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Amebiasis as a sexually transmitted infection: A re-emerging health problem in developed countries

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Abstract: Amebiasis, which is caused by *Entamoeba histolytica* (*E. histolytica*), is the second leading cause of parasite-related death worldwide. It manifests from asymptomatic carriers to severe clinical conditions, like colitis and liver abscesses. Amebiasis is commonly seen in developing countries, where water and food are easily contaminated by feces because of the poor sanitation. However, a recently challenge in many developed countries is the increase in domestic cases of invasive amebiasis as a sexually transmitted infection (STI amebiasis). In contrast to food-/waterborne transmission of *E. histolytica* in developing countries, transmission of STI amebiasis occurs directly through human-to-human sexual contact (*e.g.*, men who have sex with men and people who engage in oral-anal sex); in this setting, asymptomatic infected individuals are the main reservoir of *E. histolytica*. The Development of screening methods for the early diagnosis of asymptomatic *E. histolytica* infection is the key to epidemiologic control. Moreover, delay in diagnosis of severe cases (*e.g.*, fulminant amebiasis) leads to death even in developed countries. It is also important to increase clinical awareness of domestically transmitted STI amebiasis in the clinical settings. This review considers the changing epidemiology and clinical manifestations of STI amebiasis, and finally discusses the future strategies for the better practice.

Keywords: STI amebiasis, *Entamoeba histolytica*, asymptomatic carriers, epidemiological control, high-risk populations, serological screening

Introduction

Amebiasis is caused by *Entamoeba histolytica* (*E. histolytica*), which is transmitted *via* the fecal-oral route and is the second leading cause of parasite-related death worldwide (*1*). Transmission could occur *via* the oral ingestion of the transmissible cystic form of *E. histolytica*, which is continuously shed in the stool (*2,3*). Therefore, amebiasis was thought to be prevalent only in developing countries due to poor sanitation, but it is increasingly being reported as a sexually transmitted infection (STI amebiasis) in developed nations (*4,5*). Additionally, amebiasis sometimes causes a life-threatening disease called fulminant amebiasis, which presents as an acute abdomen from the perforation of the large intestinal and mimics acute appendicitis (*6-11*). If such cases are not treated with amebicidal drugs and resection of the perforated intestine, they are often critical and result in death. In fact, many cases of fulminant amebiasis are only diagnosed postmortem. This is because clinicians in developed nations may not be fully aware of the increasing risk posed by *E. histolytica* infection.

In this review, we discuss the epidemiology and clinical manifestations of STI amebiasis, as well as the measures required for the epidemiological control of this infection.

Epidemiology

Amebiasis is a disease caused by the oral ingestion of the transmissible cystic form of *E. histolytica* found in human feces. In developing countries, transmission typically occurs *via* the ingestion of food and water contaminated with feces. It was previously believed that amebiasis only occurred in people who had visited an endemic area, causing imported infections in developed countries. However, it was recently recognized that the pathogen can also be transmitted directly from person to person. Instances of *E. histolytica* strain clustering have been found as a result of sexual contact (*4,5*) or within institutions caring for individuals with cognitive

impairment (*12,13*). Food- or waterborne infections occur in areas with poor sanitation. *E. histolytica* remains a leading cause of parasitic-infection-related mortality and morbidity worldwide (*1*). However, over the past two decades, amebic infection has been increasingly reported as a sexually transmitted infection (STI) in developed countries of East Asia and in Australia (*2,14-21*). One study conducted at a human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS) voluntary counseling testing (VCT) center in Taiwan, reported that men who have sex with men (MSM) and people engaging in oral-anal sex, were more likely to be seropositive for *E. histolytica* (*22*). Our group reported that *E. histolytica* seropositivity was over seven times higher than that of HIV-1, and the same as that of syphilis, at a VCT center in Tokyo (*23,24*). The incidence of invasive amebiasis (symptomatic cases) in Japan, based on national surveillance data from 2000 to 2013, is on the rise (*25*). Additionally, the proportion of domestic cases has increased to 85%. Moreover, since 2008, there has been a shift in transmission patterns, with cases associated with heterosexual contact surpassing those derived from homosexual contact (*25*). In addition to the shift in disease epidemiology, the recent rise in the number of amebiasis cases in Japan is thought to be due to an increase in accidentally diagnosed cases by colonoscopy (*25*), highlighting the importance of the active surveillance to reveal the accurate disease prevalence including asymptomatic carriers. Of note, only symptomatic invasive amebiasis cases are currently included in the national surveillance data in Japan.

In addition, genotyping studies of *E. histolytica* clinical isolates have reported that multiple original Japanese genotypes are currently prevalent as STI strains in Japan (*26,27*). Furthermore, whereas older studies from the United States and United Kingdom reported that amebiasis among MSMs arose from nonpathogenic *E. dispar* infection (*28,29*), more recently, specific sexual behaviors among MSM, particularly oral-anal intercourse, have emerged as a key risk factor for amebiasis-related mortality in the United States (*30*). *E. histolytica* infection has also been recently reported to occur as a comorbidity among HIV-infected MSM or as a domestic STI in developed European countries (*5,31-34*). Thus, it is important for primary care physicians to take detailed histories of sexual behavior (*e.g.*, oral-anal sexual contact) as well as travel history, whenever amebiasis is suspected. Furthermore, there are a considerable number of asymptomatic infected people in the high-risk community, which is the main transmission source for STI amebiasis. An effective epidemiological strategy should therefore efficiently identify and treat asymptomatic individuals in high-risk communities to decrease the infection reservoir of STI amebiasis.

