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Case Series

Cryptosporidiosis in HIV-seronegative patients: A case series

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ABSTRACT

Enteric infections by *Cryptosporidium spp* are a frequent cause of diarrhea among immunocompromised and HIV infected patients but there are limited reports of their occurrence in immunocompetent patients. Cryptosporidiosis is a faeco-orally transmitted protozoan infection and is one of the leading causes of chronic parasitic diarrhea. Here we report a case series of cryptosporidiosis from three different patients where two patients are immunocompetent and one is a post renal transplant case. All the patients came with watery diarrhea for which stool samples were collected and examined by modified Ziehl Neelsen acid fast staining (Kinyoun's method). On microscopic examination of smear, multiple, pink, round to oval acid fast oocysts of 4-6 μ m diameter were seen against a blue background morphologically resembling *Cryptosporidium spp*. Early diagnosis and prompt initiation of antiparasitic drugs aids in timely treatment and prevention of disseminated cryptosporidiosis.

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1. Introduction

Cryptosporidium is a coccidian parasite of many animal species and is a common cause of diarrhea in virtually all human populations with a particular predilection for those who have contact with animals, patients with the acquired immunodeficiency syndrome (AIDS), international travelers, and persons living in tropical regions of the developing countries.^{1,2}

Although the disease is largely self-limiting in the immunocompetent hosts but in the immunocompromised populations such as organ transplant recipients treatment is particularly challenging due to its propensity to cause significant morbidity and mortality. Diagnosis of the disease in the susceptible population is dependent on having a high index of suspicion especially in low-prevalence areas as routine stool sampling for ova, cysts, and parasites is not diagnostic.³

Cryptosporidiosis is usually transmitted by poor sanitary conditions with low levels of personal hygiene and by ingestion of water tainted with human waste. Centers for Disease Control and Prevention (CDC) reports have shown that outbreaks due to this organism have increased even in the developed countries such as the United States owing to contamination of recreational and municipal water supplies.⁴ The oocysts are quite durable and can remain contagious under moist conditions for even 6-8 months.⁵ Studies have shown that the median infectious dose is just 132 oocysts and infected cases can excrete up to 10⁸ oocysts per day.^{2,6}

Enteric parasitic infestation is a major public health problem especially in the tropical regions of the developing countries. *Cryptosporidium spp* can cause severe diarrhea and malnutrition leading to fatal outcomes in the immunocompromised. There are numerous reports of their occurrences in immunocompromised and AIDS patients but there is paucity of data in HIV sero-negative and immunocompetent cases. In light of the above, we present

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three cases of cryptosporidiosis- one in a post renal transplant patient and two in immunocompetent patients.

2. Case 1

A 51-year old male patient was admitted to the in-patient department of our tertiary care hospital with complaints of loose watery stools for 2 days. He also experienced epigastric pain, nausea, fatigue and decreased appetite. He experienced up to 3-4 episodes of non-bloody watery diarrhea per day with weight loss of about 6 kg over the past 4 months. Patient was hypertensive, diabetic and had chronic kidney disease for which renal transplant was performed in 2017 outside our facility. He had also suffered from COVID-19 infection two years back. There was no history of fever, shortness of breath, rash, cyanosis, and edema. Systemic examination was essentially normal. He was conscious, well oriented and his vitals were satisfactory. Aerobic cultures for blood, urine and stool were negative for any pathogenic bacteria. However, the patient continued to receive broad spectrum antibiotics, steroids, probiotics, immunity boosters and multivitamins for his prevailing illnesses. Viral markers for HIV, Hepatitis B and Hepatitis C tested outside were also negative and there was no history of any high risk behavior.

His blood sample was subjected to the biochemistry, pathology, and microbiology laboratories for routine testing. The laboratory work-up revealed white blood cell count: 8000/ μl with 82% neutrophils, 8% lymphocytes, 8% monocytes, 0.7% eosinophils; hemoglobin: 12.6 g/dL; platelets: 138,000/ mm^3 ; the liver and kidney function tests were within normal limits.

A stool sample was collected which was subjected to stool examinations such as wet mount and modified Ziehl Neelsen (mZN) acid fast stain staining. In wet mount no pus cells, RBC, ova, cyst or trophozoites were seen. Smear was prepared and stained by modified Ziehl Neelsen acid fast stain and seen under 100X oil immersion. On microscopic examination, multiple, pink, round to oval acid fast oocysts of 4-6 μm diameter were seen against a blue background morphologically resembling *Cryptosporidium spp* (Figure 1). Patient was treated with an antiparasitic drug Nitazoxanide 500mg twice a day orally for 6 days and was normally discharged. However, the stool sample was positive for *Cryptosporidium* oocysts even after 21 days of follow up. He was continued on Nitazoxanide and advised follow up visits.

