

Atypical Presentation of *Blastocystis*-Related Dysentery in an HIV-Infected Patient

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Abstract: *Blastocystis hominis* (*B. hominis*) is a protozoan parasite that inhabiting the gastrointestinal tract of humans and animals. Often considered a commensal parasite, severe manifestations are uncommon and isolated. This case report describes a case of *B. hominis* infection in an HIV-seropositive individual manifesting as dysentery. The diagnosis was confirmed through both microscopic and quantitative Polymerase Chain Reaction (qPCR), which proved to be highly sensitive and specific for detecting this parasite. The patient was treated with cotrimoxazole and metronidazole in addition to antiretroviral therapy. The findings underscore the importance of utilizing advanced diagnostic techniques to accurately identify *B. hominis* in immunocompromised patients, thereby facilitating prompt and appropriate management.

Keywords: *Blastocystis hominis*, HIV, bloody diarrhea, qPCR

Introduction

Blastocystis hominis (*B. hominis*) is an intestinal protozoan parasite frequently identified in humans and a variety of animals.^{1,2} It is estimated that one billion of the world population harbours *Blastocystis* sp. although the prevalence of *B. hominis* varies significantly depending on population demographics and geographical location, with higher rates reported in regions with inadequate sanitation.^{3,4} Most infections are asymptomatic though occasionally it can cause gastrointestinal symptoms such as diarrhea, abdominal discomfort, and nausea.⁵

In individuals with compromised immune responses, such as those infected with the Human Immunodeficiency Virus (HIV), the risk of opportunistic infections is markedly increased.⁶ Despite not being categorized as a classical opportunistic infectious agent, *B. hominis* may behave opportunistically in patients with impaired immune systems due to increased potentials of colonization and symptom development.⁶⁻⁸ Nevertheless, it is still unclear how exactly *B. hominis* infection affects the course of HIV and its treatment efficacies.

Conventional diagnostic methods for *B. hominis* using microscopic examination of stained stool samples often lack the sensitivity and specificity required for accurate detection. To this end, quantitative Polymerase Chain Reaction (qPCR) has emerged as a superior diagnostic technique, offering heightened accuracy in identifying parasitic infections, including those caused by *B. hominis*.⁹

This case report presents atypical presentation of *Blastocystis*-related dysentery in an HIV-infected patient, emphasizing the efficacy of qPCR for rapid and accurate confirmation of this parasite's diagnosis. It also describes the response to

combination therapies. This report highlights the critical role of advanced diagnostic techniques in the management of infections in immunocompromised individuals.

Case Description

A 20-year-old male was admitted to the outpatient clinic of Dr Saiful Anwar General Hospital in Malang, East Java, Indonesia, with a complaint of diarrhea about seven times per day which was experienced for the past four days. The diarrhea contained blood and mucus and was accompanied by stomach ache and nausea, with no vomiting. He also experienced fever for the past two weeks which co-existed with coughing and shortness of breath. There was a painless enlargement of the cervical gland on the left side due to cervical lymphadenopathy. The patient also suffered general malaise, which left him feeling of discomfort, illness, or fatigue. His appetite decreased, leading to significant weight loss. His skin was dry and itchy, and rashes appeared on his hands, feet, and almost all over the body surface.

As immunosuppression was suspected, the patient was subjected to HIV testing using three HIV rapid tests, including ViroCheck HIV ½ (RV-H25A, PT Indec Diagnostic, Indonesia), d3TEKS1 HIV 1/2 Antibody Rapid Test (PT Merah Putih Manufaktura, Indonesia), and Arkan Medical One Step Anti-HIV (1/2) and Syphilis Test Combo (PT Arkanindoplast Utama, Indonesia), all of which showed positive results. The CD4⁺ count was down to 78 cells/ μ L. These results ensuring that diagnosis HIV is established.

