Management of *Entamoeba histolytica* infection – treatment strategies and possible new drug targets

Susanna Haapanen, Seppo Parkkila

Faculty of Medicine and Health Technology, Tampere University and Fimlab Laboratories Ltd, Tampere University Hospital, Tampere, Finland

e-mail:

susanna.haapanen@tuni.fi;

seppo.parkkila@tuni.fi

Contents

Biology and pathogenesis of *Entamoeba histolytica*

Epidemiology of *E. histolytica* infection

Diagnosis of *E. histolytica* infection

Current treatment options for *E. histolytica* infections

Future therapeutics and vaccine development

Concluding remarks

References

Abstract

Entamoeba histolytica infection, amoebiasis, is a major cause of morbidity and mortality in developing countries. It is also a significant causative agent of traveler´s diarrhea. It has been estimated that amoebiasis may affect 10% of the global population. The most common

infection route is via ingestion of contaminated food and water. About 90% of infected individuals are asymptomatic, but the infection may also lead to severe complications, such as colitis with bloody diarrhea, liver abscesses, and colonic perforation. The classical gold standard for diagnosis is the detection of trophozoites from stool samples by microscopy, although this method is labor-intensive and has low sensitivity. Several other diagnostic methods, based on parasite culture, serologic tests, antigen detection, and polymerase chain reaction, have been developed. In the future, multiplex PCR methods will be widely used for the simultaneous detection of various pathogenic microorganisms including *E. histolytica*. Treatment of amoebic colitis typically involves a combination therapy with so called luminal agents (paromomycin, diloxanide furoate, iodoquinol) combined with tissue amoebicides (metronidazole, tinidazole). Even though the present treatment options are mostly effective, new drugs are needed to treat all patients with amoebiasis, and different vaccine candidates are under development to eradicate *E. histolytica* from population.

Keywords: Entamoeba histolytica, amoebiasis, drug, diagnosis, treatment, therapy

Biology and pathogenesis of *Entamoeba histolytica*

Entamoeba histolytica is a unicellular pathogenic protozoan causing amoebiasis which mainly occurs as an intestinal infection [1,2]. Bloody diarrhea (amoebic colitis) and liver abscess are the most common consequences [3]. The clinical manifestations are often divided into three groups depending on the symptoms and spreading of the parasite in the human body: 1) Intraluminal amoebiasis covers the first weeks of the infection when there are no symptoms, but the diagnosis could be made. 2) Amoebic colitis is the most common appearance of the disease which includes diarrhea, sometimes bloody stools, fever, abdominal cramps and weight loss. 3) The most severe form is a disseminated amoebiasis in which the parasite forms abscesses in internal organs, although the intestinal symptoms may be absent or mild [4-6]. The most common site for abscess is the liver, and other extraintestinal lesions have been reported in the brain, lung and peritoneum [7,8].

E. histolytica is closely related to another species of the *Entamoeba* family, *E. dispar*. For years it was though possible to be an asymptomatic carrier of *E. histolytica* [6,9]. Detailed studies showed that asymptomatic carriers were, in fact, infected with *E. dispar* instead of *E.*

histolytica. Thus, it is now concluded that *E. histolytica* infection leads to a symptomatic disease, but nevertheless, the symptoms may be mild [10].

The life cycle of *E. histolytica* has two different stages including cysts and trophozoites. Transmission occurs via fecal-oral-route. Infection is usually contracted by eating food contaminated with quadrinucleated cysts, more rarely directly by person-to-person contact [6]. Excystation occurs in the small intestine where one cyst releases eight motile trophozoites. Trophozoites migrate to the large intestine, adhere the mucous wall through multi-unit Gal/GalNAc lectins, form new cysts, and invade through the intestine wall [11]. *E. histolytica* is capable of lysing human tissues, killing immune effector cells by contact-dependent cytolysis and with amoebapores and can degrade the host extracellular matrix with cysteine proteases. Trophozoites are easily destroyed if they encounter the gastric fluid or environment outside the human body. However, cysts may survive up to weeks outside the body with an ability to cause infection. Hence, the cysts secreted to stool are ready to transmit amoebiasis to other people [6].

