Le Infezioni in Medicina, n. 1, 125-129, 2025
doi: 10.53854/liim-3301-12

A new autochthonous case of amebiasis in Italy in a patient without risk factors: an alert for clinicians and laboratorians

Lucia Corich[1], Igor Bramuzzo[2], Francesca Perandin[3], Davide Treggiari[3], Salvatore Scarso[3], Silvia Rigo[1], Shamanta Grosso[1], Paola Diamante[1], Maria Teresa Urbano[4], Massimo Crapis[2], Giancarlo Basaglia[1], Manuela Avolio[1].

[1] Laboratory of Microbiology and Virology, Azienda Sanitaria Friuli Occidentale Santa Maria degli Angeli, Pordenone, Italy;
[2]Department of Infectious Diseases, Azienda Sanitaria Friuli Occidentale Santa Maria degli Angeli, Pordenone, Italy;
[3]Department of Infectious-Tropical Diseases and Microbiology, IRCCS Sacro Cuore Don Calabria Hospital, Negrar di Valpolicella, Verona, Italy;
[4] Gastroenterology Unit, Azienda Sanitaria Friuli Occidentale Santa Maria degli Angeli, Pordenone, Italy.

Article received 18 October 2024 and accepted 10 January 2025

SUMMARY

We describe a rare case of intestinal amebiasis of unknown source, in an immunocompetent patient with no risk factors. The detection of an autochthonous *Entamoeba histolytica* strain causing ulcerative colitis (UC) in a healthy individual was unexpected, but it was the second time it had occurred in Italy in a few months therefore, it highlighted the urgency to establish a new approach in the diagnosis of UC and more generally in the diagnosis of inflammatory bowel disease, involving routine parasitological investigation even in a non-endemic area.

Keywords: amebiasis, *Entamoeba histolytica*, inflammatory bowel disease, polymerase chain reaction, sequencing.

INTRODUCTION

Amebiasis is caused by *Entamoeba histolytica* and is the second leading cause of death from parasitosis worldwide [1]. It has a cosmopolitan distribution, but is widespread mainly in tropical and subtropical countries or in regions with low socio-economic levels and poor hygiene. In developed countries, most cases occur among immigrants and travelers back from endemic regions.
The infection is mainly contracted via the fecal-oral route, therefore, through ingestion of water or food (especially fruit and vegetables) contaminated by faeces containing amoebic cysts. Amebiasis can also be transmitted sexually by oral-anal contact.
E. histolytica infection is asymptomatic in about 90% of cases and it can occur in invasive forms in less than 1% of cases [2]. In symptomatic forms, it presents clinical pictures of variable severity ranging from moderate chronic diarrhea, alternating with periods of constipation, to acute fulminant dysentery. Secondary symptoms such as fever, mucorrhoea, weight loss, weakness, cramping abdominal pain may also appear.
There are several pathogens that can cause enterocolitis with symptoms very similar to those caused by amebiasis, such as *Clostridioides difficile*, *Shigella spp.*, *Escherichia coli*, *Salmonella spp.*, *Campylobacter*

Corresponding author
Lucia Corich
E-mail: lucia.corich@asfo.sanita.fvg.it

spp., *Vibrio spp.*, *Giardia intestinalis* and *Cytomegalovirus* (CMV).
Here, we report an autochthonous case of intestinal amebiasis, of which it was not possible to establish the source of infection, in an immunocompetent patient with no risk factors.

