Entamoeba histolytica: Clinical Update and Vaccine Prospects

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Amebiasis is extraordinarily common in children of the developing world. This realization has come from application of diagnostic techniques that distinguish the nonpathogenic parasite *Entamoeba dispar* from *Entamoeba histolytica*. *E. histolytica* infection is found in children with dysentery, diarrhea, and in many cases in children with no gastrointestinal symptoms. Genetically distinct strains of *E. histolytica* exist but evidence is too preliminary to judge if some strains are more virulent than others. A provocative study from Tanzania has shown that pregnant women with HIV infection who are coinfected with *E. histolytica* are at greater risk of delivering a low birth weight infant. Perhaps the most exciting new data is the identification of acquired immunity to recurrent *E. histolytica* infection. Acquired immunity is linked to a mucosal immune response against a major virulence factor of the parasite, a Gal/GalNAc lectin responsible for adherence and killing of the host. Prospects for a vaccine are thus brightening at the same time that the true burden of disease is comprehended.

Introduction

The goal of this review is to synthesize data published in 2001 on amebiasis in humans. Space limitations prevent this review from being comprehensive, and I apologize to authors whose work is not referenced. The intent is to stimulate the interested reader to delve more deeply into the literature of this fascinating and important parasite. The discussion is divided into seven sections that start with diagnosis and end with treatment.

Diagnosis of Amebiasis

Entamoeba histolytica has been reclassified into pathogenic (*E. histolytica* sensu strictu) and nonpathogenic (*Entamoeba dispar*) species [1]. The classic stool ova and parasite examination, whereby *E. histolytica* is identified by its appearance in trichrome or iron hematoxylin-stained stool specimens, cannot differentiate *E. histolytica* from the nonpathogenic but identical appearing parasite *E. dispar*. The stool ova and parasite exam is also insensitive and underrepresents the true incidence of *E. histolytica* infection. For example Kevin Kain and colleagues (University of Toronto) have estimated that the sensitivity of stool ova and parasite as performed in community labs is under 10%.

Over the past several years, in collaboration with TechLab, Inc., Blacksburg, VA, we have developed US Food and Drug Administration (FDA)-approved stool antigen detection assays that are specific and sensitive for the pathogenic parasite *E. histolytica*. These antigen detection assays capture and detect the parasite's Gal/GalNAc lectin from stool or serum samples. When used to screen stool samples from children in the Mirpur camp of Dhaka, Bangladesh, the TechLab *E. histolytica* test was three times more sensitive than stool ova and parasite for the identification of *E. histolytica*. This past year we reported that the sensitivity of this antigen detection test had been improved in a second-generation FDA-approved test (the TechLab *E. histolytica* II test). The second-generation antigen detection test identified *E. histolytica* infection in 100% (16/16) of culture-positive specimens, and overall identified infection in 4.3% (50/1164) of asymptomatic preschool children aged 2 to 5 years from Mirpur, Bangladesh. Agreement of antigen detection with polymerase chain reaction (PCR) tests on stool was also demonstrated [2••].

Antigen detection in serum can serve as a noninvasive diagnostic test for amebic liver abscess. Amebic and bacterial abscesses appear identical on ultrasound or CT, and it is rarely possible to identify *E. histolytica* in stool specimens from patients with amebic liver abscess. We used the detection in serum of circulating *E. histolytica* Gal/GalNAc lectin to diagnose amebic liver abscess in patients from Dhaka, Bangladesh. The TechLab *E. histolytica* II test detected Gal/GalNAc lectin in the serum of 22 of 23 (96%) amebic liver abscess patients tested prior to treatment with the anti-amebic drug metronidazole, and zero of 70 (0%) controls. After 1 week of treatment with metronidazole, nine of 11 (82%) patients became serum lectin antigen negative (Table 1).

Other approaches for the diagnosis of amebiasis that were tested in 2001 include the Ridascreen Entamoeba (R-Biopharm, Germany), and the Triage Micro Parasite Panel (Biosite Diagnostics, Inc., San Diego, CA) [3,4].

