# Original Article

# Stealthy Invader: Cryptosporidium's Unseen Prevalence in Western Maharashtra

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## Abstract

**Background:** In developing countries, enteric parasitic protozoa of the genus *Cryptosporidium* have emerged as a major source of diarrhoea. They can cause both acute and chronic diarrhoea in immune-compromised as well as immunocompetent individuals. The purpose of this study was to ascertain the prevalence of *Cryptosporidium* spp. as an enteric pathogen responsible for diarrhoea in immunocompetent individuals.

**Objective**: To estimate the prevalence of *Cryptosporidium* infection among immunocompetent individuals suffering from gastrointestinal (GI) symptoms.

**Materials and Methods:** A cross-sectional study was conducted at the Microbiology Laboratory of a tertiary care hospital in Western Maharashtra from March 2022 to February 2023. Stool specimens from patients presenting with GI symptoms were collected. To identify *Cryptosporidium* oocysts, direct stool smears were prepared, air-dried, and stained using a modified Kinyoun's acid-fast technique. Oval and round acid-fast bodies (dark pink), 4-5 µm in size, were identified as *Cryptosporidium* oocysts after being examined by two observers.

**Results:** A total of 132 samples were included in this study, of which 110 (83.3%) tested positive for *Cryptosporidium* oocysts. The study found a high prevalence (83.3%) of *Cryptosporidium* spp. in immunocompetent patients with GI symptoms.

**Conclusion:** The significant prevalence identified in this study underscores the necessity of routine screening for *Cryptosporidium* parasites as part of diagnostic protocols for patients presenting with GI symptoms.

***Keywords:***

*Immunocompetent, Cryptosporidium, prevalence, oocyst*

## Introduction

Cryptosporidium spp., a protozoan parasite, gained recognition as a human pathogen in 1976 and is a major cause of waterborne illness worldwide. Cryptosporidium spp. belongs to the phylum Apicomplexa and infects the microvillous border of the gastrointestinal epithelium in a wide range of hosts, including humans. The most common route of transmission for cryptosporidiosis is the faecal-oral route, which involves consuming live oocysts of animal or human origin, emitted with faeces that contaminate food or water [1]. It is one of the major causes of diarrhoeal disease in humans globally.

Cryptosporidium causes self-limited diarrhoeal illness in immunocompetent hosts and persistent or chronic diarrhoea in

immunocompromised hosts, and its prevalence may indirectly indicate sanitation standards. The incidence of Cryptosporidium-associated severe, life-threatening diarrhoea in immunocompromised individuals, as well as in children, markedly increased with the emergence of the human immunodeficiency virus (HIV) and the pandemic of acquired immunodeficiency syndrome (AIDS) [2].

Self-limiting diarrhoea in immunocompetent hosts often presents as acute, profuse, watery diarrhoea associated with abdominal cramps or pain, nausea, vomiting, low-grade fever, malaise, weakness, fatigue, weight loss, and loss of appetite. Symptoms generally begin 2–10 days (average 7 days) after infection with the parasite and last for about 1 to 2 weeks in individuals with healthy immune systems [3].

While there are many reports detailing occurrences in immunocompromised and AIDS patients, data concerning cases in immunocompetent individuals is lacking. This study aims to estimate the prevalence of Cryptosporidium in immunocompetent individuals reported with gastrointestinal (GI) symptoms.

## Materials and Methods

**Case Definition:** *Immunocompetent* – Individual with a healthy immune system and no prior comorbidities. *Immunocompromised* – Individuals such as people living with HIV (PLHIV), transplant recipients, carcinoma patients, or those with neutropenia.

**Study Population and Duration:** A cross-sectional study was conducted from March 2022 to February 2023 in the Department of Microbiology at a tertiary care hospital in Western Maharashtra. A total of 132 stool samples were collected from patients in both outpatient and inpatient departments who presented with GI symptoms, including diarrhea, abdominal pain, vomiting, fever, loss of appetite, and weight loss.

**Sample Collection and Processing:** The stool samples were collected in clean, wide-mouthed, sterile, screw-capped disposable containers. The container label, which included the name, date, location data, and number of containers, was matched with the requisition form. A unique ID was given upon receiving the sample at the microbiology laboratory, and the samples were processed, preferably within 1 hour, for routine microscopy using normal saline and iodine mounts, as well as the modified Kinyoun's acid-fast stain (cold method).

**Exclusion Criteria:** Stool samples received from immunocompromised patients, such as people living with HIV (PLHIV), transplant recipients, carcinoma patients, TB patients, or those with neutropenia.