Clinical manifestations of *E. histolytica* infection and the associated disease forms

Asymptomatic self-limiting infection is the most common form of amebiasis. Up to 80–90% of people exposed to *E. histolytica* cysts have no symptoms or mild symptoms and clear the infection spontaneously. However, asymptomatic infection persists for more than a year in some individuals, who are reported as asymptomatic cyst passers (*2,3*) or as fecal occult blood (FOB)-positive individuals incidentally diagnosed during colonoscopy (*25,35,36*). These asymptomatic infected individuals can be a source of new infections in the community in the poor sanitary condition. Some of these patients develop symptomatic invasive disease, usually within one year of latent infection (*3,37-39*). However, in some cases, *E. histolytica* also causes severe, life-threatening amebiasis.

Amebic colitis and liver abscess are the most common clinical forms of invasive amebiasis. These common disease forms of invasive amebiasis respond well to therapy with tissue-active agents, such as metronidazole. However, in some cases, amebic colitis is complicated by the secondary bacterial peritonitis owing to a fistula or perforation of the large intestine, which leads fulminant amebiasis (*6-8*). The use of immunosuppressive agents, such as corticosteroids, is reported as a risk factor for the development of fulminant amebiasis (*8*). However, because no risk factors can be identified in the majority of fulminant amebiasis cases, this condition is considered to mostly occur in immunocompetent hosts (*8*). Furthermore, fulminant amebiasis presents as an acute abdomen, which often mimics appendicitis; thus, this form of the disease is sometimes called amebic appendicitis (*6,9-11*). It is very difficult for physicians to differentiate between amebic and nonamebic appendicitis using clinical and laboratory findings (*10*). Moreover, amoebic infection is frequently overlooked by routine histological examination (using hematoxylin and eosin [H&E] staining) of the resected intestinal tissue. Instead, immunohistochemistry with a monoclonal antibody against *E. histolytica* or periodic acid–Schiff (PAS) staining are recommended for the identification of *Entamoeba* species. Although PAS non-specifically stains polysaccharides, it can be used to easily distinguish *Entamoeba* species (positive staining) from leukocytes (negative staining) in the resected tissue (Figure 1).

Appendectomy without amebicidal treatment may result in severe postoperative complications, such as abdominal sepsis, gastrointestinal fistula, or hemorrhage (*8-10,40*). The inaccurate diagnosis and treatment of amebic appendicitis result in poor clinical outcomes. Indeed, many cases of amebic appendicitis are only diagnosed at autopsy and the mortality rate for this condition is estimated to be as high as 3.2–33%, even in developed countries (*9,40,41*). Moreover, a systematic review of 174 cases revealed that most patients with amebic appendicitis experienced several months of asymptomatic infection with *E. histolytica* before

developing an acute abdomen, as the time from exposure to symptom onset ranges from months to years (*9*). These findings emphasize the importance of early detection to protect patients from life-threatening invasive disease. Thus, the underestimation of the epidemiology of STI amebiasis cases, as well as the wide range of clinical presentations, pose considerable challenges in primary care settings in developed countries, which need to be overcome.

Diagnosis of *E. histolytica* infection (Figure 2)

Diagnosis of invasive "symptomatic" disease

For symptomatic cases, a combination of antigen detection in the stool and serological testing is currently recommended (*42,43*). When amebic colitis is suspected (owing to diarrhea and/or dysentery), antigen detection and direct microscopic examination are reliable tests, with sensitivities and specificities comparable to those of the polymerase chain reaction (PCR) (*44,45*). Enzyme-linked immunosorbent assay (ELISA) and immunochromatography (IC) antigen detection kits for stool samples are available in many countries. These immunoassays, especially IC, are relatively simple to perform and yield rapid results. Moreover, the most frequently used ELISA (Techlab *E. histolytica* II) and IC (Techlab *E. histolytica* QUIK CHEK) kits can distinguish *E. histolytica* from other *Entamoeba* species because they use antibodies targeting an *E.-histolytica*-specific Gal/GalNAc lectin (*46,47*). PCR (especially real-time PCR) testing of stool or infected abscess/tissue samples is the most sensitive and specific diagnostic method for *E. histolytica* amebiasis. However, PCR protocols, including the need to extract DNA from various clinical samples, remain too technically complex for general dissemination (*43*). In Japan, there is no insurance coverage to be used for the routine clinical diagnosis of *E. histolytica* infection and primarily considered as a research tool. Serum antibody testing is useful in cases when amebic liver abscess is suspected without intestinal symptoms. Antibodies are detectable in over 70% of patients within 5–7 days of acute infection, and in over 90% of patients at 2–3 weeks after infection acquisition. However, patients remain seropositive for years after treatment (*48*). Therefore, although a negative serological result is helpful in ruling out disease, a positive result cannot be used to distinguish between present and past infection.

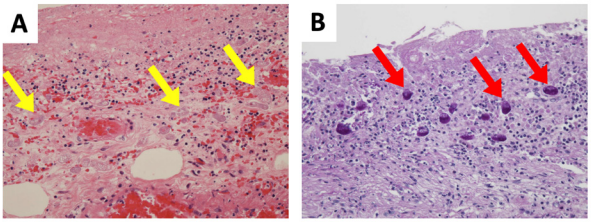

Figure 1. Representative histopathological images of *Entamoeba*-positive colonic tissue (Original photo from National Center for Global Health and Medicine; magnification, 400×). (A) In submucosal tissue, the cytoplasm of the invading *Entamoeba* trophozoites (yellow arrows), identified using hematoxylin and eosin (H&E) staining, is very similar to that of leukocytes. **(B)** Periodic acid-Schiff (PAS) staining differentiates PAS-positive *Entamoeba* trophozoites (red arrows) from PAS-negative leukocytes.

