Significance of amebiasis: 10 reasons why neglecting amebiasis might come back to bite us in the gut

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Introduction

Nearly 150 years since the first detailed description of the invasive, tissue-destroying intestinal parasite, Entamoeba histolytica, amebiasis remains an infection of consequential global importance. Infection with E. histolytica can lead to amebic colitis, amebic dysentery, and amebic liver abscess. Even though amebiasis is a leading cause of diarrhea globally, a lack of basic science research to improve our understanding of the complex pathogenesis of this parasite hampers progress. Renewed attention is required to help combat this infection of poverty in order to develop innovative and inexpensive point-of-care diagnostic and surveillance tools, novel treatment options, and effective preventive strategies to help end amebiasis transmission. This viewpoint summarizes 10 reasons why amebiasis is a global health problem in need of further attention (Fig 1).

Reason #1: Amebiasis remains an infection of top global importance, particularly in poverty-stricken settings

Amebiasis is a leading cause of severe diarrhea worldwide [1, 2], though estimates of actual disease burden may be prone to reference test bias, so some heed is warranted. In a large, multinational, prospective case-based study of children with moderate-to-severe diarrhea using molecular methods to identify etiology, however, amebiasis ranked among the top 15 causes of diarrhea in the first two years of life in children living in developing countries, where diarrhea remains the fifth leading cause of death in children under the age of five years [1, 3–5]. While amebiasis occurs worldwide, it is largely an infection of impoverished communities, particularly when sanitation is poor. Amebiasis remains endemic in several developing areas of Central and South America, Asia, and Africa [2]. Advances in molecular technology have improved our understanding of this infection by leading to the recognition and separation of E. histolytica from other morphologically identical but less-pathogenic or nonpathogenic species of Entamoeba, including E. dispar, E. moshkovskii, and E. bangladeshi [6]. Despite the ability to distinguish E. histolytica by molecular methods, prevalence data on amebiasis remain scarce and imprecise because of inadequate utilization and access to surveillance and diagnostic tools with superior sensitivity and specificity. Some reports that attempt to describe the incidence and prevalence of amebiasis may be inaccurate, especially if the poorly sensitive method of microscopy is used. Experts estimate that millions of people continue to be infected with E. histolytica each year, and there are several recent reports that help to illustrate the
10 Reasons Not to Neglect Amebiasis

1. Top 15
   - cause of diarrhea globally, affecting millions of impoverished people annually

2. 55,000
   - people die of amebic colitis each year

3. Travelers
   - cause of diarrhea in travelers who return ill

4. STD
   - oral-anal sexual transmission increasingly recognized

5. Outbreak
   - ease of dissemination facilitates outbreaks among military and general public

6. Mimics IBD
   - misdiagnosis can lead to fulminant amebic colitis

7. Hypervirulent
   - strains recently reported

8. Errors
   - in diagnosis due to use of insensitive laboratory tests

9. Only 1
   - class of drugs available to effectively treat invasive forms of disease, with no alternatives if resistance develops

10. No vaccine
    - available to prevent infection or decrease disease severity

Amebiasis is a global problem. New innovations for diagnosis, discovery of drug targets and strategies for control are needed.

Fig 1. Significance of amebiasis.

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current disease burden of amebiasis. In Mexico, for example, over 8.8 million cases of amebiasis were reported to their National Epidemiological Surveillance System between 2000–2010 [7]. The seroprevalence of amebiasis in some rural areas of Mexico reaches as high as 42% [8, 9], although it should be noted that detectable antibodies to *E. histolytica* may persist for years, so seroprevalence may overestimate the true disease burden. In Asia, leishmaniasis and amebiasis represent the neglected tropical protozoal infections of highest burden, particularly in the Indian subcontinent [10]. *E. histolytica* was detected by molecular methods in nearly 15% of fecal samples submitted for analysis in northeast states of India for instance [11], although again the true disease burden for much of Asia remains unknown. Amebiasis prevalence data from Africa are particularly limited, but it appears widespread. Up to one-third of the population in Vhembe, South Africa had reactive serology, for example [12], while 38% of patients presenting for medical care with acute diarrhea in Egypt were diagnosed with amebiasis by stool antigen study [13]. In the large Global Enteric Multicenter Study (GEMS) of children under the age of five years with moderate-to-severe diarrhea living in seven countries of sub-Saharan Africa and South Asia, *E. histolytica* was among the top seven pathogens causing dysentery [4].

