

Original Article



Incidence of Cryptosporidium a Neglected Parasite in Immunocompetent Adults in Swabi

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Author's Contribution

^{1,2}Substantial contributions to the conception or design of the work; or the acquisition, ^{3,6}Active participation in active methodology, analysis, or interpretation of data for the work, ^{1,4,5}Drafting the work or revising it critically for important intellectual content

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ABSTRACT

Objective: To highlight the increasing incidence of Cryptosporidium in immunocompetent adults and the resultant altered epidemiology.

Methodology: This study was conducted over a period of one year, from 1st January 2023 to 1st of January, 2024 at Microbiology Laboratory MTI-GKMC/BKMC, Swabi. Modified Ziehl Neelsen staining was performed on the received stool samples from patients presenting with wide range of symptoms associated with Irritable Bowel Syndrome (IBS). Abdominal pain, recurrent episodes of diarrhea and weight loss were the main symptoms. Modified ZN stain being one of the most reliable, quick and cost effective methods for the detection of cysts of Cryptosporidium using 40X and oil immersion lens was used for diagnosis.

Results: A total of 62 samples were received from immunocompetent patients in the said period out of which 34% were positive for the oocysts of Cryptosporidium species. Out of a total of 21 positive samples 62% were males and 38% were females. The age range of the patients spanned between 20 to 60 years.

Conclusion: Knowing this high incidence 34% of Cryptosporidium spp. in the healthy adults, necessitates to prioritize steps for the prevention of this infection by implementation of public health measures.

Key words: Cryptosporidium, immunocompetent, filtration, cysts, diarrhea.

Introduction

Although associated with persistent potential life threatening diarrhea in immunocompromised patients, the unnoticed incidence of this intracellular protozoan parasite in the immunocompetent individuals is a point of concern, attributed especially to its ability to be highly resistant to chemical disinfection and filtration.

Cryptosporidium is a protozoan parasite, known for causing diarrhea worldwide, in humans as well as in animals.¹ More than 40 species are known so far, with Cryptosporidium parvum and hominis being responsible for more than 90% of the human cases via ingestion of oocysts contaminating food and water among humans and

animals and between them.² Infectious sporozoites are released from the oocysts in the small intestine which then infect intestinal epithelial cells. After repeated cycles of asexual replication, followed by sexual reproduction, mature oocysts are formed which are then released in the feces.³

Cryptosporidium along with Giardia, both being waterborne pathogens were included in the “Neglected Disease Initiative” by the World Health Organization.⁴

Cryptosporidium got highlighted as a zoonotic parasite of medical concern, in the year 1982, after the publication of a report by CDC on diarrhea caused by Cryptosporidium in patients infected with Human Immunodeficiency Virus (HIV).⁵ Its upswing as a public health concern was in the

year 1993 when a surprisingly large outbreak due to contamination of public water supplies was reported in the USA, UK and Sweden infecting more than 400,000 people with *Cryptosporidium hominis*.⁵ Besides being asymptomatic carriers, the patients of cryptosporidiosis may present with a wide range of symptom including acute gastroenteritis with profuse, watery diarrhea, associated with abdominal cramps, vomiting and even weight loss over long term illness.⁶

Irritable Bowel Syndrome is a common gastrointestinal disorder but its complex etiology and pathogenesis makes it difficult to diagnose. The most commonly encountered four symptoms include bloating, loose stools with increased frequency with the onset of pain and relief of pain with bowel movements based on Rome IV criteria.⁷

There exists an association between Cryptosporidiosis and IBS along with long term effects that are associated with both the prevalent species⁸. The association between IBS and Cryptosporidiosis in healthy adults is a point of major concern.⁹ Frequently reported in patients with IBS, infection with *Cryptosporidium parvum* produces symptoms similar to those described by IBS patients, proposing that this parasite is a potential cause of post inflammatory-IBS.¹⁰

The only FDA approved antiparasitic agent against Cryptosporidiosis for humans is Nitazoxanide.¹¹ Since Nitazoxanide in immunocompetent individuals does not guarantee treatment efficacy, and can only reduce the load of parasite, prevention of Cryptosporidiosis can be achieved by reducing the prevalence of infection, by breaking the transmission pathways both between and among animals and humans and lastly by maintaining good hygiene for both.¹² Also by knowing the major sub-species prevailing in a certain area can definitely help in effective infection prevention.¹²

Despite being a globally prevalent disease it is not equally appreciated as a point of global health concern. Obtaining this epidemiological information, and its association with IBS which is quite overlooked, along with One Health approach, will definitely help in accurate estimation of burden of disease and controlling cryptosporidiosis in immunocompetent patients.

