The Prevalence and Risk Factors of *Cryptosporidium parvum* in Diarrheic Stool of Children in Rivers State University Teaching Hospital

Nyenke, C. U. [1*], Ikpeama, R. A. [1] Achi G.I[2], Erasmus M.A[3]. and Lionel-Pepple, V. T. [1]

[1] Department of Medical Laboratory Science, Pamo University ofMedical Sciences, Port Harcourt, Nigeria

[2] School of Medical Laboratory Science, Rivers State College of Health Science Management , Port Harcourt, Nigeria.

[3] Department of Medical Laboratory Science, Rivers State University Teaching Hospital, Port Harcourt, Nigeria.

*Corresponding Author's email: clemyenke@pums.edu.ng; Tel:+2348037098294

Article type: Original research

Cite as: Nyenke CU, Ikpeama RA, Achi GI, Erasmus MA, Lionel-Pepple VT. The Prevalence and Risk Factors of Cryptosporidium parvum in Diarrheic Stool of Children in Rivers State University Teaching Hospital. Nexus Med. Lab. Sci. J. 2024;1(3):8-15

Abstract

Cryptosporidium parvum is an intracellular obligate protozoan parasite that lives in the intestine of their host. They can cause acute to severe diarrhea in humans mostly in children. This study was aimed at investigating the prevalence and risk factors associated with *Cryptosporidium parvum* in the diarrheal stool of children admitted to Rivers State University Teaching Hospital. A cross sectional study was carried out on stool samples collected from 150 diarrheic children admitted in the children's emergency ward and children's ward in Rivers State Teaching Hospital after obtaining parental consent. Ethical approval was obtained from the Ethics Committee of the Rivers State Teaching Hospital, Port Harcourt. The diagnostic methods employed included the Modified Ziehl Neelsen staining technique and direct wet mount using normal saline and iodine. Well-structured questionnaires were used to obtain information on socio-demographics and risk factors. Data obtained were analysed using Statistical Package for the Social Sciences (SPSS). The study revealed an overall prevalence of *Cryptosporidium parvum* in children with diarrhea to be (8.0%) and this prevalence were found to hold significant statistical importance within the study. Gender and awareness, however, did not demonstrate any statistically significant correlation with cryptosporidiosis. On the other hand, among children under the age of 5 years, there was a markedly elevated prevalence, and this was established to be statistically significant. Furthermore, the study highlighted that certain risk factors, particularly personal hygiene and nutritional practices, exhibited a statistically significant association with cryptosporidiosis. The level of cryptosporidia oocysts in children were significantly high therefore, health campaigns should be carried out to educate parents and guardians on healthy practices to abduct in order to control the rate of cryptosporidiosis in children.

