Enterocytozoon Bieneusi Infects Children With Inflammatory Bowel Disease Undergoing Immunosuppressive Treatment

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Objectives: Patients with inflammatory bowel disease (IBD) are susceptible to intestinal opportunistic infections due to both defective mucosal immunity and altered immune response resulting from immunosuppressive treatment. Microsporidia infecting the gastrointestinal tract and causing diarrhoea can potentially affect the course of IBD.

Methods: Stool samples (90 IBD children and 121 healthy age-matched controls) were screened for Encephalitozoon spp. and Enterocytozoon bieneusi by microscopy and polymerase chain reaction followed by sequencing.

Results: E. bieneusi genotype D was found in seven out of 90 (7.8%) IBD children. No children from the control group were infected, making the pathogen prevalence in the IBD group significant (P = 0.002). Furthermore, infection was confirmed only in patients receiving immunosuppressive treatment (P = 0.013).

Conclusions: Children with IBD are at risk of intestinal E. bieneusi infection, especially when receiving immunosuppressive treatment. Therefore, microsporidia should be considered as a significant infectious agent in this group of patients.

Keywords: Enterocytozoon bieneusi, inflammatory bowel disease, children, immunosuppressive treatment, molecular characterization

INTRODUCTION

Inflammatory bowel disease (IBD) is a complex, chronic inflammatory condition of the gastrointestinal tract developing as a result of the interaction of genetic and environmental factors. The two main types of IBD are Crohn's disease (CD) and ulcerative colitis (UC). Although the aetiology of IBD is still poorly understood, the role of the gut microbiota combined with genetic susceptibility in disease pathogenesis is highlighted (1). Since immunomodulators are commonly used in the treatment of IBD, patients' immune response is compromised making them more susceptible to opportunistic infections (2). Currently, IBD has evolved into a worldwide disorder with rising prevalence in paediatric populations and age is an additional, independent risk factor for opportunistic infections (3). To date, the majority of reported infections among IBD patients have been caused by bacteria and viruses (4). Reports on fungal infections in the course of IBD are comparatively rare (5, 6).
**Materials and Methods**

**Patients**

Samples from two groups of HIV-negative patients under the age of 18 years were analysed between 2014 and 2017: (i) children with IBD (study group; n = 90), including children with CD (57/90) and UC (33/90), who had been under the care of the 2nd Department and Clinic of Paediatrics, Gastroenterology and Nutrition (Wrocław Medical University, Poland) and (ii) healthy children (control group; n = 121) matched by age with the study group. The revised Porto Criteria recommended by the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPghan) were used for the diagnosis of IBD (14). Clinical CD and UC activity were assessed according to the Paediatric Crohn’s Disease Activity Index (PCDAI) and the Paediatric Ulcerative Colitis Activity Index (PUCAI), respectively. IBD patients were divided according to three clinical frameworks: (i) 43 (47.8%) patients with active disease presenting at, or shortly after, diagnosis with no previous treatment for IBD, (ii) 20 (22.2%) patients with remission (PCDAI/PUCAI scores <10 for at least 12 months) and (iii) 27 (30%) patients with active disease (mild: PCDAI/PUCAI scores 11–25; moderate: PCDAI/PUCAI scores 26–50; severe: PCDAI/PUCAI scores >51 and symptoms of disease).

The immunosuppressive treatment was administered in 52.2% (47/90) of all IBD children, according to current clinical practise. Four groups of immunosuppressive drugs: (i) glucocorticoids, (ii) azathioprine, (iii) methotrexate and (iv) infliximab or adalimumab were implemented in various configurations as shown in **Table 1**. Of 47 patients with immunomodulatory treatment, 38 were treated with one drug only (80.9%), eight with two drugs (17%) and one with three drugs (2.1%). Moreover, at the time of the study, 16.7% (15/90) of IBD children were being treated with metronidazole. Adequate antibiotics were administered in case of five children diagnosed with bacterial infection.

Apart from the diagnosis of IBD, there were no other criteria for participation in the study group. Among clinical data, diarrhoea (defined as at least 3 watery bowel movements per day) and fever (body temperature above 38°C) at the time of examination were taken into account. Additionally, standard laboratory microbiological examinations were carried out among IBD children to identify infections with bacteria of the genus *Salmonella*, *Shigella*, *Campylobacter*, *Yersinia* and toxins A and B of *Clostridium difficile* during acute exacerbation of the basic disease. The following exclusion criteria have been applied for control group: (i) acute infections, (ii) inflammatory, autoimmune or immunodeficiency diseases, and (iii) no previous immunosuppressive treatment history of note.

