

The diagnostic implications of the separation of *Entamoeba histolytica* and *Entamoeba dispar*

J P ACKERS

Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine,
Keppel Street, London, WC1E 7HT, UK

(Fax, 44(0)20-7636-8739; Email, john.ackers@lshtm.ac.uk)

For much of the last hundred years most cases of amoebiasis have been diagnosed by light microscopy. Only relatively recently have we become aware that this technique is usually incapable of distinguishing between two species – *Entamoeba histolytica* and *E. dispar* – only the first of which is a pathogen. The implications of this for patient management and, even more, for the validity of epidemiological surveys, are only slowly being addressed. What is clear is that methods are urgently required to distinguish between infections with these two species and this review attempts to summarise some of those, which have been developed to meet this need.

[Ackers J P 2002 The diagnostic implications of the separation of *Entamoeba histolytica* and *Entamoeba dispar*; *J. Biosci. (Suppl. 3)* 27 573–578]

1. Introduction

In 1875 Fedor Lösch (Kean *et al* 1978) described the case of a young farmer who had been admitted to his clinic in St Petersburg, Russia, two years earlier. The man had been suffering from chronic dysentery and Lösch found such large numbers of amoeba in his faeces that he became convinced that they were responsible for the dysentery. He named the organism *Amoeba coli* and showed that it produced colonic ulceration and dysentery in dogs. Subsequently Schaudinn renamed the organism *Entamoeba histolytica*.

2. Invasive amoebiasis

This patient of Lösch's was the first person known to have died from amoebiasis. Clinical disease results from the ability of *E. histolytica* to penetrate the wall of the large bowel and to spread extraintestinally. In brief, penetration of the gut wall may lead to ulceration which, if extensive enough, produces the classical signs and symptoms of amoebic dysentery. Extraintestinal spread most frequently involves the liver, causing hepatic amoebiasis or amoebic liver abscess; spread to other distant

organs such as the brain is known but is very rare. This is not a clinical article and current texts should be consulted for advice on patient management (Ravdin and Petri 1994; Petri and Singh 1999; Hughes and Petri 2000).

The accurate diagnosis of hepatic amoebiasis (amoebic liver abscess) has always been easier, even with limited resources, than that of amoebic dysentery – a consequence of a characteristic clinical picture, significantly raised antibodies detectable with simple whole-antigen tests and lesions easily detected by widely-available ultrasonography (Petri and Singh 1999). Invasive intestinal amoebiasis, on the other hand, has always presented more difficult challenges.

3. The asymptomatic cyst-passer

By the start of the 20th century it had become very clear that many persons shedding cysts of "*E. histolytica*" had no symptoms of disease and in 1925 Emile Brumpt (Brumpt 1925) suggested that there were two separate but indistinguishable types of amoeba. However, because Walker and Sellards (1913) had already demonstrated that cysts isolated from asymptomatic patients could, on occasion, cause disease when fed to volunteers, and

Keywords. Amoebiasis; antigen detection; *Entamoeba dispar*; *Entamoeba histolytica*; diagnosis; polymerase chain reaction

because there was no way of distinguishing between the cysts of the pathogenic and non-pathogenic species, Brumpt's suggestion was largely ignored. Indeed, in 1969 WHO (WHO 1969) defined amoebiasis as "infection with *Entamoeba histolytica*, with or without clinical manifestations", implying that all strains were potentially pathogenic.

For many years it was far from clear whether the outcome of infection was due to differences in host or parasite, but since the pioneering observation of Martínez-Palomo and colleagues on lectin-mediated agglutination (Martínez-Palomo *et al* 1973), it has become more and more apparent that there are fundamental differences between the organisms recovered from patients with invasive disease and those parasitising asymptomatic cyst passers. Subsequently a very large amount of isoenzyme work by Sargeant and Williams demonstrated consistent differences between pathogenic and non-pathogenic isolates (Sargeant 1988). In particular "fast" hexokinase bands became and remain the simplest marker of pathogenicity, while many of the original distinct zymodemes are now known to result from variation in culture conditions (Blanc and Sargeant 1991).

