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PREVALENCE OF CRYPTO-SPORIDIOSIS AND ASSOCIATED RISK FACTORS AMONG PATIENTS WITH ACUTE DIARRHOEA ATTENDING ABUJA TEACHING HOSPITAL, NIGERIA.

Idris Nasir ABDULLAHI, Anthony Uchenna EMERIBE, Kabir UMAR, Hafeez Aderinsayo ADEKOLA, Peter Elisha GHAMBA, Nafiu FARUKU, Nkechi Blessing ONUKEGBE, Chisom Emmanuel OKE-CHUKWU, Adamu BABAYO, Dele Ohinoyi AMADU.

ABSTRACT

Background: Cryptosporidiosis is a zoonotic and foodborne parasitic infection which has become a major subject of public health concern in developing countries. This study aimed to determine the prevalence and associated risk factors of cryptosporidiosis in patients with acute diarrhea (<5 days post onset) attending University of Abuja Teaching Hospital (UATH), Abuja, Nigeria. **Materials and Methods:** 160 patients presenting with acute diarrhea (40 <5 years children, 40 HIV patients, 40 patients with active pulmonary tuberculosis (pTB) and 40 persons with type-2 diabetic mellitus (T2DM)). Fresh fecal samples were collected and immediately analyzed for *Cryptosporidium spp* using modified Ziehl Neelsen (mZN) protocol and Cryptosporidium antigen (CA) based-ELISA. **Results:** The detection rate of CA by ELISA was 26.3% while *Cryptosporidium spp* oocytes detection by mZN was 13.7%. The prevalence of cryptosporidiosis was 7.5% in pTB infected patients, 20% in HIV patients, 62.5% in <5 years children and 27.5% in T2DM patients. The prevalence of cryptosporidiosis was significantly associated with gender, socioeconomic status and marital status ($p< 0.05$) Possession of house pets (OR=9.21, 95% CI=4.15-20.4), practice of backyard poultry (OR=8.81, 95% CI=3.27-23.73), daily vegetable consumption (OR=4.42, 95% CI=2.02-9.63) and household size (OR= 2.49, 95% CI=1.14-5.43) were significant risk factors of cryptosporidiosis. **Conclusion:** Compared to the 15% national prevalence of cryptosporidiosis in Nigeria, it can be inferred that the present study revealed a significantly high prevalence of cryptosporidiosis among paediatric patients, HIV patients and T2DM patients presenting with acute diarrhea to Abuja Teaching Hospital.

Keywords: Cryptosporidiosis, Children, Type 2 Diabetes Mellitus, Diarrhea, HIV, Intestinal parasite, Risk factors, Tuberculosis.

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Keywords: Cryptosporidiosis, Children, Type 2 Diabetes Mellitus, Diarrhea, HIV, Intestinal parasite, Risk factors, Tuberculosis.

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INTRODUCTION

Cryptosporidium is an apicomplexan parasite, which is common among domestic and wild animals.[1] Transmission to humans occur mainly through fecal-oral route when they consume food and water contaminated with oocysts, or during recreational events (such as swimming pools).[1,2] On several occasions cryptosporidiosis has been implicated in diarrhea outbreaks in many countries.[2]

Cryptosporidiosis often manifests as self-limiting diarrhea in immunocompetent persons, and as a progressively life-threatening diarrhea in people who are immunocompromised. These include persons with human immunodeficiency virus infection (HIV), active pulmonary tuberculosis (pTB), younger children and the elderly, persons on cancer chemotherapy, and any other condition that could compromise the immune function such as malnutrition.[3]

There are several studies which reported disproportionate prevalence of cryptosporidiosis in Nigeria. However, a recent meta-analysis revealed a national prevalence of cryptosporidiosis of 15% in Nigeria.[4] Of these studies, few used enzyme immunoassays and compared results with that of the modified Zeihl Neelsen (mZN) technique. In addition, none simultaneously compared the prevalence of cryptosporidiosis across different study population. Hence, this study aims to determine the prevalence and associated risk factors of cryptosporidiosis among various categories of immunocompromised patients presenting with acute diarrhea at University of Abuja Teaching Hospital, Gwagwalada, Abuja, Nigeria.

