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Case Report

First case report of Cyclosporiasis from eastern India: Incidence of *Cyclospora cayetanensis* in a patient with unusual diarrheal symptoms

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Abstract

Cyclospora cayetanensis, a recently described coccidian parasite causes severe gastroenteric disease worldwide. Limited studies are found on the incidence of *C. cayetanensis* infection from India; hence remains largely unknown. To date, no case of cyclosporiasis from eastern India has been reported. In this study, we described an incidental case of *C. cayetanensis* in a 30 years old Bengali female patient with no travel history from eastern India. In June 2022, the patient presented with a history of diarrhoea persisting for more than two months with continuous passage foul smelling stools for which she took multiple antibiotics that were ineffective. There were no Salmonella, Shigella, or Vibrio-like organisms in the patient's faecal sample, and Toxin A/B of Clostridium difficile was also not detected by ELISA. The patient was HIV-negative. Finally, UV autofluorescence and DNA-based diagnosis confirmed the presence of *C. cayetanensis*, and the treatment with a combination of appropriate antibiotics was successful. This case report could raise awareness about *C. cayetanensis* associated diarrhoeal cases in India.

Key words: Cyclosporiasis; *Cyclospora cayetanensis;* diarrhoea; India.

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Background

Cyclospora cayetanensis is a recently described intestinal protozoan parasite belonging to the family *Eimeriidae*, subclass Coccidia, phylum Apicomplexa [1,2]. It was first reported to be an unidentified Isospora-like coccidian parasite causing diarrhoea in patients of Papua New Guinea by Ashford (1979) [2]. Later, in 1994, Ortega, Gilman & Sterling proposed the name *C. cayetanensis* after observing the sporulation and excystation of the organism and the unique morphological features of its oocysts via both light and electron microscopy [2]. Until now, *C. cayetanensis* has been identified solely from the human enteric tract and is now considered an emerging organism, able to cause a severe gastro-enteric disease called cyclosporiasis, especially in immunocompromised individuals, children, and the elderly [2]. Human cyclosporiasis can range from asymptomatic to severe, and if not treated, clinical symptoms can persist for several weeks to a month or more [3,4]. The clinical outcomes are associated with the age and immune response of the host [2,4]. Presently, the biology, risk factors, and routes of transmission of *C. cayetanensis* remain poorly understood [5]. *C. cayetanensis* transmitted to new human hosts via the faecal-oral route or through food and water contaminated by oocysts [6]. Non-sporulated oocysts of *C. cayetanensis* require a maturation period of 7–14 days outside the host body under favourable environmental conditions and thus become infectious [7]. The infective oocysts contain two ovoid sporocysts, each containing two sporozoites. Therefore, the oocysts in fresh stool are non-infectious and it is highly unlikely to be infected by this parasite via fresh stool through faecal-oral contact [8].

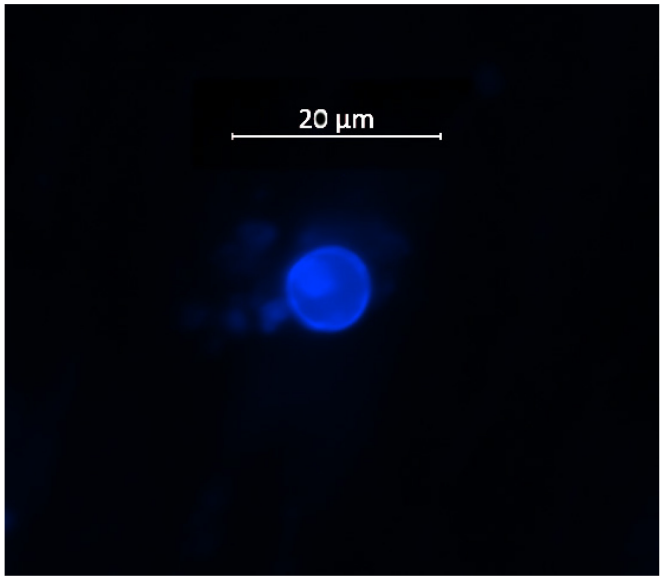
Cyclospora is responsible for several outbreaks worldwide in the last two decades, despite having endemicity in only tropical and sub-tropical areas [8]. Although, most of the cases reported in non-endemic

areas have been linked with travellers returning from endemic countries or through food imported from said countries [8]. *Cyclospora* infections have been reported over the years in travellers and residents of endemic areas such as Peru, Haiti, Guatemala, Nepal, India, the USA, Central America, South Asia, and Eastern Europe [9]. There have been reports of *C. cayetanensis* cases from different parts of India but none from eastern India [10]. In the following study, we present an incidence of *C. cayetanensis* in a female patient who has been experiencing diarrhoeal symptoms for more than two months from a suburban area of Kolkata in eastern India.

