyi, 1983; Agbakwuru *et al.*, 1998; Brown *et al.*, 2003). Mbae *et al.*, (2013) reported a low prevalence of *Ascaris*, (1.6%) *Trichuris* (0.2%) and hookworm (0.1%) in an informal settlement in Nairobi, Kenya. These differences confirm the extent of local variations that may exist because of climatic conditions and partly because of general hygiene in such localities. In Lagos, the low level of environmental hygiene and sanitation could make diarrhoea one of the most reported cases in our hospitals (Ademola, 1994). Therefore, concerted efforts must

be geared towards improved personal hygiene and environmental sanitation.

The presence of *E. histolytica/dispar* and *Giardia* as diarrhoea causing parasites could exacerbate diarrhoea when it occurs concurrently with *Cryptosporidium* and increasing findings of *Cryptosporidium* in immunocompetent individuals' calls for improvement of personal hygiene (Franco and Neto, 2002).

Reports of concurrent intestinal parasitic infection with *Cryptosporidium* in hospital-based studies in patients with diarrhoea and other gastrointestinal presentations are very few. The study of Akujobi and Oguniola (2006) that was conducted in one of the tertiary hospitals in Lagos reported a *Cryptosporidium* prevalence of 18.7%. Our concurrent study presents a broad range of intestinal protozoan and helminthic parasitoses. In our view, most individuals with diarrhoea hardly seek medical attention while request for *Cryptosporidium* specific tests are rare because this test is not done routinely. This current study demonstrated the co-occurrence of *Cryptosporidium* species with other parasitoses and thus, the contribution of *Cryptosporidium sp* to the presenting gastroenteritis could be missed.

There is therefore, need for the examination of clinical samples in clinical laboratories in order to detect the presence of *Cryptosporidium* species. The possibility that these organisms could be confirmed in clinical samples is higher if tests for *Cryptosporidium* parasites were included among the routine tests for patients with diarrhoea, especially in patients with HIV/AIDs.

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