Epidemiology of *E. histolytica* infection

Worldwide, *E. histolytica* infections lead to the death of over 55 000 people annually [12,13] and approximately 50 million people have a symptomatic infection each year [4,14]. According to the World Health Organization *E. histolytica* is the third leading cause of death from parasitic disease; only malaria and *Schistosoma mansoni* cause more mortality [15]. Fortunately, there is some indication that the mortality rates of amoebiasis are gradually decreasing.

Amoebiasis is endemic in tropical and subtropical areas, which mostly involve developing countries. However, globalization and travelling brings the parasite to developed countries, and the prevalence has been estimated to be as high as 4 % in the USA [4]. For comparison, the seroprevalence is up to 42 % in rural areas of Mexico. Higher incidence and prevalence figures are strongly associated with the lower quality and availability of sanitation in the area.

Diagnosis of *E. histolytica* infection

There are multiple methods with different characteristics to diagnose amoebiasis. The classical golden standard has been microscopy. Although it is labor-intensive and requires skilled technicians, its simplicity and low cost has outweighed the obvious limitations. Therefore, microscopy still remains widely used, especially in resource-limited laboratories of endemic, high-prevalence areas [16]. Microscopy has low sensitivity and specificity, and it is time-consuming as it often requires multiple samples to reach the final diagnosis [17].

A wide variety of quantitative real-time polymerase chain reaction (qPCR) assays have been recently developed for the diagnosis of enteric viral, bacterial and parasitic agents. PCR has also become a widely recommended method as the primary tool for diagnosing *E. histolytica* infection. To reach a more comprehensive view of the infection from a single specimen, there has been a trend towards multiplex approach that allows simultaneous identification of multiple pathogens [18,19]. Several multiplex gastrointestinal pathogen panel tests are already commercially available, some of them involving fully integrated robotic systems incorporating DNA extraction, amplification, detection, and analysis directly from stool samples [20,21]. Food and Drug Admistration (FDA, USA) has approved several gastrointestinal panels involving *E. histolytica* detection to clinical practice and recommends them as golden standard, and the World Health Organization (WHO) also advocates PCR as the primary method [22,4]. On one hand, PCR is sensitive (sensitivity 92-100 %), specific (specificity 89-100 %) and rapid, but on the other hand, it requires equipment, kits and an educated technician [20,23].

Stool antigen detection, serology, culture, isoenzyme analysis and point-of care (POC) tests are other options which have been widely investigated [20,4,17]. Often none of them alone leads to the final diagnosis, but they are certainly useful as complementary tests. As an example, stool antigen detection has been used as a complementary test for microscopy, which can overcome the limited sensitivity and specificity of the classical microscopy test. Serology is particularly useful for detecting the cases with extraintestinal infections, when the stool sample was negative. Unfortunately, serology does not separate an active infection from past infection [4]. Culture and isoenzyme analyses are additional tools to differentiate *E. histolytica* from *E. dispar*, but the success rate of the culture is only 50-70 %, the risk of false negative is high, and the methods are time-consuming. Therefore, PCR has largely replaced culture in diagnostic use [17]. POC tests are typically commercial test assays

which are based on antigen detection, serology or PCR. Their characteristics and costs vary enormously. Nonetheless, a sensitive and specific POC may bring significant advantage in endemic areas, allowing mass screening of population.

Current treatment options for *E. histolytica* infections

The medication used to treat amoebiasis can be divided in three groups depending on their point of action: intraluminal, tissue, and mixed amoebicides [24]. Intraluminal amoebicides are effective against cysts in the gut, tissue amoebicides treat the symptomatic disease in intestines and other tissues, and mixed treatments have both actions.

Recommended treatment options according to Haque et al. are described in Table 1 [25]. The traditional treatment against *E. histolytica* infection is metronidazole, a widely used antiparasitic and anti-anaerobic bacteria drug [26]. The recommended first-line treatment includes three daily doses of 750 mg of metronidazole for 5 (or 7) - 10 days or three daily doses of 800 mg of tinidazole for 5 days [25,27]. Oral administration is usually sufficient even in invasive infections as the bioavailability of metronidazole is approximately 80 %. Nevertheless, intravenous administration is also an option in hospital setting, if the response to oral treatment was found inadequate.