**Sample Collection**

Single stool samples were collected into disposable sterile containers from all of the patients. In the case of three

**Table 1** | Comparison of Enterocytozoon bieneusi-positive and -negative IBD children’s basic characteristics.

| Characteristic | Enterocytozoon bieneusi-positive (N = 7) | Enterocytozoon bieneusi-negative (N = 83) | P-value |
|---------------|-----------------------------------------|------------------------------------------|---------|
| Age<br>Median years (range) | 11 (10-17) | 14.5 (6-17) | 0.117 |
| Sex<br>Male | 3 (42.9) | 48 (57.8) | 0.461 |
| Female | 4 (57.1) | 35 (42.2) | 0.461 |
| Type of IBD<br>CD | 5 (71.4) | 52 (62.7) | 1.000 |
| UC | 2 (22.2) | 31 (37.3) | 1.000 |
| Symptoms<br>Fever | 0 | 2 (2.4) | 1.000 |
| Diarrhoea | 2 (28.6) | 28 (33.7) | 1.000 |
| Microbiological diagnosis<br>Yersinia | 0 | 1 (2.4) | 1.000 |
| Salmonella group D | 0 | 1 (2.4) | 1.000 |
| Clostridium difficile toxins | 0 | 3 (7.1) | 1.000 |
| Metronidazole | 2 (28.6) | 13 (15.7) | 0.330 |
| Immunosuppressive regimen<br>general immunosuppression | 7 (100) | 40 (48.2) | 0.015* |
| glucocorticoids monotherapy | 0 | 5 (6.0) | 1.000 |
| azathioprine monotherapy | 6 (85.7) | 22 (26.5) | 0.003* |
| adalimumab/infliximab monotherapy | 0 | 5 (6.0) | 1.000 |
| azathioprine + glucocorticoids | 1 (14.3) | 7 (8.4) | 0.491 |
| glucocorticoids + methotrexate + infliximab | 3 (1.2) | 1 (1.2) | 0.000* |

Data represent number (%) unless otherwise indicated; CD, Crohn’s disease; UC, ulcerative colitis; *P-value < 0.05.
IBD children additional sampling after implementation of immunosuppressive treatment was performed. Each sample was split into two parts, one part was immediately fixed in high quality methanol for microscopy and second part was stored, without preservatives at −20°C for 1–2 weeks before molecular analyses.

Microscopic Examination
Standard Calcofluor M2R staining method was used to detect microsporidial spores (15). Briefly, dry methanol-fixed smears were stained with 0.1% Calcofluor M2R (Sigma-Aldrich, St. Louis, MO, USA) in phosphate-buffered saline (PBS) for 10 min, rinsed gently with PBS, stained with 0.5% Evans blue (Sigma-Aldrich, St. Louis, MO, USA) in distilled water for 30 s, rinsed again in PBS, and allowed to air dry. Stained slides were examined by fluorescence microscopy using UV light and at a magnification of 1,000x.

DNA Isolation
For DNA extraction, a total of 200 mg of stool was initially homogenised by bead disruption using a Precellys 24 Instrument (Bertin Technologies, France). Genomic DNA (gDNA) was extracted using the GeneMATRIX Stool DNA Purification Kit (EurX, Gdańsk, Poland) according to the manufacturers’ instructions and stored at −20°C for molecular analyses.

Molecular Examination
gDNA was analysed by genus-specific nested polymerase chain reaction (nested-PCR) protocols amplifying a partial sequence of a small subunit of ribosomal RNA gene (16S rRNA), the entire ITS (internal transcribed spacer) region, and a partial sequence of the 5.8S rRNA gene of E. bieneusi and Encephalitozoon spp. Among all IBD children (n = 90) the median age of patients was 14 years, and ranged between six and 17 years, while in children of the control group (n = 121) the median age was 10 years, and ranged between three and 16 years. Overall, the male-to-female ratio was 51 (56.7%) to 39 (43.3%) in the study, and 58 (47.9%) to 63 (52.1%) in the control group.

RESULTS
Among all IBD children (n = 90) the median age of patients was 14 years, and ranged between six and 17 years, while in children of the control group (n = 121) the median age was 10 years, and ranged between three and 16 years. Overall, the male-to-female ratio was 51 (56.7%) to 39 (43.3%) in the study, and 58 (47.9%) to 63 (52.1%) in the control group.