CASE REPORT

In January 2024, a 60-year-old Italian man with bloody diarrhea and rectorrhagia (up to 12 discharges/day) for 1 month performed a colonoscopy at the General Surgery Department of San Vito al Tagliamento Hospital (Italy), which detected procto-colitis in the medium activity phase in a context of suspected ulcerative colitis (UC). Therefore, mesalazine (800 mg 3 caps/die) and subsequently oral prednisone (50 mg/die) were administrated, but without any benefit.
At the end of February 2024, he was admitted to the Gastroenterology Department of Santa Maria degli Angeli Hospital in Pordenone (Italy), due to the persistence of abdominal pain and bowel changes.
The crampy abdominal symptoms had already appeared in 2020 and 2022, so two colonoscopies had been performed resulting within normal limits.
The patient also reported significant weight loss (8-10 kg) in the previous two months, while he had never been feverish.
Blood laboratory tests showed: leukocytes 9.79 x 10^3/µL (reference interval (r.i.) 4.0–11.0), with neutrophils 9.04 x 10^3/µL (r.i. 1.8–8.0) and lymphocytes 0.54 x 10^3/µL (r.i. 1.0–4.5), Hb 13.8 g/dL (r.i. 14.0–18.0), platelets 287 x 10^3/µL (r.i. 140–440), creatine 0.6 mg/dL (r.i. 0.7–1.2), C-reactive protein 10.6 mg/dL (r.i. 0.0–0.5), procalcitonin 0.12 µg/L (r.i. 0.05–0.5 local infections), albumin 2.1 g/dL (r.i. 3.4–5.0) and transferrin 159 mg/dL (r.i. 200-375). Serology for HIV, HBV, HCV, EBV, and VZV was negative. *Mycobacterium tuberculosis* infection was excluded due to the negativity of the Quantiferon TB-Test.
Suspecting a flare-up of inflammatory bowel disease (IBD), intravenous methylprednisolone (40 mg/die) and oral metronidazole (250 mg every 8 hours) were administered, with benefits for the bowel.
Colonoscopy was repeated (Figure 1) with biopsy resampling which confirmed the suspicion of active UC without dysplasia, but also the positivity of CMV by immunohistochemistry was found. The ultrasound of the abdomen excluded liver lesions.

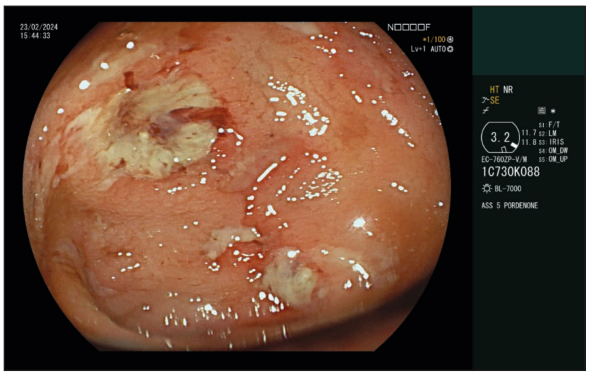

Figure 1 - Rectosigmoidoscopy performed at admission. Rounded ulcers (fairly typical of CMV infection).

Two multiplex real-time RT-PCR tests for bacteria, parasites and viruses (Allplex Gastrointestinal Panel Assays, Seegene and Filmarray Gastrointestinal Panel, Biomerieux) were performed in 3 different stool samples collected before starting therapy and resulted negative for all pathogens but *E. histolytica*, definitively confirmed by DNA sequencing of SSU rRNA gene using forward primer 239F (5-ATTGTCGTGGCATCCTAACTCA-3) and reverse primer 88R (5-GCGGACGGCTCATTATAACA-3) that amplified a 172-bp fragment [3] (Figure 2). Sequencing reactions were loaded into the ABI Prism 3100 Genetic Analyzer and the sequence obtained was used to run a BLAST search

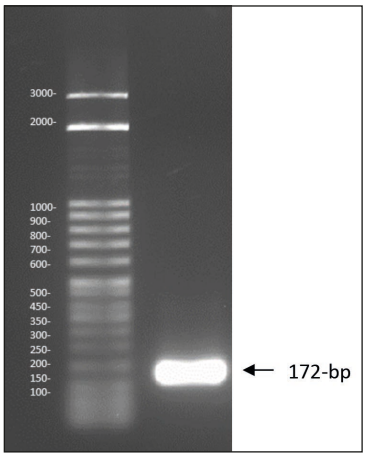

Figure 2
Stained agarose gel with PCR product amplified using primers 239F/88R from a stool sample (lane 2); molecular weight marker (lane 1).

