

ENTAMOEBA HISTOLYTICA AND GIARDIA LAMBLIA INFECTIONS : CURRENT DIAGNOSTIC STRATEGIES

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Summary :

Entamoeba histolytica and *Giardia lamblia* constitute, in a world context, the two commonest intestinal protozoan parasites to affect man. Therefore accurate diagnosis is of paramount importance if resultant infections are to be adequately managed. Demonstration of the cyst or trophozoite stage in a faecal sample(s) (several newer techniques are available) remains the lynch-pin of diagnostic strategies; however, excretion of cysts, especially, is intermittent and evidence of infection is not always manifest in a single examination. A limited range of other techniques is also available for a 'parasitological diagnosis'. Within the last decade, serological techniques (largely dependent on invasive properties of the organism) have attained levels of diagnostic competence. Therefore, a very high index of suspicion now ensues from indirect evidence of infection.

KEY WORDS : *Entamoeba histolytica*. *Giardia lamblia*. diagnostic tests. serological diagnosis.

Diagnosis of *Entamoeba histolytica* and *Giardia lamblia* infections – in a world context the two most common gastrointestinal protozoan infections to affect *Homo sapiens* (Cook, 1994) – is very largely dependent on diagnostic parasitological techniques. Efficacy of diagnosis is dependent first and foremost on investigational methods. A major diagnostic problem lies however, in the erratic nature of cyst-excretion. Experience, dexterity, and diligence of the investigator are of paramount importance, and this applies especially with microscopic techniques. Whilst serological techniques have, within the last decade, attained levels of relative excellence, many of them remain research procedures, and the brunt of investigation devolves on demonstration of either trophozoite and/or cyst of the respective organism (with or without use of a concentration technique).

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Résumé : INFECTIONS À ENTAMOEBA HISTOLYTICA ET GIARDIA LAMBLIA : STRATÉGIES DIAGNOSTIQUES ACTUELLES

E. histolytica et *G. lamblia* constituent, à l'échelle mondiale, les deux protozoaires affectant le plus souvent l'homme. Un diagnostic précis est de ce fait primordial si l'on veut contrôler de manière adéquate les infections qui en résultent. La mise en évidence de kystes ou de trophozoïtes dans un échantillon de fèces (plusieurs techniques récentes sont disponibles) reste la clé du diagnostic; cependant, l'excrétion des kystes, tout particulièrement, est intermittente et l'infection n'est pas toujours manifeste lors d'un seul examen. Un certain nombre d'autres techniques sont disponibles pour un « diagnostic parasitologique ». Au cours de la dernière décennie, les techniques sérologiques (largement dépendantes des propriétés invasives du micro-organisme) ont atteint un niveau de compétence diagnostique. Désormais, la probabilité de l'infection est très élevée après une mise en évidence indirecte.

MOTS CLES : *Entamoeba histolytica*. *Giardia lamblia*. tests diagnostiques. diagnostic sérologique.

GIARDIA LAMBLIA : THE MAJOR SMALL- INTESTINAL PROTOZOAN PARASITOSIS

CLINICAL SCENARIO

Clinical manifestations are varied, but the majority of infections are acquired during overseas travel (Cook, 1994; Farthing, 1994). A travellers' diarrhoea-like illness – to be differentiated from other causes of this clinical syndrome – is commonplace. Persisting diarrhoea (> 10 days) especially in the traveller who has returned from a tropical/sub-tropical environment constitutes a further possibility. At the extreme end of chronicity, on-going diarrhoea/malabsorption (> 10 weeks), is a further clinical sequel; this syndrome must be differentiated from other conditions with an absorptive defect, including post-infective tropical malabsorption ('tropical sprue'). Therefore, the clinical presentation varies; the physician must raise the 'index of suspicion' for this protozoan parasitosis (Cook, 1994; Farthing, 1994; Davis & Reynoldson, 1994).