***Keywords**: children, Cryptosporidium parvum, diarrheic stool, prevalence, risk factors*

Introduction

Cryptosporidium is an apicomplexan parasite first described from the gastric epithelium of laboratory mice by Tyzzer in 1907 and later named *Cryptosporidium muris*. The first *Cryptosporidium* species associated with diarrhea and mortality was described by Slavin from turkeys in 1955 [1]. Until 1970, *Cryptosporidium* species were not considered economically or medically important, but the veterinary importance of *Cryptosporidium* was highlighted by the association of *Cryptosporidium parvum* with bovine diarrhea [1]. *Cryptosporidium parvum* is an intracellular parasite that can infect healthy children with a strong immune system, resulting in temporary diarrhea [2]. Diarrhea is a prevalent gastrointestinal condition responsible for more than 3.1 million deaths annually among children below 15 years of age, primarily affecting regions with limited resources and healthcare access [3,4,5]. *Cryptosporidium parvum* are a type of Apicomplexan parasites that undergo sexual reproduction within the lining of the intestines. *Cryptosporidium species* are recognized as prominent contributors to waterborne disease outbreaks occurring across the globe. Despite the widespread presence of cryptosporidiosis, it has not received adequate attention, prompting its inclusion by the WHO in 2004 among the "neglected diseases" with a clear association to poverty, particularly within many developing nations [6]. These parasites are typically transmitted through the fecal-oral route, often by consuming contaminated food or water containing their oocysts [1, 2]. These oocysts, which are tough and resilient to acid and alcohol, come in two main types: thick-walled and thin-walled [2, 3]. Over time, the presence of *Cryptosporidium parvum* has been increasingly associated with malnutrition and fatalities resulting from diarrheal complications, particularly among children residing in developing nations [6]. Cryptosporidiosis primarily targets the jejunum and ileum within the small intestine, leading to a watery diarrhea that can endure for up to two weeks and potentially reoccur [6,7]. It has been demonstrated to induce a series of significant health impacts, encompassing weight loss, growth impairment, long-lasting effects on child development, and an elevated case fatality rate [2,3]. Transmission of *Cryptosporidium parvum* occurs through fecal-oral route by ingesting food or water contaminated with oocysts. *Cryptosporidium* parvum can also be transmitted person to person through feces, consumption of contaminated food, additionally it has been described that water constitutes a vehicle for this parasite, since it resists the purification techniques. Oocysts can cause autoinfection [6,8]. Biopsy of an infected organ such as the intestine through an endoscopy, flotation method, electrophoresis procedures, sourthern blot assays, and PCR techniques can be used to diagnose the disease and determine [6,8]. In Nigeria, coccidiosis treatment in humans is through administration of medications called coccidiostats, examples include; Amprolium (thiamine analogue), decoquinate, lasalocid, roxarsone, salinomycin, spiramycin, and toltrazuril [7,9]. Water treatment and good water management practices coupled with good nutrition and access to quality healthcare has migrated its impact in developed world. Conventional water treatment may not be sufficient to clear *Cryptosporidium parvum* because its small size (4-6micrometer) makes oocysts more difficult to filter. Solar and UV light disinfection are efficient in reducing oocysts viability and infectivity [8,9]. Avoid oral or anal sex, avoid touching farm animals or coming in contact with their faeces. Proper washing and cooking of foods and vegetables, avoid swallowing or drinking pool, stream or lake water, when travelling some places have poor water treatment and food sanitation therefore, avoid raw fruits and vegetables, tap water, unpasteurized milk or dairy products [8,9,10].

Materials and Methods

Study Area

The Rivers State University Teaching Hospital (RSUTH) formerly known as Braithwaite Memorial Specialist Hospital (BMSH) served as the site of this study. Within latitudes $4^0$78 N and $7^0$01 E, RSUTH can be found. As a Tertiary Health Faculty, the hospital accepts referrals from both public and private institutions in Port Harcourt and the surrounding area. This study was carried out between January 2023 to July 2023

Study Design and Population

A cross-sectional study was carried out using the sample of the selected participants. A total of 150 children between the ages of 1month - 15years who met the inclusion criteria were enrolled in this study. The children enrolled include those admitted in children emergency ward and children ward. Data was generated from well -structured questionnaires that were distributed to the participants who volunteered to partake in the study. The questionnaires entailed questions on socio-demographics and risk factors associated with coccidian infection.

Ethical Considerations

Ethical approval was obtained from Rivers State Ministry of Health and Rivers State Hospital Management Board ethical committee (RSUTH/REC/2022271). Written consent to participate in the study was obtained from participants [5.11]

Eligibility criteria

The study included children between 1 month-15 years who were not currently on antiparasitic medication, healthy children who were not immunocompromised, immunocompromised children and all those who gave their consent while children that those who were not between the ages of 1 month to 15years or who did not belong to the study population were excluded in addition participants who provided informed or written consent but were later determined by preliminary tests to be ineligible for the study were also excluded

Sample collection & Data Collection

A total of 150 Parents/guardians of children with gastrointestinal symptoms (with diarrhea) were given labeled stool containers to collect one stool sample at the time of collection. They were guided on how to collect a suitable amount of stool in the containers The date and time of passage were provided for each specimen. The data of the patients were obtained from a properly filled questionnaire which was given to each of them. The structured questionnaire entailed questions participant's socio-demographics, such as (age, educational level, medical history, etc.) and clinical presentations such as (diarrhea, abdominal pain and dehydration)