		Clinical form of amebiasis				Comments for each diagnostic test
		A. Asymptomatic carrier	B. Amebic colitis	C. Amebic liver abscess	D. Fulminant amebiasis	
Pathogen detection tests	Stool, direct microscopy	△	△	△	△	Not generally recommended because testing accuracy highly depends on the skill and experiences of the examiner.
	Stool, antigen test	△	◎	△	△	Detection of trophozoite surface antigen. Sensitivity is high for loose stool but low for formed stool.
	Histopathology	○	△	×	◎	Morphological identification in resected intestinal tissue. Periodic acid Schiff (PAS) staining should be performed.
	Nucleic acid detection test (PCR)	◎	△	△	△	High sensitivity and specificity; however not widely implemented because of high cost and technical complexity.
Blood test	Serological antibody test	○	○	◎	○	Highly sensitive in the convalescent phase of infection; however, seropositivity remains for 2–3 years post-infection.

Figure 2. Diagnostic methods for different clinical forms of amebiasis. (A) Asymptomatic carriers can be screened using serology (~90% sensitivity); however, serology can also detect past infections, which had occured within the last 2–3 years. PCR of stool samples or colonoscopy have a high diagnostic value for current *Entamoeba* infection. **(B)** If the patient has diarrhea and/or dysentery, a stool antigen test should be performed for amebic colitis; serology is useful as an adjunct to the diagnosis. **(C)** For amebic liver abscess, a serological test is non-invasive and highly sensitive. A stool antigen test can be performed for patients with diarrhea and/or dysentery. **(D)** For the diagnosis of fulminant amebiasis, *Entamoeba* invasion into the submucosal tissue can be confirmed by histopathology of resected intestinal tissue. Periodic acid-Schiff (PAS) staining should be performed alongside routine hematoxylin and eosin (H&E) staining to differentiate between *Entamoeba* and leukocytes. Marks on the table: ×, cannot be performed; △, generally low diagnostic value or too labor intensive; ○, used alonside other diagnostic tools, ◎, highly recommended.

A diagnosis should be made by combining serological methods with the evaluation of clinical manifestations and the identification of liver abscess using imaging. In patients with diarrhea, antigen testing of stool samples is also a useful method for diagnosing *E. histolytica* infection (*43*). As mentioned above, because *Entamoeba* species have a similar morphology to leukocytes in the resected colonic tissue, routine pathological examination using H&E staining is often ineffective. Thus, if there is even the slightest suspicion of intestinal amebiasis, PAS staining should additionally be performed.

Diagnosis of noninvasive "asymptomatic" infection

It is challenging for clinicians to diagnose *E. histolytica* infection in an asymptomatic carrier, as stool antigen tests and direct microscopy have insufficient sensitivities in these individuals (*44,45,49-51*). Although serological testing is highly sensitive, even in asymptomatic carriers (*52,53*), a positive result may indicate past and not necessarily present infection. To distinguish between present and past infections in asymptomatic individuals with positive serological test results, PCR of stool samples is commonly performed. Moreover, colonoscopy has a high diagnostic value (*35,54-57*). In asymptomatic cases, visible infective ulcers can be found but usually limited within the proximal side (from cecum to ascending colon), whereas ulcers are distributed to the multiple sites of large intestine (from cecum to rectum) in symptomatic colitis cases (*58,59*). Interestingly, even in the asymptomatic cases, trophozoites are identified at infection sites whereas cystic forms are commonly detected in the stools (*58,59*). Importantly, these ulcerative lesions are not found in the small intestine (terminal ileum), which helps differentiate amebiasis from other inflammatory diseases, such as Crohn disease, tuberculosis, and cytomegalovirus colitis (*35,60*). In Japan, an increasing number of cases of asymptomatic *E. histolytica* infection have been accidentally diagnosed by colonoscopy in people with positive FOB results who had been referred for colon cancer screening (*25,36*). In another report from a Japanese HIV-1 cohort, amebic colitis was pathologically identified in 11.2% of asymptomatic HIV-1-positive individuals, of whom 87.5% were seropositive for *E. histolytica* (*52*). At present, the diagnosis of *E. histolytica* infection, and especially asymptomatic infection, is often challenging. However, we believe that in high-risk patients, employing a highly sensitive serological antibody test to screen for asymptomatic infection, followed by PCR or colonoscopy and reach a definitive diagnosis, would prove effective.

Treatment of *E. histolytica* infection

The recommended treatment regimens differ for the invasive and noninvasive forms of *E. histolytica* infection.

Treatment of invasive infection

For invasive infections, such as those leading to amebic colitis and amebic liver abscesses, tissue-active agents should be administered prior to lumen-active agents because the latter are poorly absorbed in the intestine. Metronidazole (Flagyl®) or tinidazole (Tindamax®) are widely used as tissue-active agents; although only metronidazole is approved for use in patients with amebiasis in Japan. Treatment with tissue-active agents should be followed by lumen-active agents because parasites persist in the intestine in up to 40–60% of patients who achieve complete symptom recovery with tissue-active agents (*61*). In addition to tissue-active agents, administration of broad-spectrum antibiotics and/or surgery should be considered in patients with fulminant colitis and amebic appendicitis, as described in detail above (see: Clinical manifestations of *E. histolytica* infection and the associated disease forms). Therapeutic needle aspiration or catheter drainage are not routinely required for uncomplicated liver abscesses (*62*). These interventions are recommended in addition to treatment with medication if the patient experiences clinical deterioration, does not respond to the initial medication, or if alternative diagnoses need to be ruled out. Additionally, some reports suggest that clinicians should consider these interventions in patients with a high risk of abscess rupture, as defined by a cavity with a > 5 cm diameter or by the presence of lesions in the left lobe of the liver; although these criteria have proved inconclusive in case-control studies (*43*).