**Reason #2: Re-emergence of amebiasis in developed countries**

Traditionally, the incidence of amebiasis has been low in industrialized and developed countries, but recent trends have shown reemergence associated with travel to endemic areas, immigration, and sexual transmission [14]. For example, in a large case series from Paris, France; all of the 90 patients with amebic liver abscess identified between the years 2002–2006 were imported, with about half occurring in European-born travelers to tropical areas and the other half in foreign-born immigrants [15]. Amebiasis was the third most frequently isolated pathogen among returning travelers presenting to one of 42 GeoSentinel Surveillance Network sites globally and seeking medical attention for gastrointestinal infection [14]. Travelers to South Asia, the Middle East, and South America appear to be at highest risk, particularly those engaging in missionary and other types of volunteering work [14]. Other reports indicate that a smaller proportion of travelers are affected by amebiasis, and these differences may reflect variations in the epidemiologic risk of groups studied, methods used for detection, duration of travel, and duration of symptoms prior to performing diagnostic testing [16–18]. Here in the United States, the prevalence of amebiasis is about 4%, and, surprisingly, at least five people die in the US from this infection each year [19]. In the state of California, an average of 329 cases of amebiasis are reported annually [20]. In the state of Texas, there are nearly 200 cases of amebiasis reported each year [21]. These two instances in the US underscore an underappreciated problem affecting the poor living among the wealthiest nations, where amebiasis represents one of the most common neglected tropical infections affecting people living in developed countries [22]. Awareness of amebiasis in these settings is important, as lack of familiarity with this infection has led to failure to recognize amebic colitis with resultant fulminating and even fatal outcomes [23].

**Reason #3: Amebiasis is also a sexually transmitted disease**

Transmission of amebiasis is through ingestion of the infective cyst, often through fecally contaminated food and water, but direct person-to-person contact, including oral–anal sexual practices, has been recognized increasingly as an alternative means of transmission [24]. Homosexual men, bisexual men, and other groups of men who have sex with men (MSM) in particular are at higher risk of acquisition of amebiasis [25], as highlighted by several countries in Asia, Europe, North America, and Australia [24–29]. In Taiwan for example, HIV-infected
MSM had 15 times the odds of having infection with \textit{E. histolytica} than other risk groups [26]. In the Beijing and Tianjin provinces of China, amebiasis seroprevalence reached as high as 41\% among MSM [25]. A report from Canada recently showed that transmission is also associated with heterosexual and female homosexual activity as well [30].

\textbf{Reason #4: Amebiasis can be easily transmitted in outbreak settings}

Outbreaks of amebiasis continue to be reported among military and general populations [31, 32]. In a recent outbreak of amebiasis in the Meru county of central Kenya, at least 38 people were hospitalized with four fatalities [33]. Several properties of the \textit{E. histolytica} cyst, such as low infectious dose and relative resistance to chlorine, facilitate the ease with which it can be disseminated through contamination of food and water supplies, even in low incidence areas. Thus, \textit{E. histolytica} is classified as a category B priority biodefense pathogen by the National Institute of Allergy and Infectious Diseases.

\textbf{Reason #5: Severe amebic disease is associated with high fatality}

Following ingestion, infective cysts transform to invasive trophozoites, which leads to the development of mucosal inflammation and colonic ulcers [34]. Most of those infected will have asymptomatic amebiasis. For poorly understood reasons, about 10\%–20\% will develop symptomatic disease, characterized by diarrhea and dysentery. Invasion and dissemination to extraintestinal sites such as the liver can follow. Fulminant disease is rare, occurring in 1\%–2\% of infections, but carries a high fatality, exceeding 50\% in those with severe colitis [23]. \textit{E. histolytica} was one of the top 10 causative agents of moderate-to-severe diarrhea in children under the age of five years at two of the GEMS study sites in Bangladesh and Mali. Diarrhea with \textit{E. histolytica} was associated with a relatively greater risk of death across all GEMS sites and was the enteric pathogen with the highest hazard ratio for death in the second year of life [5]. It is estimated that amebic colitis kills more than 55,000 people each year [35]. While the incidence of amebic liver abscess is much lower than intestinal amebiasis, the associated morbidity and fatality can also be quite high [15, 36].