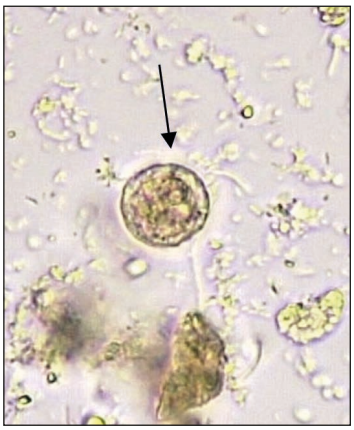

Figure 3
Cyst of *E. histolytica/ E. dispar.*, 40X.

against the GenBank database which identified *E. histolytica*.
According to our molecular-based screening approach, the laboratory required 3 stool samples to be sent for microscopic parasite examination [4]. It showed the presence of cysts of *E. histolytica/dispar* (Figure 3), and the antigen assay (E. HISTOLYTICA QUIK CHEK", TECHLAB) detected *E. histolytica*. The test for IgG antibodies against *E. histolytica* in serum (Bordier Affinity Products) was positive (ratio 4,717).
The patient reported no recent travel abroad, no risky oral-anal sexual intercourse, and no previous corticosteroid or immunosuppressive therapy. He was not a smoker.
The dosage of metronidazole was adjusted to 500 mg orally every 8 hours for 7 days, followed by paromomycin 500 mg orally every 8 hours for another 7 days and valganciclovir 900 mg orally every 12 hours for 21 days. The steroid therapy was gradually reduced and then suspended. Mesalazine was continued for several months.
At subsequent outpatient follow-ups, the patient reported well-being, regularization of the bowel movement with no more bloody diarrhea, and weight recovery of the 10 kg previously lost. Fecal calprotectin had normalized from an initial value of 654 µg/g (r.i <50) to 22 µg/g, and systemic inflammation indices were within range (C-reactive protein 0.5 mg/dL, procalcitonin 0.03 µg/L). The search for *E. histolytica* DNA in stool samples was negative.
In April 2024, he repeated a colonoscopy which still showed ulcerations with fibrin and pancolic erosions, but in the left colon and rectum, there were also signs of cicatricial phenomena (Figure 4). The biopsy performed at the same time showed a histological picture compatible with infectious colitis in the resolution phase.

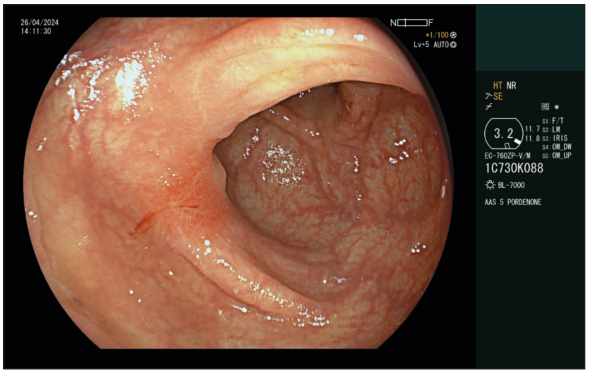

Figure 4 - Rectosigmoidoscopy in post-hospitalization at the end of therapy. Previous ulcerations with erythematous and retracted mucosa are visible.

DISCUSSION AND CONCLUSIONS

Cases of amebiasis have been reported in Italy for several years, but mostly in patients coming from endemic areas and/or presenting predisposing factors [5].
On admission, our patient presented with bloody diarrhea and rectorrhagia. UC was initially suspected, but later the diagnosis changed to infectious pancolitis caused by *E. histolytica*.
This is the second case of autochthonous amebiasis found in Italy in a few months; the previous one had been identified in Brescia in May 2023 [6]. Both patients were immunocompetent and had no risk factors or evident sources of infection, but our patient did not develop a liver abscess, while amebiasis may have predisposed him to secondary CMV infection, which was shown by biopsy.
CMV infection is very common in the intestine and can contribute to inflammatory processes through an increase in the production of IL-6 [7]. It often affects patients with UC, who usually exhibit a flare-up of the disease or an increase in its severity.
Weng MT et al. describe the unfortunate progression of amebiasis with UC and co-infection of CMV and fungi in an HIV-positive patient [8]. On the other hand, in immunocompetent patients, CMV colitis may be self-limiting once the previous