Sequencing and Phylogenetic Analyses
Secondary PCR products were sequenced in both directions using the Sanger sequencing method. Amplification and sequencing of each sample were repeated twice. Nucleotide sequences were edited using the program ChromasPro 2.1.5 and aligned with each other and with reference sequences from GenBank (www.ncbi.nlm.nih.gov/blast) using MAFFT version 7 (http://mafft.cbrc.jp/alignment/software/). Phylogenetic analyses were performed using MEGA7 software and trees were inferred by the maximum likelihood (ML) method. Bootstrap support for branching was based on 1,000 pseudoreplicates.

Statistical Analysis
The Fisher’s exact test was used to compare categorical variables (sex, type and activity of IBD, presence of fever and/or diarrhoea, applied immunosuppressive treatment and metronidazole) between microsporidia-positive and -negative patients, while the continuous variable (age) was compared using Student’s t-test. A value of P < 0.05 was considered significant.

Ethics Approval Statement
The study was approved by the Human Research Ethics Committee of Wrocław Medical University (KB-24/2014). Written consent was provided by the parents or legal guardians on behalf of all the paediatric patients involved in the study.

TABLE 2 | Sequences of oligonucleotides used for specific Enterocytozoon bieneusi and Encephalitozoon spp. rDNA amplification and direct sequencing.

| Species            | Reaction step | Primer                  | Sequence (5' - 3')                  | Final amplicon (bp) |
|-------------------|---------------|-------------------------|-------------------------------------|---------------------|
| Enterocytozoon bieneusi | Primary       | EBIT3                   | GGTCTATAGGGATGAAGAG                 | ~390 – depending on genotype |
|                   |               | EBIT4                   | TTOGAGTTCCTTCCGCCTC                 |                     |
|                   | Secondary     | EBIT1*                  | GGTCTGAAATCTATGCTGCT               |                     |
|                   |               | EBIT2_4*                | ATTCGCCAGCGGATCCAAAGTG             |                     |
| Encephalitozoon spp. | Primary      | INT580F                 | TGCAATTAAATGTCGTAAGT               | E. cuniculi (~305), E. intestinalis (289) |
|                   |               | INT580R                 | TTTCACTGGCGCTACTCAAG               |                     |
|                   | Secondary     | MANT_3*                 | GGAATTTCAACCCCGCGCTCwTAT           |                     |
|                   |               | MANT_4*                 | CCAAGCTTTAGCTAAAGTymAArGGGT        | E. hellem (~315)    |

*oligonucleotides used for direct sequencing.
No infection caused by these microsporidia was confirmed among children from the control group, making the *E. bieneusi* prevalence in IBD patients statistically significant (*P* = 0.002; data not shown). Phylogenetic analysis revealed the presence of *E. bieneusi* genotype D in all samples. Spores were confirmed microscopically in five out of seven (71.4%) samples (Figure 1). No co-infection with other screened bacteria was observed in any *E. bieneusi*-positive patient.

The *E. bieneusi* infection was confirmed exclusively in a group of children receiving immunosuppressive treatment, which is 14.9% (7/47) of the patients in that group (*P* = 0.013), regardless of its duration (1.5–43 months). Six out of seven (85.7%) *E. bieneusi*-positive IBD patients received azathioprine as a single drug (*P* = 0.003), while one patient received simultaneous therapy with azathioprine and glucocorticoids. Out of three patients diagnosed before and after azathioprine implementation, two were positive for *E. bieneusi*, and infection was confirmed only after immunosuppressant treatment. *E. bieneusi* infection was observed in patients with various activity of IBD (Table 3).

No significant associations were found for the other tested variables (*P* > 0.05; Table 1).

Detailed clinical and demographic characteristics of *E. bieneusi*-positive patients are shown in Table 3.