Further laboratory investigations, including routine blood tests, serology of *Toxoplasma gondii* (ECLIA, Elecsys Toxo IgG.07028008501.V4.en; Elecsys Toxo IgM.07028024501.V4.en), Cytomegalovirus/CMV (ECLIA, Elecsys CMV IgG.07027117500.V4.en; Elecsys CMV IgM.07027133501.V2.en) and HBsAg (ECLIA, Elecsys HBsAg II.08814848500.V5.en), failed to identify any common pathogens. Given the patient's immunocompromised status, a more comprehensive parasitological examination was conducted. Stool samples were collected and prepared using direct smear, Giemsa, and Trichrome stain. Microscopic examination using direct smear identified amoeboid form of protozoa which showed prominent, extended pseudopodia of varying shape, while Giemsa and Trichrome stained samples showed cells containing large central bodies (vacuoles), with thin bands of cytoplasm around and the cells appearing to have "beaded rim". The protozoa appearances were abundantly identified using microscopic examination, with more than five forms per high powered fields observed (Figure 1).

To further investigate the protozoan species, the DNA extraction was performed from his stool samples using (QIAamp DNA Mini Kit, Qiagen, Hilden, Germany). Following this, quantitative polymerase chain reaction (qPCR) was performed to target *Giardia intestinalis*, *Entamoeba histolytica*, *Cryptosporidium* spp., and *Dientamoeba fragilis* as previously published. An additional detection targeting *Blastocystis hominis* was performed using primers 5'-CCTGAGAGATGGCTACCACATCC -3' and 5'- ATAGTCCCGCATTGTGATTATTGT -3', and a Quasar 705-based probe (5'- CGCGTAAATCACCCAATCCTGACACA -3'). The qPCR setup and cycles were performed according to the previously described protocol.¹⁰ The cycle threshold (Ct) was analyzed using CFX Maestro Software (Bio-Rad

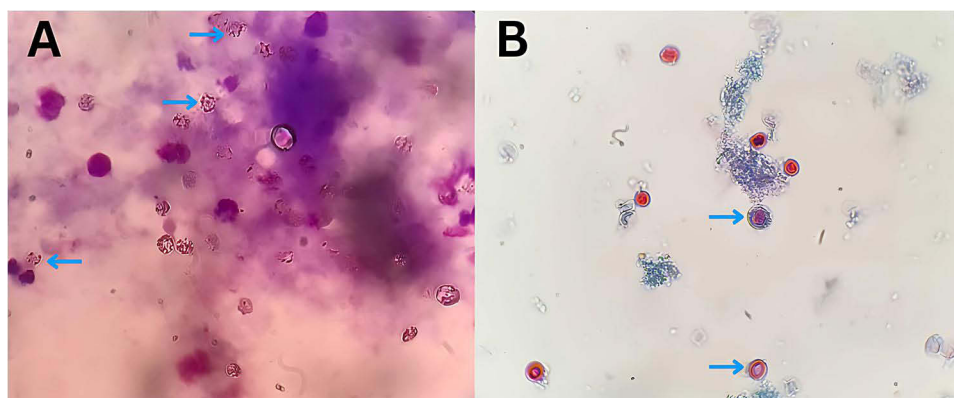


Figure 1 Amoeboid, granular, and vacuolar-like protozoan's form on (A) Giemsa staining and (B) Trichrome staining.

