Case Report: Cutaneous Amebiasis: The Importance of Molecular Diagnosis of an Emerging Parasitic Disease

Patricia Morán, Liliana Rojas, René Cerritos, Valeria Zermeño, Alicia Valadez, Griselda Montes de Oca, Miguel Ángel Reyes, Enrique González, Oswaldo Partida, Eric Hernández, Miriam Nieves, Tobías Portillo, Marco Gudiño, Manuel Ramiro, and Cecilia Ximénez*

Department of Experimental Medicine, Faculty of Medicine, National Autonomous University of Mexico (UNAM), Mexico City, Mexico; Dermatology Department, General Hospital of Mexico, Ministry of Health, Mexico City, Mexico; Urology Department, General Hospital of Mexico, Ministry of Health, Mexico City, Mexico; Unit of Education, Scientific Research and Health Policy Direction of Medical Benefits, Mexican Institute of Social Security Mexico City, IMSS, Mexico

Abstract. Cutaneous amebiasis is the least common clinical form of human amebiasis in Mexico, sexual amebiasis was only occasionally observed before the late 1980s. However, in the last few decades, most of the documented cases of cutaneous amebiasis from around the world are sexually transmitted. We present two cases of sexually transmitted genital amebiasis. The molecular characterization of the *Entamoeba* species in the affected tissues underlines the importance of an etiological diagnosis using specific and sensitive techniques that avoid the rapid destruction of tissues and the irreversible sequelae to the anatomy and function of the affected organs. In addition, for those interested in the study of the human-amoebic disease relationship and its epidemiology, the detection of a new, mixed infection in an invasive case of amebiasis reveals new perspectives in the study of the extraordinarily complex host-parasite relationship in amebiasis.

INTRODUCTION

Cutaneous amebiasis was first described in 1892 by Nasse,¹ in a patient with an amoebic liver abscess that was complicated after drainage and caused extensive ulceration and necrosis of abdominal skin, subcutaneous tissue, and muscles. No evidence of the presence of trophozoites is mentioned in the original article, but Nasse's clinical diagnosis was that of an amoebic liver abscess and cutaneous amebiasis. This condition is the least common clinical form of human amebiasis and is generally the consequence of an *Entamoeba* infection of previously damaged skin. Ngai and Frazier² reviewed the available literature published before 1936 and found 27 reported cases from China, Indochina, and the United States; thereafter, the number of reported cases of cutaneous amebiasis decreased substantially to 11 cases in 1941.^{3–9} Magaña and others¹⁰ recorded 26 documented cases of cutaneous amebiasis from the clinical files of the Pediatric Dermatology Department of the General Hospital of Mexico of the Health Ministry, and from the Pathology Department of the Hospital of Medical Specialties of the Mexican Institute of Social Security (IMSS) at the XXI Century Medical Center. However, the majority of the reported cases occurred from 1969 to the late 1980s. Cases occurred in patients with deep skin lesions in the anal, perineal, vulval, vaginal, and genital regions, as well as the diaper area of babies with diarrhea caused by *Entamoeba histolytica*. In adults, the most common location of cutaneous amebiasis is the abdominal area, caused by the involvement of the abdominal wall during a spontaneous amoebic liver abscess or as a complication during an amoebic liver abscess drainage procedure.^{11–15} Another type of transmission of amoebic infection is sexual. In 1978, this type of amebiasis was well documented by Hurwitz and Owen in San Francisco.¹⁶ The authors

mention that during 1976, the incidence of reported of laboratory confirmed cases of amebiasis in San Francisco was 101, compared with eight cases in 1970¹⁶; thereafter, the documented cases of this clinical form of amebiasis decreased substantially.

Although cutaneous amebiasis is a rare disease, most of the documented cases of this condition that have been reported in the international literature in the last few decades have been sexually transmitted amebiasis.^{17–21} In the last 12 months, two cases of sexually transmitted perianal amebiasis were documented by Medina-Murillo and Rodríguez-Wong (2011) in Mexico.²²

Our group has been interested in the study of the molecular epidemiology of human amebiasis, especially the molecular characterization of *E. histolytica* and *Entamoeba dispar* genetic variants, which are the most prevalent in Mexico, and is important from an epidemiological point of view and their relation to the clinical outcome of the infection.^{23–26} With regard to the cases of sexually transmitted genital cutaneous amebiasis presented here, the goal is to stress the importance of this clinical form of amoebic disease in the differential diagnosis of both genital and perineal ulcerative lesions in both males and females. We aim to use two cases of cutaneous amebiasis to bring attention to this clinical syndrome and to also review the complexity of the diagnostic process involved. A timely diagnosis and directed treatment strategy are essential to avoiding serious anatomical damage and to ensuring the healing of lesions without future functional sequelae.