3. Case 2

A 59-year-old male patient came to the outpatient department of our hospital with chief complaints of loose stools 3-4 episodes per day since last 3-4 months. The diarrhea was watery, copious, non- bloody with pain abdomen, nausea and chest pain. Fever and other

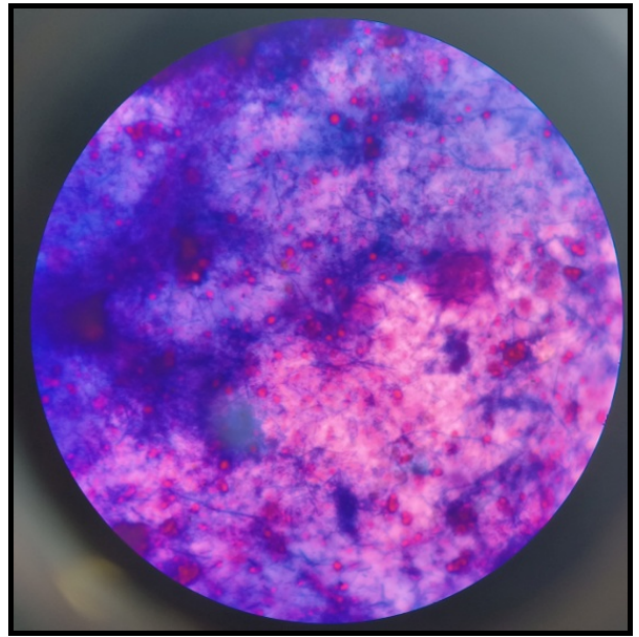


Fig. 1: Showing acid fast oocysts of *Cryptosporidium spp* in Kinyoun's acid fast staining case 1

systemic symptoms were absent. Patient also experienced weight loss of 5-6 kg in last 3-4 months. There was no history of fever, shortness of breath, rash, cyanosis, and edema. Systemic examination was essentially normal. The patient was deemed immunocompetent as there was no history of diabetes, tuberculosis, hypertension or other immunosuppressing illnesses. Patient was not on any medication for systemic illnesses. Mantoux test for TB was negative. Chest X-ray was satisfactory. Viral markers for HIV, Hepatitis B and Hepatitis C tested outside were also negative and there was no history of any high risk behavior. However, the patient was living in an area with unsatisfactory water and sanitation practices.

The laboratory work-up revealed white blood cell count: 7880/ μl with 63% neutrophils, 30% lymphocytes, 6% monocytes, 0.8% eosinophils; hemoglobin: 12.7 g/dL; platelets: 219,000/ mm^3 ; the liver and kidney function tests were within normal limits.

Diarrhea patients are subjected to laboratory tests only upon clinical suspicion. Patient had chronic diarrhea and did not respond to antibiotics hence common bacterial causes were ruled out. Also, viral etiologies are more common in childhood so parasitic agents were suspected and stool samples were sent for the same. Stool wet mount revealed absence of pus cells, RBC, ova, cyst and trophozoites. Stool for occult blood was also negative. Smear for microbiological testing was prepared and stained by modified Ziehl Neelsen (mZN) acid fast stain and seen under 100X oil immersion. On microscopic examination, multiple, pink, round to oval acid fast

ooocysts of 4-6 μ m diameter were seen against a blue background morphologically resembling *Cryptosporidium spp* (Figure 2). Patient was prescribed antiparasitic drug Nitazoxanide 500 mg twice a day orally with food for 3 days. Unfortunately the patient did not follow up due to likelihood of relief of symptoms hence clearance of oocysts from stool sample could not be ascertained.

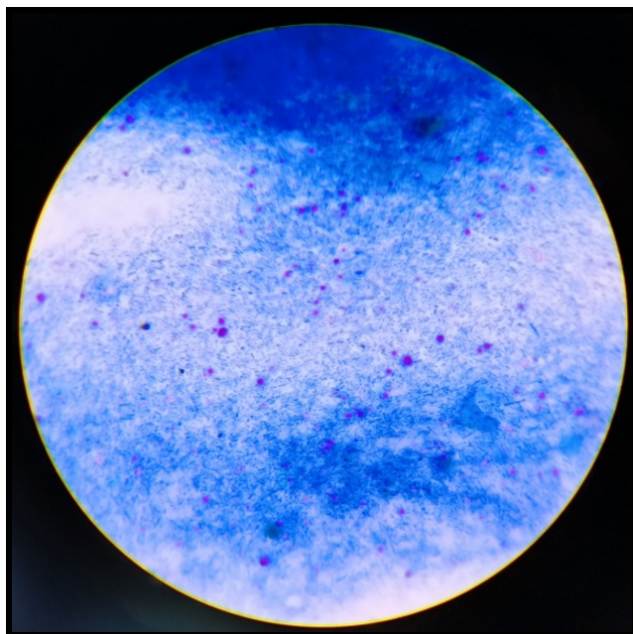


Fig. 2: Showing acid fast oocysts of *Cryptosporidium spp* in Kinyoun's acid fast staining case 2