Table 1. Suggested treatment options for amoebiasis according to Haque and coworkers [25].

DIAGNOSIS AND DRUG	ADULT DOSAGE	PEDIATRIC DOSAGE
Amoebic liver abscess		
Metronidazole	750 mg orally x 3, 7 – 10 days	35 – 50 mg/kg/day in 3 divided doses, 7 – 10 days
	OR	
Tinidazole	800 mg orally x 3, 5 days	60 mg/kg/day (maximum 2 g), 5 days
	FOLLOWED BY A LUMINAL AGENT	
Paromomycin	25 – 35 mg/kg/day in 3 divided doses, 7 days	25 – 35 mg/kg/day in 3 divided doses, 7 days
	OR SECOND-LINE AGENT	
Diloxanide furoate	500 mg orally x 3, 10 days	20 mg/kg/day in 3 divided doses, 10 days
Amoebic colitis		

Metronidazole	750 mg orally x 3, 7 – 10 days	35 – 50 mg/kg/day in 3 divided doses, 7 – 10 days
	FOLLOWED BY A LUMINAL AGENT AS FOR AMOEBIC LIVER ABSCESS	
Asymptomatic intestinal colonization		
Paromomycin	25 – 35 mg/kg/day in 3 divided doses, 7 days	25 – 35 mg/kg/day in 3 divided doses, 7 days
	OR SECOND-LINE AGENT	
Diloxanide furoate	500 mg orally x 3, 10 days	20 mg/kg/day in 3 divided doses, 10 days

Metronidazole is effective against trophozoites but is usually inadequate to eradicate cysts from the gut [26]. Therefore, in the management of all forms of invasive disease, including amoebic colitis, the standard recommendation is to give a tissue amoebicide (metronidazole or tinidazole) followed by an intraluminal amoebicide (diloxanide furoate, paromomycin or iodoquinol) [26,28]. This treatment procedure would optimally eradicate both the live parasites and intraluminal cysts. It is notable, however, that some controversy still exists whether cyst eradication is always needed after metronidazole or tinidazole treatment, especially in endemic areas, where re-infection is frequent [29]. The increased complexity of combination regimens, additional drug costs, more frequent side events, and the restricted availability of intraluminal amoebicides on the local market, all reduce compliance with combination therapy.

As all pharmaceutical agents, metronidazole has adverse side-effects, for instance nausea, diarrhea, loss of appetite and metallic taste in the mouth [27,30]. Comparison of metronidazole and tinidazole has not revealed any major difference concerning the subjective side-effect profiles of these drugs [31]. Notably, metronidazole inhibits the action of hepatic CYP2C9 enzyme which leads to many undesirable interactions with other drugs, such as frequently used anticoagulant warfarin [32,33]. Hence, the inhibition of CYP2C9 may lead to decreased or increased concentrations of other drugs in the blood stream, potentially leading to drug related adverse side effects or loss of action.

It is noteworthy that only few creditable clinical trials exist in the medical literature considering the pharmacological treatment of *E. histolytica* infection. Gonzales and coworkers published a meta-analysis of randomized controlled trials of antiamoebic drugs given alone or in combination, compared with placebo or another antiamoebic drug, for

amoebic colitis [29]. In total, they were able to include 41 trials (4999 participants) which met the inclusion criteria. However, many trials were old and only one used adequate randomization and allocation concealment, was blinded, and analyzed all randomized participants. Moreover, the diagnostic methods used in those trials were not always reliable. Despite these uncertainties, they concluded that compared with metronidazole, 1) tinidazole may be more effective in reducing clinical failure, 2) tinidazole may be associated with fewer adverse events, and 3) combination drug therapy may be more effective for reducing parasitological failure.

E. histolytica resistance against metronidazole has been considered rare. Wassmann et al. [34] and Samarawickrema et al. [35] induced resistance in axenic *E. histolytica* cultures up till lethal doses of metronidazole. The mechanism of resistance has been shown to involve increased activity of iron-containing superoxide dismutase (Fe-SOD) and peroxiredoxin and decreased expression of flavin reductase and ferredoxin 1 [34,35]. The activation of Fe-SOD is usually a reaction to various stress inducing situations, for instance overpopulation of cells, and thus not only the drug effect of metronidazole [35]. In the case of metronidazole, the activation of SOD may be linked to the protection of microorganisms from a variety of toxic radicals.