CASE REPORTS

Patient 1 was a 38-year-old male with a secondary school level of education and a history of chronic smoking and alcoholism. The disease started after sexual intercourse with a male partner. The first symptoms were preputial and penile edema, areas of induration, constant pain, urethral blood discharge, and blood clots in the urine. The patient was studied as an outpatient by the Urology Department of Mexico's General Hospital SSa in Mexico City D.F. The initial antibiotic therapy

*Address correspondence to Cecilia Ximénez, Departamento de Medicina Experimental, Facultad de Medicina, UNAM, Dr. Balmis No 149 Col. Doctores, Mexico D.F. CP 06726, Mexico. E-mail: cximenez@servidor.unam.mx

was intravenous (IV) as follows: levofloxacin, 500 mg every 24 hr for 10 days; ceftriaxone, 1 g twice daily for 5 days; and clindamycin, 300 mg for 10 days. After 3 days, one of the indurations on the prepuce spontaneously drained a bloody and purulent material without odor. The patient returned to the hospital with no fever or general discomfort, and the clinical examination showed localized dermatosis affecting the trunk, the genital region including the penis at its shaft and the distal portion of the glans, the scrotum and the pubis. Dermatitis was characterized by a painful and progressively growing ulcer with an erythematous base and irregular borders. Additionally, the ulcer was necrotic in appearance, was covered by a fibroid material, and produced a discharge. Deformation of the penis was evident, giving the impression of a circumcised penis. The patient underwent surgical debridement and biopsy of the lesion. The routine clinical laboratory tests were within normal limits. The patient was discharged after 7 days of antibiotic treatment with cefuroxime (750 mg IV, every 8 hr). The biopsy showed balanitis with acute and chronic inflammatory cell infiltration, multiple pyogenic microabscesses, and the absence of neoplastic cells. Over the next 2 weeks, the lesion enlarged and the patient returned to the hospital where he was admitted to the Urology Department. The physical examination showed severe erosion of the prepuce skin and areas of extended necrosis. The posterior side of the penis showed a large area of skin erosion with serous and purulent material. There was an open abscess in the suprapubic area draining an odorless, bloody, and purulent

material, and the scrotum was also affected, with multiple ulcers (Figure 1A). At this point, Department of Experimental Medicine from the Faculty of Medicine was consulted regarding a possible *E. histolytica* infection. The patient was tested for levels of serum anti-amoebic antibodies through an enzyme-linked immunosorbent assay (ELISA) technique described previously.²⁷ The patient was clearly positive by ELISA (optical density [OD] at 490 nm = 0.60, cut-off point OD at 490 nm = 0.525), by microscopic detection of *E. histolytica*/*E. dispar* in the biopsy specimen (Figure 1B),²⁸ and by polymerase chain reaction (PCR) characterization of *Entamoeba* species extracted from the environmental DNA obtained from the purulent material of the damaged tissue.²⁹ After the diagnosis of cutaneous amebiasis, the patient was treated with metronidazole (500 mg IV, every 8 hr) and ceftriaxone (1 g IV, every 12 hr). After 30 days of anti-amoebic and antibiotic treatment, the abscesses healed and large areas of granulation tissue were observed; however, the loss of skin was severe and involved the entire penis. The patient is scheduled for reconstructive surgery and an autologous skin transplant.