Sample Analysis

All universal precautions were strictly followed when carrying out laboratory analyses. The physical characteristic of a fresh fecal specimen was examined in determining the types of organisms present. Modified Ziehl-Neelsen staining method was used[8] it uses carbolfuchsin as a primary stain which was heated followed by decolorization with an acid-alcohol solution and methylene blue as a counterstain. When viewed under a microscope, Ziehl-Neelsen stained slides shows acid-fast organisms as reddish pink and non-acid-fast organisms as blue[8]. Physiological saline and iodine was also used to detect the presence of oocysts or sporocysts.[8]

Data analysis

Questionnaires were checked for completeness. The data was analyzed using Statistical Package for Social Sciences (SPSS). Prevalence was expressed in percentages. The association between socio-demographics and variables were tested using Pearson chi-square. Statistical significance was accepted at $p< 0.05$ (95% confidence interval).

Results

Out of 150 stool samples that were examined, *Cryptosporidium parvum* was found in 12 stool samples having the prevalence of (8.0%). 150 structured questionnaires were also distributed for analysis containing their socio-demographics, medical history, personal hygiene and nutritional practice.

Table 1 shows the prevalence of *Cryptosporidium parvum* based on their sex and age groups. Male shows a high prevalence of *Cryptosporidium parvum* (8.6%) while female has a low prevalence of *Cryptosporidium parvum* (7.2%). The prevalence of *Cryptosporidium parvum* (0.54) is >0.05, therefore, there is no statistical relationship between sex and cryptosporidiosis. Among the age groups, <5years has the highest positive number for cryptosporidiosis with *Cryptosporidium parvum* having (12.6%). Age group 11 – 15years has the lowest prevalence of *Cryptosporidium parvum* (0%). The prevalence of *Cryptosporidium parvum* (0.07) based on age is <0.05, therefore, there is a statistical relationship between age and cryptosporidiosis.

Table 1 – The prevalence of Cryptosporidium parvum based on sociodemographics.

Socio-demographic	Number examined (%)	Number positive (%)	df	Chi square	P value
		C. parvum			*C. parvum*
Sex					
Male	81 (54)	7 (8.6)	1	0.842	0.54
Female	69 (46)	5 (7.2)			
Age Group					
< 5 years	63 (42)	8 (12.6)	2	9.149	0.07
6 - 10 years	45 (30)	4 (8.9)			
Total	150	12 (17.9)			

C. parvum= Cryptosporidium parvum, df= difference, P < 0.05.

Table 2 shows the association between awareness, personal hygiene and nutritional practice as risk factors and infection with *Cryptosporidium parvum.* The risk factors associated with cryptosporidiosis in this study were consumption of unwashed fruits and vegetables, consumption of untreated water, absence of regular deworming, consumption of infected raw or partially cooked meat and children whose guardians or parents lacked awareness about the transmission of *Cryptosporidium parvum* this may be due to the absence of proper awareness and educational initiatives resulting in deficient knowledge about the parasite's transmission, and a lack of understanding about the clinical symptoms associated with cryptosporidiosis.

Table 2- Association between risk factors and cryptosporidiosis

Risk Factors	Response	Number Examnined (%)	Number Positive (%)	P. Value	Chi Square	df
			C. parvum	*C. parvum*	*C. parvum*	*C. parvum*
Knowledge of coccidian	Yes	25 (16.7)	2 (8.0)	1.00	0.000	1
	No	125 (83.3)	10 (8.0)			
Aware that is causes diarrhea	Yes	23 (15.3)	3 (13.0)	1.00	0.018	1
	No	127 (84.7)	9 (7.1)			
Wash fruits and vegetables	Yes	110 (73.3)	1 (0.9)	0.00	28.181	1
	No	40 (26.7)	11 (27.5)			
Treats drinking water	Yes	108 (72.0)	0 (0)	0.00	33.540	1
	No	42 (28.0)	12 (28.6)			
Deworms 3-6 months ago	Yes	111 (74.0)	1 (0.9)	0.00	29.233	1
	No	39 (26.0)	11 (28.2)			

C belli= Cystoisospora belli, df= difference, P < 0.05

Discussion

The prevalence of *Cryptosporidium parvum* was (8.0%), this is close to the prevalence observed in a related study conducted by Lucero-Garzón *et al.* (2015), wherein a prevalence of (7.0%) for *Cryptosporidium parvum* was recorded. This is in contrast with the findings of some scholars whose respective research reported notably higher prevalence rates of (19.4%), (17.8%), and (13.40%) respectively for *Cryptosporidium parvum* this may be due to differences in geographical locations and environmental conditions [2,12,13].