Future therapeutics and vaccine development

Sulfolipid metabolism is necessary for the parasitic lifestyle of *E. histolytica* [36]. The sulfate activation is performed through two sequential reactions producing adenosine 5′-phosphosulfate (APS) and 3′-phosphoadenosine 5′-phosphosulfate (PAPS) with the catalysts ATP sulfurylase (AS) and APS kinase (APSK), respectively. PAPS is used as a sulfate donor in a variety of reactions which provide crucial molecules for trophozoite proliferation and encystation. Sulfate activation takes place in mitochondrial-related organelles called mitosomes from where PAPS is transferred to cytosol where sulfolipids are generated with the catalyzing help of sulfotransferases (SULTS) and sulfatases (SF). From these enzymes the APSK has been considered the most promising target of antiamoebic drug development, as it is unique to *E. histolytica* physiology in the early steps of sulfate activation. 2-(3-fluorophenoxy)-N-[4-(2-pyridyl)thiazol-2-yl]-acetamide (A-D-11), 3-phenyl-N-[4-(2-pyridyl)thiazol-2-yl]-imidazole-4-carboxamide (A-H-11), and auranofin have been found to

halt trophozoite proliferation as well as encystation [37,38]. A-D-11 and A-H-11 have no cytotoxic effect in human cells, in contrast to auranofin which is, in fact, already in human use as an oral drug for rheumatoid arthritis [39,37]. Auranofin also inhibits thioredoxin reductase, enhancing sensitivity of trophozoites to reactive oxygen-mediated killing [38]. Thioredoxin reductase of *E. histolytica* (EhTrxR) is an important enzyme in the redox system and for intracellular oxygen detoxification. Martínez-Pérez and coworkers recently showed that rabeprazole, a proton pump inhibitor, inhibits the EhTrxR enzyme [40]. Rabeprazole also affected amoebic proliferation and several other functions required for parasite virulence. In a hamster model of liver infection, sublethal rabeprazole concentration (600 µM) promoted parasite death. The authors concluded that the molecular structure of rabeprazole can be useful as a scaffold to design new amoebicides.

Nitazoxanide is a novel antiparasitic agent, which has been shown to be effective against *E. histolytica* in both the intraluminal and invasive forms of infection and has been suggested to represent a potential successor to metronidazole [41].

Flavonoids, such as kaempferol, catechin and isoquerticin, have antiamoebic activity, which has been demonstrated only *in vitro* [42]. Therapeutic dosage, administration route as well as pharmacokinetics and –dynamics are yet to be determined.

E. histolytica has a single β-carbonic anhydrase (EhiCA) [43]. EhiCA was produced as a recombinant protein which was used in kinetic and inhibition studies using different sulfonamides and anions [44,45]. Bua *et al.* discovered 4-hydroxymethyl/ethyl-benzenesulfonamide to have the best inhibitory action against EhiCA (K_Is of 36–89 nM) with weaker inhibition impact on human carbonic anhydrase I and II (K_Is of 21 µM and 125 nM, respectively) [44]. Several carbonic anhydrase inhibitors, clinically used for other conditions, were also tested. Among these compounds, acetazolamide, methazolamide, ethoxzolamide and dichlorphenamide showed good inhibitory effects (K_Is of 509–845 nM), while they also inhibited efficiently human CA I and II (K_Is ranging 8–1200 nM) [44]. Thus, these compounds provided no selectivity against EhiCA. In addition, some anions had good inhibition properties: sulfamide, phenylarsonic acid, phenylboronic acid and fluorosulfonate showed K_Is of 28 µM, 38 µM, 47 µM, and 86 µM, respectively [45]. Furthermore, their inhibitory effects against human carbonic anhydrase I and II were weaker than against EhiCA (K_Is ranging 310 nM –49.2 µM), which makes them slightly selective against the amoeba

carbonic anhydrase. These results clearly opened new avenues for further investigations to determine the effects of carbonic anhydrase inhibitors *in vivo* and to design novel compounds specifically targeting β-carbonic anhydrases.