Patient 2 was a 66-year-old adult male with a primary school education level. The symptoms began after sexual intercourse with a female partner. The patient detected edema of the scrotum and the penis and a painful ulcer in the scrotal area. The original ulcer evolved into a painful and progressively growing ulcerative lesion and made urination virtually impossible. The patient was admitted to the Urology Department of the General Hospital of Mexico SSa in Mexico

FIGURE 1. Macroscopic and microscopic study. (A) Macroscopic lesions in patient 1. A view of the dorsal region of the penis showing a large ulcerative lesion with irregular borders. Part of the ulcer is covered with a fibrinoid discharge. Anterior view of the genital region showing the ulcerative lesion on the pubis, an ulcerative lesion on the penis with necrotic lesions at the borders and the total absence of the prepuce. (B) Microphotography of the biopsy specimen obtained after 10 days of metronidazole treatment. The tissue slides were stained with periodic acid-Schiff (PAS). An infiltration of mononuclear inflammatory cells and the presence of red stained trophozoites of *Entamoeba histolytica*/*Entamoeba dispar* were observed. (C) Macroscopic lesions in patient 2. The arrows show the exposition of testes, and upper rows indicate sites of necrotic lesions of the skin. (D) Slide of an imprint of ulcerative lesions showing the presence of trophozoites. The slide was stained using the PAS technique.

City D.F., and the physical examination of the genital area showed a large ulcerated area with an erythematous base and irregular borders with large necrotic areas covered by a fibrinous and purulent discharge. The looseness of the skin exposed the testes and the urethra, which was sectioned because of the necrotic damage (Figure 1C). The patient first underwent surgical debridement and suprapubic drainage of the bladder. Out of concern for cutaneous amebiasis, the patient was tested for levels of serum anti-amoebic antibodies through the ELISA technique mentioned previously.²⁷ However; this patient was seronegative for anti-amoebic antibodies. Microscopic detection of *Entamoeba* parasites in the biopsy specimens is shown in Figure 1D.²⁸ The DNA was extracted from the purulent material and molecular characterization of the *Entamoeba* infecting species was performed.²⁹ After the etiological diagnosis was established, intravenous metronidazole (500 mg, every 8 hr for 10 days), ceftriaxone (1 g BID for 5 days), and clindamycin (300 mg for 10 days) were administered. The clinical evolution of the patient was satisfactory. He is currently waiting for genitals and the urethra reconstructive surgery procedure.

Microscopic detection of *Entamoeba* trophozoites. Biopsy specimens from the ulcer borders located on the penis and scrotum were obtained in both cases and fixed in paraformaldehyde for 24 hr. The tissue was then segmented, dehydrated, and embedded in paraffin. The tissues (4–5 μ M) were cut with a microtome Leica RM 2145 (Buffalo Grove, IL), mounted on slides, and stained with hematoxylin-eosin (HE) and periodic acid-Schiff (PAS) techniques.²⁸ The slides were observed at 10 $\times$ and 40 $\times$ magnifications with a light microscope.

Genotyping of *Entamoeba* species and phylogenetic reconstructions of genetic markers. The QIAamp DNA Mini Kit (QIAGEN, Valencia, CA) was used to extract DNA, fol-

lowing the manufacturer's instructions, from the purulent and bloody secretion specimens taken from different sites of the genital region. One transfer RNA (tRNA) gene-linked short tandem repeat was amplified from the DNA using species-specific primers for *E. histolytica* and *E. dispar*, as previously described.²⁹ The primer used to amplify the molecular marker for *E. dispar* was NKD3-D5, and the primer NK2H3-H5 was used for amplification of the *E. histolytica* molecular marker. The reaction and PCR conditions for amplification of these molecular targets were described previously.²⁹ The sequencing reactions had a total volume of 15 μ L, consisting of 2 μ L of the Large Dye Terminator Sequencing Kit (Applied Biosystems, San Francisco, CA), 1.6 μ M of primer, and 5 μ L of the purified, amplified product. The amplification conditions were as follows: 1 cycle of 5 min at 95°C, 45 cycles of 10 sec at 95°C, 10 sec at 50°C, and 4 min at 60°C. Sequencing was performed in a capillary sequencer (ABI-Avant 100, University of Washington). Sequences were manually verified with the BioEdit program.³⁰ Taxonomic identity was established by comparing the obtained sequences against the GenBank database (NCBI). Sequences were aligned manually based on the short tandem repeat patterns previously reported for the locus NK2H3-H5.³¹ Phylogenetic reconstruction for the molecular marker, NK, was carried out with the unweighted pair group method with arithmetic mean (UPGMA) using the Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL) Program, version 17.

Ethical concerns. During the admission process, the physician in charge asked the patients to sign an informed consent letter so that the cases could be documented through photography during treatment and progression of the illness and later published. The two cases were approved by both the Research and Ethical Committee of the Faculty of Medicine, UNAM and by the General Hospital of Mexico City.