The current study gender was not significantly associated with prevalence of coccidian parasites however; there were differences in the prevalence rates reported for males and females. In males, the prevalence of *Cryptosporidium parvum* stood at (8.6%) in comparison, females exhibited slightly lower prevalence rates, with *Cryptosporidium parvum* at (7.2%). Molloy *et al.* (2010) in a related study made similar observation where it was noted that girls from families with higher socioeconomic statuses and greater maternal education levels demonstrated lower infection rates.[12]. This association suggests that parents with higher educational attainment might engage in behaviors like promoting hygiene awareness that could reduce the risk of infections in their children. The potential for educated mothers to pass on knowledge, particularly to their daughters, is suggested as a contributing factor. This suggests that there is an intricate dynamic interaction between gender, socioeconomic factors, and behavioral practices in the transmission of coccidian

infections.[12]. However, other similar studies conducted by some other researchers reported higher prevalence rates in females, with figures of (65.0%) and (79.1%) respectively, compared to males with (60.0%) and (78.8%).[13,14]

This study found out that there is a significant association between prevalence of *Cryptosporidium parvum* and age emphasizing that age plays a meaningful role in the dynamics of cryptosporidiosis. Children aged less than 5 years recorded the highest prevalence of (12.6%) for *Cryptosporidium parvum*. This shows the vulnerability of children in this age group to *Cryptosporidium parvum*, and this can be attributed to their natural inclination to explore their surroundings, potentially encountering animal excreta, contaminated edibles, and contaminated objects that often find their way into their mouths. Similar pattern of vulnerability among children under 5 years was observed in related studies [13,14]. Age group of 11–15 years recorded the lowest prevalence rates of (0%) for *Cryptosporidium parvum*. This observation aligns with the findings from similar studies where they identified the lowest prevalence of coccidian parasites among children above 11 years of age this may be due to the fact that children at this certain age are educated on personal hygiene and environmental sanitation[13,14,15].

An association was observed between awareness and the prevalence of *Cryptosporidium parvum* in children. Specifically, children whose guardians or parents were not aware that the parasite *Cryptosporidium parvum* causes diarrhea exhibited a higher prevalence ,this observation may be due to the absence of proper awareness and educational initiatives resulting in deficient knowledge about the parasite's transmission, and a lack of understanding about the clinical symptoms associated with coccidiosis. Tombang *et al.* (2019) made similar findings in their own study, further supporting the observation that poor awareness and limited knowledge about *Cryptosporidium parvum* can contribute to higher infection rates.[2.] Despite these findings, the current study found no statistically significant link between awareness and cryptosporidiosis, indicating that while awareness may contribute to the prevalence of coccidiosis, it does not hold a strong statistical influence.

A significantly high occurrence of *Cryptosporidium parvum* (28.6%) was noted among children who consume unwashed fruits and vegetables, as well as those who consume untreated water,. This could be attributed to ingesting contaminated produce from unhygienic sources like open markets, consuming untreated water from sources like sachet water, boreholes, and wells, and neglecting hand hygiene practice after using the restroom. This invariably indicates that inadequate personal hygiene can influence the susceptibility of children to coccidian Infections Similar observations were made by scholars in prior related research studies conducted. [2,15,16] . In this study, the absence of regular deworming interventions, occurring at least every three months, emerged as a significant factor influencing the prevalence of *Cryptosporidium parvum* . This occurrence might be attributed to the continuous consumption of infected raw or partially cooked meat and the potential contamination of children's water bottles, plates, and utensils due to unclean hands. Paul et al 2019 and Tamomh et al 2021 aligned with this observations in related studies.[15,16] Hence, it becomes evident that nutritional practice contributes substantially to the progression of coccidian infections in children .

Conclusion

Cryptosporidium parvum is of public health significance in Nigeria especially among school children. Regular deworming (chemotherapy),provision of good protective footwears, frequent health education and improved sanitary measures (provision of pipe-borne water and good toilet facilities) will help control the spread of *Cryptosporidium parvum* infection.