Treatment of noninvasive infection

Noninvasive infections (*i.e.*, those in asymptomatic infected individuals) can be treated using a lumen-active agent and do not require a tissue-active agent (*43*). Paromomycin (Humatin®) is currently recommended because of its high potency in asymptomatic cyst passers (*3*). However, because the criteria of disease severity in amebic colitis have not been clarified, the decision to use a tissue-active agent before a lumen-active agent in such situations has been left to the discretion of the treating clinician. Tissue-active agents, and especially high-dose metronidazole, can cause severe side effects, such as encephalopathy (*63-67*). Older reports published before the era of PCR differentiation of *E. histolytica* from *E. dispar* and *E. moshkovskii* described the efficacy of paromomycin, even for symptomatic amebic colitis (*68-70*). According to recent case reports of endoscopically diagnosed asymptomatic or mild chronic amebic colitis, tissue-active agents are generally used for the initial treatment of infection (*31,71-73*). However, in a case report of two asymptomatic or mildly symptomatic patients with ulcerative lesions, the ulcerative lesions

were completely cured with paromomycin alone, without prior treatment with tissue-active agents (*34*). Although insufficient conclusions have been reached regarding the optimal treatment of endoscopically diagnosed asymptomatic or mild amebic colitis, it may be possible to treat noninvasive infections using only luminal activators. Further investigations will be needed to determine the appropriate treatment for the increasing number of patients with amebic colitis diagnosed *via* colonoscopy, especially in developed countries.

Monitoring after treatment and disease prevention

Although routine monitoring after amebiasis treatment is not recommended, reinfection with food-/waterborne and sexually transmitted *E. histolytica* frequently occurs in endemic areas/situations (*27,74-76*). A retrospective study of a Japanese HIV-1 cohort, which investigated the risk factors of invasive amebiasis recurrence (*27*), found that acquiring hepatitis C and syphilis during the follow-up period after the first episode of invasive amebiasis was associated with a higher rate of invasive amebiasis recurrence. This finding suggests that the reacquisition of cysts due to new infections rather than the reactivation of remaining cysts after use of tissue-active agents (in the absence of a lumen-active agent) played a greater role in the recurrence of invasive amebiasis. Thus, primary care physicians working in developed countries should inform their patients of factors and behaviors that will place them at risk of *E. histolytica* cyst acquisition and educate them about how these can be avoided (*e.g.*, avoiding potentially contaminated water or food in developing countries, unsafe homosexual contact, and unsafe oral-anal sexual contact).

Future directions for the control of STI amebiasis

Increasing physician awareness and strengthening epidemiology research programs

The incidence of ameba-related deaths is increasing worldwide. As mentioned above, amebiasis is no longer only highly prevalent in developing counties but is also re-emerging in many developed nations. Furthermore, an effective vaccine for amebiasis is not currently available. To ensure that patients receive an early diagnosis and appropriate treatment access, it is important to raise awareness and increase knowledge of STI amebiasis among clinicians, especially in developed countries, where this disease may not be well known. Addressing this issue requires the development and dissemination of educational materials and training programs, which should be regularly updated in accordance with the latest research findings. Improving diagnosis and treatment will lead to better patient outcomes. In addition, an effective epidemiological strategy should be implemented. The strategy outlined below underscores the importance of adopting an epidemiological approach for managing STI amebiasis. Continuous monitoring of the epidemiology of *E. histolytica* infection will be necessary to confirm the efficacy of the adopted strategy. This would involve strengthening existing surveillance systems, investing in research to better understand *E. histolytica* biology, and establishing effective infection control measures.

Proposed epidemiological strategy against STI amebiasis

In developing countries, contaminated food/water is the main source of *E. histolytica* infection (Figure 3). Therefore, people living in an affected area are at an equally high risk of exposure to this pathogen. In such a scenario, infection control is dependent on the improvement of sanitation and the provision of clean water and food to the community. On the other hand, most residents of developed countries are not at high-risk of *E. histolytica* infection. Thus, in this setting, person to person transmission occurs primarily within a high-risk population/community and often involves asymptomatic infected individuals. Thus, to develop an effective epidemiological strategy to control *E. histolytica* transmission, it is crucial to first identify the population/community at high-risk for amebiasis. Once these individuals are identified, they can be screened of asymptomatic infection and treated to reduce the reservoir of *E. histolytica* in the community. Our previous studies have already identified that MSM and people living with HIV/AIDS (PLWHA) are high-risk populations for STI amebiasis. By contrast, despite the fact that 10–20% of invasive amebiasis cases are reported in women (according to national surveillance program in Japan), the detailed epidemiological data collected from this group are limited. To conduct an effective epidemiological survey, it is important to acknowledge that the majority of the infected individuals are asymptomatic, which leads to the underestimation of STI amebiasis cases. A serological

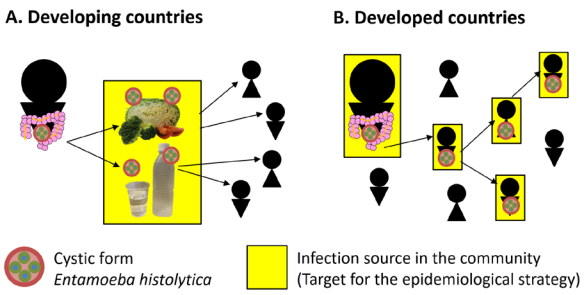

Figure 3. *Entamoeba histolytica* transmission routes in different settings. (A) In developing countries, transmission occurs *via* contaminated water and food. Thus, improvement of sanitation and provision of clean water and food is the recommended epidemiological strategy (yellow rectangle). **(B)** In developed countries, person to person transmission through sexual contact is most common. Identification of asymptomatic infected people, followed by treatment initiation, is the recommended epidemiological strategy (yellow rectangle).

