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ORIGINAL ARTICLE

Cryptosporidiosis: An Ignored Cause of Persistent Diarrhea among Children

Faryal Manzoor [1], Farhan Rasheed [2], Muhammad Saeed [3], Junaid Rashid [4], Nazia Mahmood [5], Ambereen Anwar Imran [6]

[1] Medical Lab Technologist, Department of Pathology, Shaukat Khanum Memorial Cancer Hospital Lahore
[2] Assistant Professor, Department of Pathology, Allama Iqbal Medical College and Jinnah Hospital Lahore
[3] Medical Technologist, Manager-Pathology Department, DHQ Hospital Mandi Bahauddin Pakistan
[4] Professor, Department of Paediatrics, The Children's Hospital and The Institute of Child Health, Lahore
[5] Assistant Professor, Department of Paediatrics, Allama Iqbal Medical College and Jinnah Hospital Lahore
[6] Professor, Department of Pathology, Allama Iqbal Medical College and Jinnah Hospital Lahore

ABSTRACT

Objective: To evaluate the frequency of Cryptosporidiosis among children presenting to a tertiary care hospital with persistent diarrhea.

Materials and Methods: This cross-sectional study was conducted from September 2016 to February 2017 at Microbiology Department, Allama Iqbal Medical College, Lahore. Non-probability consecutive sampling technique was used and a total of 71 stool samples were collected from children admitted to Paediatric ward of Jinnah Hospital, Lahore, and full filling the inclusion criteria. Every sample was collected according to standard protocol, brought to the lab within 3 hours, and processed for modified ZN staining. Cryptosporidium oocysts (bright red against a blue background) were identified.

Results: Among 71 enrolled children (male=41, female =30) Cryptosporidium was detected in 9.85% (n=7) stool samples via modified ZN stain. Majority of the children with Cryptosporidium species cyst infection were less than 5 years old age. All the children presented with watery diarrhea abdominal pain and fever. Only one case of Cryptosporidium species cyst in diarrheal stools of children below 6 months of age was presented. The frequency of Cryptosporidium infection was twice in males 12.5% as compared to females 6.6 %.

Conclusion: The present study highlights the importance of Cryptosporidium detection as a cause of diarrhea, especially in children.

Key words: Cryptosporidium, Modified ZN staining, Persistent Diarrhea.

Author`s Contribution
[1] Conception, synthesis, planning of research and manuscript writing Interpretation and discussion
[2-4] Data analysis, interpretation and manuscript writing, [5,6] Active participation in data collection.

Address of Correspondence
Muhammad Saeed
Email: mian.scientist@yahoo.com

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Introduction

Cryptosporidiosis *is a* highly contagious intestinal infection caused by, emerging pathogen, tiny microscopic parasite Cryptosporidium. A protozoan among the phylum Apicomplexa. E*arly* human cases were detected in 1976 at United States (US) among Immuno-compromised adults and normal or immunocompetent children.[1] Among

young children cryptosporidiosis typically presents with watery diarrhea. Passage of abnormally liquid or unformed stools at an increased frequency.[2]

In the early 1980s, diarrheal disorders were the biggest child killers, responsible for an estimated 4.6 million deaths worldwide every year. Despite widespread use of oral rehydration therapies and an increased understanding of the pathogenesis of diarrhea, 2.5 million children still die from these illnesses every year, almost all of them in developing countries. Cryptosporidium species are leading agents of chronic or persistent diarrhea worsened by specific risk factors such as malnutrition or immune deficiency. Outbreaks of Cryptosporidiosis have been reported in several countries. In 1993, more than 400,000 peoples were affected by the waterborne outbreak in Milwaukee-Wisconsin.[3]

Numerous of Cryptosporidium hominis cases are reported from developed nations like North and South America, Australia and among developing countries [4] pediatric populations very high prevalence is reported from Perù [5] Malawi [6] Kenya [7] India[8] Haiti [9] and Brazil.[10] In case of children, adults and hospitalized HIV-infected children South Africa[11,12] and Uganda.[13] Whereas *Cryptosporidium parvum* infection reported from Europe, especially in UK.[4]

