

Leading article – Tropical infection of the gastrointestinal tract and liver series

Diagnosis of invasive amoebiasis – time to end the morphology era

Amoebiasis, human infection by *Entamoeba histolytica*, has many clinical presentations. Most of the intestinal infections are asymptomatic and result from non-pathogenic organisms. The most common clinical presentations of invasive amoebiasis are acute colitis or acute right upper quadrant pain with fever, each have a broad differential diagnosis. Epidemiological clues and the characteristics of the clinical presentation are very helpful for clinicians expert in tropical diseases. Physicians in developed countries, however, are in general unfamiliar with the presentation and treatment of amoebiasis and are overly dependent on the laboratory. In the United States the ability of clinical laboratories to correctly diagnose *E. histolytica* in faecal samples is suspect and serology for anti-amoebic antibodies is rarely immediately available. Time and again patients with invasive amoebiasis are diagnosed or treated incorrectly, the second often in an overly aggressive manner. Diagnostic methodology of high sensitivity and specificity that is less susceptible to human error would be helpful for clinicians and clinical researchers. The purpose of this paper is to briefly review clinical amoebiasis and the latest information on development of new diagnostic methods that may eliminate the need to perform microscopic examination of faeces.

E. histolytica infection and invasive amoebiasis

Extensive studies of starch gel electrophoretic patterns of *E. histolytica* faecal isolates, termed zymodemes, by Sargeant *et al.*^{2,3} showed that there may be distinct pathogenic and non-pathogenic strains of *E. histolytica*. Recent studies of antigenic specificity,³ differences in genomic DNA⁴ and ribosomal RNA,⁵ and clinical epidemiology⁶ confirm the existence of distinct *E. histolytica* strains.

E. histolytica is highly endemic in areas of India, Africa, Mexico, South and Central America, and Asia. Spread is by the faecal-oral route and lower socioeconomic status with inadequate sanitation predisposes to infection. Oral-anal sexual practices and longterm institutionalisation with psychiatric illness or mental retardation are established risk factors. Travel or immigration from endemic areas are often responsible for physicians in developed countries encountering patients infected with this parasite.

Subjects who asymptotically harbour non-pathogenic *E. histolytica* (termed *E. dispar*) in their large bowel have never been recorded as developing invasive amoebiasis. In addition, they lack amoebic antigens in serum samples and do not mount a serum anti-amoebic antibody response.⁶⁻⁸ Asymptomatic infections clear spontaneously in 9–12 months, possibly because of a local mucosal immune response or a change in colonic microflora. It is not known whether such asymptomatic infections are in any way deleterious to the host, perhaps by impaired nutrition or frequent episodes of uncharacterised diarrhoeal illness. Given that non-pathogenic infection has a prevalence of up to 50% in highly endemic areas, reinfection is possible and anti-protozoal treatment is certainly not indicated. There is no evidence that any form of effective immunity exists to prevent recurrent luminal infection by *E. histolytica*.

Most infections with pathogenic organisms are also asymptomatic,⁶ accounting for 10–40% of all subclinical infections. There is a wide variation between geographical areas in the percentage of infections that are pathogenic. Asymptomatic subjects harbouring pathogenic *E. histolytica* develop a serum anti-amoebic antibody response, have amoebic antigens in serum samples,⁷ and may have pathological evidence of colonic invasion. Only a few, as low as 10%, present with an invasive amoebiasis syndrome. It is not clear if host or parasite factors determine the virulence of the infection. Apparently, the prevalence of serum anti-amoebic IgG antibodies in the uninfected population of an endemic area (20–25%) results from previous asymptomatic infection with pathogenic organisms rather than a remote episode of symptomatic invasive amoebiasis.⁷ Clearly, the risks of pathogenic *E. histolytica* to the host and by transmission of infection to close associates, merits anti-amoebic treatment.⁹