An increasing number of biochemical, immunological and genomic differences between the two species are now recognized and this information finally led to the formal separation of the two species (Diamond and Clark 1993) with the name *Entamoeba histolytica* being retained for the pathogenic species and Brumpt's name *E. dispar* being revived for the non-pathogen.

4. Biological characteristics that distinguish *E. histolytica* from *E. dispar*

- Isoenzyme patterns, particularly hexokinase.
- Specific epitopes, recognized by reaction with several monoclonal antibodies.
- Sequence differences in the rDNA episome.
- Significant (2–18%) sequence differences between homologous genes.
- A small number of genes, for example *ariel* (Willhoeft *et al* 1999a) and *cp5* (Bruchhaus *et al* 1996; Willhoeft *et al* 1999b) appear so far to be unique to *E. histolytica*.
- It has proved much easier to adapt *E. histolytica* to axenic growth. Axenic culture of *E. dispar* proved extremely difficult and has so far been achieved for only one strain (Clark 1995).
- Scanning electron microscopic examination of axenic cultures of both species show significant differences – particularly in the appearance of the surface (Clark *et al* 2000). This may be linked to the apparent lack of surface lipophosphoglycan (LPG) from *E. dispar* (Bhattacharya *et al* 2000).

So, in summary, we now believe that four, not three species of *Entamoeba* (*E. histolytica*, *E. dispar*, *E. coli*, *E. hartmanni*) may regularly be found in the human large bowel, only one of which is a pathogen. There are also a few rare species: "atypical," "low temperature" or "Laredo" strains of *E. histolytica*, now known to be the normally free-living species *E. moshkovski* (Clark and Diamond 1991), *E. polecki*, *E. chattoni* and *E. gingivalis*, which will not be considered further here. Of the principal four, the important point is that the cysts of *E. coli* and *E. hartmanni* may be distinguished by light microscopy applying well-understood criteria from those of *E. histolytica* and *E. dispar* but that the latter two are indistinguishable from each other.

5. The Mexico meeting

In 1997 WHO convened a meeting in Mexico City (WHO 1997) to consider the implications of the separation of the two species. The definition of amoebiasis as "infection with *Entamoeba histolytica*, with or without clinical manifestations" was reaffirmed but the name *Entamoeba histolytica* is now to be used only for the pathogenic species, clearly separated from *E. dispar*. Amongst the conclusions of the meeting were:

- The criteria (such as size) used to form the classical taxonomic description of *E. histolytica* cannot distinguish between *E. histolytica* and *E. dispar* and, of particular importance in diagnostic microscopy, cysts of the two species are identical.
- When diagnosis is made by light microscopy, since the cysts of the two species are indistinguishable, they should be reported as *E. histolytica/E. dispar*.
- In asymptomatic individuals treatment is not appropriate when *E. histolytica/E. dispar* has been specifically detected
- Optimally, *E. histolytica* should be specifically identified and infections, if present, treated.

What this means in practice is that while it would be ideal if every infected patient had a specific diagnosis of either *E. histolytica* or *E. dispar* made, the diagnostic procedure most frequently employed in disease-endemic areas (light microscopy) is unable to do this.

6. Species-specific diagnosis of *E. histolytica* and *E. dispar*

Before considering the various methods available for distinguishing between the two species in faeces, it is worth noting that there are two situations (both acknowledged at the Mexico meeting) where a presumptive diagnosis of infection with *E. histolytica* can be made without actually identifying the organism present.

The first is where amoebic trophozoites containing ingested red blood cells (“haematophagous trophozoites”) can be identified in the faecal specimen (González-Ruiz *et al* 1994). For this to be even possible the specimen must either be examined as soon as possible after being passed (within 30 min at most), meanwhile being kept warm if necessary or it must immediately be fixed and subsequently stained, usually with Trichrome. Once fixed, specimens may be stored for many months without deterioration. Detailed protocols may be found in standard reference books (Garcia and Bruckner 1997) or obtained from the Centers for Disease Control (<http://www.dpd.cdc.gov/dpdx/HTML/DiagnosticProcedures.htm>). Although *E. dispar* is perfectly capable of ingesting red blood cells *in vitro* this does not appear to occur in the colon even if blood is present as a result of a second infection such as shigellosis. It is also worth noting that for this reason the presence of trophozoites without ingested red blood cells, even in a specimen of bloody diarrhoea, does not enable the diagnosis of amoebic dysentery to be made.