FIGURE 2. Phylogenetic reconstruction using the unweighted pair group method with arithmetic mean (UPGMA) from the intergenic STR associated with the transfer RNA (tRNA) genes. (A) NK2H3-H5 region (GenBank accession nos. JN191599 and JQ828978) for *E. histolytica*. Each species was found in the same cluster as the reference strain *E. histolytica* HM:IMSS. (B) NKD3-D5 region (GenBank accession no. JN191598) for *E. dispar* showing a distinct genotype from the reference strains *E. dispar* SAW760 and to others previously reported sequences.

RESULTS

Microscopic detection of *Entamoeba* species. The presence of *Entamoeba* trophozoites was observed in the biopsy specimens taken from the largest ulcer borders of both patients. For patient 1, the biopsy was performed after 2 days of metronidazole therapy. For patient 2, the imprint specimen was obtained before treatment; however, in both cases, the presence of trophozoites was clearly observed by the characteristic dark red staining of the slides stained with the PAS technique.

Genotyping of *Entamoeba* species. The characterization of *E. histolytica* and *E. dispar* variants in both patients was performed using the intergenic tRNA region NK2H3-H5 (Embank accession nos. JN191599 and JQ828978) for *E. histolytica* (Figure 2A), and NKD3-D5 (Embank accession no. JN191598) for *E. dispar* (Figure 2B), as the molecular targets.

Entamoeba histolytica was detected in the lesions of both patients, and the phylogenetic reconstruction for the molecular marker NK2H3-H5 shows that in both cases, the sequences are in the same cluster, indicating that both isolates have the same genotype as the reference strain, *E. histolytica* HM:IMSS (Figure 2A). Although analysis of the molecular marker NKD3-D5 was performed in both patients, *E. dispar* was only detected in the sample from patient 1, and the *E. dispar* species in this case has a distinct genotype from the reference strain, *E. dispar* SAW760 (Figure 2B).

DISCUSSION

The cytolytic ability of *E. histolytica* has been well documented since the first reported cases of cutaneous amebiasis.¹⁰⁻¹³ Both cases presented here are representative of the evolution of lesions without specific anti-amoebic treatment and show the irreversible character of the damage inflicted by this parasite. Although cutaneous amebiasis is relatively rare, it should be considered in the differential diagnosis of ulcerative lesions of human genitalia, and in particular, in amebiasis-endemic areas and in high risk groups. Considering the recent reports of genital cutaneous amebiasis in endemic countries,^{17-20,22} sexual transmission of this disease currently can be considered the major form of transmission. From an epidemiological point of view, the extensive study of such cases allows us to trace the transmission and sources of infection and to also characterize prevalent genotypes of *E. histolytica* and *E. dispar* variants in endemic countries. These cases highlight the complexity of the *Entamoeba*-human relationship with regard to infection. Patient 1 is the first case of cutaneous amebiasis in which the presence of both species of *Entamoeba* has been demonstrated. Previously, our group reported cases of amoebic liver abscesses where the presence of both *E. histolytica* and *E. dispar* infection was demonstrated by PCR using different molecular markers.³² Coincidentally, the amoebic lesions in these patients were also co-infected by pyogenic bacteria, *Enterobacter cloacae* and *Staphylococcus aureus* in patient 1, and *Escherichia coli* in patient 2. This outcome was similar to the case of pyogenic and mixed liver abscess (amoebic-pyogenic) reported previously.³² We propose that the pathogenic behavior of *Entamoeba* species in human infection could be potentiated by the co-infection with bacteria.

The phylogenetic reconstruction of the genetically studied targets showed that the *Entamoeba* species detected in these

patients were located in the same cluster for the marker NK2H3-H5 and shared the same genotype as the reference strain *E. histolytica* HM:IMSS (Figure 2A). On the other hand, an analysis of the molecular marker NKD3-D5 showed that the *E. dispar* species detected in patient 1 had a distinct genotype from the reference strains *E. dispar* SAW760 and to other previously reported sequences (Figure 2B). The detection of both *Entamoeba* species (*E. histolytica* and *E. dispar*) suggests that mixed infections are more than an isolated observation in human amebiasis.