MATERIALS AND METHODS

This hospital-based cross-sectional study was conducted at the University of Abuja Teaching Hospital, Gwagwalada, located at a surburb of Abuja, the federal capital territory (FCT), Abuja, Nigeria. According to the 2016 Nigerian National Population census, less than 10% of Gwagwalada area council had access to portable tap water. Ethical approval was obtained from the Human Ethical Research Committee (HREC) of UATH. The study was explained to patients or parents/guardians of children, and were enrolled into the study upon giving written and / or verbal informed consent. Structured questionnaire was used to collate sociodemographic variables and possible cryptosporidiosis risk factors from patients or parents/guardians. This was conducted in accordance with the Declaration of Helsinki. Data generated were anonymously analyzed throughout the study.

Between 2nd April to 30th November, 2018, patients were randomly recruited based on the following inclusion criteria; patients who have active pTB, HIV, T2DM or less than 5 years, presenting with acute diarrhea, and tested negative for intestinal parasites by routine wet preparation microscopy and have not started anti-protozoan therapy. However, those that who did not provide consent to participate in the study were excluded.

Interviewer-administered questionnaire was used to assess sociodemographic variables of participants, such as age, occupation, education level, civil status, monthly income, household, conditions, water availability, supply, washing fruit and vegetables. Indices of socioeconomic status (SES) was defined by educational attainment, income, housing, and employment variables into 3 groups: Upper SES, Middle SES and Lower SES (See APPENDIX I for full definition of indices of socioeconomic status).

Fresh diarrheic stool samples uncontaminated with urine were collected in a properly labeled (with patient's study number), leak proof, clean, sterile plastic containers with screw cap lid from patients. Samples

were transported to the laboratory and safety precautions were observed throughout the period of processing the specimens. Fresh samples were kept at 2 - 8º C and tested within 24 hours of collection.

A direct sample of the stool was made with both saline and iodine mounts on clean grease free slides and examined under the microscope first with 10× and then 40× objective lens for ova and cysts of parasites. A sample of the stool was concentrated using formol ether method concentration technique.[5] Detection of *Cryptosporidium* oocysts in the concentrated stool was done using the modified cold Ziehl Neelsen staining technique, as previously described (See Appendix II).[5] Determination of Fecal Cryptosporidia Antigen was also performed by Sandwich ELISA using Para-Tech® ELISA kit from *Medical Chemical Corp, California, USA* (See APPENDIX III).

The study sample size was determined using fishers' expression. Using the 4.8% prevalence of cryptosporidiosis from a previous cross-sectional study in North-central Nigeria, a minimum sample size of 72 was calculated.[6] However, the number was increased to 160 (comprising of 40 <5years children, 40 HIV patients, 40 patients with active pTB and 40 patients with T2DM) to improve the statistical credence of the study. Data were presented as mean +/- SD and percentages. Categorical variables were compared with prevalence of cryptosporidiosis using chi-square test. Data was submitted for univariate logistic regression analyses to compute Odd Ratios (OR) at 95% confidence interval (95%CI) as a measure of risk factor for cryptosporidiosis. The various tests were carried out as two-tailed and outcomes with probability value below 0.05 were considered to be statistically significant.

RESULTS

The detection rate of Cryptosporidiosis by ELISA was 42/160 (26.3%) while *Cryptosporidium spp* was detected by mZN in 22/160 (13.7%) (Figure 1). The prevalence of cryptosporidiosis was 7.5% (3/40) in pTB patients, 20% (8/40) in HIV infected patients, 62.5% (25/40) in <5 years children and 27.5% in T2DM patients (Table I). There was significant association between prevalence of cryptosporidiosis with the various patients' groups (χ^2=32.15; p<0.0001).