**Inclusion Criteria:** Stool samples received from immunocompetent patients, with no previously known comorbidities and meeting the general microbiology preanalytical sample acceptance criteria.

**Formol-Ether Concentration Technique:** The samples underwent concentration using the formol-ether sedimentation technique[5]. After centrifugation, the supernatant was carefully removed and discarded, leaving behind a concentrated sediment. This sediment was then subjected to staining using a modified acid-fast method, specifically Kinyoun's stain, to detect oocysts belonging to *Cryptosporidium* spp.

**Modified Kinyoun's Acid-Fast Stain (Cold Method):** The stool sample was uniformly distributed in the center of the slide with constant rotational movement. Measures were taken to avoid making the smear too thick (one should be able to see through the wet material before it dries). The slides were air-dried for 10 minutes with the smeared surface facing up in the dryer. Absolute

methanol was used to fix the dry smear for 1 minute. The entire smear was covered with Kinyoun's carbol-fuchsin solution for 5 minutes. Tap water was used to gently wash the slide. Subsequently, the smear was treated with 1% sulfuric acid for decolorization, and the slide was rinsed with tap water again. A 1% methylene blue solution was added as a counterstain for 4-5 minutes. The smear was allowed to air dry for 5 minutes on the draining rack. The smear was examined microscopically, using 40X and 100X (oil immersion lens) objectives, and scanned thoroughly for parasite identification[4].

**Oocyst Identification:** Oocysts were identified based on their characteristics: typically round to ovoid, with a diameter of approximately 4–6 µm. These structures exhibit acid-fast staining properties, although staining intensity may vary. Some oocysts may remain unstained, while fully sporulated structures may display red coloration and crescentic shapes or bodies. Additionally, sporozoites may be visible within the unstained wall of the oocyst. Quantification of oocysts was performed as follows: 1+ indicating 1–10 oocysts/smear, 2+ indicating 11–50 oocysts/smear, and 3+ indicating more than 50 oocysts/smear[5].

**Statistical Analysis:** The data was collected and subjected to analysis using the Statistical Package for the Social Sciences (SPSS) version 24.0 (SPSS Inc., Chicago, IL, USA) software. Chi-square tests were applied to assess the significance of cryptosporidiosis in males and females.

## Results

A total of 132 stool samples were included in the study (Table 1), of which 68% (90/132) were received from males and 32% (42/132) from females (Fig. 1). The mean age of the patients presenting with diarrhoea was 38.64 years (SD ±14.63). Out of 132 samples examined using the modified Kinyoun's acid-fast staining method, 83.3% (110/132) were positive for *Cryptosporidium* oocysts (Fig. 2), while 16.7% (22/132) yielded negative results (Fig. 3).

In the present study, no significant difference was noted between males (85.5%) and females (78.6%) in contracting cryptosporidiosis (p-value > 0.05). Among the 110 cases where *Cryptosporidium* oocysts were detected, the age group of 21–30 years exhibited the highest prevalence at 27.2% (30/110), while the lowest prevalence of 2.7% (3/110) was found in the age group of ≤10 years (Table 2). This study also noted an escalation in disease incidence during the warm, rainy season and in humid climates. The peak period for cases was from May to September, constituting 69% (76/110) of the reported positive cases during this time frame (Fig. 4).