**DISCUSSION**

Routine examination of infectious agents among IBD individuals includes mainly bacteria and viruses. In our study we found *E. bieneusi*—intracellular parasitic fungus—in 7.8% of children with IBD, while none of the patients from the control group was infected, and this difference was statistically significant. Similar results have been shown in stool samples collected from adults with IBD receiving immunosuppressive agents, where 12.7% of patients were infected with *E. bieneusi* (19).
One of the reasons for increased susceptibility of IBD patients to such opportunistic infections and/or the reactivation of latent infection might be the immune deterioration as a result of immunosuppressive treatment implemented to achieve IBD remission and/or to prevent disease flares. Indeed, in our study E. bieneusi was confirmed exclusively in children with IBD receiving immunosuppressive treatment. Since the duration of immunosuppressive therapy in E. bieneusi-positive patients varied from 1.5 to 43 months, even temporary immunomodulatory treatment may be a risk factor for E. bieneusi infections. This may be confirmed by the detection of E. bieneusi infection in two of our patients after, but not before treatment with immunosuppressive drug, azathioprine.

On the other hand, the ongoing inflammatory process in the course of IBD might facilitate inhabitation of the intestine by pathogens. Since IBD affects the gastrointestinal tract, the influence of this disorder on increased susceptibility to infection may result from the degradation of the intestine and defective mucosal immunity (5). In the course of CD as well as of UC inflammatory infiltrates appear within the intestinal mucosa, destroying mucosal and, in case of UC, also submucosal membrane. Moreover, mucosal inflammation as a result of alterations in the structure and/or quantity of mucins and claudins, could make the intestine more vulnerable to penetration of pathogen (20, 21). It would allow the pathogen to persist in the intestinal mucosa, making the process chronic (5). Furthermore, microsporidia could persist and replicate inside macrophages and in this form, they can be transported throughout the host (22). Brdičková et al. (23) have shown that other microsporidial species, E. cuniculi genotype II, migrates to inflammatory foci probably with the contribution of immune cells participating in its formation (23). If this is the case in patients suffering from chronic inflammatory process, subsequent worsening of the IBD clinical picture might occur.

Epidemiological studies have shown that microsporidiosis among individuals with an impaired immune system is symptomatic, including primarily diarrhoea and fever (12). Since diarrhoea might be a symptom of IBD as well, the difficulties in differentiation the causative agent in cases of infection is generally highlighted (24). Nevertheless, only a minority of microsporidia-positive samples were diarrheal and no significant association between infection and diarrhoea or fever was observed in patients in this study. However, as microsporidia can persist as a latent infection (25), there is a risk of development of symptoms, for instance as a result of increasing the immunosuppressive treatment dose. Moreover, infection with intracellular pathogens in patients with chronic inflammatory process may cause deepening in tissue damage in the place where inflammation persists and worsening of the clinical picture of the disease. Therefore, the need for diagnosing of such infections, especially in immunosuppressed patients, even asymptomatic, is emphasised by the risk of development of more severe symptoms, their incomprehensible impact on the course of the disease and the complexity or the effectiveness of the therapy process.

Interestingly, all of our patients were infected with E. bieneusi, similarly as in the report of Hasani et al. (19). Genotype D which is widespread among different groups of patients, both immunosuppressed an immunocompetent (12) and has a broad geographic range (26), was identified in all these samples. However, regardless of the high E. bieneusi prevalence among immunocompetent people (25), intestinal microsporidial infections, especially in immunosuppressed patients, can be associated also with Encephalitozoon species, mainly E. intestinalis and E. cuniculi. There are studies suggesting that metronidazole, drug used in IBD therapy, might be able to reduce the number of cells infected with Encephalitozoon species in vitro (27) and E. bieneusi responds only temporarily to treatment with metronidazole (28). However, according to the study by Lallo et al. (29) metronidazole is not effective against microsporidia. A similar conclusion can be drawn from clinical studies (30). Therefore, it is difficult to conclude if metronidazole treatment applied in 16.7% of IBD children had an influence on the microsporidial species identified in this study. Moreover, the question as to whether or not processes taking place in the intestine of IBD patients promote in some way inhabitation selectively by E. bieneusi remains unknown and requires detailed investigation. Nevertheless, detailed characterisation of microsporidial species is essential due to the fact that microsporidia treatment is limited and genus-specific. Albendazole is the drug of choice for Encephalitozoon spp. treatment and even though it is relatively well-tolerated, this drug has limited efficacy against E. bieneusi, especially in patients with impaired immune systems (30). Fumagillin reveals higher efficacy against E. bieneusi but shows toxicity and may not be fully effective in eradicating the pathogen (31). In such cases, rehabilitation of the immune system by dose reduction or temporary withdrawal of the immunosuppressive drug may be beneficial and lead to the pathogen elimination without the need for specific treatment (32).