Table 1. Diagnostic tests for amebic colitis and liver abscess

	Sensitivity, %	Specificity, %
Microscopy*		
Colitis	10–60	10–50
Liver abscess	10–20	10–50
Stool antigen detection†		
Colitis	> 90	> 90
Liver abscess	40	> 90
Serum antigen detection†		
Liver abscess	> 90	> 90
Stool PCR‡		
Colitis	> 90	> 90
Serum antibody		
Colitis	50–70	§
Liver abscess	80–95	§

**Entamoeba histolytica* and *Entamoeba dispar* appear identical by microscopic ova and parasite exam of stool, accounting for the low specificity of this test.
†TechLab *E. histolytica* II test.
‡PCR tests are based on the unique DNA sequence of the multicopy small subunit ribosomal RNA gene of *E. histolytica*.
§Seropositivity rates of greater than 25% to 50% in some regions in the developing world limit the specificity of serologic tests in distinguishing acute from past infection.
PCR—polymerase chain reaction.

Figure 1. The Mirpur refugee camp in Dhaka, Bangladesh.

Neither of these tests can distinguish *E. histolytica* from *E. dispar*, so they would at best serve as screening tools, with additional specific testing for *E. histolytica* required for all positive results. The Triage Panel has the advantage of coupling testing of *E. histolytica*/*E. dispar* with *Giardia lamblia* and *Cryptosporidium parvum*, covering the three most common parasites that cause diarrhea in the United States. Both of these tests detected all of the *E. dispar*/*E. histolytica* infections detected by microscopy, but unfortunately the sensitivity was not compared with the new "gold standards" for diagnosis (either the TechLab *E. histolytica* II kit or PCR). As a final note, it is not only the distinction between *E. histolytica* and *E. dispar* that is confusing to some clinicians. Many laboratories that report the identification of other nonpathogens (such as *Endolimax coli*, *Entamoeba coli*, *Entamoeba hartmanii*, and *Iodamoeba hartmanni*) do not report these as nonpathogens, likely leading to unnecessary additional testing or treatment [5•].

Diagnosis of amebiasis at a minimum should include a combination of *E. histolytica*-specfic stool (or in the case of liver abscess serum) antigen detection or PCR coupled with serum antibody tests. Reliance on solely a stool ova and parasite test will lead to inaccurate diagnosis in many cases.

Genetic variation within *Entamoeba histolytica*

Graham Clark of the London School of Hygiene and Tropical Medicine has pioneered techniques for genetic fingerprinting of *E. histolytica* isolates. He has used a variety of polymorphic genes from the parasite to type strains by differences in the number of repeat units [6]. The varied organ tropism and clinical presentations of infection by *E. histolytica* have stimulated interest on the role of parasite genetic diversity in virulence. Ayeh-Kumi *et al.* [7] investigated genetic diversity among 54 *E. histolytica* isolates from the Mirpur refugee camp in Dhaka, Bangladesh (Fig. 1). Polymorphisms in the *E. histolytica* serine-rich gene were measured from the products of nested PCR on DNA extracted from stool and liver aspirate pus. Both size and restriction site polymorphisms were detected among the isolates within this endemic area (Fig. 2). Approximately half of the isolates had unique polymorphisms. Interestingly, the majority of *E. histolytica* from the liver had polymorphisms that were not present in intestinal isolates from the same geographic area. This data is consistent with the existence of genetic differences between *E. histolytica* that cause intestinal and hepatic disease [7]. The correlation of genetic differences with the pathogenic potential of *E. histolytica* strains, and the implications of genetic diversity for the immunoprophylaxis of amebiasis, require further study.

Natural History of *Entamoeba histolytica* Infection

The field use of rapid and specific antigen detection tests for amebiasis is providing new insights into the frequency, duration, and consequences of *E. histolytica* infection. The