4. Case 3

A 29-year-old female patient came to the outpatient department of our hospital with chief complaints of watery, non bloody diarrhea for 3 days. She experienced intermittent bouts of diarrhea lasting for 3-4 days over a period of 2 months. The episodes were of copious watery diarrhea with fatigue, vomiting, abdominal discomfort, loss of appetite and malaise. There was no history of blood in stools or vomitus, weight loss, fever or other systemic illnesses. There was no history of fever, shortness of breath, rash, cyanosis, and edema. Systemic examination was essentially normal. The patient was otherwise immunocompetent as there was no history of diabetes, tuberculosis, hypertension or other immunosuppressing conditions. However, the patient was living in a hostel with strained access to clean drinking water and improper food and personal hygiene. She was not on any medications and no past surgical history was found. We also tested her blood for viral markers-HIV, Hepatitis B and Hepatitis C in our facility which were negative and there was no history of any high risk behavior.

The laboratory work-up revealed white blood cell count: 5830/ μ l with 53% neutrophils, 36% lymphocytes, 10% monocytes, 0.1% eosinophils; hemoglobin: 12 g/dL; platelets: 326,000/ mm^3 , and the liver and kidney function tests were within normal limits.

Similar to the case 2, bacterial and viral etiologies were ruled out and sample was subjected to parasitic stool examination. Stool wet mount revealed absence of pus cells, RBC, ova, cyst and trophozoites. Smear for microbiological testing was prepared and stained by modified Ziehl Neelsen (mZN) acid fast stain and seen under 100X oil immersion. On microscopic examination, multiple, pink, round to oval acid fast oocysts of 4-6 μ m diameter were seen against a blue background morphologically resembling *Cryptosporidium spp* (Figure 3). Patient was prescribed antiparasitic drug Nitazoxanide 500mg twice a day orally with food for 3 days. Unfortunately the patient did not follow up due to likelihood of relief of symptoms hence clearance of oocysts from stool sample could not be ascertained.

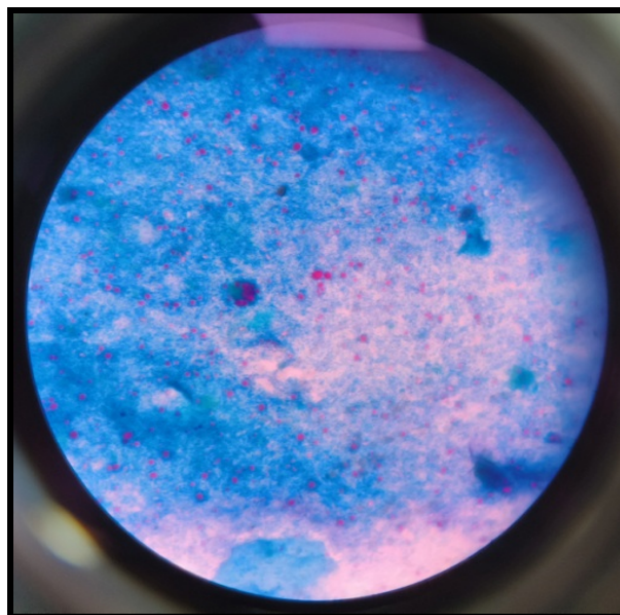


Fig. 3: Showing acid fast oocysts of *Cryptosporidium spp* in Kinyoun's acid fast staining case 3

A comparative analysis of the three cases is shown in Table 1.

5. Discussion

Cryptosporidium spp are associated with chronic and life-threatening diarrhea in patients with AIDS or other immunosuppressive conditions. In immunocompetent hosts, cryptosporidiosis is usually self limiting but can be associated with acute or persistent diarrhea with low grade fever, weight loss, nausea, vomiting, and abdominal discomfort.^{4,7} Although numerous species have been

Table 1: Showing comparative analysis of the three cases

Features	Case 1	Case 2	Case 3
Clinical Presentation	Loose watery non-bloody stools, weight loss, epigastric pain, nausea, fatigue, decreased appetite	Copious, non-bloody diarrhea, pain abdomen, nausea and chest pain, weight loss	Intermittent bouts of diarrhea, fatigue, vomiting, abdominal discomfort, loss of appetite and malaise
Viral Markers	Negative	Negative	Negative
Additional Tests (CBC/LFT/KFT/Cultures)	Unremarkable	Unremarkable	Unremarkable
Chronic Illness	Hypertensive, diabetic, chronic kidney disease, history of SARS CoV2, post renal transplant	None	None
Immune status	Immunocompromised	Immunocompetent	Immunocompetent
Hygiene status	Satisfactory	Unsatisfactory	Unsatisfactory
Follow up	Failure to clear oocysts from stool even after 21 days	Lost to follow up due to likelihood of relief of symptoms.	Lost to follow up due to likelihood of relief of symptoms.