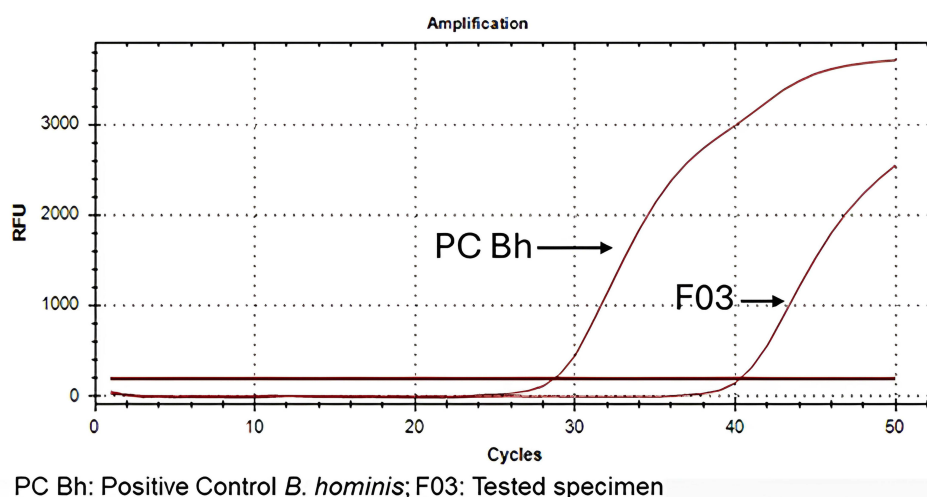


Figure 2 The rt-PCR detection of *Blastocystis hominis*.

Laboratories, Inc., Singapore). The qPCR analysis revealed the presence of *B. hominis* DNA, with a Ct value of 40.29 (Figure 2).

Both microscopy and molecular examinations confirmed the presence of *B. hominis* in the stool sample of the outpatient. The two positive methods for *Blastocystis* (microscopy and qPCR), showed a much stronger association. Thus, our results established a diagnosis of Blastocystosis for the patient. This result is significant considering the rarity of symptomatic *B. hominis* infection.

The patient was then treated with cotrimoxazole 960 mg twice a day and metronidazole 750 mg three times daily for ten days in addition to antiretroviral (ARV) treatment. The ARV prescribed for the patient using the combination of tenofovir 300 mg, lamivudine 300 mg, and dolutegravir 50 mg, per day. After the course of anti-parasitic treatment and ARV, the patient experienced a marked improvement of symptoms. Follow-up microscopic stool examination five days post-treatment showed negative result for *B. hominis* identification, and the patient reported a complete resolution of gastrointestinal symptoms after ten days.

Discussion

Blastocystis hominis is an intestinal protozoan parasite that is often overlooked due to its generally asymptomatic nature. Occasionally, it can cause gastrointestinal symptoms, particularly in immunocompromised patients^{5,11} The case of a *B. hominis* infection in an HIV-infected patient, as described in this report, underscores several important considerations in the diagnosis and management of such infections.

This case is notable because of the atypical *Blastocystis*-related dysentery, as opposed to the commonly asymptomatic feature, found in an HIV-infected patient. Previous study on HIV patients have reported the presence of *B. hominis* in stool samples without clinical symptoms.¹² However, there were others who reported gastrointestinal symptoms related to *B. hominis* infection, which contribute in accelerating the progression of HIV/AIDS.^{13,14}

Although mostly asymptomatic, *B. hominis* infection may cause a range of clinical symptoms, including diarrhea, nausea, vomiting, stomach ache, flatulence, tenesmus, constipation, anorexia, weight loss, lethargy, fever, chills, dehydration, and insomnia. In this case, the patient experienced bloody and mucous diarrhea, with faecal leucocytosis, which was potentially caused by the amoeboid form of the parasite. It has been postulated that amoeboid forms of *B. hominis* contributed to *Blastocystis* pathology due to their capacity to adhere to intestinal epithelial cells.¹⁵ Other report demonstrated that vacuolar forms of *Blastocystis* sp. can reach the lamina propria, submucosa, and muscle layers in mice infected orally with this parasite.¹⁶