Amoebic colitis usually presents subacutely over days to weeks with bloody diarrhoea and abdominal pain, only a few patients are febrile. The differential diagnosis includes all the bacterial causes of inflammatory colitis including infection with *Escherichia coli*, invasive shigella, campylobacter, salmonella, *Vibrio parahaemolyticus*, and yersinia, or toxin mediated colitis resulting from *Clostridium difficile*. A chronic or intermittent presentation of colonic amoebiasis is common and clinically indistinguishable from idiopathic inflammatory bowel disease (IBD). This is an important consideration, mistakenly prescribing corticosteroids (for IBD) to a patient with chronic amoebic colitis may lead to acceleration of disease, fulminant colitis, and toxic megacolon requiring colectomy.¹⁰ Amoebiasis should be ruled out in all patients in which IBD is being considered, especially when risk factors are present.¹¹ Fulminant amoebiasis is also more common in neonates, pregnant women, and the malnourished. Rare cases have been reported in subjects with the acquired immune deficiency syndrome (AIDS)¹²; however, an increase in the frequency or severity of invasive amoebiasis has not been seen.

Amoebic liver abscess is the most common extraintestinal form of the disease. Patients may present acutely with right upper quadrant pain and fever or subacutely with symptoms of pain and weight loss greater than 10 days.¹³ In the acute group of patients, amoebic liver abscess is often mistaken for pyogenic diseases of the gall bladder, biliary tract or liver. The subacute presentation resembles the signs and symptoms of malignancy, especially hepatocellular carcinoma. An ultrasound of the right upper quadrant is helpful in rapidly distinguishing biliary from hepatic disease and defining the extent of disease. In acute amoebic disease, multiple abscesses involving both lobes are common.¹³ The classic presentation of a solitary abscess of the right lobe of the liver is more commonly found in patients with greater than 10 days of symptoms. Expensive diagnostic studies, such as computed tomography or magnetic resonance imaging, offer little benefit beyond sonography. Most patients will not have concurrent diarrhoeal symptoms. New information shows, however, that most patients with amoebic liver abscess harbour pathogenic *E. histolytica* in the

gut lumen that must be eradicated to prevent a recurrence.¹⁴ Needle aspiration of the liver should only be used if it is necessary to rule out bacterial abscess, if rupture of the amoebic abscess seems imminent, or if there is an inadequate response to specific anti-amoebic treatment after three to five days.

Diagnosis of *E histolytica* intestinal infection and invasive amoebiasis

Currently, examination of three separate stool specimens is required to attain a 90% sensitivity for detection of *E histolytica* intestinal infection.¹⁵ A single examination identifies only 40–60% of infections, although a purged stool sample may have a higher yield. As mentioned, the false positive rate for stool microscopy is high, often because of incorrect identification of leucocytes as amoebas. Culture of a single stool sample for *E histolytica* in Robinson's media has a yield equivalent to microscopy of three samples. This technique, however, is not generally available in clinical pathology laboratories. Unfortunately, microscopy requires skilled personnel and laboratory support and does not differentiate pathogenic from non-pathogenic *E histolytica*. Finding haematophagous trophozoites is indicative of invasive amoebiasis, but it must be verified that the ingested material are erythrocytes and not yeasts. The need for multiple stool samples confounds diagnosis in areas where it is unrealistic for patients to return many times to the clinic. The absence of faecal leucocytes in patients with amoebic colitis may be misleading and results from the cytolytic abilities of trophozoites.

Serological examination for anti-amoebic antibodies is a very useful diagnostic study if properly interpreted. After seven days of symptoms, over 95% of patients with amoebic colitis or liver abscess will be seropositive.¹³ Serology is especially helpful in non-endemic areas in which a positive test is highly predictive of current disease. Conversely, a negative serology in an endemic area is very useful in reducing the probability of invasive amoebiasis. For asymptomatic subjects who have positive faecal microscopy for *E histolytica*, a negative serology for anti-amoebic antibodies points to infection with a non-pathogenic zymodeme.^{6,7} Given the 20–25% prevalence of serum anti-amoebic IgG antibodies in most endemic areas, a positive serology in an asymptotically infected subject is inconclusive. In general, amoebic serology studies are not rapidly available, multiple techniques and non-standardised complex antigen preparations are used, and the cost of the test is high because of low demand. All these factors reduce the use of serology in clinical decision making, relegating it to being a late confirmatory study.