As *E. histolytica* is an important cause of morbidity and mortality especially in low-income countries, the need of vaccine is real. Humans and non-human primates are the only reservoirs of *E. histolytica,* which makes the eventual goal to eradicate the disease plausible [10]. *E. histolytica* triggers many immune pathways of the host, which has further led to attempts to develop a vaccine against this parasite [24,46,2]. A Gal/GalNAc lectin-based vaccine has been the most widely investigated candidate; also a serine-rich *E. histolytica* protein and an attenuated strain of *E. histolytica* have been investigated in rodent models. Nevertheless, none of these theoretically promising vaccines have reached clinical trials. We hope that the interest in novel vaccines against *E. histolytica* will increase along with the new era in vaccinology that has recently been witnessed during the COVID-19 pandemic. The eradication of E. histolytica should be considered both an important goal for better global health and an investment for the global sustainable development goals.

Concluding remarks

Entamoeba histolytica is the third leading cause of mortality of parasite infections, which causes pressure to have tools for rapid diagnosis as well as affordable and effective treatment. The clinical manifestation of amoebiasis varies from an asymptomatic infection to colitis and even to life-threatening invasive infection. Fortunately, we have good treatment options for different clinical situations, although there is already some indication of emerging drug resistance. Vaccination would represent the most effective option to reduce the global disease burden in long term, but no such preventive option is available at this moment.

Compliance with Ethical Standards

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Original research of our team is funded by the Academy of Finland and Jane & Aatos Erkko Foundation.

Ethical Approval: This chapter does not contain any studies with human participants or animals performed by any of the authors.

References

1. Hashmey R, Genta RM, White Jr AC (1997) Parasites and Diarrhea. I: Protozoans and Diarrhea. J Travel Med 4 (1):17-31. doi:10.1111/j.1708-8305.1997.tb00769.x
2. Kantor M, Abrantes A, Estevez A, Schiller A, Torrent J, Gascon J, Hernandez R, Ochner C (2018) Entamoeba Histolytica: Updates in Clinical Manifestation, Pathogenesis, and Vaccine Development. Can J Gastroenterol Hepatol 2018:4601420. doi:10.1155/2018/4601420
3. Debnath A, Rodriguez MA, Ankri S (2019) Editorial: Recent Progresses in Amebiasis. Front Cell Infect Microbiol 9:247. doi:10.3389/fcimb.2019.00247
4. Shirley DT, Farr L, Watanabe K, Moonah S (2018) A Review of the Global Burden, New Diagnostics, and Current Therapeutics for Amebiasis. Open Forum Infect Dis 5 (7):ofy161. doi:10.1093/ofid/ofy161
5. Showler AJ, Boggild AK (2013) Entamoeba histolytica. CMAJ 185 (12):1064. doi:10.1503/cmaj.121576
6. Stanley SL, Jr. (2003) Amoebiasis. Lancet 361 (9362):1025-1034. doi:10.1016/S0140-6736(03)12830-9
7. Wells CD, Arguedas M (2004) Amebic liver abscess. South Med J 97 (7):673-682. doi:10.1097/00007611-200407000-00013
8. Cheepsattayakorn A, Cheepsattayakorn R (2014) Parasitic pneumonia and lung involvement. Biomed Res Int 2014:874021. doi:10.1155/2014/874021