The outcome of the infection in both cases underlines the importance of a timely, specific, and sensitive diagnosis to avoid the extensive tissue damage and irreversible anatomic sequelae of cutaneous amebiasis. Moreover, underline the physician necessity to be aware of genital ulcers, that at the beginning may not be impressive but the earlier molecular diagnosis can detect or discard the presence of *Entamoeba* parasites, and as we mentioned before can produce fast and serious tissue damage without opportune treatment.

Received May 2, 2012. Accepted for publication July 29, 2012.

Published online December 3, 2012.

Acknowledgments: We thank Elena Ortiz for her secretarial assistance and Pedro Balderas for technical assistance with the microscopic analysis of stool samples. We would also like to acknowledge Miss. Angélica L. Serrano for the informatics assistance.

Financial support: This work was partially supported by the National Council for Science and Technology (CONACyT), grants 140990 and 79220, and grants IN 226511 and IN-204208 from the PAPIIT program for Scientific Research Development (DGAPA) UNAM.

Authors' addresses: Patricia Morán, Liliana Rojas, René Cerritos, Valeria Zermeño, Alicia Valadez, Enrique González, Eric Hernández, Oswaldo Partida, Miriam Nieves, Tobías Portillo, Marco Guadío, and Cecilia Ximénez, Department of Experimental Medicine, Faculty of Medicine, National Autonomous University of Mexico (UNAM), Mexico City, Mexico, E-mails: patricia_morans@yahoo.com.mx, rojasvelazquezliliana@gmail.com, renecerritos@gmail.com, flos_aquae@hotmail.com, alicia.vs17@gmail.com, rivasenrimx@yahoo.com.mx, ericghdz@yahoo.com.mx, oswpartida@yahoo.com.mx, mirnieves@yahoo.com.mx, tobiassportillo@gmail.com, gudino@servidor.unam.mx, and cximenez@servidor.unam.mx. Griselda Montes de Oca, Dermatology Department, General Hospital of Mexico, Ministry of Health, Mexico City, Mexico, E-mail: grismos@yahoo.com.mx. Miguel Ángel Reyes, Urology Department, General Hospital of Mexico, Ministry of Health, Mexico City, Mexico, E-mail: marg3@prodigy.net.mx. Manuel Ramiro, Ministry of Health, Mexico City; Unit of Education, Scientific Research and Health Policy Direction of Medical Benefits, Mexican Institute of Social Security Mexico City, IMSS, Mexico, E-mail: mramiroh@yahoo.com.mx.

REFERENCES

1. Nasse D, 1892. Coincidental amoebic liver abscess; dysentery and nosocomial gangrene. *Arch F Klin Chir* 43: 40-53.
2. Nagai SK, Frazier CN, 1933. Cutaneous amebiasis: a review and report of three cases observed in North China. *China Med J* 47: 1154-1167.
3. Kouri P, Bolaños JM, Fuentes C, Rodríguez, 1933. Cutaneous amebiasis by *Entamoeba histolytica*. Case Report: especially studied histological and proto-zoological. *Rev Med Ciruj de la Habana* 38: 83-89.
4. Crawford S, 1933. Amebiasis cutis: report of case. *Arch Dermat Syph* 28: 363-374.
5. Meleney FL, Meleney HE, 1935. Gangrene of the buttock, perineum and scrotum due to *Entamoeba histolytica*. *Arch Surg* 30: 980-989.
6. Carini A, 1936. Progressive destruction in cutaneous amebiasis. *Bull Soc Pathol Exot* 29: 584-592.