An ideal diagnostic test for *E histolytica* would be rapid, would differentiate a pathogenic from non-pathogenic *E histolytica*, would distinguish current from remote infection, and would not require expert microscopy. In addition the test should be highly reproducible, accurate, quantitative, and applicable to developing world situations. I present some relevant studies from my laboratory and summarise where the field stands today.

We have identified and purified a highly conserved *E histolytica* antigen, the 260 kDa galactose inhibitable adherence protein (GIAP).^{16,17} This surface protein mediates parasite binding to colonic mucins, epithelial cells, and host inflammatory cells; attachment by the GIAP is required for amoebic cytolytic activity. The GIAP consists of 170 kDa heavy and 35 kDa light subunits; the gene for the heavy subunit has recently been cloned and sequenced.^{18,19} This well characterised antigen has a central role in disease pathogenesis and seems well suited for application to diagnostic studies. We determined by immunoblotting that over

95% of serum samples from subjects with invasive amoebiasis possessed antibodies to the 170 kDa GIAP heavy subunit.⁷ In collaboration with Terry Jackson's group in Durban, South Africa we used purified GIAP in an enzyme linked immunosorbent assay (ELISA) and found that 99% of convalescent serum samples from a large group of patients with amoebic liver abscess had anti-GIAP antibodies, while none of 69 USA controls with or without other parasitic infection were positive.⁷ The prevalence of anti-GIAP serum IgG antibodies (25%) was identical in all asymptomatic South Africans studied regardless of whether they were infected with non-pathogenic *E histolytica*. Studies of patients from Cairo, Egypt confirmed the utility of the GIAP ELISA.²⁰ Eighty nine per cent of 37 subjects with invasive amoebiasis, who were symptomatic for at least one week, possessed serum anti-GIAP IgG antibodies. Only four of 71 with acute colitis of less than 10 days duration, however, had serum anti-GIAP IgG antibodies, pointing to a need for other diagnostic methods. We used an ELISA to study serum IgM antibodies to the purified GIAP-antigen, and found anti-GIAP IgM present in samples from 55% of colitis patients and 78% of amoebic liver abscess patients in Egypt. Importantly, in the acute colitis patients who lacked serum IgG antibodies to the GIAP, 41% had serum anti-GIAP IgM antibodies present. These studies established that a single well characterised *E histolytica* antigen could be used effectively to detect serum anti-amoebic antibodies.

To deal with the current limitations in diagnosis of acute amoebic disease in endemic areas, especially the lack of distinction between pathogenic and non-pathogenic strains in asymptomatic hosts, we developed an antigen detection ELISA using anti-GIAP monoclonal antibodies to assay for GIAP antigen in serum and faeces.⁸ These antibodies were produced in our laboratory and characterised by Petri *et al*³ to be specific for two heavy subunit epitopes shared by both pathogenic and non-pathogenic strains and four epitopes found exclusively in pathogenic isolates. We found that 42% of Egyptian subjects with asymptomatic *E histolytica* infection and 57% with amoebic colitis had serum GIAP antigen. For comparison, only two of 50 USA controls (4%), 8% of healthy Egyptian controls, one of 22 Egyptians infected with other enteric parasites, four of 20 (20%) with bilharzial colitis, and one of 21 USA subjects with IBD were positive for serum GIAP antigen. The specificity of the monoclonal antibodies for the GIAP heavy subunit in serum samples was shown by immunoblotting. Stool cultures and zymodeme analysis were not performed on the Egyptian subjects. We studied a number of serum samples from South African subjects with well characterised faecal isolates. Only two of 34 with asymptomatic non-pathogenic *E histolytica* intestinal infection had GIAP antigen present in serum (Fig 1). In contrast, three of four samples from subjects with asymptomatic pathogenic intestinal infection possessed GIAP antigen. Amoebic liver abscess results exclusively from infection by pathogenic *E histolytica*, 75% were found to have serum GIAP antigen (Fig 1). All of the positive serum samples were obtained from patients having an acute presentation with right upper quadrant pain and fever. The sensitivity of serum antigen detection in identification of pathogenic *E histolytica* infection was 68.7%. The specificity was higher at 94.2%, providing a positive predictive value of 0.733 and, importantly, a negative predictive value of 0.071.