A related but much more invasive technique is the examination of fixed and stained biopsy specimens. The identification of trophozoites deep within the tissues and containing ingested red blood cells enables a definitive diagnosis of invasive amoebiasis due to *E. histolytica* to be made; unfortunately normal haematoxylin and eosin staining is not ideal for these parasites. Periodic acid-Schiff (PAS) or direct fluorescent antibody staining is much superior (Gilman *et al* 1980).

Secondly, in symptomatic individuals the presence of high titres of specific antibody is strongly correlated with the presence of invasive amoebiasis. In patients normally resident in non-endemic areas with low or undetectable levels of pre-existing antibody, serological testing using simple techniques and crude antigens (such as ELISA or indirect haemagglutination) is an effective diagnostic technique for intestinal as well as hepatic invasive amoebiasis (Pillai *et al* 1999). Results are much less clear-cut in disease-endemic areas; while virtually everyone infected with *E. histolytica* will be seropositive whether or not they are symptomatic (Jackson *et al* 1985), these antibodies will persist for months or years following spontaneous or drug-induced loss of the infection. This, plus the fact that titres tend to be lower than in cases of hepatic amoebiasis, makes simple serology of limited diagnostic value where infection is common (Hossain *et al* 1983; Shetty *et al* 1988).

The use of recombinant antigens and defined epitopes reduces but does not completely eliminate the problem of persisting seropositivity (Lotter *et al* 1995; Stanley *et al* 1998; Haque *et al* 1999). The specific measurement of IgM rather than IgG may also help to distinguish current from past infections (Abd-Alla *et al* 1998).

7. Asymptomatic carriage of *E. histolytica*

When *E. dispar* was separated from *E. histolytica* it was assumed that the vast majority of asymptomatic cyst shedders would turn out to be infected with *E. dispar* and that all those infected with *E. histolytica* were either clinically ill or probably would become so if not treated. In fact this has not turned out to be the case and surveys in South Africa (Gathiram and Jackson 1987), Bangladesh (Haque *et al* 2001) and Vietnam (E Tannich, personal communication) have shown that, surprisingly, only a few percent of those genuinely infected with *E. histolytica* ever go on to develop clinical amoebiasis. Why this should be is not clear and there are many possible explanations but it does not reduce the need for the accurate, species-specific diagnosis of *E. histolytica* in faecal specimens. This is needed firstly because, since there is no way of knowing which infected persons will progress to clinical amoebiasis, all should be treated; secondly because they are (as Walker and Sellards showed all those years ago) excreting cysts which can cause clinical amoebiasis in others and thirdly, because only in this way can we obtain accurate epidemiological data from which to make a better estimate of the burden of amoebiasis on the health of the world (Petri *et al* 2000).

8. Specific detection of *E. histolytica* and *E. dispar* in faeces

8.1 Isoenzyme analysis

This technique, which was amongst the earliest to suggest that “pathogenic” and “non-pathogenic” *E. histolytica* were in fact two separate species, had until recently been applied to more specimens than any other and thus deserved its reputation as the “gold standard” against which newer methods need to be validated (Sargeant 1988). In practice, however, it has several disadvantages: culture of microscopically cyst-positive faeces is by no means always successful (Sehgal *et al* 1995); it may take seven to fourteen days to grow enough trophozoites to prepare the lysates for analysis and the process itself is somewhat cumbersome. Even though the original procedure involving starch-gel electrophoresis of four enzymes is often simplified to merely examining hexokinase mobility in agarose mini-gels (Strachan *et al* 1988), isoenzyme electrophoresis is now rarely used in clinical diagnosis.