Accurate parasitological diagnosis is essential if a *G. lamblia* infection is to be differentiated from, for example, the following – all of which can be causatively related to on-going diarrhoea/malabsorption : persisting *Salmonella* spp., *Campylobacter* spp., and

<i>Giardia lamblia</i>	duodenal/jejunal aspirate 'Enterotest' histology : duodenal/jejunal [haematoxylin + eosin] faecal-sample
<i>Entamoeba histolytica</i>	faecal-sample rectal-scrape/biopsy histology : colo-rectal [haematoxylin + eosin] hepatic aspirate

Table I. – Diagnostic material

Trophozoites	motile Field's stain
Cysts	Thompson's (negative stain) specific FA
Antigen-detection (Smithyman, 1994)	specific FA ELISA fluorescence-activated cell sorting (FACS) gene probe dipstick (ICA)

Table II. – *Giardia lamblia* – parasitology/faecal antigen detection

≡ small intestinal mucosal damage	IFAT ELISA
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Table III. – *Giardia lamblia* – serology

Shigella spp. infections, other small-intestinal parasitoses, HIV enteropathy, ileo-caecal tuberculosis, 'tropical sprue', and gluten-induced enteropathy. Symptoms associated with these various entities can be exacerbated by the presence of hypolactasia – which results in super-added intolerance to milk and dairy produce.

MATERIAL AVAILABLE FOR DIAGNOSIS

Table I summarises the nature of diagnostic material(s) available to the parasitologist. Whilst duodenal/jejunal aspirate, 'Enterotest' (string-test), and/or histology will most likely yield trophozoites of *G. lamblia*, a faecal-sample will in all probability reveal cysts, although trophozoites are occasionally present. In a recent study involving individuals exposed to this infection in India, a positive diagnosis was made from duodenal-aspirate in 44 % and from a faecal-sample in 85 % – thus indicating that the two diagnostic approaches are indeed complimentary (Goka *et al.*, 1990). Trophozoites in a duodenal/jejunal biopsy-specimen are best detected in flecks of mucus adherent to the biopsy-fragment or capsule; in histological sections, they can be visualised on or near the epithelial surface.

PARASITOLOGY

Table II summarises techniques currently in use for detecting trophozoites/cysts of *G. lamblia*. In a fresh film (of duodenal/jejunal fluid), trophozoites can be

seen to be motile; Field's stain is a valuable technique. Although cysts may also be visible on a direct film (usually involving a faecal-sample), Thompson's (negative staining) technique is of value, as is a specific fluorescent antibody technique. Immunological (IFA, ELISA, fluorescence-activated cell sorting [FACS], gene probe, and dipstick [ICA]) techniques have also been used to detect *Giardia* antigen in a faecal-sample (Ungar *et al.*, 1984; Green *et al.*, 1985; Janoff, 1989; Addiss *et al.*, 1991) (table II). DNA-based faecal detection assays should be possible with the development of specific DNA probes for *G. lamblia* (Char & Farthing, 1991; Perez *et al.*, 1994). Perez *et al.* have reported 100 % sensitivity and 98 % specificity using commercially available ELISA and DIF, compared with conventional light microscopy. However, difficulties have arisen resulting from incomplete liberation of DNA from the cyst-stage (Perez *et al.*, 1994). In order to circumvent this problem, Smithyman has used a capture ELISA method based on a stable *Giardia*-specific coproantigen – common to both cyst and trophozoite stages (Smithyman, 1994). Sensitivity can be enhanced using the polymerase chain reaction (PCR).

SEROLOGY

Table III summarises serological investigations of value in the detection of a *G. lamblia* infection. Immunological responses utilise serum antibody measurements (Farthing *et al.*, 1987; Farthing, 1990); field assays have produced sensitivities and specificities > 90 %; however, evidence of efficacy in the routine

laboratory is awaited (Farthing, 1994). Unfortunately, most studies indicate that detection of anti-*Giardia* IgG does not readily distinguish between present and past infection (Farthing, 1994; Davis & Reynoldson, 1994). Both IgM and IgA responses are however relatively short-lived, and these may be of value in diagnosing an on-going infection (Goka *et al.*, 1986; Nash *et al.*, 1987; Goka *et al.*, 1989).