Fifteen stool samples from Egyptian amoebic colitis subjects were studied, in comparison with 26 American control samples.⁸ All 15 Egyptian colitis patients had GIAP antigen in faeces, compared with one of 26 USA controls (Fig 2). The monoclonal antibodies used in this ELISA (designated 3F4 and 8A3) detect GIAP antigen present in both pathogenic and non-pathogenic *E histolytica*.³ A correlation of results in serum and faeces confirmed that serum

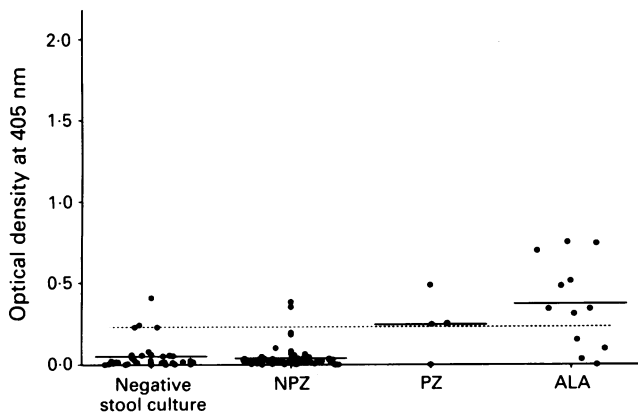


Figure 1: Detection of adherence protein antigen in serum samples of subjects with asymptomatic *E histolytica* intestinal infection or with amoebic liver abscess (ALA). (NPZ, non-pathogenic zymodeme infection; PZ, pathogenic zymodeme infection). Note that only two of 35 with negative cultures and two of 34 with NPZ intestinal infection had serum antigen compared with three of four with PZ intestinal infection and eight of 12 with amoebic liver abscess. (Reprinted with permission from *J Clin Microbiol* 1993; 31: 2845-50.)

GIAP antigenemia occurs only during pathogenic *E histolytica* infection. Studying two different monoclonal antibody systems (3F4 primary and 8A3 secondary compared with 8C12 primary and 1G7 secondary) to differentiate pathogenic from non-pathogenic *E histolytica* in faeces, we found that faeces from eight of 15 Egyptian subjects had pathogenic zymodeme specific GIAP epitopes present. Seven of these eight subjects had GIAP antigen in their serum samples, compared with none of seven with non-pathogenic organisms in faeces.

ELISA with polyclonal anti-amoebic antibodies or uncharacterised monoclonal antibodies has been used by other researchers to detect *E histolytica* antigen in faeces.²¹⁻²² These reports did not differentiate pathogenic from non-pathogenic infection and therefore have limited clinical utility. Use of a monoclonal antibody to 84 and 81 kDa *E histolytica* proteins in immunofluorescence studies of organisms cultured from stool was successful in identifying pathogenic infection.²³ Culture and immunofluorescence are cumbersome, time consuming procedures requiring specialised laboratory support. Use of polymerase chain reaction and hybridisation of polymerase chain reaction products with faecal DNA samples holds promise for use in diagnosis.²⁴ This technology, however, currently seems even further removed than antigen detection in development for application in the field. While our studies were in progress, Haque *et al*²⁵