9. Oliveira FM, Neumann E, Gomes MA, Caliari MV (2015) Entamoeba dispar: Could it be pathogenic. Trop Parasitol 5 (1):9-14. doi:10.4103/2229-5070.149887
10. Stauffer W, Ravdin JI (2003) Entamoeba histolytica: an update. Curr Opin Infect Dis 16 (5):479-485. doi:10.1097/00001432-200310000-00016
11. Cornick S, Chadee K (2017) Entamoeba histolytica: Host parasite interactions at the colonic epithelium. Tissue Barriers 5 (1):e1283386. doi:10.1080/21688370.2017.1283386
12. Carrero JC, Reyes-Lopez M, Serrano-Luna J, Shibayama M, Unzueta J, Leon-Sicairos N, de la Garza M (2020) Intestinal amoebiasis: 160 years of its first detection and still remains as a health problem in developing countries. Int J Med Microbiol 310 (1):151358. doi:10.1016/j.ijmm.2019.151358
13. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, Abraham J, Adair T, Aggarwal R, Ahn SY, Alvarado M, Anderson HR, Anderson LM, Andrews KG, Atkinson C, Baddour LM, Barker-Collo S, Bartels DH, Bell ML, Benjamin EJ, Bennett D, Bhalla K, Bikbov B, Bin Abdulhak A, Birbeck G, Blyth F, Bolliger I, Boufous S, Bucello C, Burch M, Burney P, Carapetis J, Chen H, Chou D, Chugh SS, Coffeng LE, Colan SD, Colquhoun S, Colson KE, Condon J, Connor MD, Cooper LT, Corriere M, Cortinovis M, de Vaccaro KC, Couser W, Cowie BC, Criqui MH, Cross M, Dabhadkar KC, Dahodwala N, De Leo D, Degenhardt L, Delossantos A, Denenberg J, Des Jarlais DC, Dharmaratne SD, Dorsey ER, Driscoll T, Duber H, Ebel B, Erwin PJ, Espindola P, Ezzati M, Feigin V, Flaxman AD, Forouzanfar MH, Fowkes FG, Franklin R, Fransen M, Freeman MK, Gabriel SE, Gakidou E, Gaspari F, Gillum RF, Gonzalez-Medina D, Halasa YA, Haring D, Harrison JE, Havmoeller R, Hay RJ, Hoen B, Hotez PJ, Hoy D, Jacobsen KH, James SL, Jasrasaria R, Jayaraman S, Johns N, Karthikeyan G, Kassebaum N, Keren A, Khoo JP, Knowlton LM, Kobusingye O, Koranteng A, Krishnamurthi R, Lipnick M, Lipshultz SE, Ohno SL, Mabweijano J, MacIntyre MF, Mallinger L, March L, Marks GB, Marks R, Matsumori A, Matzopoulos R, Mayosi BM, McAnulty JH, McDermott MM, McGrath J, Mensah GA, Merriman TR, Michaud C, Miller M, Miller TR, Mock C, Mocumbi AO, Mokdad AA, Moran A, Mulholland K, Nair MN, Naldi L, Narayan KM, Nasseri K, Norman P, O'Donnell M, Omer SB, Ortblad K, Osborne R, Ozgediz D, Pahari B, Pandian JD, Rivero AP, Padilla RP, Perez-Ruiz F, Perico N, Phillips D, Pierce K, Pope CA, 3rd, Porrini E, Pourmalek F, Raju M, Ranganathan D, Rehm JT, Rein DB, Remuzzi G, Rivara FP, Roberts T, De Leon FR, Rosenfeld LC, Rushton L, Sacco RL, Salomon JA, Sampson U, Sanman E, Schwebel DC, Segui-Gomez M, Shepard DS, Singh D, Singleton J, Sliwa K, Smith E, Steer A, Taylor JA, Thomas B, Tleyjeh IM, Towbin JA, Truelsen T, Undurraga EA, Venketasubramanian N, Vijayakumar L, Vos T, Wagner GR, Wang M, Wang W, Watt K, Weinstock MA, Weintraub R, Wilkinson JD, Woolf AD, Wulf S, Yeh PH, Yip P, Zabetian A, Zheng ZJ, Lopez AD, Murray CJ, AlMazroa MA, Memish ZA (2012) Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 380 (9859):2095-2128. doi:10.1016/S0140-6736(12)61728-0
14. Chou A, Austin RL (2020) Entamoeba Histolytica. In: StatPearls. Treasure Island (FL),
15. Amoebiasis (1997). Wkly Epidemiol Rec 72 (14):97-99
16. Paulos S, Saugar JM, de Lucio A, Fuentes I, Mateo M, Carmena D (2019) Comparative performance evaluation of four commercial multiplex real-time PCR assays for the detection of the diarrhoea-causing protozoa Cryptosporidium hominis/parvum, Giardia duodenalis and Entamoeba histolytica. PLoS One 14 (4):e0215068. doi:10.1371/journal.pone.0215068
17. Saidin S, Othman N, Noordin R (2019) Update on laboratory diagnosis of amoebiasis. Eur J Clin Microbiol Infect Dis 38 (1):15-38. doi:10.1007/s10096-018-3379-3
18. van Lieshout L, Verweij JJ (2010) Newer diagnostic approaches to intestinal protozoa. Curr Opin Infect Dis 23 (5):488-493. doi:10.1097/QCO.0b013e32833de0eb
19. Verweij JJ, Stensvold CR (2014) Molecular testing for clinical diagnosis and epidemiological investigations of intestinal parasitic infections. Clin Microbiol Rev 27 (2):371-418. doi:10.1128/CMR.00122-13
20. Ryan U, Paparini A, Oskam C (2017) New Technologies for Detection of Enteric Parasites. Trends Parasitol 33 (7):532-546. doi:10.1016/j.pt.2017.03.005