The mechanism of Cryptosporidium associated diarrhea includes a combination of increased intestinal permeability, chloride secretion, and malabsorption. Characteristics of Cryptosporidium associated infection consist of prolonged persistent diarrhea, abdominal cramps, vomiting, low-grade fever, generalized malaise, weakness, fatigue, loss of appetite and nausea.[14] The genus Cryptosporidium has 14 well-defined species, while *Cryptosporidium parvum, Cryptosporidium muris, Cryptosporidium felis, Cryptosporidium meleagridis, Cryptosporidium canis, Cryptosporidium suis,* and *Cryptosporidium cervine* are generally pathogenic for human beings.[15] However, *Cryptosporidium hominis* and *Cryptosporidium parvum* are a common cause of human cryptosporidiosis both in immunocompetent and in immunocompromised individuals, with the difference in prevalence in different regions around the globe.[16]

Diagnosis can be made by several techniques, such as histopathology, modified acid-fast stains, fluorescent stains, immunofluorescent assays using oocyst-specific monoclonal antibodies, antigen-detection assays and molecular techniques like polymerase chain reaction (PCR) for *Cryptosporidium parvum* DNA. Although PCR is most sensitive and specific technique, however, these techniques are unavailable in developing countries due to high cost and lack of expertise. Most diagnostic laboratories in developing countries are using conventional techniques, modified Ziehl Neelsen (ZN) stain for the detection of Cryptosporidium oocyst, as it is rapid, easiest and cheapest method. The present study was planned to evaluate the frequency of Cryptosporidiosis among children presenting to a tertiary care hospital with persistent diarrhea.

Materials and Methods

This cross-sectional study was conducted from September 2016 to February 2017 at Microbiology lab, Allama Iqbal Medical College, Lahore. The sample size was calculated with the help of formula stated below:[17] (CI 95%) n= Pq/ (E/1.96)^2

Non-probability consecutive sampling technique was used and a total of 71 stool samples were collected from patients admitted in pediatric wards of Jinnah hospital, Lahore. Children with persistent diarrhea (>14 days and >3 episodes per day): up to 12 years and both genders were included in the study. Duplicate samples from the same patient during the same episode of illness were excluded. Specimen processing delayed for more than three hours after collection were also excluded. Every sample was collected according to standard protocol, brought to the lab within 3 hours, and processed by modified ZN staining. Cryptosporidium oocysts appear bright red against a blue background in this method.

Modified ZN Staining Method

Appropriate smears were prepared on glass slides from stool specimens with the help of wire loop, air dried it, fixed with 70% ethanol and finally covered with carbol fuchsin stain. The slide was heated until vapors begin to rise. The stain was allowed to cover the smear for 5 minutes and washed in running tap water, decolorized with 3% sulphuric acid for 30 seconds and again wash with running tap water. The smear was then covered with methylene blue for 5 minutes and wash in running tap water, dry the slide and examined under oil immersion objective (100x). *Cryptosporidium* oocysts appear bright red against a blue background in this method.[18] (Figure 1)

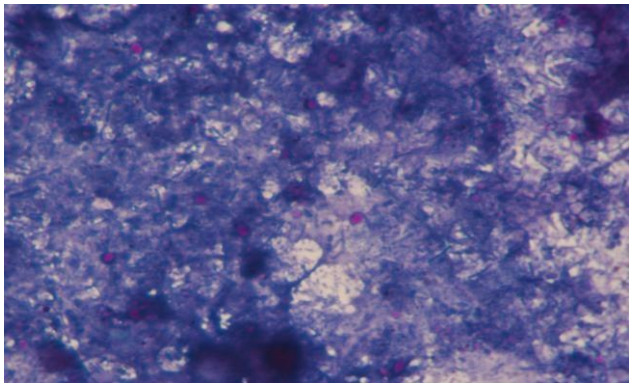

Figure 1: Cryptosporidium oocyst in Modified ZN Stain

Results

Among 71 enrolled children, 41 were males and 30 were females Cryptosporidium was detected among 9.85% (n=7) stool samples via modified ZN stain. Majority children with Cryptosporidium species cyst infection were more than 5 years old age, Mean age was 4±1. All the children presented with watery diarrhea abdominal pain and fever. Only one case of Cryptosporidium species cyst in diarrheal stools was below 6 months of age. Frequency of Cryptosporidium infection was twice in males 12.5% as compared to females 6.6%. Age group wise frequency distribution is showed in Table:1.