\textbf{Reason #6: Devastating consequences may arise if amebic colitis is misdiagnosed as inflammatory bowel disease}

Patients with amebic colitis may present acutely or chronically with abdominal pain, diarrhea, bloody stools, and weight loss [2]. Many of these symptoms overlap with those of inflammatory bowel disease, and the two conditions may be indistinguishable even by stool inflammatory markers, imaging, endoscopic findings, and lesion distribution [23]. Patients with both symptomatic and asymptomatic forms of amebiasis treated with corticosteroid therapy are at high risk of developing severe and even fatal amebic colitis [23]. This is complicated by the fact that corticosteroids are a mainstay in the management of inflammatory bowel disease, so it is important to consider the diagnosis of amebiasis in patients prior to the administration of corticosteroid treatment. In Turkey, for example, the prevalence of \textit{E. histolytica} detected by stool antigen assay was 32\% in patients presenting with ulcerative colitis, underscoring the importance of considering this diagnosis particularly in those who reside in endemic areas [37]. Caution is needed here in the face of the increasing global incidence and prevalence of inflammatory bowel disease [38].
Reason #7: New emergence of hypervirulent strains of *E. histolytica*

Of grave concern has been the report of the highly virulent nature of the *E. histolytica* strains that were being transmitted in the recent outbreak of amebiasis in Canada [30]. There were substantial differences noted from other *E. histolytica* isolates [30]. It is possible that the noted genetic alterations could have either allowed the parasite to more readily evade host immune responses or be associated with higher virulence, resulting in more severe clinical presentations. Ongoing vigilance is needed to identify other hypervirulent strains.

Reason #8: Inaccurate tests continue to be used for diagnosis

Despite advances in methodologies to diagnose infection with *E. histolytica*, lack of access prohibits the use of these more accurate tests. Recommended diagnostic modalities available to assist with diagnosing amebiasis, include molecular assays, stool antigen detection assays, and serology. Molecular assays (considered the gold standard for diagnosis) have high sensitivity and specificity approaching 100%, can be combined with multiplex panels to detect multiple enteric pathogens simultaneously, and can also be used for both stool and fluid samples [2, 39]. Molecular methods, however, are expensive and require specialized instruments and kits for analysis, as well as trained personnel, making this diagnostic method virtually inaccessible to endemic, resource-limited settings. The stool antigen detection tests also have very good sensitivity and specificity in identifying intestinal amebiasis but are expensive, also require expertise to perform, and remain heavily underutilized in endemic areas [2]. Serology can be a useful adjunctive test, particularly for the diagnosis of amebic liver abscess and other extraintestinal manifestations of the disease, but antibodies remain detectable for years after treatment, making it difficult to distinguish between active and past infection [2, 8]. While stool microscopy is more widely available, visualization of cysts and trophozoites can be easily missed by the examiner and when seen cannot be differentiated from other *Entamoeba* species [2, 40]. Stool microscopy has a sensitivity of <60%, and its use should be avoided when other modalities are available [2]. This highlights the need for accessible, inexpensive, point-of-care tests that can diagnose intestinal and extraintestinal forms of amebiasis with high sensitivity and specificity in endemic areas in order to identify those in need of treatment and decrease spread by asymptomatic carriers.

Reason #9: Lack of drug development makes us ill-prepared to deal with the development of potential resistance

All patients with amebiasis need treatment to mitigate disease and prevent spread, but only a small number of drugs are available to effectively do this. Treatment of amebiasis is complicated, and there is no single agent available that reliably treats both the invasive and intestinal carriage stages of infection. Hence it is recommended that those suffering with symptomatic disease require treatment with two different drugs, an amebicidal tissue-active agent followed by a luminal cysticidal agent. This combination may result in fewer parasitologic failures than use of a tissue-active agent alone [41]. Those with asymptomatic amebiasis only require treatment with a luminal cysticidal agent. A single class of drugs, the nitroimidazoles, currently serve as the mainstay of effective treatment for symptomatic forms of amebiasis [41, 42]. Toxicity can be associated with this class, including peripheral neuropathy, encephalopathy, and cerebellar ataxia [41, 43]. The nitroimidazoles, namely metronidazole and tinidazole, are also the treatment of choice for other anaerobic protists infections, such as giardiasis and trichomoniasis. Given that resistance to these agents has already been documented among *Trichomonas*
and the ease of generating resistance strains in the laboratory [48], it is feared that it is only a matter of time before we start seeing the emergence of drug resistant amebiasis. Laboratory induced metronidazole resistant \( E. \) \textit{histolytica} has already been described through increased expression of iron-containing superoxide dismutase and peroxiredoxin and decreased expression of ferredoxin 1 and flavin reductase, for example [49]. The thiazolide agent, nitazoxanide, has been used by some to treat the tissue invasive form of amebiasis, based on the results of a small, single-center trial conducted in Cairo, Egypt [50]. The high response rate in the placebo arm raises methodologic concerns, and further studies are needed to clarify efficacy before nitazoxanide can be widely recommended as a treatment option for amebiasis. Drugs with cysticidal activity for the treatment of luminal carriage include paromomycin, iodoquinol, and diloxanide furoate. Diloxanide furoate is of very limited availability and significant safety concerns exist with the use of iodoquinol, which has been associated with the development of optic neuritis and peripheral neuropathy [51]. The revival of drugs historically found to have antiamebic activity, such as anisomycin and prodigiosin, and the repurposing of existing drugs already approved for other therapeutic indications with secondary antiamebic properties, such as mefloquine, are alternative strategies currently under active investigation [52]. Furthest along into clinical drug development among these repurposed drugs is the gold compound, auranofin, which targets thioredoxin reductase in \( E. \) \textit{histolytica}, rendering it susceptible to oxidative stress. Auranofin was previously used in the management of selected patients with rheumatoid arthritis, but further safety analysis is needed to inform whether established concerns such as diarrhea, rash, and bone marrow toxicity will limit its use as an antiparasitic agent [53]. Lastly, several bioactive natural compounds, such as flavinoids and curcuminoids, have potential antiamebic effect but are much in need of further characterization and are a long way from potential clinical application [54], as recently reviewed [52]. There has really been little progress made in drug development over the past 60 years [55], despite priorities set by the National Institute of Allergy and Infectious Diseases to develop drugs for the treatment of category B biodefense pathogens [52]. The ability to successfully treat amebiasis in the future is unpredictable, and there is an urgent need for effective therapies.