intestinal damage caused by amebiasis has resolved [9].
Some studies have established that there is an association between the *E. histolytica* genotype and the clinical outcome of the infection [10-12]. In fact, the distribution of *E. histolytica* genotypes is significantly different among patients with no symptoms, or with diarrhea/dysentery or with liver abscess [10]. In particular, not all genotypes have the same ability to cause liver abscess, and the genotypes found in liver abscess differ from those found in the intestine [10, 11].
Thus, we might speculate that the difference between the genotypes of the two *E. histolytica* strains recently identified in Italy could underlie the different evolution of the disease in the two patients mentioned.
One of the risk factors for the development of amebiasis is the treatment with steroids, which can also lead to a fulminant progression of amoebic colitis [13, 14]. However, our patient was treated with steroids neither at the time of symptom onset nor at the time of sample collection.
A recent study highlights that many patients diagnosed with UC presented protozoan infections and supported the importance of a routine parasitological investigation in patients with UC [15]. Our case confirms this need and directs attention in particular to patients who do not present the typical risk factors, such as origin from endemic areas, precarious hygienic conditions, homosexuality, immunosuppression, and steroid therapy and, more specifically, extends the alert to clinicians and laboratorians working in Italian hospitals.
Following the diagnostic suspicion of ulcerative pancolitis, our patient had been treated with steroids and mesalazine. Only microbiological investigations have made it possible to set up the correct therapy and to provide for the reduction until the suspension of steroid therapy, which, in the case of amebiasis, is strongly contraindicated [8, 13, 14].
The unexpected finding of *E. histolytica* in the stool samples highlights the fundamental role of the multiplex PCR panel able to identify a pathogen that is absolutely unusual in a patient without risk factors and to simultaneously exclude 21 other enteropathogens (many of which etiological agents of colitis), allowing the appropriate therapy to be promptly started and thus contributing to the success of the ulcerative pancolitis treatment.
A further aspect that emerges from our experience is the high possibility, in cases of UC and more generally in cases of IBD, of getting to a wrong or incomplete diagnosis if the etiopathogenetic complexity of this disease group is not considered [16]. Indeed, the finding of CMV positivity without the contextual parasitological investigation could have directed our diagnosis and therefore, the therapy towards that single target, risking to witness an even more severe evolution of the disease [13, 14]. Thus, in support of a recent study, we also highlight the need to suspect an *E. histolytica* infection in the case of patients diagnosed with CMV enterocolitis, especially if no improvement occurs despite treatment with antiviral and effective reduction in antigenic concentration [14].
In conclusion, our work highlights that:
- recently, cases of autochthonous amebiasis of unknown source in patients without risk factors are emerging in Italy, thus suggesting that this infection may be underestimated;
- even in Italy, it is necessary to introduce a routine parasitological investigation in the case of IBD;
- it is recommended to look for *E. histolytica* before administering therapy with immunosuppressants or immunomodulators, such as corticosteroids, even in patients without risk factors and even in non-endemic areas, such as Italy;
- the use of the vaccine (not yet developed) could be the winning weapon to prevent these rare autochthonous cases from multiplying, seriously endangering the resolution of several clinical cases, both of symptomatic and asymptomatic patients;
- the identification of the aforementioned "undefined sources of infection" could help us select, in the population considered not at risk, the groups to which a possible future vaccination should be recommended.

Conflict of interest
The authors declare they have no conflict of interest.

Funding
None.

Acknowledgements
The authors are grateful to Mrs Maria Luisa Pittini for editing the English text.