References

1. Bouzid M. Waterborne parasites: detection of food- and waterborne parasites. Encyclopedia of Food Microbiology. 2014; 2:773-781.
2. Tombang AN, Ambe NF, Bobga TP, Nkfusai CN, Collins NM, Ngwa SB. Prevalence and risk factors associated with cryptosporidiosis among children within the ages 0–5 years attending the Limbe regional hospital, southwest region, Cameroon. Biomedcentral Public Health, 2019;19(1):1144.
3. James KT, Kekitinwa A, Nabukeera N, Akiyoshi DE, Stephen MR, Giovanni W, Xiaochuan F, Saul T. *Cryptosporidium parvum* in children with diarrhea in mulago hospital, Kampala, Uganda. American Society of Tropical Medicine and Hygiene. 2003; 68(6):710 - 715.
4. Wogu MN, Onosakponome EO, Harry UA. Prevalence of Geohelminths among Primary School Children in Edoh Community, Esit Eket Local Government Area, Akwa Ibom State, Nigeria. Asian Journal of Medicine and Health. 2022;18(9):123-127
5. Onosakponome EO, Adedokun AA, Michael NW. Assessment of Risk Factors and Outcome of Co-Infection of Soil Transmitted Helminths and *H. pylori* among School Age Children Living in Riverine Slum Settlements in Port Harcourt, Nigeria. International Journal of Tropical Disease & Health. 2022;43(1): 12-17,
6. García-Sánchez E, Valladares-Carranza B, Talavera-Rojas M, VelázquezOrdóñez V. Cryptosporidiosis. Importancia en salud pública. Revista Electronica de Veterinaria. 2014;(15):271 - 280.
7. Gordon JL, Qing H. Cryptoporidiosis – an overview. The Journal Biomedical Research. 2011;25(1):1 - 16.
8. Joseph AA, Popoola GO. Comparism of various staining techniques in the diagnosis of coccidian parasitosis in HIV infection. Medical Journal of Zambia. 2007;1(44):1-8.
9. Anosa GN, Obetta VC, Okorie-kanu CO, Eze JI. Comparative efficacy of Toltrazuril, Amprolium and Embazine Fort against mixed *Eimeria Species* Infection Broilers. Nigerian Veterinary Journal. 2011;32(3):214 - 217.
10. Collinet AS, Ward HD. Cryptosporidiosis: Environmental, therapeutic, and preventive challenges. European Journal of Clinical Microbiology of Infectious Disease. 2010;29:927-935.
11. Ohanu EC, Onosakponome EO, Nyenke CU, Abah AE. The Sero-Epidemiology and Risk Factors of *E. histolytica* Infection in Calabar, Nigeria. South Asian Journal of Parasitology. 2022;6(2):19-26.
12. Lucero-Garzón TA, Álvarez-Motta LA, Chicue-López JF, López-Zapata D, Mendoza-Bergaño CA. Parasitosis intestinal y factores de riesgo en niños de los asentamientos subnormales, Florencia- Caquetá , Colombia. Revista Facultad National de Salud Pública.; 2015;33(2):171-80.
13. Molloy SF, Tanner CJ, Kirwan P, Asaolu SO, Smith HV, Nichols RAB, Connelly L, Holland CV. Sporadic Cryptosporidium

infection in Nigerian children: risk factors with species identification. Epidemiology & Infection; 2011;139(6):946 –954.
14. Nassar SA, Oyekale TO, Oluremi AS. Prevalence of Cryptosporidium infection and related risk factors in children in Awo and Iragberi, Nigeria. Journal of Immunoassay and Immunochemistry. 2017;38(1):2-9.
15. Paul OO, Isaiah OA. Epidemology of Cryptosporidium infection in different hosts in Nigera: A meta-analysis. Parasitology Inernational. 2019;71:194-204.
16. Tamomh AG, Agena AM, Elamin E. Prevalence of cryptosporidiosis among children with diarrhoea under five years admitted to Kosti teaching hospital, Kosti City, Sudan. Biomedcentral Infectious Disease. 2021;21:349.