8.2 Antigen detection

Although only a very few genes specific to *E. histolytica* and absent from *E. dispar* have been discovered as yet, almost all homologous proteins contain amino-acid

substitutions which result in the expression of species-specific epitopes. Detection of these epitopes with monoclonal antibodies is the basis of a number of quick and convenient diagnostic methods. The target molecule which has been most intensively studied is the heavy subunit of the galactose/N-acetyl-galactosamine inhibitable lectin; of six monoclonal antibodies raised against this molecule (Petri *et al* 1990b), only two reacted with *E. histolytica* and *E. dispar* while the other four reacted only with *E. histolytica* (Petri *et al* 1990a). These antibodies are the basis of two kits manufactured by TechLab Inc. (www.Techlab.com) one of which (based on one of the non-specific monoclonal antibodies) identifies *E. histolytica*/*E. dispar* while the other, based on a specific one can identify *E. histolytica*. Sequential application of these two kits can specifically identify both species although they cannot distinguish mixed infection with *E. histolytica* and *E. dispar* from infection with *E. histolytica* alone. Evaluation in Bangladesh shows clearly that these kits are more sensitive and specific than either wet-film microscopy or culture (Haque *et al* 1998, 2000). Because the gold-standard test requires cultivation of the organism which is known not to be 100% sensitive it is difficult to assess whether the kits produce false-positive results, but PCR (see below) suggests that most culture or microscopy negative but antigen positive samples are true positives (Haque *et al* 1998). The speed and convenience of these kits is also a strong point in their favour (Evangelopoulos *et al* 2001). Other workers have used similar monoclonal antibodies with equal success for species specific diagnosis in Egypt (Abd-Alla *et al* 2000b; Abd-Alla and Ravdin 2002).

Other kits are available from Cellabs in Australia (<http://www.cellabs.com.au/>) (detects *E. histolytica* and *E. dispar*); R-Biopharm in Germany (<http://www.r-biopharm.com/Human/HumanFrame.html>) (*E. histolytica*/*E. dispar* only; Schunk *et al* 2001) and Remel in the USA (<http://www.remel.com/products/clinical/level2/MicrowellFormat.cfm>) (*E. histolytica* only; Ong *et al* 1996). A dipstick test for *E. histolytica*/*E. dispar*, *G. intestinalis* and *C. parvum* is also available commercially (<http://www.biosite.com/products/micro.asp>).

8.3 DNA blotting

The use of species-specific DNA probes to hybridise with unamplified DNA isolated from faecal samples has the great attraction of simplicity, particularly if non-radioactive labels are used. The method has been applied successfully (Samuelson *et al* 1989; Agarwal *et al* 1998) but not widely taken up, probably because of doubts about its sensitivity. The polymerase chain reaction (PCR) method, however, has been as widely tested for

amoebiasis as for most other diagnostic problems and forms the subject of much of the remainder of this review.

8.4 PCR based methods

The ability of the PCR to specifically amplify minute amounts of pathogen DNA has revolutionised the diagnosis of many infectious diseases, and the numerous sequence differences between homologous genes in *E. histolytica* and *E. dispar* make it a natural candidate for identifying these two species. A number of methods have been published (Tannich and Burchard 1991; Acuna-Soto *et al* 1993; Katzwinkel-Wladarsch *et al* 1994; Rivera *et al* 1996; Britten *et al* 1997; Troll *et al* 1997; Evangelopoulos *et al* 2000; Verweij *et al* 2000). Most, but not all, rely on amplifying unique regions of the SSUrRNA episome, its high copy number providing increased sensitivity. Although in the original procedures the product was often detected by gel electrophoresis followed by ethidium bromide staining, colorimetric detection using specific probes is now frequently employed. This provides the advantage of an easy-to read and familiar microtitre plate format. Light Cycler PCR has now been applied to the diagnosis of amoebiasis (E Tannich, personal communication; see also <http://www.artus-biotech2.de/>). PCR has also proved capable of detecting *E. histolytica* DNA in liver abscess contents (Tachibana *et al* 1992; Britten *et al* 1997; Zaman *et al* 2000).

