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# *Entamoeba histolytica* and *Cryptosporidium* co-infections in an HIV-infected, viral suppressed patient with a normal CD4 count

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*Dear Editor,*

*Entamoeba histolytica* and *Cryptosporidium* are significant enteric pathogens affecting men who have sex with men (MSM) and people with HIV (PWH).[1,2] While molecular diagnostic tools have enhanced parasitic detection in PWH with normal CD4 counts in Western countries,[3] such data remains limited in Taiwan.

Here we present a 32-year-old male with HIV diagnosed since January 2018 and currently under antiretroviral therapy with dolutegravir and lamivudine, presented in January 2024 with persistent watery diarrhea. His CD4 count was 398 cells/mm$^3$ with undetectable viral load. His stool samples tested negative for *Campylobacter*, *Salmonella*, *Shigella*, and Shiga-toxin-producing *Escherichia coli* by multiplex PCR (BD Max™ Enteric Bacterial Panel, New Jersey, US). Additionally, tests for Rotavirus and Norovirus were negative. Due to the persistence of symptoms and elevated serum C-reactive protein levels, antibiotics were initiated covering bacterial gastroenteritis.

Despite empiric antibiotics, his symptoms worsened over two months, developing bloody stools, abdominal cramping, 4-kg weight loss, and urinary incontinence. Colonoscopy revealed hyperemic mucosa with small ulcers, and biopsy showed focal active colitis. His serum anti-*E. histolytica* antibody (*Entamoeba histolytica* ELISA IgG, NovaTec Immundiagnostica GmbH, Dietzenbach, Germany) was negative. Multiplex PCR (BD Max Enteric Parasite Panel, New Jersey, US) was employed and positive for *E. histolytica* and *Cryptosporidium*, confirmed and validated by in-house PCR and Sanger sequencing. The primer sets were AWA995F/AWA1206R for *Cryptosporidium* and En_outer1/En_outer1R/Eh1/Eh2 for *E. histolytica* (Fig. 1).[4,5] *Giardia* was detected in the multiplex PCR panel, although the in-house PCR was negative. The patient was treated with metronidazole 750 mg three times daily for one week, and his symptoms completely resolved. His urine sample tested positive for gonorrhea but negative for chlamydia by nucleic acid amplification tests. After treatment for amebiasis, the patient declined intensive follow-up visits due to improvement in diarrheal symptoms. It was not until his scheduled routine visit for HIV three months later that he received ceftriaxone treatment for his gonococcal urethritis. During this visit, multi-site surveillance testing for sexually transmitted infections (STI) was performed, which revealed concurrent rectal gonorrhea. He did not receive the following luminal amebicide, paramomycin.

This case presents several aspects. First, it demonstrates an unusual co-infection of amebiasis and cryptosporidiosis in a PWH with normal CD4 count. Prolonged watery diarrhea was more consistent with cryptosporidiosis, while bloody stools and ulcerative lesions suggested amebiasis. Second, the negative serological test but positive PCR for *E. histolytica* emphasizes that early diagnosis of amebiasis requires comprehensive testing, including molecular diagnostics and potentially endoscopic evaluation. Third, the patient's recovery without anti-*Cryptosporidium* treatment suggests either self-limited cryptosporidiosis or a possible bystander co-infection (though less likely). Finally, the discrepancy between multiplex PCR and in-house PCR for *Giardia* shows potential limitations and possible false-positivity of multiplex panels. Subsequent testing revealed gonococcal urethritis. Given the significant improvement in diarrheal symptoms following metronidazole, concurrent rectal gonorrhea was less likely to be the primary cause of the patient's diarrhea.

In Taiwan, where cryptosporidiosis is rare due to usual water-boiling practices, this case underscores the importance of enhanced parasitic pathogen detection in MSM and PWH with gastrointestinal symptoms. It also emphasizes the value of molecular diagnostic tools and multi-site STI screening in managing such high-risk populations.

## CRediT authorship contribution statement

**Chun-Hsien Chen:** Writing – original draft, Data curation, Conceptualization. **Wei-Hung Cheng:** Methodology, Investigation, Data curation, Conceptualization. **Ling-Shan Syue:** Validation, Methodology, Investigation, Conceptualization. **Ming-Chi Li:** Investigation, Data curation, Conceptualization. **Chin-Shiang Tsai:** Writing – review & editing, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

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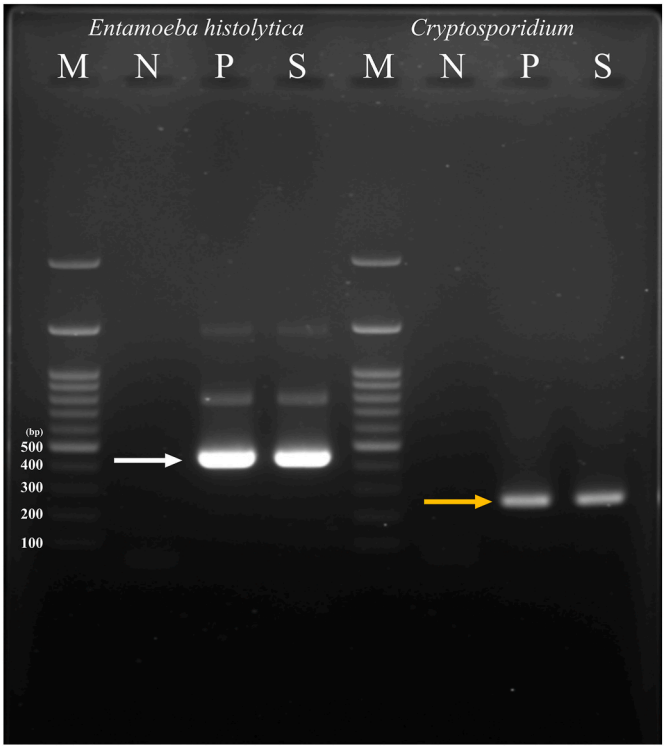


**Fig. 1.** Detection of *E. histolytica* and *Cryptosporidium* in stool specimens using agarose gel electrophoresis. The figure showed the positive detection of *E. histolytica* and *Cryptosporidium* in stool specimens. M represents the DNA 100-bp molecular size markers; P is the positive control containing DNA of *E. histolytica* and *Cryptosporidium*; N is the negative control using $ddH_2O$. The arrows indicate the expected target products, with the blue arrow representing *E. histolytica* and the yellow arrow representing *Cryptosporidium*.

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