Children ≤5 years had the highest prevalence of cryptosporidiosis, 25 (62.5%) followed by those between 35 – 44 years, 3 (21.4%). However, none of those between 45 – 54 years had cases of cryptosporidiosis. The prevalence of cryptosporidiosis was relatively higher in males, 27(37.5%) than the female counterpart, 15 (17.0%). Patients without formal education had the highest prevalence of cryptosporidiosis, 22 (33.8%) and least among those with tertiary education, 3 (12.5%). The prevalence of cryptosporidiosis was highest among patients who were married, 27 (37.5%), followed by the singles, 14 (18.4%) and least in widows/ widowers, 1 (8.3%).

In regards to the residential areas of patients, rural residents had the highest prevalence of cryptosporidiosis, 21 (45.6%), followed by those who reside in urban communities, 8 (24.2%), and least in suburb settlers, 13 (16.0%). Patients who were unemployed had the highest prevalence of cryptosporidiosis, 12 (38.7%) and least among civil servants, 3 (10.3%). Patients who were at lower socioeconomic category had the highest prevalence of cryptosporidiosis, 29 (39.7%), and least in those with high socioeconomic status, 3 (7.3%). The prevalence of cryptosporidiosis was significantly associated with sex, socioeconomic status and marital status (p< 0.05), but not with education level, occupation, age and residential area (p>0.05) (Table II).

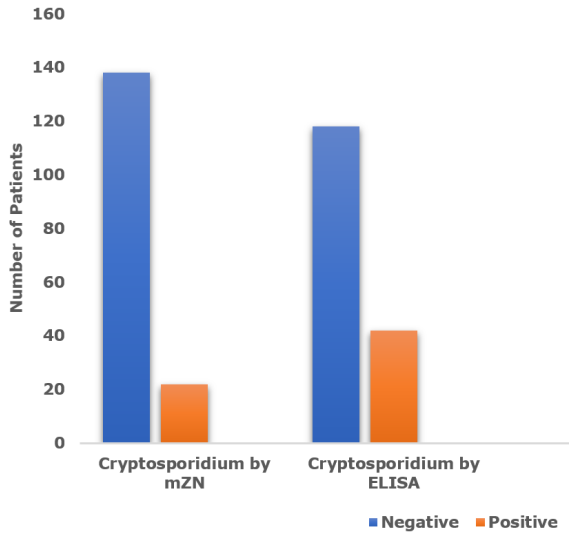

Figure 1: Cryptosporidium detection rates of modified Zeihl Neelsen stain and ELISA.

In regards to the risk factors of cryptosporidiosis, patients who had pets in their houses had relatively higher cases of cryptosporidiosis, 29 (55.8%) than those who did not, 13 (12.0%). Those that consumed vegetable foods on daily basis had relatively higher cases of cryptosporidiosis, 31 (40.2%) than who did not, 11 (13.3%). Patients who had boreholes/ wells as their sole sources of drinking water had relatively higher cases of cryptosporidiosis, 23 (27.1) than those who outsourced their drinking water from taps, 19 (25.3%). Persons who practiced solely open bush defecation had higher cases of cryptosporidiosis, 22 (32.4%) than those who defecate using only pit latrines and water closet, 20 (21.7%). Patients who resided in a densely populated households had higher cases of cryptosporidiosis, 31 (33.7%) than those who resided in sparsely populated houses, 11 (16.9%). Persons who had backyard poultry in their houses had higher cases of cryptosporidiosis, 15 (68.2%) than those who do not, 27 (19.6%).

After univariate logistic regression, possession of house pets (OR=9.21, 95% CI=4.15-20.4), practice of backyard poultry (OR=8.81, 95% CI=3.27-23.73), daily vegetable consumption (OR=4.42, 95% CI=2.02-9.63) and household size (OR= 2.49, 95% CI=1.14-5.43) were significant risk factors of cryptosporidiosis (Table III).