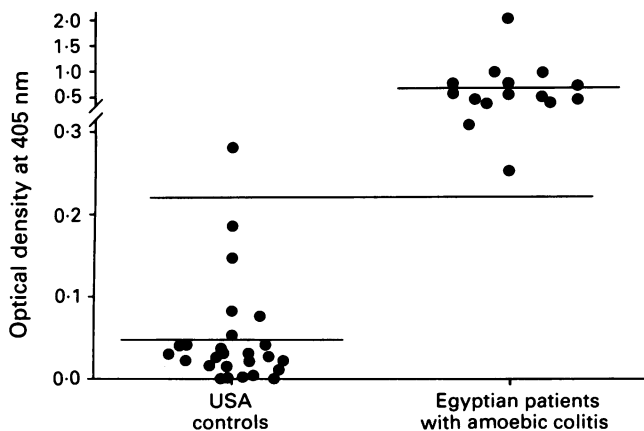


Figure 2: Detection of galactose-inhibitable adherence protein (GIAP) antigen in faeces by ELISA. Monoclonal antibodies in this study (3F4 and 8A3) will detect both GIAP antigen from pathogenic and non-pathogenic isolates. Only one of 26 USA controls were positive compared with all 15 Egyptian patients with amoebic colitis ($p < 0.01$). (Reprinted with permission from *J Clin Microbiol* 1993; 31: 2845-50.)

An approach for diagnosis of amoebiasis in the future. Patients should have appropriate epidemiological risk factors and clinical presentations

	ELISA for faecal GIAP antigen		ELISA for serum GIAP antigen	ELISA for serum anti-GIAP antibodies	
	PZ specific	NPZ specific	PZ specific	IgM	IgG
	Asymptomatic NPZ infection	(-)	(+)	(-)	(-)
Asymptomatic PZ infection	(+)	(-)	(+)	(+)	(+)
Amoebic colitis*					
acute (<10 days)	(+)	(-)	(+)	(+)	(-)
chronic	(+)	(-)	(+)	(±)	(+)
ALA†					
acute (<10 days)	(±)	(-)	(+)	(+)	(-)
chronic	(±)	(-)	(+)	(±)	(+)

* Stool should be positive for occult blood; † ultrasound should defect in the liver; ‡ may be positive in an endemic area because of previous PZ infection.

reported use of ELISA to detect GIAP antigen in faeces, using rabbit polyclonal anti-GIAP 'catching antibodies' and the anti-GIAP monoclonal antibodies as secondary antibodies. Their system was specific for pathogenic zymodemes and detected GIAP antigen in the faeces from 12 subjects with pathogenic infection.

The Table illustrates a future approach to the diagnosis of amoebiasis. Combining studies of faecal GIAP antigen, serum GIAP antigen, and serum IgM and IgG antibodies to GIAP provides enhanced specificity. Currently, these methods are in development but are not commercially available. Obviously, other *E histolytica* antigens may be shown to be useful alone or in combination with the GIAP. Use of recombinant antigens, as reported by Zhang *et al* in the studies of the GIAP heavy subunit, would be preferable for standardisation and commercial application. In the future, ELISA technology could be abandoned in favour of rapid agglutination methods that are even more applicable to field situations. Efforts should be made to develop panels as described in the Table, which will eliminate the need for stool microscopy in the diagnosis and treatment of *E histolytica* infection.

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Editorial

Gut index change

There will be a change in the indexing of the journal starting with the 1994 index. Papers and proceedings will be indexed using a keyword system. Authors will be asked to select up to five keywords for each paper at manuscript stage, which will then be used to compile the annual index. A keyword is a word (or phrase) that will identify the subject matter of a written paper or proceeding in an index. The index will be published, as usual in the December issue. The format will be different, with the title of the paper repeated after each keyword entry. The author index will no longer include the title of the paper and will become a list of authors only.

Authors should scan papers for headings that may not be in the title, to use British approved names rather than pharmaceutical names for drugs, and to avoid general terms such as clinical, complications, adverse

effects, and patient. As the subject of the journal is the 'digestive system' this should not be used as a heading. In general, it is preferable not to split accepted concepts. For instance, upper gastrointestinal tract is preferable as a keyword, rather than tract, upper gastrointestinal.

Some shortened forms can be accepted such as DNA, AIDS, HIV, and cAMP, which are universally known, but mostly the full form should be used as the keyword. Alpha fetoprotein, alpha and beta receptor blockades for example are usually submitted in full. Greek letters are not generally used in alphabetisation. Other examples include the use of oesophagus rather the oesophageal, but growth factors should be placed under the specific type, for example, epidermal, fibroblast. There will be no cross references in the keyword index.