In *in vitro* testing with cultured trophozoites PCR was about one hundred times more sensitive than antigen detection (Mirelman *et al* 1997); it also has the advantage that it can be developed to provide strain (as well as species) identification. However, it is important to be aware of the disadvantages of the method. Firstly, three separate steps are required – DNA extraction, amplification and product detection. While the necessary equipment is now widely available the process is not as quick or as simple as the use of an antigen detection kit. Secondly, as with all PCR-based methods, great care has to be taken to eliminate the risk of false positives due to contamination from product prepared earlier – although the Light Cycler technique greatly reduces this danger. Neither PCR nor antigen detection kits are fully sensitive if the faeces have been frozen or preserved and neither method is affordable for routine use in most disease-endemic countries. In practice, the theoretically higher sensitivity of PCR is balanced by the speed and convenience of a kit (Evangelopoulos *et al* 2001) and the method chosen will usually depend on local resources and preferences.

9. Specific detection of *E. histolytica* antigens in other samples

Some interesting results have been published suggesting that it may be possible to detect *E. histolytica* antigen in

the saliva (Abd-Alla *et al* 2000a) and circulating in the blood (Abd-Alla *et al* 1993) of infected patients. This is provided either of these specimens may be more acceptable to patients and these are interesting reports, although sensitivity might be a problem.

10. Conclusions

Nearly ten years after Diamond and Clark redefined *E. histolytica*, we now have a number of well-validated methods for distinguishing between it and *E. dispar*. While none are cheap and it is unlikely that they will be in routine use in disease-endemic areas in the near future, they do provide the tools to re-examine the important question raised by Julia Walsh (Walsh 1986) – just how common is *E. histolytica* in the world?