identified but *C. parvum* and *C. hominis* are the most common species to infect humans.^{4,8} Various studies have revealed that *C. hominis* is the dominant species across the world.^{8–10}

Cryptosporidium is the second most prevalent diarrhea causing parasite after Giardia and is a leading cause of water and food borne enteritis especially in the developing nations.^{4,11–13} Infection is transmitted by faeco-oral route by ingestion of oocysts with contaminated drinking water. However, zoonotic and anthropogenic transmission has also been implicated in certain cases.¹³ Cryptosporidium only infects the brush border epithelium of the intestine as opposed to the deep tissue areas.⁴ Incubation period ranges from 2-10 days following exposure. Each oocyst releases four sporozoites that develop into trophozoites in the gut which get encapsulated in the intestine. These multiply asexually and form micro and macro gametes which then fuse to give rise to diploid oocysts that are excreted in stool (thick oocysts). These oocysts are the infective form of the parasite and can also be transmitted via autoinfection (thin oocysts).¹⁴

In the first case that we have reported, the patient failed to clear oocysts from his GIT on account of his immunodeficiency which may be due to organ transplant, diabetes or consumption of immunosuppressive drugs. Appropriate anti-cryptosporidial therapy for the immunocompromised patients still remains a challenging task. The second and third cases were otherwise healthy but may have acquired cryptosporidiosis from faeco-oral transmission by consumption of contaminated drinking water or improper personal hygiene. *C. parvum* oocysts are known to contaminate even treated drinking water as they can resist disinfection, chlorination and ozonation of water sources. However, treatment and filtration have been known to reduce the number of oocysts in drinking water.² The second and third cases were likely relieved of their symptoms and since there are financial constraints

of multiple visits in a corporate hospital, it can possibly explain why they did not come for follow up visits. In view of the above, repeat testing of stool samples could not be carried out and clearance of oocysts from stool samples after treatment could not be ascertained. Only the first case came for follow up as his symptoms persisted due to likely immunosuppression. Since Cryptosporidium diarrhea is not a notifiable disease hence its occurrences around this area could not be ascertained. However, only second case is presently residing in Delhi and the other is a hostel student in Haryana who had only sought treatment in Delhi upon referral from a relative.

There is scanty data of opportunistic enteric parasitic infection in immunocompetent patients with diarrhea. Few studies have reported frequency of *Cryptosporidium spp* to be 21% in Chennai and 66% in Maharashtra.^{15,16} Prevalence in north India ranges from 36% to 50% in adult diarrhea cases.^{10,17} A review of 78 reports has shown that *Cryptosporidium spp* prevalence varies in developed and developing nations with seropositivity has high as 90% in Brazil and 50% in China to 22% in high income countries.^{4,8,17} High temperatures, exposure to farm animals and more rainfall have been associated with increased risk for cryptosporidiosis.⁴

Although, molecular methods such as PCR and immunofluorescence microscopy are considered the gold standard still light microscopy using modified acid fast stain continues to be the most commonly used method for diagnosing coccidian parasites such as *Cryptosporidium spp* in the stool samples.¹¹ However, stool copro-antigen detection by immunochromatographic tests (ICT) and ELISA based methods are also available for easy and rapid diagnosis. Stool FilmArray gastrointestinal panel (GIP) is also increasingly becoming popular owing to its quick turn-around time.³ ICT has a disadvantage of detecting only *C. parvum* antigens and ELISA results need to be confirmed by mZN staining. In light of the above, microscopy

continues to be the preferred method of diagnosis in low prevalence areas owing to its good sensitivity and specificity than other rapid diagnostic methods.⁹

Progress for newer anti-cryptosporidial drug development has been slow due to the challenges of in-vitro culture of this organism.¹² At present, Nitazoxanide, a thiazolide antiprotozoal, is a Food and Drug Administration (FDA) approved treatment that reduces the load of parasites and is recommended as first line drug for Cryptosporidiosis in immunocompetent individuals.^{6,7} Therapy still remains a challenge for the immunocompromised.

6. Conclusion

Opportunistic enteric parasites such as *Cryptosporidium* are causative agents of diarrhea not only in the immunocompromised but also in immunocompetent hosts. The aforementioned cases highlight the need for effective diagnostic techniques such as light microscopy that aid in timely diagnosis and prompt initiation of anti-cryptosporidial therapy. Adequate hand washing, good personal hygiene, use of bottled water coupled with filtration of drinking water sources are essential components of an anti-cryptosporidial bug wagon.

7. Source of Funding

None.

8. Conflict of Interest

The authors declare no conflicts of interest.

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