The other possibility of dysentery in this case was due to co-infection of *Blastocystis* with other pathogens, especially bacteria. Gastrointestinal symptoms, including abdominal discomfort, loss of appetite, and relative eosinophilia, were reportedly linked to *Blastocystis* sp. and pathogenic bacteria co-infection.¹⁷ In this case, the patient was screened for

common intestinal parasites, however this does not rule out the possibility of bacteria or microsporidia infection. Previous study was revealed that *B. hominis* was positively associated with *Campylobacter jejuni* (*C. jejuni*)/*Escherichia coli* (*E. coli*), *Giardia* spp., Enterotoxigenic *E. coli* (ETEC), Enteropathogenic *E. coli* (EPEC), Shigella/Enteroinvasive *E. coli* (EIEC), and *Cryptosporidium* spp. infection.¹⁸

Thus far, microscopic examination of stool samples is the gold standard and the most common method used in detecting intestinal parasites, particularly in Indonesia. This method is also beneficial for the identification of other findings including white blood cells, Charcot-Leyden crystals, etc.¹⁹ In addition, this conventional diagnostic method is useful to differentiate four distinct morphological forms of *B. hominis*, including vacuolar, granular, amoeboid, and cyst. However, microscopy is frequently reported to have low sensitivity and specificity, which may lead to potential misdiagnosis of *B. hominis*.⁴ In this case, we observed around 7–15 parasites per field of view. The amoeboid, vacuolar, and granular forms of the protozoa are clearly identifiable using conventional microscopy, but the parasite species was unable to be confirmed due to morphological similarity of the amoeboid form with that of *Entamoeba*.

The use of qPCR proved to be pivotal in accurately identifying *B. hominis*, demonstrating higher sensitivity and specificity of molecular techniques compared to traditional methods.^{5,16} The excellent performance of PCR compared to other diagnostic tools lies in its capability to detect low amount of parasite DNAs. Previous research has shown that PCR-based diagnostic approaches are more effective and sensitive for detecting *B. hominis*, with qPCR being the most sensitive. In this case, the qPCR identifies the parasite's small subunit rRNA (SSU-rDNA) gene.⁴ In the future, further determination of *Blastocystis* subtypes in the sample using specific PCR primers or DNA sequencing methods could also provide important information on the molecular epidemiology and pathogenic potential of this parasite.

This case illustrates the need for advanced diagnostic techniques in clinical settings, especially for immunocompromised patients who may present atypical manifestations. Moreover, this case prompts further discussion about the role of routine qPCR screening for gastrointestinal pathogens in HIV-infected patients presenting with persistent gastrointestinal symptoms. While qPCR may not be universally available or cost-effective in all settings, its targeted use in specific cases could improve diagnostic accuracy and patient outcomes.

The successful treatment of the patient with cotrimoxazole and metronidazole, following accurate diagnosis using qPCR, led to the resolution of symptoms, elimination of parasites, and emphasizing the importance of accurate and timely diagnosis in guiding appropriate treatment.^{20–22}

Conclusion

In conclusion, *Blastocystis hominis* is a poorly understood intestinal protozoan, with continuously debatable properties of its pathogenicity. The occurrence of atypical *Blastocystis*-related dysentery in an HIV-infected patient and the successful use of qPCR for its diagnosis highlight critical aspects of managing parasitic infections in immunocompromised individuals. The integration of advanced molecular diagnostics into routine clinical practice can significantly enhance the detection and treatment of such infections, ultimately improving patient care leading to effective treatment and improved patient outcomes, particularly in immunocompromised individuals.

Informed Consent for Publication

Written consent for publication of case details was obtained from the patient. Institutional approval to publish the case details has been obtained from the Health Research Ethics Commission of the Faculty of Medicine Universitas Brawijaya (Approved on August 8, 2023, with the approval number 253/EC/KEPK/08/2023).

Acknowledgments

The authors would like to acknowledge to the patient for his willingness and Faculty of Medicine Universitas Brawijaya, Malang, Indonesia, for supporting this study.

Funding

This research received funding by a grant from the Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia (grant number: 3173.2/2/UN10.F08/PN/2023).

Disclosure

The authors report no conflicts of interest in this work.

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