References

- Abd-Alla M D, Jackson T F H G, Gathiram V, el Hawey A M and Ravdin J I 1993 Differentiation of pathogenic *Entamoeba histolytica* infections from nonpathogenic infections by detection of galactose-inhibitable adherence protein antigen in sera and feces; *J. Clin. Microbiol.* **31** 2845–2850
- Abd-Alla M D, Jackson T G and Ravdin J I 1998 Serum IgM antibody response to the galactose-inhibitable adherence lectin of *Entamoeba histolytica*; *Am. J. Trop. Med. Hyg.* **59** 431–434
- Abd-Alla M D, Jackson T F, Reddy S and Ravdin J I 2000a Diagnosis of invasive amebiasis by enzyme-linked immunosorbent assay of saliva to detect amoebic lectin antigen and anti-lectin immunoglobulin G antibodies; *J. Clin. Microbiol.* **38** 2344–2347
- Abd-Alla M D, Wahib A A and Ravdin J I 2000b Comparison of antigen-capture ELISA to stool-culture methods for the detection of asymptomatic entamoeba species infection in Kafer Daoud, Egypt; *Am. J. Trop. Med. Hyg.* **62** 579–582
- Abd-Alla M D and Ravdin J I 2002 Diagnosis of amoebic colitis by antigen capture ELISA in patients presenting with acute diarrhoea in Cairo, Egypt; *Trop. Med. Int. Health* **7** 365–370
- Acuna-Soto R, Samuelson J, De Girolami P, Zarate L, Millan Velasco F, Schoolnick G and Wirth D 1993 Application of the polymerase chain reaction to the epidemiology of pathogenic and nonpathogenic *Entamoeba histolytica*; *Am. J. Trop. Med. Hyg.* **48** 58–70
- Agarwal S K, Guleria P, Gupta S, Goel V and Bhattacharya S 1998 Role of DNA probes in characterization of pathogenic and non-pathogenic *E. histolytica*; *J. Assoc. Phys. India* **46** 701–703
- Bhattacharya A, Arya R, Clark C G and Ackers J P 2000 Absence of lipophosphoglycan-like glycoconjugates in *Entamoeba dispar*; *Parasitology* **120** 31–35
- Blanc D and Sargeant P G 1991 *Entamoeba histolytica* zymodemes: exhibition of gamma and delta bands only of glucose phosphate isomerase and phosphoglucomutase may be influenced by starch content in the medium; *Exp. Parasitol.* **72** 87–90
- Britten D, Wilson S M, McNerney R, Moody A H, Chiodini P L and Ackers J P 1997 An improved colorimetric PCR-based method for detection and differentiation of *Entamoeba histolytica* and *Entamoeba dispar* in feces; *J. Clin. Microbiol.* **35** 1108–1111
- Bruchhaus I, Jacobs T, Leippe M and Tannich E 1996 *Entamoeba histolytica* and *Entamoeba dispar*. differences in numbers and expression of cysteine proteinase genes; *Mol. Microbiol.* **22** 255–263
- Brumpt E 1925 Etude sommaire de l' "*Entamoeba dispar*" n.sp. Amibe a kystes quadrinuclees, parasite de l' homme; *Bull. Acad. Med. (Paris)* **94** 943–952
- Clark C G 1995 Axenic cultivation of *Entamoeba dispar* Brumpt 1925, *Entamoeba insolita* Geiman and Wichterman 1937 and *Entamoeba ranarum* Grassi 1879; *J. Euk. Microbiol.* **42** 590–593
- Clark C G and Diamond L S 1991 The Laredo strain and other '*Entamoeba histolytica*-like' amoebae are *Entamoeba moshkovskii*; *Mol. Biochem. Parasitol.* **46** 11–18
- Clark C G, Espinosa-Cantellano M and Bhattacharya A 2000 *Entamoeba histolytica*: an overview of the biology of the organism; in *Amebiasis* (ed.) J I Ravdin (London: Imperial College Press) 1st edition, pp 1–45
- Diamond L S and Clark C G 1993 A redescription of *Entamoeba histolytica* Schaudinn 1903 (Emended Walker 1911) separating it from *Entamoeba dispar* Brumpt 1925; *J. Euk. Microbiol.* **40** 340–344
- Evangelopoulos A, Legakis N and Vakalis N 2001 Microscopy, PCR and ELISA applied to the epidemiology of amoebiasis in Greece; *Parasitol. Int.* **50** 185–189
- Evangelopoulos A, Spanakos G, Patsoula E, Vakalis N and Legakis N 2000 A nested, multiplex, PCR assay for the simultaneous detection and differentiation of *Entamoeba histolytica* and *Entamoeba dispar* in faeces; *Ann. Trop. Med. Parasitol.* **94** 233–240
- García L S and Bruckner D A 1997 *Diagnostic medical parasitology* 3rd edition (Washington DC: ASM Press)
- Gathiram V and Jackson T F H G 1987 A longitudinal study of asymptomatic carriers of pathogenic zymodemes of *Entamoeba histolytica*; *S. Afr. Med. J.* **72** 669–672
- Gilman R, Islam M, Paschi S, Goleburn J and Ahmad F 1980 Comparison of conventional and immunofluorescent techniques for the detection of *Entamoeba histolytica* in rectal biopsies; *Gastroenterology* **78** 435–439
- González-Ruiz A, Haque R, Aguirre A, Castanon G, Hall A, Guhl F, Ruiz-Palacios G, Miles M A and Warhurst D C 1994 Value of microscopy in the diagnosis of dysentery associated with invasive *Entamoeba histolytica*; *J. Clin. Pathol.* **47** 236–239
- Haque R, Ali I K, Akther S and Petri W A Jr 1998 Comparison of PCR, isoenzyme analysis, and antigen detection for diagnosis of *Entamoeba histolytica* infection; *J. Clin. Microbiol.* **36** 449–452
- Haque R, Ali I M and Petri W A Jr 1999 Prevalence and immune response to *Entamoeba histolytica* infection in preschool children in Bangladesh; *Am. J. Trop. Med. Hyg.* **60** 1031–1034
- Haque R, Ali I M, Sack R B, Farr B M, Ramakrishnan G and Petri W A Jr 2001 Amebiasis and Mucosal IgA Antibody against *Entamoeba histolytica* Adherence Lectin in Bangladeshi Children; *J. Infect. Dis.* **183** 1787–1793
- Haque R, Mollah N U, Ali I K, Alam K, Eubanks A, Lyerly D and Petri W A Jr 2000 Diagnosis of amoebic liver abscess and intestinal infection with the TechLab *Entamoeba histolytica* II antigen detection and antibody tests; *J. Clin. Microbiol.* **38** 3235–3239