21. Binnicker MJ (2015) Multiplex Molecular Panels for Diagnosis of Gastrointestinal Infection: Performance, Result Interpretation, and Cost-Effectiveness. J Clin Microbiol 53 (12):3723-3728. doi:10.1128/JCM.02103-15
22. Qvarnstrom Y, James C, Xayavong M, Holloway BP, Visvesvara GS, Sriram R, da Silva AJ (2005) Comparison of real-time PCR protocols for differential laboratory diagnosis of amebiasis. J Clin Microbiol 43 (11):5491-5497. doi:10.1128/JCM.43.11.5491-5497.2005
23. Shnawa BH (2017) Molecular diagnosis of Entamoeba histolytica, Entamoeba dispar, and Entamoeba moshkovskii: an update review. Annu Res Rev Biol 21 (5):1-12. doi:10.9734/ARRB/2017/37086
24. Nagaraja S, Ankri S (2019) Target identification and intervention strategies against amebiasis. Drug Resist Updat 44:1-14. doi:10.1016/j.drup.2019.04.003
25. Haque R, Huston CD, Hughes M, Houpt E, Petri WA, Jr. (2003) Amebiasis. N Engl J Med 348 (16):1565-1573. doi:10.1056/NEJMra022710
26. Freeman CD, Klutman NE, Lamp KC (1997) Metronidazole. A therapeutic review and update. Drugs 54 (5):679-708. doi:10.2165/00003495-199754050-00003
27. Hernandez Ceruelos A, Romero-Quezada LC, Ruvalcaba Ledezma JC, Lopez Contreras L (2019) Therapeutic uses of metronidazole and its side effects: an update. Eur Rev Med Pharmacol Sci 23 (1):397-401. doi:10.26355/eurrev_201901_16788
28. Kikuchi T, Koga M, Shimizu S, Miura T, Maruyama H, Kimura M (2013) Efficacy and safety of paromomycin for treating amebiasis in Japan. Parasitol Int 62 (6):497-501. doi:10.1016/j.parint.2013.07.004
29. Gonzales MLM, Dans LF, Sio-Aguilar J (2019) Antiamoebic drugs for treating amoebic colitis. Cochrane Database Syst Rev 1:CD006085. doi:10.1002/14651858.CD006085.pub3
30. Bernstein LH, Frank MS, Brandt LJ, Boley SJ (1980) Healing of perineal Crohn's disease with metronidazole. Gastroenterology 79 (2):357-365
31. Schwebke JR, Desmond RA (2011) Tinidazole vs metronidazole for the treatment of bacterial vaginosis. Am J Obstet Gynecol 204 (3):211 e211-216. doi:10.1016/j.ajog.2010.10.898
32. O'Reilly RA (1976) The stereoselective interaction of warfarin and metronidazole in man. N Engl J Med 295 (7):354-357. doi:10.1056/NEJM197608122950702
33. Tirkkonen T, Heikkila P, Huupponen R, Laine K (2010) Potential CYP2C9-mediated drug-drug interactions in hospitalized type 2 diabetes mellitus patients treated with the sulphonylureas glibenclamide, glimepiride or glipizide. J Intern Med 268 (4):359-366. doi:10.1111/j.1365-2796.2010.02257.x
34. Wassmann C, Hellberg A, Tannich E, Bruchhaus I (1999) Metronidazole resistance in the protozoan parasite Entamoeba histolytica is associated with increased expression of iron-containing superoxide dismutase and peroxiredoxin and decreased expression of ferredoxin 1 and flavin reductase. J Biol Chem 274 (37):26051-26056. doi:10.1074/jbc.274.37.26051
35. Samarawickrema NA, Brown DM, Upcroft JA, Thammapalerd N, Upcroft P (1997) Involvement of superoxide dismutase and pyruvate:ferredoxin oxidoreductase in mechanisms of metronidazole resistance in Entamoeba histolytica. J Antimicrob Chemother 40 (6):833-840. doi:10.1093/jac/40.6.833
36. Mi-Ichi F, Yoshida H (2019) Unique Features of Entamoeba Sulfur Metabolism; Compartmentalization, Physiological Roles of Terminal Products, Evolution and Pharmaceutical Exploitation. Int J Mol Sci 20 (19). doi:10.3390/ijms20194679
37. Mi-Ichi F, Ishikawa T, Tam VK, Deloer S, Hamano S, Hamada T, Yoshida H (2019) Characterization of Entamoeba histolytica adenosine 5'-phosphosulfate (APS) kinase; validation as a target and provision of leads for the development of new drugs against amoebiasis. PLoS Negl Trop Dis 13 (8):e0007633. doi:10.1371/journal.pntd.0007633
38. Debnath A, Parsonage D, Andrade RM, He C, Cobo ER, Hirata K, Chen S, Garcia-Rivera G, Orozco E, Martinez MB, Gunatilleke SS, Barrios AM, Arkin MR, Poole LB, McKerrow JH, Reed SL (2012) A