DISCUSSION

Cryptosporidiosis, which is rarely seen in immunocompetent individuals is commonly found at relatively higher rate in immunocompromised persons. Cryptosporidiosis in children and immune-compromised individuals such as HIV-infected persons have been reported from many parts of the world. In Nigeria, this too have been reported in children, malnourished and HIV infected individuals, but there is limited data in patients with pTB and T2DM.[6,7,8]

This study reported very high prevalence of cryptosporidiosis of 26.3% and 13.7%, according to ELISA and mZN technique, respectively. This prevalence is significantly higher than previously estimated

Table I: Prevalence of Cryptosporidiosis among various group of Patients.

Study group	No. of Subjected tested	No. (%) Positive by mZN stain Microscopy	No. Positive (%) by Cryptosporidium antigen ELISA	χ^2	*p* value
Children (< 5 years)	40	9 (22.5)	25 (62.5)		
HIV infected persons	40	6 (15.0)	8 (20.0)		
Persons with active pTB	40	1 (2.5)	3 (7.5)		
Persons with T2DM	40	6 (15.0)	11 (27.5)		
Total	160	22 (13.8)	42 (26.3)	32.15	*<0.0001*

mZN - modified Zeihl Neelsen; pTB - Pulmonary Tuberculosis; T2DM—Type 2 Diabetes Mellitus.

Table II: Prevalence of Cryptosporidiosis by Sociodemographic variables of patients presenting with acute diarrhea (n =160).

Sociodemographic characteristic		Negative cases	Positive cases	OR (95%CI)	*p* value
Age range (years)	<5	15	25	1.67 (0.21-13.01)	0.627
	5 – 14	13	2	0.13 (0.01- 1.80)	0.136
	15 – 24	43	6	0.14 (0.16- 1.18)	0.071
	25 – 34	22	4	0.18 (0.02 -1.69)	0.134
	35 – 44	11	3	0.27 (0.03-2.82)	0.276
	45 – 54	12	0	0.04 (0.00-1.11)	0.057
	≥55	2	2	Referent	
Gender	Male	45	27	2.92 (1.40-6.07)	0.004*
	Female	73	15	Referent	
Educational Level	No Formal education	43	22	3.58 (0.96-13.3)	0.057
	Primary	33	9	.91 (0.46-7.87)	0.371
	High School	21	8	2.67 (0.62-11.46)	0.187
	Tertiary	21	3	Referent	
Residential Area	Rural	25	21	2.62 (0.98-7.03)	0.053
	Suburb	68	13	0.59 (0.22-1.69)	0.309
	Urban	25	8	Referent	
Socioeconomic status	Low	44	29	8.3 (2.36-29.59)	0.0001*
	Middle	38	8	2.67 (0.66-10.8)	0.170
	High	38	3	Referent	
Occupation	Farmer	30	16	0.84 (0.33-2.17)	0.726
	Civil servant	26	3	0.18 (0.05-0.74)	0.017*
	Self-employed	24	6	0.39 (0.13-1.25)	0.114
	Student	19	3	0.25 (0.06-1.03)	0.055
	Unemployed	19	12	Referent	
Marital status	Married	45	27	2.36 (1.10 -5.06)	0.027*
	Widowed	11	1	0.40 (0.05-3.38)	0.402
	Single	62	14	Referent	

Table III: Risk factors of cryptosporidiosis in patients presenting with acute diarrhea (n =160).