7. Manson-Bahr P, 1938. Amoebic invasion of skin and subcutaneous tissues. *Trans R Soc Trop Med Hyg* 32: 223–231.
8. Fingerland A, 1930. Cutaneous amebiasis (contribution to the etiology of postoperative progressive gangrene of the skin). *Bull Soc Franc Dermat Syph* 46: 903–911.
9. Wyatt TE, Buchholz RR, 1941. Amebiasis cutis. *Ann Surg* 113: 140–152.
10. Magaña ML, Fernández- Diez J, Magaña M, 2008. Cutaneous amebiasis in pediatrics. *Arch Dermatol* 144: 1369–1372.
11. Biagi F, Martuscelli AR, 1963. Cutaneous amebiasis in Mexico. *Int J Dermatol* 2: 129–136.
12. Chacon RA, 1975. Cutaneous amebiasis. *Mod Probl Paediatr* 17: 259–261.
13. Sunarwan IA, 1975. Case of cutaneous amebiasis. *Dermatologica* 151: 253–256.
14. Adams EB, MacLeod IN, 1977. Invasive amebiasis. I. Amebic dysentery and its complications. *Medicine (Baltimore)* 56: 315–323.
15. Wynne JM, 1980. Perineal amebiasis. *Arch Dis Child* 55: 234–236.
16. Hurwitz AL, Owen RL, 1978. Venereal transmission of intestinal parasites. *West J Med* 128: 89–91.
17. Omoniyi-Esan OG, Osasan SA, Ojo OS, 2006. Non-neoplastic diseases of the cervix in Nigerians: a histopathological study. *Afr Health Sci* 6: 76–80.
18. Richens J, 2004. Genital manifestations of tropical diseases. *Sex Transm Infect* 80: 12–17.
19. Stark D, van Hal SJ, Matthews G, Harkness J, Marriott D, 2008. Invasive amebiasis in men who have sex with men, Australia. *Emerg Infect Dis* 14: 1141–1143.
20. Monhanty I, Mohanty P, Patnaik S, Panda P, 2010. Amoebic ulcer of the male genital: a rare case report. *Indian J Sex Transm Dis* 31: 116–117.
21. Owen RL, Dritz SK, Wibbelsman CJ, 1979. Venereal aspects of gastroenterology. (Medical Staff Conference, University of California San Francisco, Mar 1979). *West J Med* 130: 236–246.
22. Medina-Murillo GR, Rodríguez-Wong U, 2011. Perianal cutaneous amebiasis. Report of two cases. *Rev Gastroenterol Mex* 76: 60–63.
23. Ramos F, García G, Valadez A, Morán P, González E, Gómez A, Melendro EI, Valenzuela O, Ximénez C, 2005. *E. dispar* strain: analysis of polymorphism as a tool for study of geographic distribution. *Mol Biochem Parasitol* 141: 175–177.
24. Morán P, Gómez A, Valadez A, García G, Ramos F, González E, Limón A, Riebeling C, Valenzuela O, Rojas L, Melendro EI, Ximénez C, 2009. Periodicity and patterns of *Entamoeba histolytica* and *E. dispar* infection in HIV⁺/AIDS patients in Mexico. *Ann J Trop Med Hyg* 103: 307–315.
25. Ximénez C, Morán P, Rojas L, Valadez A, Gómez A, 2009. Reassessment of the epidemiology of amebiasis: state of the art. *Infect Genet Evol* 9: 1023–1032.
26. Ximénez C, Morán P, Rojas L, Valadez A, Gómez A, Ramiro M, Cerritos R, González E, Hernández E, Partida O, 2011. Novelty on amebiasis: a neglected tropical disease. *J Global Infect Dis* 13: 166–174.
27. Morán P, Gómez A, Valadez A, Ramos F, González E, García G, Limón A, Valenzuela O, Ramiro M, Hidalgo H, Riebeling C, Melendro EI, Ximénez C, 2007. Amebic and pyogenic liver abscess: importance of differential diagnosis in areas of endemic amebiasis. *Trop Med Int Health* 12: 57–64.
28. Torres F, 2002. *Manual de Técnicas en Histología y Anatomía Patológica*. Barcelona: Ariel Press, 31–74.
29. Ali IK, Zaki M, Clark CG, 2005. Use of PCR amplification of tRNA gen-linked short tandem repeats for genotyping *Entamoeba histolytica*. *J Clin Microbiol* 43: 5842–5847.
30. Hall T, 1999. BioEdit: user-friendly sequence alignment editors and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* 41: 95–98.
31. Escueta-de Cadiz A, Kobayashi S, Takeuchi T, Tachibana H, Nozaki T, 2010. Identification of an avirulent *Entamoeba histolytica* strain with unique tRNA-linked short tandem repeat markers. *Parasitol Int* 59: 75–81.
32. Ximénez C, Cerritos R, Rojas L, Dolabella S, Morán P, Shibayama M, González E, Valadez A, Hernández E, Valenzuela O, Limón A, Partida O, Silva EF, 2010. Human amebiasis: breaking the paradigm? *Int J Environ Res Public Health* 7: 1105–1120.