survey (or seroprevalence study) is most widely used for the epidemiological assessment of amebiasis. The serological survey has a number of advantages: *i*) It is non-invasive and can be easily performed alongside syphilis and HIV testing; *ii*) Is it highly sensitive, even in asymptomatic infected individuals; *iii*) It can easily be used to compare data generated from multiple cohorts in developing and developed countries; and *iv*) It employs a regression model, which can easily identify risk factors and characteristics. Thus, active surveillance, for example in the form of a seroprevalence study, should be performed to ensure that all high-risk groups are accurately identified.

The next challenge is selecting an appropriate type of examination for individuals at high risk for STI amebiasis. As already described, a significant proportion of *E. histolytica* infections are asymptomatic. Thus, widespread screening of the whole high-risk population should be performed. Because of the large number of individuals requiring screening, the screening methods should ideally be inexpensive, non-invasive, and performable outside the hospital setting (*e.g.*, a VCT). Although PCR of stool samples is the most reliable method for detecting *E. histolytica* infection, it is expensive, time-consuming, and complex; thus, it is not ideal as a widespread screening method. Moreover, the handling of stool samples at VCT centers in developed countries is deemed inconvenient as most STI screening tests at these centers are performed using blood samples. On the other hand, serum antibody testing is a highly sensitive, easy, and inexpensive method for the screening of asymptomatic infection, which can be concurrently performed with other STI screening tests, such as those for HIV and syphilis (*53*). Moreover, in cases of liver abscesses, DNA has been identified in both saliva and serum (*77*). the development of detection methods targeting smaller molecular weight antigens could potentially improve the accuracy of amebiasis screening.

PCR of self-collected rectal swabs is widely used for *Chlamydia trachomatis* and *Neisseria gonorrhea* screening. This type of self-sampling is a highly efficient way of diagnosing current infection in one step, although it has never been evaluated for the diagnostic accuracy of self-collected rectal swab for *E. histoytica*. Thus, individuals with antibodies against *E. histolytica* should be urged to undergo subsequent PCR testing of their stool samples to differentiate between past and present infection; however, the optimal methods of administering the screening test to individuals with asymptomatic infection should be considered in future studies.

Conclusions

Amebiasis is caused by intestinal infection with the protozoan *E. histolytica*. Although 80–90% of amebiasis cases present with no symptoms or mild symptoms, *E. histolytica* sometimes causes severe, life-threatening disease. To improve the prognosis of patients, it is important to spread awareness of STI amebiasis in developed countries, where knowledge of the disease may be comparatively lower than in developing nations. In addition, efforts should be made to reduce the number of asymptomatic *E. histolytica* infections and limit their spread *via* sexual contact. Moreover, a targeted approach is needed to identify factors that predispose individuals to *E. histolytica* infection in the first place. Although MSM and/or PLWHA (especially men) have been identified as high-risk populations in East Asian countries, data on the female population at risk for STI amebiasis are limited. Of note, although only 10–20% of the reported cases of invasive amebiasis in Japan occur in women, high amebiasis-associated mortality rates are reported among this group. To date, PCR of stool samples is the gold standard for the diagnosis of asymptomatic *E. histolytica* infection. However, because PCR is expensive and technically complex, it is not feasible as a screening test for asymptomatic *E. histolytica* infection. Instead, serological screening followed by PCR of stool samples from seropositive individuals is a potentially feasible epidemiological strategy for targeting asymptomatic carriers. It is possible that more accessible and cost-effective assays will be developed to identify asymptomatic *E. histolytica* carriers in the future. Through these efforts, we can aim to reduce the global burden of amebiasis.

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References

1. Lozano R, Naghavi M, Foreman K, *et al.* 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. 2012; 380:2095-2128.
2. Blessmann J, Ali IK, Nu PA, Dinh BT, Viet TQ, Van AL, Clark CG, Tannich E. Longitudinal study of intestinal Entamoeba histolytica infections in asymptomatic adult