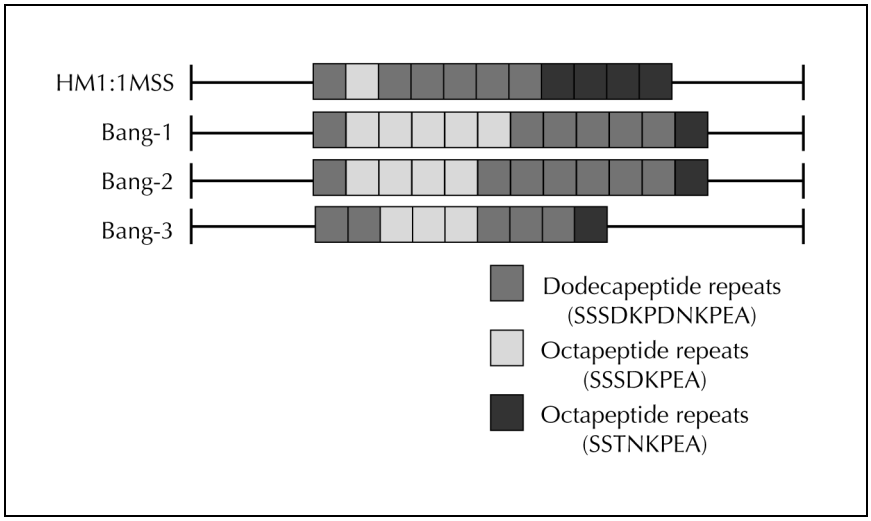

Figure 2. Genetic diversity in SREHP gene among *Entamoeba histolytica* isolates in the Mirpur refugee camp, Dhaka Bangladesh. Depiction of the protein sequences of the *SREHP* gene of three different *E. histolytica* isolates from Mirpur (Bang-1,2,3). These *SREHP* sequences are compared with the *SREHP* gene from the HM1:IMSS laboratory strain of *E. histolytica* (accession number M80910) (Li and Stanley, 1992). The internal tandem repeats are highlighted. (*Adapted from* Ayeh-Kumi *et al*. [7]; with permission.)

Table 2. Point prevalence of *Entamoeba histolytica* infection in the Mirpur refugee camp, Dhaka, Bangladesh as determined by different methods for the diagnosis of amebiasis

Test	Results of screening 680 children in Mirpur
Stool ova and parasite	4.8% *Entamoeba histolytica*/ *Entamoeba dispar*
Culture	10.4% *E. histolytica*/*E. dispar*
Antigen detection	17.3% (4.7% *E. histolytica* and 12.6% *E. dispar*)

prevalence of asymptomatic infection with *E. histolytica* was not known until recently, because without the use of *E. histolytica*-specific diagnostic tests, it was assumed that most asymptomatic infections were due to the nonpathogen *E. dispar*.

Working in an urban slum community in Fortaleza Brazil, Braga *et al*. [8•] found that amebiasis is present in one of every 10 individuals. They prospectively observed family members of index children with amebiasis. All but one of the 28 families identified had at least one other family member infected with *E. histolytica*. Stool antigen detection was more sensitive than serology for the identification of infection, with only 70% of infected individuals seropositive. Interestingly, the presence of anti-amebic serum antibodies was not associated with a lower incidence of new infections during the period of observation. Infection was self-limited (cleared within 45 days of observation), and in 70% of cases was not associated with diarrhea. This study showed that amebiasis is common in family members of an infected child and that in most cases the infection is self-limited and without symptoms.

In Dhaka, Bangladesh we studied 300 preschool children aged 2 to 5 years from Mirpur, an urban slum in Dhaka [2••]. The inhabitants of Mirpur are of Bihari ethnic origin, and settled there after the liberation of Bangladesh from Pakistan in 1971. The area is densely populated with poor sanitary and hygienic conditions. Stool specimens were collected every month for detection of *E. histolytica* infection by antigen capture and culture. The parents and child were visited and interviewed every other day by health care workers. Children with diarrhea were also detected through the parents contacting project personnel at the field clinic. When diarrheal disease was detected, a stool sample was collected for *E. histolytica* testing and the child was examined. During the first 12 months of the study, 39% (105/269) of the children had one or more new *E. histolytica* infections, with 3.1% (4/129) of the new infections associated with dysentery. We concluded that *E. histolytica* infection was a common and at times serious infection in this population (Table 2).

In Mexico City, Valenzuela *et al*. [9•] studied 39 patients with well-documented invasive amebiasis. They also observed serum anti-amebic IgG antibodies in only 70% of patients with colitis but in 100% of patients with liver abscess. There was no correlation between serum IgG and salivary IgA anti-amebic antibody levels consistent with distinct mucosal and systemic responses to the parasite. Salivary IgA could be detected up to 1 year after infection. After treatment of the initial infection, only one of the 39 patients had a second episode of invasive amebiasis during 12 months of follow-up. This study was important for its demonstration of distinct mucosal and systemic anti-amebic immune responses, and for the hint of acquired immunity provided by the 1-year follow-up of treated patients.