In conclusion we have shown that children with IBD should be considered as a group at risk of microsporidal intestinal infections, especially when receiving immunomodulatory treatment. Even though we have not found any correlation between E. bieneusi infection and course of illness, the impact of such intestinal infection on the inflammatory processes in the bowel of IBD patients cannot be excluded. Since most of E. bieneusi-infected patients were asymptomatic such an unnoticed infection might develop to a chronic condition, as a result leading to progressive intestine devastation. Therefore, it seems that microsporidia should be considered during diagnosis of infectious agents affecting children suffering from IBD.

**DATA AVAILABILITY STATEMENT**

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

**ETHICS STATEMENT**

The study was approved by the Human Research Ethics Committee of Wroclaw Medical University (KB-24/2014).
Written informed consent to participate in this study was provided by the participants’ legal guardian/next of kin.

AUTHOR CONTRIBUTIONS
ZZ and MKi designed research. ZZ, KA, MK, BS, MS, and MKi performed research and analysed data. ZZ, KA, and MKi wrote the manuscript and MK, BS, MS, AH, and BI revised of the manuscript for important intellectual content. All authors had full access to all the data in the study and had final responsibility for the decision to submit the manuscript for publication.

REFERENCES
1. Khan I, Ullah N, Zha L, Bai Y, Khan A, Zhao T, et al. Alteration of gut microbiota in inflammatory bowel disease (IBD): cause or consequence? IBD treatment targeting the gut microbiome. Pathogens. (2019) 8:1–28. doi: 10.3390/pathogens8030126
2. Seyedian SS, Nokhostin F, Malamir MD. A review of the diagnosis, prevention, and treatment methods of inflammatory bowel disease. J Med Life. (2019) 12:113–22. doi: 10.25312/jml-2018-0075
3. Gill EE, Fast NM. Assessing the microsporidia-fungi relationship: combined phylogenetic analysis of eight genes. Gene. (2006) 382.23. doi: 10.1016/j.gene.2006.02.023
4. Gill EE, Fast NM. Assessing the microsporidia-fungi relationship: combined phylogenetic analysis of eight genes. Gene. (2006) 31. doi: 10.1016/j.gene.2006.03.007
5. Didier ES. Microsporidiosis: an emerging and opportunistic infection in humans and animals. Acta Trop. (2005) 94:61–76. doi: 10.1016/j.actatropica.2005.01.010
6. Javanmard E, Mirjali H, Niyati M, Jalilzadeh E, Tabaei SJ, Aghdaei HA. Molecular and phylogenetic evidences of dispersion of human-infecting microsporidia to vegetable farms via irrigation with treated wastewater: one-year follow up. Int J Hyg Environ Health. (2018) 221:642–51. doi: 10.1016/j.ijheh.2018.03.007
7. Wasson K, Peper RL. Mammalian microsporidiosis. Vet Pathol. (2000) 37:113–28. doi: 10.1354/vp.37-2-113
8. Didier ES, Weiss LM. Microsporidiosis: current status. Curr Opin Infect Dis. (2006) 19:785–92. doi: 10.1097/01.qco.0000244055.46382.23
9. Kicia M, Wesolowska M, Kopacz Z, Jakuszkó K, Sak B, Krótonowicz D, et al. Prevalence and molecular characteristics of urinary and intestinal microsporidial infections in renal transplant recipients. Clin Microbiol Infect. (2016) 22:462.e5–9. doi: 10.1016/j.cmi.2016.01.014
10. Kicia M, Wesolowska M, Kopacz Z, Kviá M, Sak B, Sokulska M, et al. Disseminated infection of encephalitozoon cuniculi associated with osteolysis of hip periprosthetic tissue. Clin Infect Dis. (2018) 67:1228–36. doi: 10.1093/cid/ciy256
11. Levine A, Koletzko S, Turner D, Escher JC, Cucchiara S, de Ridder L, et al. The ESPGHAN revised porto criteria for the diagnosis of inflammatory bowel disease in children and adolescents. J Pediatr Gastroenterol Nutr. (2013) 58:795–806. doi: 10.1097/MPG.0b013e3182800023
12. Didier ES, Orenstein JM, Aldras A, Bertucci D, Rogers LB, Janney FA. Comparison of three staining methods for detecting microsporidia in fluids. J Clin Microbiol. (1995) 33:3138–45. doi: 10.1128/jcm.33.12.3138-3145.1995
13. Didier ES, Vosbrinck CR, Baker MD, Rogers LB, Bertucci DC, Shadduck JA. Identification and characterization of three encephalitozoon cuniculi strains. Parasitology. (1995) 111:41–21. doi: 10.1017/s0031182000056914
14. Katzwinzel-Wladarsch S, Lieb M, Helse W, Löschner T, Rinder H. Direct amplification and species determination of microsporidian DNA from stool specimens. Trop Med Int Health. (1996) 1:573–8. doi: 10.1046/j.1365-3156.1996.d01-51.x
15. Buckholt MA, Lee JH, Tzipori S. Prevalence of enterocytozoon bieneusi in swine: an 18-month survey at a slaughterhouse in Massachusetts. Appl Environ Microbiol. (2002) 68:2595–9. doi: 10.1128/AEM.68.5.2595-2599.2002
16. Hasani Z, Aghdaei HA, Balaii H, Azimirad M, Mirsamadi ES, Mirjalali H, et al. The first study on opportunistic intestinal microsporidiosis in IBD patients receiving immunosuppressive medications in Iran. Epidemiol Infect. (2017) 145:2095–9. doi: 10.1017/S0950268816000954
17. Zeissig S, Bürgel N, Grünzel D, Richter J, Mankertz J, Wahnschaffe U, et al. Changes in expression and distribution of Claudin 2, 5 and 8 lead to discontinuous tight junctions and barrier dysfunction in active Crohn’s disease. Gut. (2007) 56:61–72. doi: 10.1136/gut.2006.094375
18. Purr AE, Ranganathan S, Finn OJ. Aberrant expression of MUC1 mucin in pediatric inflammatory bowel disease. Pediatr Dev Pathol. (2010) 13:24–31. doi: 10.23508/08-06-0479.1
19. Valencakova A, Halanova M. Immune response to encephalitozoon infection review. Comp Immunol Microbiol Infect Dis. (2012) 35:1–7. doi: 10.1016/j.cimid.2011.11.004
20. Brdičková K, Sak B, Holubová N, Květonová D, Hlásková L, Kicia M, et al. Encephalitozoon cuniculi genotype II concentrates in inflammation foci. Inflamm Res. (2020) 37:133–4. doi: 10.1002/1529-0131.21811
21. Sak R, Brady D, Pelikánová M, Květonová D, Rost M, Kostka M, et al. Unapparent microsporidial infection among immunocompetent humans in the Czech Republic. J Clin Microbiol. (2011) 49:1064–70. doi: 10.1128/JCM.01147-10
22. Matos O, Lobo ML, Xiao L. Epidemiology of enterocytozoon bieneusi infection in humans. J Parasitol. (2012) 98:1424. doi: 10.1111/j.2015.11298124
23. He Q, Leitch GJ, Vivesvara GS, Wallace S. Effects of nifedipine, metronidazole, and nitric oxide donors on spore germination and cell culture infection of the microsporidia encephalitozoon hellem and