Methodology

This study was conducted over a period of one year, starting from 1st January 2023 to 1st of January, 2024 at Microbiology Laboratory MTI- GKMC/BKMC, Swabi.

Ethical approval was duly sought from institutional review board F. No. 4404/Ethical/GKMC.

Fresh stool samples were collected from patients with history of IBS at the Microbiology Laboratory of Bacha Khan Medical Complex, Swabi. Stool processing was carried out within 2 hours of collection. All of the received fresh stool samples from every patient were subjected to modified acid-fast stain. Smears were prepared and screened thoroughly for the diagnosis of the oocysts measuring 4 to 8 μ m in size, using 40 \times and 100 \times oil immersion objectives, and results were recorded. Figure 1 shows how the Cryptosporidial cysts as seen on modified ZN stain.

Patients presented with wide range of symptoms, like history of chronic diarrhea, nausea, bloating, abdominal pain and weight loss, but only those fulfilling the Rome IV criteria were included in the study. All patients not fulfilling the Rome IV criteria were excluded.

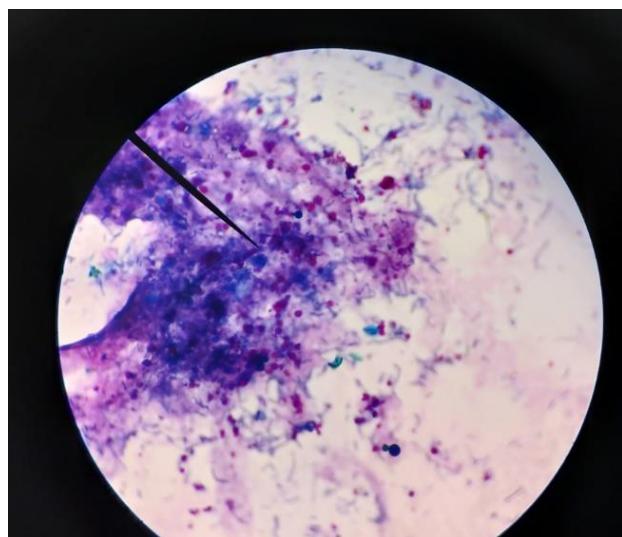


Figure 1. Cryptosporidial cysts.

Results

A total of 62 samples were received from immunocompetent patients presenting in the Medical, Gastroenterology and Infectious diseases OPD with the symptoms of IBS, in the said period of time. Out of a total of 62 samples 34% were found positive for the cysts of *Cryptosporidium* on modified Ziehl Neelsen staining.

Table 1 shows the total number of samples received with the gender wise distribution of the samples positive for *Cryptosporidium* in different age groups.

Table I: Gender-wise distribution of Cryptosporidium isolates in different age groups.

Age Group	Gender	
	Total males (n=41)	Total females (n=21)
	Positive for Cryptosporidium	Positive for Cryptosporidium
20 to 30 years	04	00
30 to 40 years	03	05
40 to 50 years	04	03
50 to 60 years	02	00
Total positive (n=21)	13	08

Table II: Speciality wise distribution of Cryptosporidium isolates.

Total number of patients positive for Cryptosporidium	Speciality (OPD)		
	Medical	Gastroenterology	Infectious diseases
21	4	7	10

19% out of the total positive samples (n=21) were received from the Medical OPD, 33% were received from the Gastroenterology OPD and 48% from the Infectious diseases OPD. Out of a total of 21 positive samples 62% were males and 38% were females as shown in Table 2.

Discussion

Cryptosporidium discovered by Tyzzer in the year 1907 and considered nonpathogenic till the year 1976 when it was recognized for the first time as an opportunistic pathogen when two human cases were reported to be suffering from diarrhea due to it. It is a zoonotic protozoan, capable of infecting a wide variety of vertebrates, including humans, causing acute gastroenteritis. As asymptomatic carriage is possible with Cryptosporidiosis, the actual incidence cannot be calculated and therefore underestimated.¹⁴ It is reported to be idiosyncratic, due to being intracellular, lacking host and organ specificity, is resistant to many antimicrobial agents and has an ability to autoinfect.¹⁴ Although, associated with diarrhea mainly in immunocompromised individuals, our study was conducted to highlight its incidence in immunocompetent people.