Acquired Immunity to Amebiasis

The first clear evidence of acquired immunity to amebiasis was provided in 2001 by our group in Bangladesh [2••]. Protection from infection was associated with intestinal IgA against the parasite's Gal/GalNAc adherence lectin. The Gal/GalNAc lectin is a surface glycoprotein complex of three subunits that is absolutely required for amebic adherence and cytotoxicity, and

Table 3. Antilectin IgA in stool is associated with an absence of *Entamoeba histolytica* colonization

	***Entamoeba histolytica* infection as determined by**			
	Stool antigen		**Stool culture**	
	(+)	**(-)**	**(+)**	**(-)**
Antilectin IgA in stool				
(+)	0*	64	0†	64
(-)	33	213	16	230
Total	33	277	16	294

*$P = 0.001$ compared with IgA (-).
†$P = 0.04$ compared with IgA (-).
Adapted from Haque *et al.* [2••]; with permission.

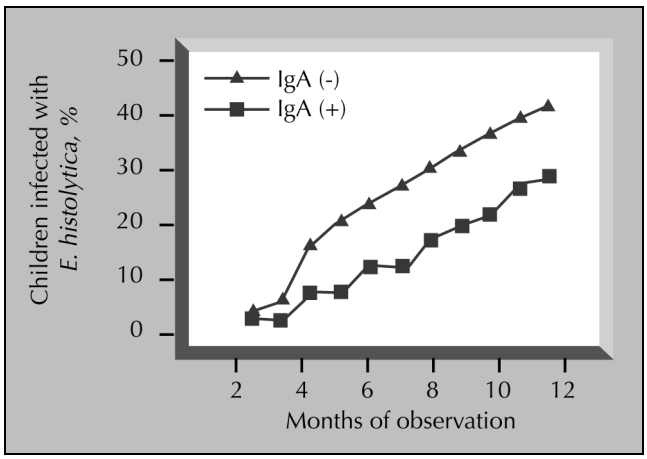

Figure 3. The presence of stool IgA antilectin antibodies is associated with a lower incidence of new *Entamoeba histolytica* infections. Children from Mirpur with ($n = 42$) or without ($n = 227$) stool lectin–specific IgA at the time of enrollment were followed for new *E. histolytica* infections, as detected by antigen detection in monthly stool samples. The two groups were statistically significantly different at 5 months ($P = 0.03$) and 7 months ($P = 0.04$). (*Adapted from* Haque *et al*. [2••]; with permission.)

which may also mediate the formation of the parasite cyst [10, 11••]. Immunity associated with an antilectin IgA response was demonstrated in several ways. First, *E. histolytica* colonization was present in 0% of children with and 13% of children without antilectin IgA (Table 3). Second, children who at entry to the study had intestinal antilectin IgA had significantly fewer new infections over a 7-month period of observation (Fig. 3). Finally, the intestinal IgA antilectin reponse was detected coincident with resolution of infection in two thirds of closely monitored new infections [2••].

Although it is not possible to prove causation in an observational study, it is tempting to speculate that the intestinal IgA response is at least partially responsible for the observed immunity. The Gal/GalNAc lectin is required for parasite adherence and cytotoxicity, and IgA purified from patients inhibited the lectin in vitro. The degree of immunity associated with the IgA response was partial and only observed up to 7 months. Explanations for the incomplete immunity include the possible requirement for cell-mediated in addition to humoral immune responses for full immunity [12••], parasite genetic variability, or the short-lived nature of the intestinal IgA response (detectable on average for only 17 days).

In contrast to the association of stool IgA antilectin antibodies with decreased *E. histolytica* infections, children with serum IgG lectin-specific antibodies at 12 months had 53% more new *E. histolytica* infections. Environmental factors were comparable between the children with and without antilectin IgG as best as could be determined, including area of residence within Mirpur, family size, age, sex, and nutritional status. Currently we are investigating whether serum IgG antilectin antibodies are a marker for an environmental or inherited susceptibility to amebiasis.

In an excellent review, Stanley [13••] highlighted major unanswered questions about immunity to amebiasis. These include determining how long protective immunity persists, asking why does protective immunity not develop in all individuals, and finally why is serum antilectin IgG associated with susceptibility to amebiasis? Much is left to be understood, but the demonstration of immunity to amebiasis represents a beachhead in the battle to control this deadly disease.