high-throughput drug screen for Entamoeba histolytica identifies a new lead and target. Nat Med 18 (6):956-960. doi:10.1038/nm.2758
39. Andrade RM, Chaparro JD, Capparelli E, Reed SL (2014) Auranofin is highly efficacious against Toxoplasma gondii in vitro and in an in vivo experimental model of acute toxoplasmosis. PLoS Negl Trop Dis 8 (7):e2973. doi:10.1371/journal.pntd.0002973
40. Martinez-Perez Y, Nequiz-Avendano M, Garcia-Torres I, Gudino-Zayas ME, Lopez-Velazquez G, Enriquez-Flores S, Mendoza E, Saavedra E, Perez-Tamayo R, Leon-Avila G, Olivos-Garcia A (2020) Rabeprazole inhibits several functions of Entamoeba histolytica related with its virulence. Parasitol Res 119 (10):3491-3502. doi:10.1007/s00436-020-06868-0
41. Chacin-Bonilla L (2012) [Current pharmacotherapy of amebiasis, advances in new drugs, and design of a vaccine]. Invest Clin 53 (3):301-314
42. Martinez-Castillo M, Pacheco-Yepez J, Flores-Huerta N, Guzman-Tellez P, Jarillo-Luna RA, Cardenas-Jaramillo LM, Campos-Rodriguez R, Shibayama M (2018) Flavonoids as a Natural Treatment Against Entamoeba histolytica. Front Cell Infect Microbiol 8:209. doi:10.3389/fcimb.2018.00209
43. Zolfaghari Emameh R, Barker H, Tolvanen ME, Ortutay C, Parkkila S (2014) Bioinformatic analysis of beta carbonic anhydrase sequences from protozoans and metazoans. Parasit Vectors 7:38. doi:10.1186/1756-3305-7-38
44. Bua S, Haapanen S, Kuuslahti M, Parkkila S, Supuran CT (2018) Sulfonamide Inhibition Studies of a New beta-Carbonic Anhydrase from the Pathogenic Protozoan Entamoeba histolytica. Int J Mol Sci 19 (12). doi:10.3390/ijms19123946
45. Haapanen S, Bua S, Kuuslahti M, Parkkila S, Supuran CT (2018) Cloning, Characterization and Anion Inhibition Studies of a beta-Carbonic Anhydrase from the Pathogenic Protozoan Entamoeba histolytica. Molecules 23 (12). doi:10.3390/molecules23123112
46. Quach J, St-Pierre J, Chadee K (2014) The future for vaccine development against Entamoeba histolytica. Hum Vaccin Immunother 10 (6):1514-1521. doi:10.4161/hv.27796