This study was conducted to highlight the importance of the increased incidence of Cryptosporidium being reported in immunocompetent adults for the first time in Swabi.

The incidence reported in our study was 34% which is higher than a study conducted in the District Buner which is in the close vicinity of the area where our study was conducted. They reported their incidence to be almost 30%.¹⁵ Khan et.al.,¹⁵ in addition to the incidence in their area, have also reported the overall prevalence of infection with Cryptosporidium in different regions of Buner district. All the figures reported by them were less than the incidence reported in our study. This prevalence was also higher than many previous studies conducted in the past and reported from different parts of the world including 18% in Canada, 21% in USA and 24% in Australia.^{16,17,18}

As per our study, no substantial evidence was seen between Cryptosporidiosis and gender groups and similar results were reported by Khan et.al.,¹⁵ Similar results were also reported in the studies conducted in Nepal and China^{19,20} and they also reported a random distribution of the positive cases among males and females.

As reported in our results the cases reported were falling in the age group starting from 20 to 60 years of age which are not in accordance with the study conducted in Nepal and Bhutan in which they reported prevalence of Cryptosporidiosis in children less than 5 years of age.²¹ This was contradicting the results in our study in which the entire positive cases were adults.

Another study conducted by Carter et.al.,¹⁴ reported sporadic cases of children, reporting the development of IBS in children being higher than in adults, contrary to our study in which it was only adults who were reported positive for infection with Cryptosporidium.

Our study authenticates the fact that infection due to Cryptosporidium spp. is predominant in all age groups of adults in Swabi. Confirmation of Cryptosporidium species in the stool samples of patients suffering from IBS and being overlooked for long, stresses on the fact that rapid, reliable, and cost-effective diagnostic tests should be developed to improve the detection, reporting, and interpretation of results, and also to include them in the workup of patients presenting with diarrhea and relevant history. This will not only help in a valid diagnosis and thorough treatment, but will also reduce the incidence of new cases and transmission of disease.

Taking these measures will definitely provide a valuable source regarding the prognostic epidemiology of Cryptosporidiosis. Since no effective drugs or vaccines are available, there is a critical need for further research for their development along with effective disinfection

methods for water supplies in order to break the chain of transmission.

Conclusion

Knowing this high incidence i.e. 34% of Cryptosporidium spp. in the healthy adults, necessitates to prioritize steps for the prevention of this infection by implementation of public health measures.

Limitations: Diagnosis till species level could not be carried out due to limited resources.