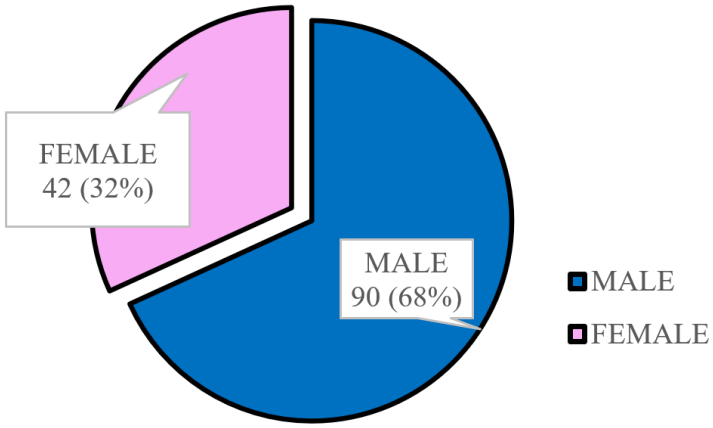


***Figure 1: Gender wise distribution of sample examined for Cryptosporidium***

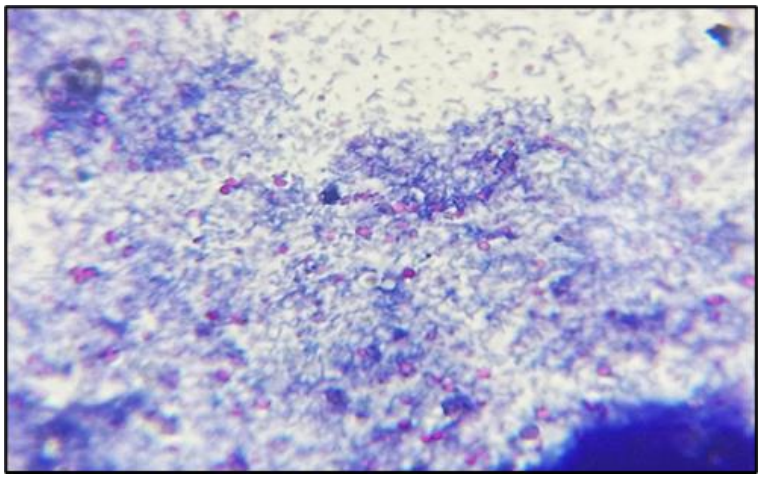

***Figure 2: Oocysts of Cryptosporidium sp. (Kinyoun's acid-fast staining)***

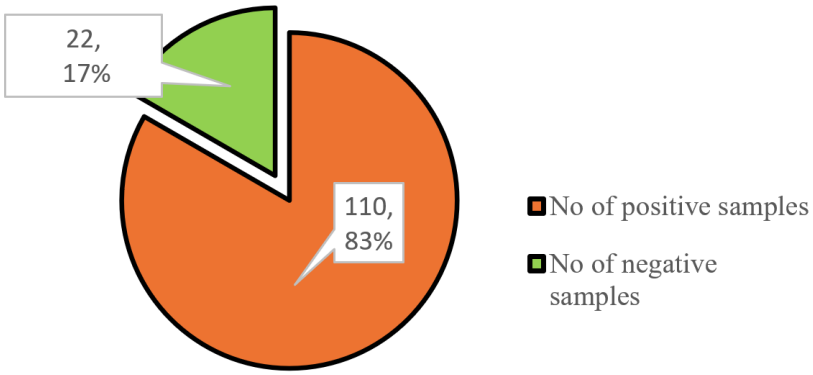


***Figure 3: Prevalence of Cryptosporidium infected cases***

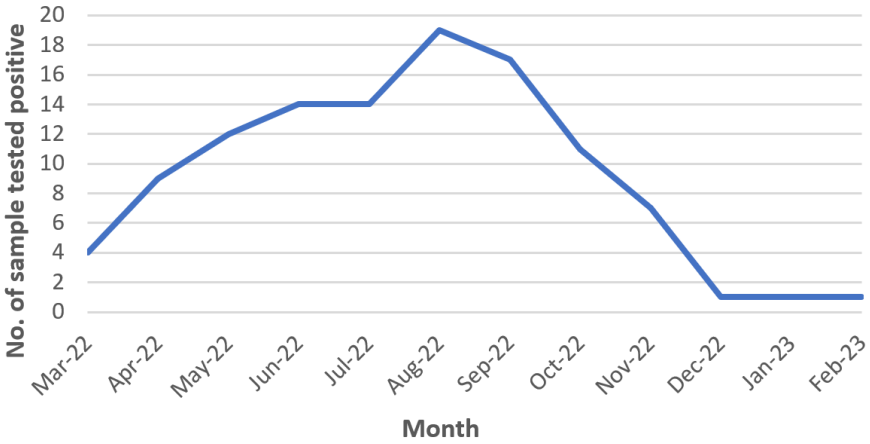


***Figure 4: Month wise distribution of positive sample***

***Table 1: Demography Data Of Enrolled Cases***

| Variable | Frequency | Percentage(%) |
|---|---|---|
| **Sex** | | |
| **Male** | 90/132 | 68 |
| **Female** | 42/132 | 32 |
| **Age Groups (Y)** | | |
| **0-10** | 4 | 3 |
| **11-20** | 12 | 9 |
| **21-30** | 36 | 27.2 |
| **31-40** | 34 | 25.7 |
| **41-50** | 17 | 12.8 |
| **51-60** | 14 | 10.6 |
| **>60** | 15 | 11.3 |