Amebiasis in the HIV-infected Patient

Although infection with the nonpathogenic parasite *E. dispar* in men who have sex with men has been common in the developed world, invasive *E. histolytica* infection has not been common. In the past year there were several published studies of amebiasis in HIV-infected individuals [14–17]. Japanese men who have sex with men have previously been observed to have a high prevalence of antiamebic antibodies indicative of prior *E. histolytica* infection. Mitarai *et al.* [15] reported six cases of amebiasis in HIV-infected men from Japan. Remarkably, five of the six cases were initially misdiagnosed and treatment delayed. Two of the five cases of colitis were fatal. The two patients with fatal colitis died from colonic perforation

Table 4. Effect of parasites on birth weight of infants born to HIV-infected mothers in Tanzania

Parasite	Adjusted birth weight decline (95% CI)
Entamoeba histolytica	-252 (-496, -7)
Malaria parasitemia	-133 (-221, -45)
Hookworm	-93 (-211, 25)
Cryptosporidium parvum	None
Ascaris lumbricoides	None
Strongyloides stercoralis	None

Data from Dreyfuss *et al.* [18••].

and peritonitis. CD4 T-cell counts were greater than 250 cells/μL in four of four patients who survived, and 6 cells/μL in one of the patients who died. CD4 T-cell determination was not done in the other fatal case. Three patients from Taiwan with HIV and amebic liver abscess were reported by Liu *et al*. [16]. Two of the three patients had CD4 T-cell counts greater than 250 cells/μL and all three patients were cured of their liver abscess with metronidazole with or without drainage. At this time too little is known to draw any conclusions. A clear research need is to investigate potential interaction of HIV and *E. histolytica* infection in regions of the world such as the Indian subcontinent and central Africa where the two infectious diseases are unfortunately common.

Low Birth Weight, HIV, and Maternal *Entamoeba histolytica* Infection

Most deaths of children in the developing world occur in the first year of life, with as many as one in 10 children dying by his or her first birthday. Low birth weight infants are more likely to die in the neonatal period and in the first year of life. Dreyfuss *et al*. [18••] assessed the risk factors for low birth weight in a cohort of 822 HIV-positive women in Tanzania. Women were enrolled in their second trimester; sociodemographic, nutritional, immunlogic, parasitologic, and infant risk factors for low birth weight were assessed; and birth weight was measured at hospital delivery. The mean birth weight was 3 kg, and 11% of the children weighed under 2.5 kg and 12% were small for gestational age. Multivariate analysis demonstrated that *E. histolytica* infection of the mother was associated with a 250-g decrease in birth weight. Hookworm was associated with a more modest decrease of 100 g. None of the other intestinal parasites tested for (*C. parvum*, *Ascaris lumbricoides*, *Strongyloides stercoralis*) were associated with a decrease in birth weight (Table 4). The decreased birth weight associated with *E. histolytica* infection was in fact greater than any other infectious disease measured in the study, including malaria. If this association of *E. histolytica* infection with low birth weight in HIV-infected mothers is independently confirmed, then it will be of great importance to test the effect of perinatal treatment of amebiasis on birth weight.

New Treatment Approaches for Amebiasis

Although metronidazole resistance is being encountered in some of the anaerobic protozoan parasites, to date this has not been the case for *E. histolytica* [19•,20]. However, since metronidazole is the only highly effective drug for invasive amebiasis, it is prudent to explore alternate therapies. Nitazoxanide is a nitrothiazolyl-salicylamide derivative with activity against some protozoa, nematodes, estodes, trematodes, and anaerobic bacteria. Rossignol *et al*. [21•] conducted a randomized, double-blinded, placebo-controlled study of nitazoxanide for diarrhea caused by *E. histolytica*. Fifty-three patients were identified from a total of 725 patients from Egypt with diarrhea who were infected with *E. histolytica*/*E. dispar*. Patients coinfected with *E. histolytica* plus *G. lamblia* or bacterial enteropathogens including toxigenic or adherent *E. coli* were excluded from the analysis. Approximately 7 days after initiation of therapy, cure of infection was assessed parasitologically and by symptom score. Treatment with nitazoxanide resulted in an 80% clinical response and 69% parasitologic cure compared with rates of 48% and 39% for placebo, respectively [21•].

Conclusions

The year 2001 saw the greatest advances in our understanding of the disease amebiasis of any year in memory. As with all of science, what was learned served to point out how much is unknown. Yet it is fair to say that the road to a vaccine is a little straighter this year than last. The magnitude of disease caused by amebiasis, coupled with the identification of acquired immunity linked to mucosal anti-Gal/GalNAc lectin IgA, suggests that now is time to invest in a targeted approach to vaccine development to prevent this lethal disease.

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- •• Of major importance

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