Experience at the Hospital for Tropical Diseases, London, indicates that serological results for *G. lamblia* infection are only positive when significant mucosal damage exists, and *G. lamblia* infection is present (Ridley & Ridley, 1976); the more severe the mucosal impairment, the greater the likelihood of antigen passage/absorption into the portal circulation. Whereas 32 out of 36 serum samples from cases of *G. lamblia* infection associated with malabsorption gave a positive result using an immunofluorescent test, those obtained from patients without malabsorption and controls were negative. From this experience, therefore, serological techniques are only likely to yield positive results when the clinical situation is advanced (florid), and diarrhoea/malabsorption already present.

RADIOLOGICAL CHANGES

When a heavy *G. lamblia* infection (accompanied by malabsorption) is present, dilatation of small-intestinal 'loops' with thickening of mucosal folds are often demonstrable on barium examination. However, these changes occur in many 'malabsorption-states' and are certainly not specific for giardiasis.

ENTAMOEBIA HISTOLYTICA INFECTION

Unlike *G. lamblia*, *E. histolytica* is (i) primarily a colo-rectal organism, and furthermore (ii) an invasive protozoan (Cook, 1994; Ravdin, 1988). Whilst, therefore, a parasitological diagnosis is usually feasible, the likelihood of a positive serological result is far greater than in a *G. lamblia* infection; it should be recognised however, that this applies only to invasive disease.

CLINICAL SCENARIO

Clinical manifestations of an *E. histolytica* infection vary widely (Cook, 1994; Ravdin, 1988). In the asymptomatic cyst-carrier state the organism is confined to the colo-rectal lumen and invasion of colonic tissue is absent. Invasive colonic disease gives rise classically to dysentery (bloody diarrhoea), the differential diagnostic list of which is substantial (see below). As a sequel to invasion into the portal circu-

lation – hepatic disease (amoebic liver 'abscess') may ensue. The differential diagnostic list here includes several other space-occupying lesions involving the liver (see below).

When colo-rectal disease is present, dysentery – following an incubation period of 7-21 days – is the likely sequel and blood and/or mucus will be present in a faecal sample; although only a few polymorphonuclear leucocytes are demonstrable, these are frequently present in abundance in a peripheral blood-specimen. Associated symptoms include: headache, nausea, fever, colic, and tenesmus. Complications of colo-rectal disease (a positive serological result is usual) include: necrotising colitis (with or without perforation), amoebic appendicitis, amoeboma, haemorrhage, and stricture.

The major differential diagnostic entity is colo-rectal shigellosis – caused by: *Shigella dysenteriae*-1 (Shiga's bacillus), *S. flexneri*, *S. boydii*, and *S. sonnei*. The incubation period is usually 1-4 days and faecal-leucocytes are usually present in abundance; a polymorphonuclear leucocytosis in peripheral blood is unusual, bacteraemia not being a feature of this disease. Systemic complications include haemolytic-uraemic syndrome (caused by *S. dysenteriae*-1 exotoxin) and Reiter's syndrome (usually in association with HLA-B27). Differentiation of amoebic colitis from shigellosis is usually straightforward on clinical grounds and parasitology/serology merely confirmatory. Other differential diagnoses – especially when diarrhoea has persisted for >10 days after return of a traveller from a tropical location – include schistosomal-colitis and inflammatory bowel disease. This latter entity usually consists of ulcerative colitis, but Crohn's disease is a distinct possibility. Whilst such a traveller has had no previous experience of colonic symptoms, these are precipitated by a colo-rectal infection acquired in a tropical/subtropical environment.

MATERIAL AVAILABLE FOR DIAGNOSIS

Table I summarises material(s) likely to be available to the parasitologist. Whether the trophozoite or cyst form is present in a faecal-sample depends largely on the symptomatic state of the individual (Fig. 1). When stools are well-formed, the cyst-stage is likely to be present; however, with an unformed specimen and/or rectal scrape, the trophozoite stage of *E. histolytica* is far more likely to be dominant.