REFERENCES

[1] Kumanan T, Sujanitha V, Sreeharan N. Amoebic liver abscess: a neglected tropical disease. *Lancet Infect Dis.* 2020; 20(2): 160-162.
[2] Mohammad FI. A general review on the amoebiasis. *Int J Res Pharm Sci.* 2019; 10: 479-483.
[3] Verweij JJ, Oostvogel F, Brienen EA, Nang-Beifubah A, Ziem J, Polderman AM. Short communication: Prevalence of Entamoeba histolytica and Entamoeba dispar in northern Ghana. *Trop Med Int Health.* 2003; 8(12): 1153-1156.
[4] Avolio M, Tedeschi R, Camporese A. Diagnosis of Acute Gastrointestinal Infections in Hospitalized Patients: A Molecular-Based Screening Approach. *J Med Diagn Meth.* 2019; 8: 284.
[5] Salzano A, Rossi E, Carbone M, et al. [Suburban amebiasis: the diagnostic aspects via computed tomography and echography and the percutaneous treatment of amebic liver abscesses]. *Radiol Med*. 2000; 99(3): 169-173.
[6] De Francesco MA, Villanacci V, Pasini M, et al. Amoebic colitis and liver abscess: A rare case of autochthonous invasive infection due to Entamoeba histolytica. *J Infect Public Health*. 2024; 17(3): 464-466.
[7] Rahbar A, Boström L, Lagerstedt U, Magnusson I, Söderberg-Naucler C, Sundqvist VA. Evidence of active cytomegalovirus infection and increased production of IL-6 in tissue specimens obtained from patients with inflammatory bowel diseases. *Inflamm Bowel Dis*. 2003; 9(3): 154-161.
[8] Weng MT, Wei SC, Leong YL, Tung CC, Shun CT, Wong JM. Amebic and cytomegalovirus colitis mimic ulcerative colitis. *Adv Dig Med.* 2016; 3: 65-68.
[9] Lee CS, Low AH, Ender PT, Bodenheimer HC Jr. Cytomegalovirus colitis in an immunocompetent patient with amebiasis: case report and review of the literature. *Mt Sinai J Med*. 2004; 71(5): 347-350.
[10] Ali IK, Mondal U, Roy S, Haque R, Petri WA Jr, Clark CG. Evidence for a link between parasite genotype and outcome of infection with Entamoeba histolytica. *J Clin Microbiol*. 2007; 45(2): 285-289.
[11] Ayeh-Kumi PF, Ali IM, Lockhart LA, Gilchrist CA, Petri WA Jr, Haque R. Entamoeba histolytica: genetic diversity of clinical isolates from Bangladesh as demonstrated by polymorphisms in the serine-rich gene. *Exp Parasitol*. 2001; 99(2): 80-88.
[12] Ali IK, Solaymani-Mohammadi S, Akhter J, et al. Tissue invasion by Entamoeba histolytica: evidence of genetic selection and/or DNA reorganization events in organ tropism. *PLoS Negl Trop Dis*. 2008; 2(4): e219.
[13] Shirley DA, Moonah S. Fulminant Amebic Colitis after Corticosteroid Therapy: A Systematic Review. *PLoS Negl Trop Dis*. 2016; 10(7): e0004879.
[14] Shijubou N, Sumi T, Kamada K, et al. Fulminant amebic colitis in a patient with concomitant cytomegalovirus infection after systemic steroid therapy: A case report. *World J Clin Cases*. 2021; 9(15): 3726-3732.
[15] Pestechian N, Tavakoli S, Adibi P, Safa AH, Parsaei R, Yousefi HA. Prevalence of Intestinal Protozoan Infection in Patients with Ulcerative Colitis (UC) in Isfahan, Iran. *Int J Prev Med*. 2021; 12: 114.
[16] Griemert T, Siegel E, Brandstetter M, Straub BK, Kreft A, Galle PR, Sprinzl MF. *Entamoeba histolytica*-associated proctitis and ileitis mimicking Crohn's disease-A case report. *Clin Case Rep.* 2023; 11(5): e6833.