carriers. J Clin Microbiol. 2003; 41:4745-4750.
3. Blessmann J, Tannich E. Treatment of asymptomatic intestinal Entamoeba histolytica infection. N Engl J Med. 2002; 347:1384.
4. Salit IE, Khairnar K, Gough K, Pillai DR. A possible cluster of sexually transmitted Entamoeba histolytica: Genetic analysis of a highly virulent strain. Clin Infect Dis. 2009; 49:346-353.
5. Escolà-Vergé L, Arando M, Vall M, Rovira R, Espasa M, Sulleiro E, Armengol P, Zarzuela F, Barberá MJ. Outbreak of intestinal amoebiasis among men who have sex with men, Barcelona (Spain), October 2016 and January 2017. Euro Surveill. 2017; 22:30581.
6. Chaturvedi R, Gupte PA, Joshi AS. Fulminant amoebic colitis: A clinicopathological study of 30 cases. Postgrad Med J. 2015; 91:200-205.
7. Nisheena R, Ananthamurthy A, Inchara YK. Fulminant amebic colitis: A study of six cases. Indian J Pathol Microbiol. 2009; 52:370-373.
8. Shirley DA, Moonah S. Fulminant amebic colitis after corticosteroid therapy: A systematic review. PLoS Negl Trop Dis. 2016; 10:e0004879.
9. Otan E, Akbulut S, Kayaalp C. Amebic acute appendicitis: Systematic review of 174 cases. World J Surg. 2013; 37:2061-2073.
10. Kobayashi T, Watanabe K, Yano H, Murata Y, Igari T, Nakada-Tsukui K, Yagita K, Nozaki T, Kaku M, Tsukada K, Gatanaga H, Kikuchi Y, Oka S. Underestimated amoebic appendicitis among HIV-1-infected individuals in Japan. J Clin Microbiol. 2017; 55:313-320.
11. Koo JS, Choi WS, Park DW. Fulminant amebic colitis mimicking pseudomembranous colitis. Gastrointest Endosc. 2010; 71:400-401; discussion 401.
12. Nishise S, Fujishima T, Kobayashi S, Otani K, Nishise Y, Takeda H, Kawata S. Mass infection with Entamoeba histolytica in a Japanese institution for individuals with mental retardation: Epidemiology and control measures. Ann Trop Med Parasitol. 2010; 104:383-390.
13. Rivera WL, Santos SR, Kanbara H. Prevalence and genetic diversity of Entamoeba histolytica in an institution for the mentally retarded in the Philippines. Parasitol Res. 2006; 98:106-110.
14. Hung CC, Chang SY, Ji DD. Entamoeba histolytica infection in men who have sex with men. Lancet Infect Dis. 2012; 12:729-736.
15. Nagata N, Shimbo T, Akiyama J, Nakashima R, Nishimura S, Yada T, Watanabe K, Oka S, Uemura N. Risk factors for intestinal invasive amebiasis in Japan, 2003-2009. Emerg Infect Dis. 2012; 18:717-724.
16. James R, Barratt J, Marriott D, Harkness J, Stark D. Seroprevalence of Entamoeba histolytica infection among men who have sex with men in Sydney, Australia. Am J Trop Med Hyg. 2010; 83:914-916.
17. Park WB, Choe PG, Jo JH, Kim SH, Bang JH, Kim HB, Kim NJ, Oh MD, Choe KW. Amebic liver abscess in HIV-infected patients, Republic of Korea. Emerg Infect Dis. 2007; 13:516-517.
18. Hung CC, Deng HY, Hsiao WH, Hsieh SM, Hsiao CF, Chen MY, Chang SC, Su KE. Invasive amebiasis as an emerging parasitic disease in patients with human immunodeficiency virus type 1 infection in Taiwan. Arch Intern Med. 2005; 165:409-415.
19. Stark D, van Hal SJ, Matthews G, Harkness J, Marriott D. Invasive amebiasis in men who have sex with men, Australia. Emerg Infect Dis. 2008; 14:1141-1143.
20. van Hal SJ, Stark DJ, Fotedar R, Marriott D, Ellis JT, Harkness JL. Amoebiasis: Current status in Australia. Med J Aust. 2007; 186:412-416.
21. Stark DJ, Fotedar R, Ellis JT, Harkness JL. Locally acquired infection with Entamoeba histolytica in men who have sex with men in Australia. Med J Aust. 2006; 185:417.
22. Hung CC, Wu PY, Chang SY, Ji DD, Sun HY, Liu WC, Wu CH, Chang SC. Amebiasis among persons who sought voluntary counseling and testing for human immunodeficiency virus infection: A case-control study. Am J Trop Med Hyg. 2011; 84:65-69.
23. Yanagawa Y, Nagashima M, Shinkai T, *et al*. Seroprevalence of *Entamoeba histolytica* at voluntary counseling and testing center in Tokyo: Data from 2017. Late breaker poster presentation at ASM in Atlanta, Georgia, USA 2018.
24. Yanagawa Y, Nagashima M, Gatanaga H, Kikuchi Y, Oka S, Yokoyama K, Shinkai T, Sadamasu K, Watanabe K. Seroprevalence of *Entamoeba histolytica* at a voluntary counselling and testing centre in Tokyo: A cross-sectional study. BMJ Open. 2020; 10:e031605.
25. Ishikane M, Arima Y, Kanayama A, Takahashi T, Yamagishi T, Yahata Y, Matsui T, Sunagawa T, Nozaki T, Oishi K. Epidemiology of domestically acquired amebiasis in Japan, 2000-2013. Am J Trop Med Hyg. 2016; 94:1008-1014.
26. Escueta-de Cadiz A, Kobayashi S, Takeuchi T, Tachibana H, Nozaki T. Identification of an avirulent Entamoeba histolytica strain with unique tRNA-linked short tandem repeat markers. Parasitol Int. 2010; 59:75-81.
27. Watanabe K, Gatanaga H, Escueta-de Cadiz A, Tanuma J, Nozaki T, Oka S. Amebiasis in HIV-1-infected Japanese men: Clinical features and response to therapy. PLoS Negl Trop Dis. 2011; 5:e1318.
28. Weinke T, Friedrich-Janicke B, Hopp P, Janitschke K. Prevalence and clinical importance of Entamoeba histolytica in two high-risk groups: Travelers returning from the tropics and male homosexuals. J Infect Dis. 1990; 161:1029-1031.
29. Allason-Jones E, Mindel A, Sargeaunt P, Williams P. Entamoeba histolytica as a commensal intestinal parasite in homosexual men. N Engl J Med. 1986; 315:353-356.
30. Gunther J, Shafir S, Bristow B, Sorvillo F. Short report: Amebiasis-related mortality among United States residents, 1990-2007. Am J Trop Med Hyg. 2011; 85:1038-1040.
31. Spinzi G, Pugliese D, Filippi E. An unexpected cause of chronic diarrhea. Gastroenterology. 2016; 150:e5-e6.
32. Roure S, Valerio L, Soldevila L, Salvador F, Fernández-Rivas G, Sulleiro E, Mañosa M, Sopena N, Mate JL, Clotet B. Approach to amoebic colitis: Epidemiological, clinical and diagnostic considerations in a non-endemic context (Barcelona, 2007-2017). PLoS One. 2019; 14:e0212791.
33. Timsit BL, Deroux A, Lugosi M, Colombe B, Bouillet L. Amoebosis: May sexual transmission be an underestimated way of contamination?. Rev Med Interne. 2018; 39:586-588. (in French)
34. Yamamoto K, Yanagawa Y, Oka S, Watanabe K. Two cases of endoscopically diagnosed amebic colitis treated with paromomycin monotherapy. PLoS Negl Trop Dis. 2020; 14:e0008013.
35. Nagata N, Shimbo T, Akiyama J, Nakashima R, Niikura R, Nishimura S, Yada T, Watanabe K, Oka S, Uemura N.