PARASITOLOGY

Table IV summarises some relevant features regarding trophozoites and cysts of *E. histolytica* (Healy, 1988). In a fresh ('wet-mount') faecal-sample, trophozoites are motile, and contain ingested erythrocytes derived

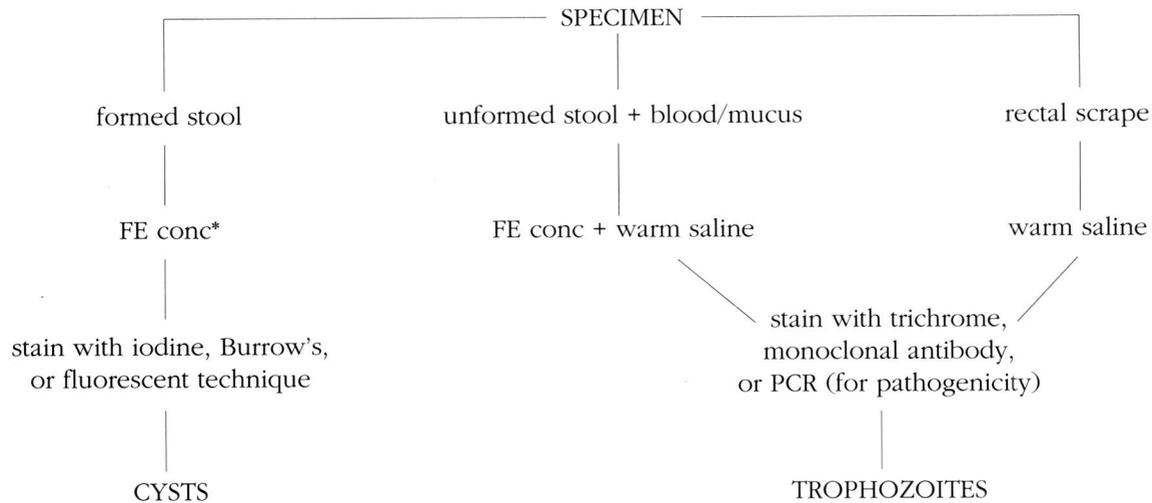


Fig. 1. – Flow-chart indicating the stage in the life-cycle of *E. histolytica* likely to be detected parasitologically, in different clinical situations. * FE conc : formal ether concentration.

from the parasitised host (the 'gold standard'). Staining techniques include the trichrome (which defines nuclei) and Wright's stain. Research techniques include a PCR – which can be carried out on a faecal sample, and a specific fluorescent antibody technique – which utilises a cultured sample, and has the potential to differentiate pathogenic from non-pathogenic strains of *E. histolytica* (see below) (Healy, 1988). An ELISA for detecting amoebic antigen in a faecal sample holds great promise, and does not require a skilled microscopist (Healy, 1988). Palacios *et al.* working in Mexico, recorded that an ELISA gave a 100 % detection-rate (Palacios *et al.*, 1978), whilst Randall *et al.*, 1984, in San Francisco, recorded a 93 % agreement between results of a commercially available ELISA and microscopy. Using a commercially prepared HK-9 monoclonal antibody as antigen captive source, Ungar *et al.*, 1985, produced variable results which might have been strain-specific. A great deal of research has lately centred on differentiation of pathogenic (*E. dysenteriae*) from non-pathogenic (*E. dispar*) strains. Whilst P.G. Sargeant, 1988, (London) has – on the basis of isoenzyme studies – championed genetic differences, D. Mirelman, 1988 (Israel) has considered environmental factors to be more relevant. Recent evidence based on DNA technology clearly indicates, however, that the two strains are genetically separate (Tannich *et al.*, 1986). In a world context the vast majority of strains of *E. histolytica* are non-invasive (*E. dispar*) and cause nothing more than a cyst-carrier state, but significant geographical variations exist – there being a high proportion of invasive (*E. dysenteriae*) cases in southern America and South Africa, for example (Sargeant, 1988).