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encephalitozoon intestinalis. *Antimicrob Agents Chemother.* (1996) 40:179–85. doi: 10.1128/AAC.40.1.179

28. Canning EU, Hollister WS. Enterocytozoon bieneusi (Microspora): prevalence and pathogenicity in AIDS patients. *Trans R Soc Trop Med Hyg.* (1990) 84:181–6. doi: 10.1016/0035-9203(90)90247-C

29. Lallo MA, Vidoto da Costa LF, Manoel de Castro J. Effect of three drugs against encephalitozoon cuniculi infection in immunosuppressed mice. *Antimicrob Agents Chemother.* (2013) 57:3067–71. doi: 10.1128/AAC.00157-13

30. Costa SF, Weiss LM. Drug treatment of microsporidiosis. *Drug Resist Updat.* (2000) 3:384–99. doi: 10.1054/drup.2000.0174

31. Molina J-M, Tourneur M, Sarfati C, Chevret S, de Gouvello A, et al. Fumagillin treatment of intestinal microsporidiosis. *N Engl J Med.* (2002) 346:1963–9. doi: 10.1056/NEJMoa012924

32. Kicia M, Wesolowska M, Jakuszko K, Kopacz Z, Sak B, Květonová D, et al. Concurrent infection of the urinary tract with encephalitozoon cuniculi and enterocytozoon bieneusi in a renal transplant recipient. *J Clin Microbiol.* (2014) 52:1780–2. doi: 10.1128/JCM.03328-13

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