Predictive value of endoscopic findings in the diagnosis of active intestinal amebiasis. Endoscopy. 2012; 44:425-428.
36. Okamoto M, Kawabe T, Ohata K, Togo G, Hada T, Katamoto T, Tanno M, Matsumura M, Yamaji Y, Watabe H, Ikenoue T, Yoshida H, Omata M. Amebic colitis in asymptomatic subjects with positive fecal occult blood test results: Clinical features different from symptomatic cases. Am J Trop Med Hyg. 2005; 73:934-935.
37. Gathiram V, Jackson TF. A longitudinal study of asymptomatic carriers of pathogenic zymodemes of Entamoeba histolytica. S Afr Med J. 1987; 72:669-672.
38. Haque R, Mondal D, Kirkpatrick BD, Akther S, Farr BM, Sack RB, Petri WA Jr. Epidemiologic and clinical characteristics of acute diarrhea with emphasis on Entamoeba histolytica infections in preschool children in an urban slum of Dhaka, Bangladesh. Am J Trop Med Hyg. 2003; 69:398-405.
39. Watanabe K, Aoki T, Nagata N, Tanuma J, Kikuchi Y, Oka S, Gatanaga H. Clinical significance of high anti-entamoeba histolytica antibody titer in asymptomatic HIV-1-infected individuals. J Infect Dis. 2014; 209:1801-1807.
40. Ito D, Hata S, Seiichiro S, Kobayashi K, Teruya M, Kaminishi M. Amebiasis presenting as acute appendicitis: Report of a case and review of Japanese literature. Int J Surg Case Rep 2014; 5:1054-1057.
41. Ichikawa H, Imai J, Mizukami H, Uda S, Yamamoto S, Nomura E, Tajiri T, Watanabe N, Makuuchi H. Amoebiasis presenting as acute appendicitis. Tokai J Exp Clin Med. 2016; 41:227-229.
42. Haque R, Huston CD, Hughes M, Houpt E, Petri WA, Jr. Amebiasis. N Engl J Med. 2003; 348:1565-1573.
43. Bennett JE, Dolin R, Blaser MJ. Mandell, Douglas, and Bennett's principles and practice of infectious diseases. Eighth edition. Philadelphia, Elsevier Saunders; 2015.
44. Fotedar R, Stark D, Beebe N, Marriott D, Ellis J, Harkness J. Laboratory diagnostic techniques for Entamoeba species. Clin Microbiol Rev. 2007; 20:511-532, table of contents.
45. Saidin S, Othman N, Noordin R. Update on laboratory diagnosis of amoebiasis. Eur J Clin Microbiol Infect Dis. 2019; 38:15-38.
46. Haque R, Faruque AS, Hahn P, Lyerly DM, Petri WA, Jr. Entamoeba histolytica and Entamoeba dispar infection in children in Bangladesh. J Infect Dis 1997; 175:734-736.
47. Haque R, Ali IK, Akther S, Petri WA, Jr. Comparison of PCR, isoenzyme analysis, and antigen detection for diagnosis of Entamoeba histolytica infection. J Clin Microbiol. 1998; 36:449-452.
48. Petri WA, Jr., Singh U. Diagnosis and management of amebiasis. Clin Infect Dis. 1999; 29:1117-1125.
49. Visser LG, Verweij JJ, Van Esbroeck M, Edeling WM, Clerinx J, Polderman AM. Diagnostic methods for differentiation of Entamoeba histolytica and Entamoeba dispar in carriers: Performance and clinical implications in a non-endemic setting. Int J Med Microbiol. 2006; 296:397-403.
50. Mohanty S, Sharma N, Deb M. Microscopy versus enzyme linked immunosorbent assay test for detection of Entamoeba histolytica infection in stool samples. Trop Parasitol. 2014; 4:136-138.
51. Tanyuksel M, Petri WA, Jr. Laboratory diagnosis of amebiasis. Clin Microbiol Rev. 2003; 16:713-729.
52. Watanabe K, Nagata N, Sekine K, Watanabe K, Igari T, Tanuma J, Kikuchi Y, Oka S, Gatanaga H. Asymptomatic intestinal amebiasis in Japanese HIV-1-infected individuals. Am J Trop Med Hyg. 2014; 91:816-820.
53. Yanagawa Y, Shimogawara R, Takano M, Aoki T, Mizushima D, Gatanaga H, Kikuchi Y, Oka S, Yagita K, Watanabe K. Identification of asymptomatic Entamoeba histolytica infection by a serological screening test: A cross-sectional study of an HIV-negative men who have sex with men cohort in Japan. PLoS Negl Trop Dis. 2022; 16:e0009793.
54. Lee KC, Lu CC, Hu WH, Lin SE, Chen HH. Colonoscopic diagnosis of amebiasis: A case series and systematic review. Int J Colorectal Dis 2015; 30:31-41.
55. Marcus VA, Ward BJ, Jutras P. Intestinal amebiasis: A diagnosis not to be missed. Pathol Res Pract 2001; 197:271-274; discussion 5-8.
56. Radhakrishnan S, Al Nakib B, Shaikh H, Menon NK. The value of colonoscopy in schistosomal, tuberculous, and amebic colitis. Two-year experience. Dis Colon Rectum 1986; 29:891-895.
57. Cooper CJ, Fleming R, Boman DA, Zuckerman MJ. Varied clinical manifestations of amebic colitis. South Med J 2015; 108:676-681.
58. Yanagawa Y, Watanabe K, Nagata N, Tsukui K, Kobayashi S, Nozaki T, Gatanaga H, Oka S. Diagnostic accuracy of amebic colitis by colonoscopy. Poster presentation at 2016 Annual Meeting of American Society of Tropical Medicine and Hygiene. Atlanta, USA, Nov, 2016.
59. Yanagawa Y, Nagata N, Yagita K, Watanabe K, Okubo H, Kikuchi Y, Gatanaga H, Oka S, Watanabe K. Clinical features and gut microbiome of asymptomatic entamoeba histolytica infection. Clin Infect Dis. 2021; 73:e3163-e3171.
60. Horiki N, Furukawa K, Kitade T, *et al*. Endoscopic findings and lesion distribution in amebic colitis. J Infect Chemother. 2015; 21:444-448.
61. Irusen EM, Jackson TF, Simjee AE. Asymptomatic intestinal colonization by pathogenic Entamoeba histolytica in amebic liver abscess: Prevalence, response to therapy, and pathogenic potential. Clin Infect Dis. 1992; 14:889-893.
62. Chavez-Tapia NC, Hernandez-Calleros J, Tellez-Avila FI, Torre A, Uribe M. Image-guided percutaneous procedure plus metronidazole versus metronidazole alone for uncomplicated amoebic liver abscess. Cochrane Database Syst Rev. 2009; (1):CD004886.
63. Clements A. Case report of metronidazole-induced encephalopathy. WMJ. 2017; 116:221-223.
64. Desai JA, Dobson J, Melanson M, Pari G, Jin AY. Metronidazole-induced encephalopathy: Case report and review of MRI findings. Can J Neurol Sci. 2011; 38:512-513.
65. Bottenberg MM, Hegge KA, Eastman DK, Kumar R. Metronidazole-induced encephalopathy: A case report and review of the literature. J Clin Pharmacol 2011; 51:112-116.
66. Orion E, Matz H, Wolf R. The life-threatening complications of dermatologic therapies. Clin Dermatol. 2005; 23:182-192.
67. Karayianni M, von Heijne A, Laska AC. Encefalopathy as a side effect of metronidazole therapy - a case report. Lakartidningen. 2017; 114. (in Swedish)
68. Chavarria AP, Hunter GW, 3rd, Redmond DL, Lizono C, Loria AR. Single dose of paromomycin (Humatin) against intestinal amebiasis. Mil Med. 1964; 129:947-951.
69. Carter CH. Paromomycin (Humatin) in the treatment