SEROLOGY

Table V summarises some serological techniques which have been applied to the diagnosis of an *E. histolytica* infection (Healy, 1988); these are only positive when invasive disease involving the colo-rectum and/or liver, is present. The immunofluorescent antibody (IFA), indirect haemagglutination (IHA), and ELISA techniques utilise whole antigen. However, countercurrent immunoelectrophoresis (CIEP) incorporates soluble antigen. The latex agglutination (LA) technique is highly sensitive. The gel-diffusion precipitation (GDP) technique is rarely used. Some ten-days after the onset of clinical disease, the IFAT is positive in approximately 95 % of cases – usually at a concentration $\geq 1:160$. The CAP and CIEP techniques are more specific, but less sensitive. Results of serological tests in amoeboma are similar to those in invasive colo-rectal disease. Whereas approximately 75 % of cases of amoebic colitis yield a positive result at low titre, the cyst-carrier state invariably provides a negative serological result. Some investigators have claimed however, that certain serological tests are positive in the presence of the cyst-carrier state; this is not the experience of the majority. T.F.H.G. Jackson *et al.*, working in South Africa have, for example, recorded positive serological results in the asymptomatic cyst-carrier state (Jackson *et al.*, 1985); this clearly implicates an element of tissue invasion and does not strictly constitute a carrier-state! These authors concluded that: 'Pathogenic zymodemes are [...] in constant contact with the host's tissues, even in symptom-free individuals [...]'.

Isolation and detection of *E. histolytica* in hepatic 'abscess' aspirate is unsatisfactory (Healy, 1988).

Trophozoites	motile ingested RBCs trichrome stain (nuclei) Wright's stain monoclonal-antibody [PCR + specific FA]
Cysts	4 nuclei; 9.5-15µ iodine stain Burrow's stain (chromatid bars : DNA) fluorescent technique

Table IV. – *Entamoeba histolytica* – parasitology

immunofluorescent antibody (IFA) indirect haemagglutination (IHA) ELISA countercurrent immunoelectrophoresis (CIEP) cellulose acetate precipitation (CAP) latex agglutination (LA) [gel-diffusion precipitation (GDP)]
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Table V. – *Entamoeba histolytica* – serology

	Sensitivity	Specificity
Ultrasound	++	++
Computed tomography	++	+
Magnetic resonance (MNR)	++	+
^{99m} Tc-sulphur colloid	+	-
⁶⁷ Ga citrate	±	±
¹¹¹ Ind WBC ^S	±	±
^{99m} Tc dis IDA	+	-

Table VI. – *Entamoeba histolytica* – imaging (Ralls *et al.*, 1988).

However, Mahajan & Ganguly, 1980, using a CIEP technique, were able to detect antigen in 115 out of 125 cases of liver ‘abscess’, whilst Bhavé *et al.* using an ELISA obtained a positive result in 23 out of 25 specimens obtained from a proven amoebic ‘abscess’ (Bhavé *et al.*, 1985).

IMAGING TECHNIQUES

Table VI summarises various imaging techniques which have been used to delineate invasive hepatic disease (Ralls *et al.*, 1988). Overall, ultrasonography seems to provide the greatest sensitivity and specificity.

CONCLUSION(S)

Despite a great deal of progress in immunological techniques, diagnosis of the two outstandingly important human intestinal protozoan parasitoses remains very largely dependent on an experienced microscopist. Faecal antigen-detection has opened great possibilities, but difficulties remain, e.g., erratic excretion of the cyst-stage (in the parasite life-cycle) and unpredictability in liberation of antigen from intact cysts. Serological diagnosis is largely dependent on inva-

sion of the colonocyte (in the case of *E. histolytica* infection); *G. lamblia* is essentially a non-invasive organism and a reliable serological method seems unlikely to be readily forthcoming. Subsequent introduction of a simple technique to distinguish pathogenic from non-pathogenic strains of *E. histolytica* is likely to revolutionise the chemotherapeutic approach to this infection.

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