Case presentation

A 30 years old Bengali female patient with no travel history, living in a suburban area of Kolkata, India, was diagnosed with Hodgkin's Lymphoma-Nodular sclerosis Stage IV-BESX (Spleen involved) in June 2008 and was treated with Chemotherapy and radiotherapy during 2008-2009. After the therapy, a complete clinical remission of the disease was ascertained. She came to Tata Medical Center, Kolkata, for a follow-up in 2011, and since then there has been no evidence of disease recurrence at regular annual follow-up visits. In June 2022, she was presented with a history of diarrhoea with continuous passage foul smelling stools for more than two months. Administration of several prescribed antibiotics (for example, Ciprofloxacin 400 mg every 12 hours / Norfloxacin 400 mg every 12 hours) by the physician showed no considerable impact. Unlike commonly occurring gastrointestinal disorders, this case had no history of definite abdominal pain, vomiting, fever, and weight loss. A history of recurrent oral or anogenital ulcers was also not noticed. The presence of *Salmonella*, *Shigella,* or *Vibrio*-like organisms was not reported after 48 hours of incubation in bacterial culture. Toxin A/B of *Clostridium difficile* was not detected by ELISA. The HIV antibody test was also negative. The stool specimen was then sent for parasite examination. Faecal smears were subjected to Modified Acid-fast stain, and microscopic examination was carried out with an Olympus CX41 microscope, and microphotographs were taken with an Olympus DP12 microscope camera system at medium (400×) and then high (1000×) magnification. Microscopic observations showed few light pink to dark red, and round 8-10 µm sized oocysts with numerous spherical bodies suggestive of *Cyclospora* species. These oocysts were noticeably larger than the oocysts of *Cryptosporidium* spp.

Figure 1. Autofluorescence of *C. cayetanensis* oocysts under UV light illumination with excitation at 330-365 nm using DAPI filter.



The stool sample was sent to the Division of Parasitology, ICMR-NICED, Kolkata for further characterisation and confirmation. Initially, the patient was prescribed Tab Nitazoxanide 500 mg orally every 12 hours for 2 days until final confirmation of *Cyclospora* species from ICMR-NICED, Kolkata. Under a UV fluorescence microscope (ZEISS Axio Observer7 ApoTome.2), the oocysts displayed typical blue autofluorescence with UV excitation set at 330-365 nm against a black background using a DAPI filter (Figure 1). The fluorescence microscope is equipped with EC Plan-Neofluar 40 × / 0.75 NA objective. DNA extraction was performed for molecular identification with the QIAmp® DNA stool mini kit (Qiagen®). PCR detection was performed using 18S rRNA as the target gene. The primer sequences employed were as follows: F1E 5'- TACCCAATGAAAACAGTT-3' (outer forward primer), R2B 5'- CAGGAGAAGCCAAGGTAG-3' (outer reverse primer), F3E 5'- CCTTCCGCGCTTCGCTGCGT-3' (inner forward primer) and R4B 5'- CGTCTTCAAACCCCCTACTG-3' (inner reverse primer) [10]. Nested PCR amplification of the sample successfully produced 294 bp amplicons, which confirmed *C. cayetanensis* DNA in the sample (Figure 2). The amplified PCR product was purified by Roche PCR Gel Extraction Kit. The purified PCR product was then sequenced in both directions using the standard BigDye terminator V3.1 sequencing kit (Applied Biosystems, USA). The Basic Local Alignment Search Tool (BLAST) of NCBI finally confirmed the identity