of intestinal amebiasis: A preliminary dose-range study. Antibiotic Med Clin Ther (New York). 1959; 6:586-588.
70. Carter CH, Bayles A, Thompson PE. Effects of paromomycin sulfate in man against Entamoeba histolytica and other intestinal protozoa. Am J Trop Med Hyg. 1962; 11:448-451.
71. Wu N, Freiman JS. Caecal ulceration in an asymptomatic man. Gut. 2017; 66:886.
72. Fleming R, Cooper CJ, Ramirez-Vega R, Huerta-Alardin A, Boman D, Zuckerman MJ. Clinical manifestations and endoscopic findings of amebic colitis in a United States-Mexico border city: A case series. BMC Res Notes. 2015; 8:781.
73. Numata A, Itabashi M, Kishimoto K, Motohashi K, Hagihara M, Kuwabara H, Tanaka M, Kato H, Chiba S, Kunisaki R, Fujisawa S. Intestinal amoebiasis in a patient with acute graft-versus-host disease after allogeneic bone marrow transplantation successfully treated by metronidazole. Transpl Infect Dis. 2015; 17:886-889.
74. Haque R, Ali IM, Sack RB, Farr BM, Ramakrishnan G, Petri WA, Jr. Amebiasis and mucosal IgA antibody against the Entamoeba histolytica adherence lectin in Bangladeshi children. J Infect Dis. 2001; 183:1787-1793.
75. Gilchrist CA, Petri SE, Schneider BN, Reichman DJ, Jiang N, Begum S, Watanabe K, Jansen CS, Elliott KP, Burgess SL, Ma JZ, Alam M, Kabir M, Haque R, Petri WA Jr. Role of the gut microbiota of children in diarrhea due to the protozoan parasite Entamoeba histolytica. J Infect Dis. 2016; 213:1579-1585.
76. Haque R, Mondal D, Duggal P, Kabir M, Roy S, Farr BM, Sack RB, Petri WA Jr. Entamoeba histolytica infection in children and protection from subsequent amebiasis. Infect Immun. 2006; 74:904-909.
77. Khairnar K, Parija SC. Detection of Entamoeba histolytica DNA in the saliva of amoebic liver abscess patients who received prior treatment with metronidazole. J Health Popul Nutr. 2008; 26:418-425.

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