- Hossain M M, Ljungstrom I, Glass R I, Lundin L, Stoll B J and Hultdt G 1983 Amoebiasis and giardiasis in Bangladesh: parasitological and serological studies; *Trans. R. Soc. Trop. Med. Hyg.* **77** 552–554
- Hughes M A and Petri W A Jr 2000 Amoebic liver abscess; *Infect. Dis. Clin. North. Am.* **14** 565–582, viii
- Jackson T F H G, Gathiram V and Simjee A E 1985 Seroepidemiological study of antibody responses to the zymodemes of *Entamoeba histolytica*; *Lancet* **1** 716–719
- Katzwinkel-Wladarsch S, Loscher T and Rinder H 1994 Direct amplification and differentiation of pathogenic and non-pathogenic *Entamoeba histolytica* DNA from stool specimens; *Am. J. Trop. Med. Hyg.* **51** 115–118
- Kean B H, Mott K E and Russell A J 1978 Löscher F A Massive development of amoebae in the large intestine (Massenhafte Entwicklung von Amöben im Dickdarm) (Translation); in *Tropical medicine and parasitology, classic investigations* (New York: Cornell University Press) vol. 1, pp. 71–79
- Lotter H, Jackson T F and Tannich E 1995 Evaluation of three serological tests for the detection of anti-amoebic antibodies applied to sera of patients from an area endemic for amoebiasis; *Trop. Med. Parasitol.* **46** 180–182
- Martínez-Palomo A, González-Robles A and de la Torre M 1973 Selective agglutination of pathogenic strains of *Entamoeba histolytica* induced by Con A; *Nat. New Biol.* **245** 186–187
- Mirelman D, Nuchamowitz Y and Stolarsky T 1997 Comparison of use of enzyme-linked immunosorbent assay-based kits and PCR amplification of rRNA genes for simultaneous detection of *Entamoeba histolytica* and *E. dispar*; *J. Clin. Microbiol.* **35** 2405–2407
- Ong S J, Cheng M Y, Liu K H and Horng C B 1996 Use of the ProSpecT microplate enzyme immunoassay for the detection of pathogenic and non-pathogenic *Entamoeba histolytica* in faecal specimens; *Trans. R. Soc. Trop. Med. Hyg.* **90** 248–249
- Petri W A Jr, Haque R, Lyerly D and Vines R R 2000 Estimating the impact of amoebiasis on health; *Parasitol. Today* **16** 320–321
- Petri W A Jr, Jackson T F H G, Gathiram V, Kress K, Saffer L D, Snodgrass T L, Chapman M D, Keren Z and Mirelman D 1990a Pathogenic and nonpathogenic strains of *Entamoeba histolytica* can be differentiated by monoclonal antibodies to the galactose-specific adherence lectin; *Infect. Immun.* **58** 1802–1806
- Petri W A Jr and Singh U 1999 Diagnosis and management of amoebiasis; *Clin. Infect. Dis.* **29** 1117–1125
- Petri W A Jr, Snodgrass T L, Jackson T F H G, Gathiram V, Simjee A E, Chadee K and Chapman M D 1990b Monoclonal antibodies directed against the galactose-binding lectin of *Entamoeba histolytica* enhance adherence; *J. Immunol.* **144** 4803–4809
- Pillai D R, Keystone J S, Sheppard D C, MacLean J D, MacPherson D W and Kain K C 1999 *Entamoeba histolytica* and *Entamoeba dispar*: epidemiology and comparison of diagnostic methods in a setting of nonendemicity; *Clin. Infect. Dis.* **29** 1315–1318
- Ravdin J I and Petri W A Jr 1994 *Entamoeba histolytica* (Amoebiasis); in *Principles and practice of infectious diseases*, 4th edition (eds) G L Mandell, J E Bennett and R Dolin (New York: Churchill Livingstone) pp 2395–2408
- Rivera W L, Tachibana H, Silva Tahat M R, Uemura H and Kanbara H 1996 Differentiation of *Entamoeba histolytica* and *E. dispar* DNA from cysts present in stool specimens by polymerase chain reaction: its field application in the Philippines; *Parasitol. Res.* **82** 585–589