Risk Factors		Negative Cases	Positive Cases	OR (95% CI)	*p* value
Possession of house pet	Yes	23	29	9.21 (4.15-20.4)	<0.0001
	No	95	13	Referent	
Daily consumption vegetables food	Yes	40	31	4.44 (2.02-9.63)	0.0001
	No	72	11	Referent	
Source of Drinking water	Borehole /well	62	23	1.09 (0.54-2.21)	0.804
	Tap	56	19	Referent	
Means of defecation	Open bush	46	22	1.72 (0.85-3.50)	0.133
	Water closet/ pit latrine	72	20	Referent	
Household population size	Dense (≥8 persons)	62	31	2.49 (1.14-5.43)	0.024
	Sparse (<8 persons)	54	11	Referent	
Presence of Household Poultry	Yes	7	15	8.81 (3.27-23.73)	<0.0001
	No	111	27	Referent	

This prevalence is comparable to previous studies reported from Canada (18.02%) and USA (21.2%).[11,12] Several factors such as age, sex, gastrointestinal conditions, personal and environmental hygiene, sewage water management, consumption of untreated water, contact with infected or carrier animals and poor economic status have been recognized predisposing factors to high prevalence of cryptosporidiosis.

In particular, our data showed that the prevalence was highest among patients aged ≤5 years, followed by elderly adults aged ≥ 55 years. This is similar to findings from Khan *et al.*[13] This age group represent the vulnerable age which usually have deficient immunity due to immature immune function in younger children or wane immunity due to old age.

Out of the 40 diabetic mellitus (DM) patients screened, 27.5% had cryptosporidiosis by ELISA, this finding is similar to the study of Baqai *et al* who reported 25% cryptosporidiosis in DM patients.[14] But lower than 10.6% and 12.9% reported by Cengiz *et al* and Ali *et al*, respectively.[15,16] The difference could be due to use of less sensitive method in detecting cryptosporidiosis. Indeed, DM is an immunosuppressive disease that could predispose affected person to cryptosporidiosis.

Until recently, cryptosporidiosis was thought to be a rare complication of intestinal disease in people with tuberculosis. Limited case reports and case series documented cryptosporidiosis in person infected with active *Mycobacterium tuberculosis* (MTB). In this study, we found cryptosporidiosis and MTB coinfection in 7.5% patients. This is in consonance to the report by Mor *et al.*[17] We found a significant association between cryptosporidiosis and the study groups in this study, which has not to our knowledge been previously reported. Given overlap in risk factors for active TB and cryptosporidiosis such as malnutrition, or other immunodeficiency conditions. It is possible that this association is confounded by these or other factors. An association with active TB would support the view that respiratory tract cryptosporidiosis is related to immunocompromised state.

The ELISA positive detectable rate was twice that of the mZN protocol. Similar findings were reported by Cengiz *et al.*[15] It may be impossible to detect the parasite in stool samples, which contain few or distorted oocysts, leading a false negativity of the mZN microscopy. The false negativity of mZN is also due to scarcity of parasite. ELISA kits, however, employing *Cryptosporidium* specific monoclonal antibodies, have a sensitivity and specificity of 93% - 100% and has better chances of detecting the antigen in the stool. The results of cryptosporidium-antigen ELISA indicate that the simple, rapid, reliable, and standardized immunoassay test is sensitive and specific for routine diagnosis and may be useful for large-scale epidemiological studies of cryptosporidiosis.

The high burden of cryptosporidiosis from this study may be attributed to poor personal hygiene and inadequate sanitary facilities. The patients were predominantly illiterates (33.8%) with no formal education, with majority being farmers (34.8%) as occupations, and of low socioeconomic status. For instance, 32.4% of the patients who had no access to a household lavatory and practiced open bush defecation had cryptosporidiosis.

Not using sanitary facilities, water treatment and poor hygiene habits of some of the patients might increase the general environmental contamination and thereby increases the risk of cryptosporidium infection for all other persons living in the same setting.[18] Therefore, the protective effects of sanitation and hygiene habits could have been underestimated. Moreover, owing to its low latitude

and high elevation, the town studied has a mild climate and belongs to the humid subtropical zone.