References

- Pogreba-Brown K, Austhof E, Armstrong A, Schaefer K, Villa Zapata L, McClelland DJ et al. Chronic gastrointestinal and joint-related sequelae associated with common foodborne illnesses: a scoping review. *Foodborne Pathog Dis* 2020; 17:67-86. <https://doi.org/10.1089/fpd.2019.2692>
- Feng Y, Ryan UM, Xiao L. Genetic diversity and population structure of Cryptosporidium. *Trends Parasitol*. 2018; 34:997-1011. <https://doi.org/10.1016/j.pt.2018.07.009>
- Jumani RS, Blais J, Tillmann HC, Segal F, Wetty D, Ostermeier C, Nuber N, Lakshman J, et al. Opportunities and Challenges in Developing a Cryptosporidium Controlled Human Infection Model for Testing Antiparasitic Agents. *ACS Infect Dis*. 2021 May 14;7(5):959-968
- Helmy Y.A., Spierling N.G., Schmidt S., Rosenfeld U.M., Reil D., Imholt C., Jacob J., Ulrich R.G., Aebischer T., Klotz C. Occurrence and distribution of Giardia species in wild rodents in Germany. *Parasites Vectors*. 2018;11:213. <https://doi.org/10.1186/s13071-018-2802-z>
- Helmy YA, Hafez HM. Cryptosporidiosis: From Prevention to Treatment, a Narrative Review. *Microorganisms*. 2022; 10(12):2456. <https://doi.org/10.3390/microorganisms10122456>
- Carter BL, Chalmers RM, Davies AP. Health sequelae of human cryptosporidiosis in industrialised countries: a systematic review. *Parasit Vectors*. 2020 Sep 4;13(1):443. <https://doi.org/10.1186/s13071-020-04308-7>
- Ghosh S, Sharma JVP. Evaluation of the Symptom-Based Diagnostic Criteria for Irritable Bowel Syndrome in Conjunction With Clinical Examinations and Laboratory Investigations. *Cureus*. 2023 May 5;15(5):e38567. <https://doi.org/10.7759/cureus.38567>
- Kalantari N, Gorgani-Firouzjaee T, Ghaffari S, Bayani M, Ghaffari T, Chehrazi M. Association between Cryptosporidium infection and cancer: A systematic review and meta-analysis. *Parasitol Int*. 2020 Feb;74:101979. <https://doi.org/10.1016/j.parint.2019.101979>
- Lacy BE, Patel NK: Rome criteria and a diagnostic approach to irritable bowel syndrome. *J Clin Med*. 2017, 6:10.3390/jcm6110099. <https://doi.org/10.3390/jcm6110099>
- Jadallah KA, Nimri LF, Ghanem RA. Protozoan parasites in irritable bowel syndrome: A case-control study. *World J Gastrointest Pharmacol Ther*. 2017 Nov 6;8(4):201-207. <https://doi.org/10.4292/wjgpt.v8.i4.201>
- Jumani RS, Bessoff KLove MS, Miller P, Stebbins EE, Teixeira JE, Campbell MA, et al. Huston CD 2018. A Novel Piperazine-Based Drug Lead for Cryptosporidiosis from the Medicines for Malaria Venture Open-Access Malaria Box. *Antimicrob Agents Chemother* 62:10.1128/aac.01505-17. <https://doi.org/10.1128/AAC.01505-17>
- Brainard J., Hammer C.C., Hunter P.R., Katzer F., Hurle G., Tyler K. Efficacy of halofuginone products to prevent or treat cryptosporidiosis in bovine calves: A systematic review and meta-analyses. *Parasitology*. 2021;148:408-419. <https://doi.org/10.1017/S0031182020002267>
- Centers for Disease Control and Prevention. Laboratory identification of parasitic diseases of public health concern. Available from: URL: <http://www.cdc.gov/dpdx/diagnosticProcedures/stool/specimenproc.html>
- Carter BL, Stiff RE, Elwin K, Hutchings HA, Mason BW, Davies AP, Chalmers RM. Health sequelae of human cryptosporidiosis-a 12-month prospective follow-up study. *Eur J Clin Microbiol Infect Dis*. 2019 Sep;38(9):1709-1717. <https://doi.org/10.1007/s10096-019-03603-1>
- Khan A, Shams S, Khan S, Khan MI, Khan S, Ali A. Evaluation of prevalence and risk factors associated with Cryptosporidium infection in rural population of district Buner, Pakistan. *PLoS One*. 2019 Jan 2;14(1):e0209188. <https://doi.org/10.1371/journal.pone.0209188>
- Thivierge K, Iqbal A, Dixon B, Dion R, Levesque B, Cantin P, et al. Cryptosporidium hominis is a newly recognized pathogen in the Arctic region of Nunavik, Canada: molecular characterization of an outbreak. *PLOS Negl Trop Dis*. 2016; 10:e0004534. <https://doi.org/10.1371/journal.pntd.0004534>
- Becker DJ, Oloya J, Ezeamama AE. Household socioeconomic and demographic correlates of Cryptosporidium seropositivity in the United States. *PLOS Negl Trop Dis*. 2015; 9:e0004080. <https://doi.org/10.1371/journal.pntd.0004080>
- Ng JS, Eastwood K, Walker B, Durrheim DN, Massey PD, Porigneaux P, et al. Evidence of Cryptosporidium transmission between cattle and humans in northern New South Wales. *Exp Parasitol*. 2012; 130:437-441. <https://doi.org/10.1016/j.exppara.2012.01.014>
- Samendra S, Dev RJ, Nabaraj A, Ram CP, Krishna PP, Madhav KC, et al. Prevalence of Cryptosporidiosis Among School Going Children in Kathmandu, Nepal. *EC Microbiol*. 2016;4: 641-646.
- Liu H, Shen Y, Yin J, Yuan Z, Jiang Y, Xu Y, et al. Prevalence and genetic characterization of Cryptosporidium, Enterocytozoon, Giardia and Cyclospora in diarrheal outpatients in China. *BMC Infect Dis*. 2014;14:14-25. <https://doi.org/10.1186/1471-2334-14-25>
- Bodhidatta L, Wongstitwilairoong B, Khantapura P, Shrestha SK, Wangchuk S, Raj PA, et al. Cryptosporidiosis: Prevalence in Children in Nepal and Bhutan. *J Vet Med Res*. 2016;3:1059.