Table1: Age group wise frequency distribution of Cryptosporidium infection

Age group	Gender	Total Sample	Crypto
<5 years n(48)	Male	28	3(10.7%)
	Female	20	0(0.0%)
>5 years n(23)	Male	13	2(15.4%)
	Female	10	2 (20.0%)
Total	Male	41	5(12.1%)
	Female	30	2(6.6%)

Discussion

Intestinal parasites are very common in developing countries and Cryptosporidium has revealed to be one of the most common parasites. Cryptosporidium infests the small intestinal epithelium, thereby, resulting in an accelerated loss of villous enterocytes, severe villous atrophy, malabsorptive and secretory diarrhea which is the most pronounced clinical feature of the infection.

The present study reported 9.85% Cryptosporidium among children; similar rate (9%) was reported in a five-year study reported from Peshawar.[14] Ullah, et al. from Skardu Pakistan reported a higher frequency of Cryptosporidium among children (20.8%) [19] while very low rate was reported in a study from Karachi 1.7% .[20] Another study was conducted in Barazjan, Iran in 2012, out of 373 samples the frequency of Cryptosporidium among 5 years old children was 13.1%.[2] Haque et al from Bangladesh reported 8.4% Cryptosporidium diarrhea.[21] Another study reported 16.3% cases ofCryptosporidium diarrhea.[22] In previous studies using microscopy, approximately 3% of Indian children were found to be asymptomatically infected with Cryptosporidium spp.[23] Nkem et al from Nigeria reported very similar results as Cryptosporidium was reported infection 14.3%.[24] Male Children were more prevalent for Cryptosporidium infection (15.4%) than female (13.4%) ($p<0.05$). Shalash et al from Egypt reported 52 (72.2%) Cryptosporidium infection.[1] These sample size was nearly same as that of our study and Cryptosporidium was detected by three different methods including modified ZN staining and antigen detection by ELISA. Geographical distribution and methods of detection of Cryptosporidium also vary. Although Modified ZN staining is a less sensitive method for detection of *Cryptosporidium* but still a useful and cheap tool for detection.

The occurrence of Cryptosporidium infection is similar to that reported in children in other studies in sub-Saharan Africa, both in the Global Enteric Multicenter Study (GEMS), and in the study of Mbae et al. in Kenya.[25,26]

The results of this study highlight the fact that unusual pathogens like Cryptosporidium should not be overlooked while treating cases with diarrhea with prolonged duration Cryptosporidium has emerged as an important cause of diarrhea worldwide. This parasite has been reported not only from Western countries but also from Nigeria, Sudan, Srilanka, Bangladesh, and India with a strong possibility also in Pakistan.[27] The difference in the reported prevalence may be attributed to differences in the study population (considering age range), diagnostic methods, environmental risk factors (public water supply), time of the study (summer vs winter), nutritional status of the children, and other risk factors including the immune status of the study population.