The effects of seasonality in both temperature and rainfall were once shown to be associated with an increased risk of cryptosporidiosis.[18] These local climatic conditions define a typical habitat area for *Cryptosporidium spp*. Alternatively, these differences may reflect the use of different diagnostic techniques. In addition, we found that patients who raised livestock or poultry were more likely to be infected with Cryptosporidium, which was consistent with previous study conducted by Yang *et al.*[19]

Findings from this study showed that cryptosporidiosis occurred more in male than female. This could be due to the fact that males were exposed to lot of community activities, farming, swimming in the river than the female counterparts in Nigeria. This study is in agreement with a study conducted by Kimani *et al* which showed a wide difference in gender distribution, of males and females.[19] However Tombang *et al* reported a higher prevalence of positive cryptosporidiosis in females than their male counterparts (females, 5.4% versus males, 3.6%), which is in contradiction to both our study and Kimani *et al* findings.[20] According to their study, the reason was attributed to the fact that persons of both sexes engage in almost the same recreational activities and so are likely to be equally exposed to similar environmental conditions.

In this study, the prevalence of cryptosporidium infection was higher in HIV infected individuals, when compared to other study in eastern and western Nigeria, this could be due to difference in laboratory testing protocols utilized in these studies and the low socio-economic status of the people in our study area as compared to other localities in the south-east and south-south regions of Nigeria.[8,21]

Findings from this study also revealed that individuals who used surface water, well and borehole (for drinking or bathing purposes) were at higher risk for infection, while those who used tap water for consumption were found lesser risk for cryptosporidiosis. Most of the patients were from villages and rural areas where birds, cats and dogs are commonly wandering freely, which may be a route for subsequent zoonotic spreading of oocysts, contaminating the soil and water with their feces. To buttress this, presence of household poultry was found to be significant risk factor of cryptosporidiosis. The observation made from this study showed insignificant association between the detection of Cryptosporidium oocysts and the source of drinking water. Though statistically insignificant, the highest number of oocysts was detected among persons from houses dependent on well, bore hole water. Wells in this study area are mostly shallow and can easily be contaminated with human and animal excreta which could serve as the reservoir of the oocysts. This agrees with the reports that contaminated water represents the major source of cryptosporidiosis for humans.[13,22]

The findings of our study are in agreement that families with low economic status are at significant risk for cryptosporidiosis in the study area. Statistically, there was no significant association observed among the parent level of education and patients' occupation. Comparable study from USA reported that cryptosporidiosis is the highest risk for people living in families with poor food adequacy.[11] The food insufficiency may push the families to access food products from places where food safety and hygiene is poor such as at open street marketplaces.[13]

To date, there is no specific therapy developed against human and animal cryptosporidiosis, however, nitazoxanide has shown

promising results and has since been approved by the U.S. FDA as anti-cryptosporidiosis.[23] However, this drug is not yet become globally available. Despite the use of nitazoxanide, its curative effects on HIV/AIDS and other immunocompromised patients are not significantly effective compared to a placebo in some experimental studies.

The strength of our study lies in the use of the more sensitive and specific Sandwich Para-Tech® ELISA kit from *Medical Chemical Corp, California, USA,* which picked up twice the number of positive cases than the usual mZN testing procedure. Thus the prevalence reported by our study using the ELIZA technique may represent the true prevalence rate. There are several limitations to our study. First is the population chosen which is specific and unique to our region and hospital and hence results may not be generalizable to the population in general in Nigeria. Second is the lack of multivariate analysis of the positive variables to remove confounding variables which were found to be significant based on univariate analysis.

CONCLUSION

Base on the 15% national prevalence of cryptosporidiosis from a recent metanalysis, it can be inferred that the present study revealed a significantly high prevalence of cryptosporidiosis among patients presenting with acute diarrhea at Abuja Teaching Hospital. It's recommended that government and healthcare policymakers should consider encourage and implement measures that will improve environmental sanitary and socioeconomic conditions of their citizens. In addition, individuals should minimize risk factors associated with personal hygiene identified in this study.

CONFLICT OF INTEREST

None

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