***Table 2: Age-wise distribution of positive cases of Cryptosporidium in immunocompetent patients reported with diarrhoea***

| Age groups (years) | Sample Examined | *Cryptosporidium* detected | | |
|---|---|---|---|---|
| | | Male (%) (n=90) | Female (%) (n=42) | Total |
| **0-10** | 4 | 1 (25) | 2 (50) | 3 |
| **11--20** | 12 | 7 (58.8) | 4 (33.3) | 11 |
| **21-30** | 36 | 25 (69.4) | 5 (13.8) | 30 |
| **31-40** | 34 | 20 (58.8) | 6 (17.6) | 26 |
| **41-50** | 17 | 9 (52.9) | 4 (23.5) | 13 |
| **51-60** | 14 | 7 (50) | 7 (50) | 14 |
| **61-70** | 11 | 5 (45.4) | 4 (36.3) | 9 |
| **71-80** | 4 | 3 (75) | 1 (25) | 4 |
| **Total** | **132** | **77 (58.3)** | **33 (25)** | **110** |

## Discussion

Cryptosporidium is recognized as one of the four primary pathogens responsible for diarrhoeal diseases, particularly in low- and middle-income countries. This parasite is acknowledged as a highly infectious enteric pathogen, predominantly transmitted through the fecal-oral route [6]. The aim of this study was to determine the prevalence of cryptosporidiosis among immunocompetent individuals who presented with gastrointestinal symptoms at a tertiary care hospital in Western Maharashtra. The prevalence of human cryptosporidiosis exhibits significant variation across different regions worldwide.

The prevalence of cryptosporidiosis was found to be 83% in our study, which is in concordance with a study conducted by A. K. Gupta (2011), who reported a prevalence of 66% in Maharashtra [7]. However, various other studies revealed a prevalence rate ranging from 1.3% to 25% across different parts of India [8–10]. S. Sethi et al. (1999) reported a prevalence of 1.3% in North India [11], and a study conducted by Ravinder Kaur et al. (2002) at Maulana Azad Medical College and Lok Nayak Hospital, New Delhi, reported a prevalence of 18.9% in Delhi [12]. A study conducted by Harmesh Manocha et al. (2014) revealed a prevalence of 35-36% in adults, with a male preponderance (69%) compared to females [10,13]. However, no such significance was noted in our study.

The study conducted by Tahira et al. (2012) revealed the highest prevalence of Cryptosporidium in the age group 2–4 years, with a rate of 18.36% and no cases of cryptosporidiosis in the age group of 8–12 years. Our study revealed the highest prevalence of

cryptosporidiosis in the age group of 21–30 years (27.2%), followed by 31–40 years (23.6%), and the lowest prevalence in the age group <10 years. This result aligns with the study conducted by Tamomh et al. (2018) [14]. The low prevalence in the age group <10 years may be due to a small sample size (n=4).

This study highlights an escalation of cryptosporidiosis incidence from May to September, as also noted by Ikiroma IA et al. (2021), P. K. Mada et al., and Jyotsna S. Jagai et al. (2009) [15–17]. There is a need for molecular research to improve the sensitivity and specificity of diagnostic methods, which can provide a more accurate estimation of the prevalence of cryptosporidiosis.

Cryptosporidiosis poses a significant burden in regions like Maharashtra and requires comprehensive strategies to address transmission and seasonal variations. Improved sanitation and water quality measures are needed to reduce environmental contamination and prevent the spread of the disease. Additionally, there must be an increased focus on preventive measures for healthcare providers and the public. Effective treatment of infections in immunocompetent individuals can decrease the overall disease burden and indirectly benefit immunocompromised patients. However, tailored treatments for immunocompromised individuals are crucial for managing severe cases.

***Limitation:*** The study was conducted in a single tertiary care hospital in Western Maharashtra, with a small sample size. Pediatric samples were not included as no samples were received from the pediatric ward/OPD/ICU during the study period. This limited geographic and demographic scope may not be representative of broader populations, potentially limiting the generalizability of the findings. The use of the modified Kinyoun's acid-fast staining method may have limitations in sensitivity and specificity compared to molecular methods like PCR, potentially leading to an overestimation of Cryptosporidium cases.

## Conclusion

The study highlights a concerning 83.3% prevalence of cryptosporidiosis among immunocompetent individuals with gastrointestinal symptoms, underscoring critical public health implications. Routine screening is essential for patients with gastrointestinal symptoms, especially in similar environmental and healthcare settings.

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