Cryptosporidium is transmitted through multiple sources. The infection may be transmitted by direct person to person, contact with infected animal or by ingestion of contaminated food, or water [28] The oocysts are highly resistant to common household disinfectants and survive for long periods in the environment. It has major public health implication because the infection can result from exposure to low doses of Cryptosporidium oocyst.[6] Cryptosporidium oocysts are very resistant to most environmental factors, with an exception of heat and desiccation. Oocysts can persist for months in water and in soil and have been shown to survive for hours on wet surfaces, including stainless steel. However, they are not resistant to drying and die rapidly on dry surfaces. Temperature sensitive Cryptosporidium oocysts are not especially heat resistant and are destroyed by conventional milk pasteurization. A temperature of greater than 73°C will cause instantaneous inactivation of oocysts. Oocysts can survive for short periods at temperatures below 0°C, especially in water, but the commercial ice cream freezing process has been shown to cause inactivation and eventual die-off occurs at temperatures below -15°C.[29]

Conclusion

The prevalence of Cryptosporidiosis in children presenting with persistent diarrhea is considerable and we suggest routine stool examination for Cryptosporidium in these children for early diagnosis and treatment to prevent it. Modified ZN staining is a cheap and less time-consuming method for diagnosis of cryptosporidiosis.

References

1. Shalash IRA, Zalat R, El-Enain G, EL-Mohandes M, Mohamed E-F, Aly E. Comparison between modified acid fast staining and antigen detection assay as diagnostic techniques for Cryptosporidium parvum. World Journal of Medical Sciences. 2016;13(1):72-8.
2. Saneian H, Yaghini O, Yaghini A, Modarresi M-R, Soroshnia M. Infection rate of Cryptosporidium parvum among diarrheic children in Isfahan. Iranian journal of pediatrics. 2010;20(3):343.
3. Huang M-Z, Li J, Guan L, Li D-Q, Nie X-M, Gui R, et al. Therapeutic effects of acetylspiramycin and garlicin on cryptosporidiosis among drug users. International Journal for Parasitology: Drugs and Drug Resistance. 2015;5(3):185-90.
4. Xiao L, Bern C, Limor J, Sulaiman I, Roberts J, Checkley W, et al. Identification of 5 types of Cryptosporidium parasites in children in Lima, Peru. The Journal of infectious diseases. 2001;183(3):492-7.
5. Cama VA, Bern C, Roberts J, Cabrera L, Sterling CR, Ortega Y, et al. Cryptosporidium species and subtypes and clinical manifestations in children, Peru. Emerging infectious diseases. 2008;14(10):1567.
6. Gatei W, Wamae CN, Mbae C, Waruru A, Mulinge E, Waithera T, et al. Cryptosporidiosis: prevalence, genotype analysis, and symptoms associated with infections in children in Kenya. The American journal of tropical medicine and hygiene. 2006;75(1):78-82.
7. Ajjampur SSR, Gladstone BP, Selvapandian D, Muliyil JP, Ward H, Kang G. Molecular and spatial epidemiology of cryptosporidiosis in children in a semiurban community in South India. Journal of Clinical Microbiology. 2007;45(3):915-20.
8. Raccurt CP, Brasseur P, Verdier RI, Li X, Eyma E, Stockman CP, et al. Human cryptosporidiosis and Cryptosporidium spp. in Haiti. Tropical medicine & international health: TM & IH. 2006;11(6):929-34.
9. Bushen OY, Kohli A, Pinkerton RC, Dupnik K, Newman RD, Sears CL, et al. Heavy cryptosporidial infections in children in northeast Brazil: comparison of Cryptosporidium hominis and Cryptosporidium parvum. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2007;101(4):378-84.
10. Samie A, Bessong P, Obi C, Sevilleja J, Stroup S, Houpt E, et al. Cryptosporidium species: preliminary descriptions of the prevalence and genotype distribution among school children and hospital patients in the Venda region, Limpopo Province, South Africa. Experimental parasitology. 2006;114(4):314-22.
11. Leav BA, Mackay MR, Anyanwu A, O'Connor RM, Cevallos AM, Kindra G, et al. Analysis of sequence diversity at the highly polymorphic Cpgp40/15 locus among Cryptosporidium isolates from human immunodeficiency virus-infected children in South Africa. Infection and immunity. 2002;70(7):3881-90.
12. Tumwine JK, Kekitiinwa A, Bakeera-Kitaka S, Ndeezi G, Downing R, Feng X, et al. Cryptosporidiosis and microsporidiosis in Ugandan children with persistent diarrhea with and without concurrent infection with the human immunodeficiency virus. The American journal of tropical medicine and hygiene. 2005;73(5):921-5.
13. Kosek M, Bern C, Guerrant RL. The global burden of diarrhoeal disease, as estimated from studies published between 1992 and 2000. Bulletin of the World Health Organization. 2003;81(3):197-204.
14. Mumtaz S, Ahmed J, Ali L. Frequency of cryptosporidium infection in children under five years of age having diarrhea in the North West of Pakistan. African Journal of Biotechnology. 2010;9(8).
15. Ryan U, Fayer R, Xiao L. Cryptosporidium species in humans and animals: current understanding and research needs. Parasitology. 2014;141(13):1667-85.