**Reason #10: There is no effective vaccine for the prevention of amebiasis**

Partially protective acquired host immunity has been demonstrated, but the relative importance of mucosal, cellular, and humoral immunity in protection is still undetermined. Gal-lectin based vaccinations appear promising in animal models [56]. Other amebic antigenic virulence factors, such as the serine-rich \( E. \) \textit{histolytica} protein, hold potential as vaccine candidates but are also a long way from clinical development for use in humans, as recently reviewed [52]. Without an effective vaccine, control relies on targeting prevention of transmission by fecal–oral spread. This is quite a daunting task when considering that 2.3 billion people in this world still do not have basic sanitation facilities such as toilets or latrines, and 1.8 billion use a source of drinking water contaminated with feces [57].

**Opportunities for progress**

Modest progress in the field has improved our understanding of host cell death, mucosal inflammation, and parasite invasion with amebic infection, but there is still much that is unknown [34, 58–61]. Further understanding of pathogenesis and host response is needed as a way forward towards enhanced treatment and prevention strategies. Increased resources should be devoted to amebiasis research with priority areas highlighted in Table 1. These
recommendations overlap with several of those made by the World Health Organization over 20 years ago, overall reflecting how little advancement there has been over the past couple of decades [62]. Greater awareness and advocacy are needed to develop funding and interest to create multitargeted approaches designed to help break the cycle of transmission and prevent severity of disease. Although amebiasis is estimated to be responsible for 2.2 million disability-adjusted life years, it not listed in the Global Funding of Innovation for Neglected Diseases (G-Finder) Report indicating negligible funding [63, 64]. Now is the time to create opportunities to build on the recent interest and success in helping to improve global health outcomes. 

Conclusions

As summarized in Fig 1, the significance of amebiasis is a global problem, with prevalence that may reach as high as 40% in some areas of the world. Disease can be severe and fatal in some, but there are no accurate disease prevalence estimates to help define the true burden. Transmission is fecal–oral and so cannot easily be prevented when there is poverty, inadequate sanitation, and insufficient hygiene. Globalization, immigration, international travel, and sexual practices place everyone at risk of infection. The hardiness of the cyst further lends to ease of spread, predisposing to risks of epidemics among military, the general population, and even as a potential biological threat. The emergence of hypervirulent strains has been newly reported. Treatment options are limited, and there are no other reliably effective options if resistance develops to the nitroimidazole agents, as has already been documented with other protozoan parasites treated by this class of drugs. We still lack a viable vaccine candidate ready to enter into clinical development. Funding agencies dedicated to support research in neglected tropical diseases control should include E. histolytica as a priority pathogen in order to encourage the development of new innovative technologies for diagnosis, discovery of new drug targets, and strategies for control, including vaccine development. Now is the time to stop neglecting amebiasis.

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Table 1. Priority areas for amebiasis research.

| Priority Area                                                                 |
|-------------------------------------------------------------------------------|
| 1. Improved use of molecular epidemiology for surveillance to understand the true prevalence of amebiasis in developing countries |
| 2. Development of inexpensive, easy to use rapid diagnostic tests appropriate for field use in developing countries |
| 3. Improved understanding of molecular biology, pathogenesis, and host immune responses to inform control strategies |
| 4. Drug development to find safe, effective alternative treatment options |
| 5. Vaccine development to identify safe and effective vaccine candidates |
| 6. Development of technologies to improve access to clean water and strategies to improve water infrastructure, as well as adequate sewage disposal |

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