- Samuelson J, Acuna-Soto R, Reed S, Mirelman D, Schoolnik G, Zarate L and Wirth D 1989 DNA probe for diagnosis of *Entamoeba histolytica*; *Abstracts, XI Seminario Sobre Amibi-asis*, Mexico p. 40
- Sargeant P G 1988 Zymodemes of *Entamoeba histolytica*; in *Amoebiasis: Human infection by Entamoeba histolytica* (ed.) J I Ravdin (New York: John Wiley) 1st edition, pp 370–387
- Schunk M, Jelinek T, Wetzel K and Nothdurft H D 2001 Detection of *Giardia lamblia* and *Entamoeba histolytica* in stool samples by two enzyme immunoassays; *Eur. J. Clin. Microbiol. Infect. Dis.* **20** 389–391
- Sehgal R, Abd-Alla M, Moody A H, Chiodini P L and Ackers J P 1995 Comparison of two media for the isolation and short-term culture of *Entamoeba histolytica* and *E. dispar*; *Trans. R. Soc. Trop. Med. Hyg.* **89** 394
- Shetty N, Das P, Pal S C and Prabhu T 1988 Observations on the interpretation of amoebic serology in endemic areas; *J. Trop. Med. Hyg.* **91** 222–227
- Stanley S L Jr, Jackson T F, Foster L and Singh S 1998 Longitudinal study of the antibody response to recombinant *Entamoeba histolytica* antigens in patients with amoebic liver abscess; *Am. J. Trop. Med. Hyg.* **58** 414–416
- Strachan W D, Chiodini P L, Spice W M, Moody A H and Ackers J P 1988 Immunological differentiation of pathogenic and non-pathogenic isolates of *Entamoeba histolytica*; *Lancet* **1** 561–563
- Tachibana H, Kobayashi S, Okuzawa E and Masuda G 1992 Detection of pathogenic *Entamoeba histolytica* DNA in liver abscess fluid by polymerase chain reaction; *Int. J. Parasitol.* **22** 1193–1196
- Tannich E and Burchard G D 1991 Differentiation of pathogenic from non-pathogenic *Entamoeba histolytica* by restriction fragment analysis of a single gene amplified *in vitro*; *J. Clin. Microbiol.* **29** 250–255
- Troll H, Marti H and Weiss N 1997 Simple differential detection of *Entamoeba histolytica* and *Entamoeba dispar* in fresh stool specimens by sodium acetate-acetic acid-formalin concentration and PCR; *J. Clin. Microbiol.* **35** 1701–1705
- Verweij J J, Blotkamp J, Brienen E A, Aguirre A and Polderman A M 2000 Differentiation of *Entamoeba histolytica* and *Entamoeba dispar* cysts using polymerase chain reaction on DNA isolated from faeces with spin columns; *Eur. J. Clin. Microbiol. Infect. Dis.* **19** 358–361
- Walker E L and Sellards W S 1913 Experimental amoebic dysentery; *Phillipine J. Sci. B. Trop. Med.* **8** 253–331
- Walsh J A 1986 Problems in recognition and diagnosis of amoebiasis estimation of the global magnitude of morbidity and mortality; *Rev. Infect. Dis.* **8** 228–238
- WHO 1969 Amoebiasis; *WHO Tech. Rep. Ser. No.* 421
- WHO 1997 *Entamoeba* taxonomy; *Bull. WHO* **75** 291–292
- Willhoeft U, Buss H and Tannich E 1999a DNA sequences corresponding to the ariel gene family of *Entamoeba histolytica* are not present in *E. dispar*; *Parasitol. Res.* **85** 787–789
- Willhoeft U, Hamann L and Tannich E 1999b A DNA sequence corresponding to the gene encoding cysteine proteinase 5 in *Entamoeba histolytica* is present and positionally conserved but highly degenerated in *Entamoeba dispar*; *Infect. Immun.* **67** 5925–5929
- Zaman S, Khoo J, Ng S W, Ahmed R, Khan M A, Hussain R and Zaman V 2000 Direct amplification of *Entamoeba histolytica* DNA from amoebic liver abscess pus using polymerase chain reaction; *Parasitol. Res.* **86** 724–728