16. Cacciò SM, Thompson RA, McLauchlin J, Smith HV. Unravelling cryptosporidium and giardia epidemiology. Trends in parasitology. 2005;21(9):430-7.
17. Vaughan JP and Morrow RH. Manual of Epidemiology for District Health Management. World Health Organization. 1989; 175-176.
18. Organization WH. Basic laboratory methods in medical parasitology. 1991.
19. Khushdil A, Murtaza F, Chattha MN. CRYPTOSPORIDIOSIS AMONG CHILDREN OF DISTRICT SKARDU, PAKISTAN. Journal of Ayub Medical College Abbottabad. 2016;28(3):575-7.
20. Shoaib S, Tauheed S, Hafiz A. Frequency of Cryptosporidium in childhood diarrhoea importance of modified acid fast technique. J Ayub Med Coll Abbottabad. 2003;15:3-5.
21. Haque R, Mondal D, Kirkpatrick BD, Akther S, Farr BM, Sack RB, et al. Epidemiologic and clinical characteristics of acute diarrhea with emphasis on Entamoeba histolytica infections in preschool children in an urban slum of Dhaka, Bangladesh. The American Journal of Tropical Medicine and Hygiene. 2003;69(4):398-405.
22. Tellevik MG, Moyo SJ, Blomberg B, Hjøllo T, Maselle SY, Langeland N, et al. Prevalence of Cryptosporidium parvum/hominis, Entamoeba histolytica and Giardia lamblia among Young Children with and without Diarrhea in Dar es Salaam, Tanzania. PLoS neglected tropical diseases. 2015;9(10):e0004125.
23. Palit A, Sur D, MitraDhar K, Saha MR. Asymptomatic cryptosporidiosis in a periurban slum setting in Kolkata, India—a pilot study. Japanese Journal of Infectious Disease. 2005;58:110-1.
24. Benjamin N, Uchechukwu C, Ikechukwu D, Oliver A, Muodebe N. Cryptosporidiosis among children in some rural parts of Imo state, Nigeria. Journal of Public Health and Epidemiology. 2013;5(11):440-4.
25. Kotloff KL, Nataro JP, Blackwelder WC, Nasrin D, Farag TH, Panchalingam S, et al. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. The Lancet. 2013;382(9888):209-22.
26. Mbae CK, Nokes DJ, Mulinge E, Nyambura J, Waruru A, Kariuki S. Intestinal parasitic infections in children presenting with diarrhoea in outpatient and inpatient settings in an informal settlement of Nairobi, Kenya. BMC infectious diseases. 2013;13(1):243.
27. Baqai R, Anwar S, Kazmi S. Detection of cryptosporidium in immunosuppressed patients. J Ayub Med Coll Abbottabad. 2005;17(3):38-40.
28. Khan NU, Saleem MH, Durrani AZ, Ahmad N, Hassan A, Ayaz S, et al. Cryptosporidium: An Emerging Zoonosis in Southern Khyber Pakhtunkhwa (KPK), Pakistan. Pakistan Journal of Zoology. 2017;49(4):1455-61.
29. Li X, Atwill ER, Dunbar LA, Tate KW. Effect of daily temperature fluctuation during the cool season on the infectivity of Cryptosporidium parvum. Applied and environmental microbiology. 2010;76(4):989-93.