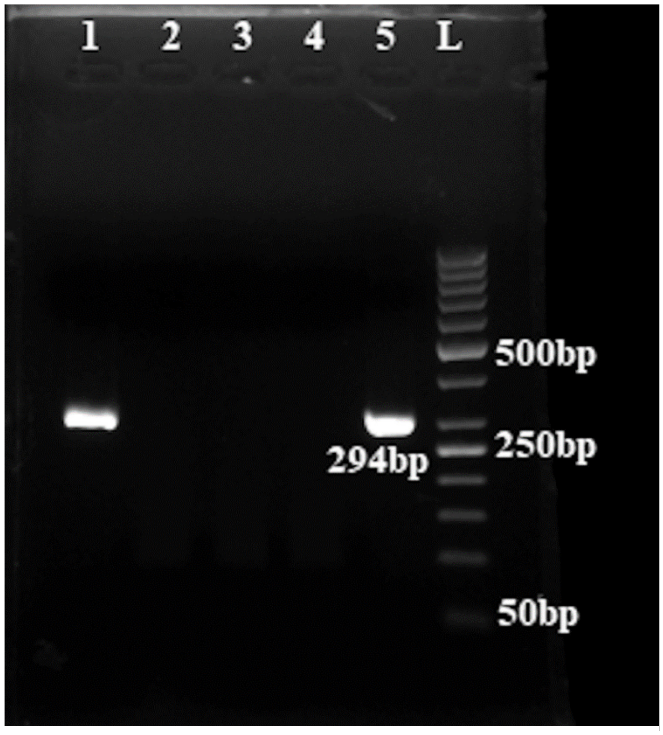
of this organism as *C. cayetanensis.* The obtained sequence showed 100% homology with previously described isolates (GenBank with accession numbers MN893894.1, KY770759.1, GQ292779.1, MN893894.1, and FJ009129.1). *C. cayetanensis* sequence identified in the case study has been deposited in GenBank under the accession number OP364971. After confirmation of *Cyclospora* via PCR and DNA sequencing from the Division of Parasitology, ICMR-NICED, treatment was switched to Tab. Septran DS (960 mg) 1 tab twice a day for 10 days. The symptoms of diarrhoea disappeared after the administration of the drug and the patient recovered.

Discussion

C. cayetanensis is endemic in Indian subcontinent [10]. However, the epidemiology of this emerging coccidian parasite remains largely unknown in India. In endemic regions, *Cyclospora* infection is generally self-limiting in immunocompetent peoples because of their acquired immunity, and only occasional sporadic relapses occur [11-13]. Until now, cyclosporiasis outbreaks in eastern India have not been recorded. This is the first study that reports the incidental detection of *C. cayetanensis* in Eastern India. Environmental infection sources of *Cyclospora* infection also have not been investigated in this region. This case report indicates that *C. cayetanensis* could become a public health issue in this region. Community-based studies are highly recommended to determine endemic status in both asymptomatic carriers and symptomatic patients. *C. cayetanensis* infections are often reported by travellers (traveller's diarrhoea) returning from endemic areas [4]. The patient has no history of travel. Therefore, the source of infection and transmission route was not determined in this case. Still, contaminated water and raw fruits or fresh vegetables might have been the suspected source of the infections. Recently a study from Italy identified *C. cayetanensis* in dogs, domestic birds, and monkeys (*Mulatta mulatta*), and the possible zoonotic nature of *C. cayetanensis* was supported by this evidence [14]. Therefore, zoonotic transmission also might be associated with an infection route, and understanding the range of this species in its actual natural hosts and its transmission should have critical implications for the control and prevention of cyclosporiasis.

The patient had suffered from Hodgkin's Lymphoma-Nodular sclerosis, a group of blood cancers that usually develops in the lymphatic system [15]. She received chemotherapy and radiotherapy, which was a predisposing factor for immunosuppression. Certain types of chemotherapy and radiotherapy have a higher likelihood of causing immunosuppression up to nine months after treatment, leaving patients vulnerable to opportunistic pathogens than the general population [16-17]. The patient in the present case report was not an immunocompromised individual as she was treated for cancer about 14 years ago. Yet, she had been suffering from cyclosporiasis for more than two months, which is unusual. We recommend an accurate diagnosis of unusual diarrheal disease to obtain effective and empiric treatment. *C. cayetanensis* infects both immunocompetent and immunocompromised individuals. In most cases, Cyclospora-infected individuals recover without problems [18]. It is more severe in individuals with poor health (an immunocompromised host), particularly in HIV-infected patients. Therefore, many questions remain unanswered about the incidence of infection in the presented case report.

Figure 2. Amplification of 18S rRNA locus of *C. cayetanensis* using nested PCR assay with template DNA isolated from the patient's stool sample.



Nested PCR product size: 294 bp. Lanes 1: sample DNA, Lanes 2: stool DNA positive for *Cryptosporidium parvum*. Lanes 3: stool DNA positive for *Cryptosporidium hominis.* Lanes 4: Negative control; Lanes 5: Positive Control for *C. cayetanensis.* L: 50 bp DNA ladder.

Conclusions

This finding is alarming and requires the attention of all health professionals, physicians, and health

authorities for urgent planning and implementation in terms of prevention and control strategies against the disease.

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Ethical approval
The protocol of this study was approved by the Institutional Human Ethics Committee of ICMR-National Institute of Cholera and Enteric Diseases, Kolkata. Informed consent was obtained from the patient.

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