Tropical Biomedicine 28(2): 194–222 (2011)

Review Paper

Public health and clinical importance of amoebiasis in Malaysia: A review

Tengku, S.A. and Norhayati, M*.
Department of Parasitology and Medical Entomology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300, Kuala Lumpur, Malaysia
* Corresponding author email: hayati@medic.ukm.my
Received 6 Jan 2011; received in revised form 14 Marxh 2011; accepted 20 March 2011

Abstract. *Entamoeba histolytica*, the causative agent of human amoebiasis remains a significant cause of morbidity and mortality in developing countries and is responsible for up to 100,000 deaths worldwide each year. *Entamoeba dispar*, morphologically indistinguishable from *E. histolytica* is more common in humans in many parts of the world. Similarly *Entamoeba moshkovskii*, which was long considered to be a free-living amoeba is also morphologically identical to *E. histolytica* and *E. dispar*, and is highly prevalent in some *E. histolytica* endemic countries. Humans are the host of infection and there would not appear to be other meaningful animal reservoirs of *E. histolytica*. *Entamoeba. histolytica* can be present in sewage and contaminated water. The infection is mainly transmitted via ingestion of water or food contaminated by faeces containing *E. histolytica* cysts. Clinical features of amoebiasis range from asymptomatic colonization to amoebic dysentery and invasive extraintestinal amoebiasis, which is manifested most commonly in the form of abscesses in liver and lungs. The epidemiology of amoebiasis has dramatically changed since the separation of *E. histolytica* and *E. dispar* species and the worldwide prevalence of these species has not been estimated until recently. Morever, *E. moshkovskii*, another morphologically indistinguishable human parasitic *Entamoeba* was not mentioned or considered as a contributor to the prevalence figures in endemic areas. Amoebiasis is still a major health problem especially in aboriginal settlements and amongst people living in remote area in Malaysia. However, until now there is only one data currently available to indicate the true prevalence and incidence of *E. histolytica* and *E. dispar*. Further studies are needed to determine the burden of *E. histolytica*, *E. dispar* and *E. moshkovskii* infections in Malaysia. In the present review, we briefly summarize all methods use in diagnosing *Entamoeba* species, ranging from microscopic identification to molecular detection such as culture and isoenzyme analysis, antibody detection tests, antigen detection tests, immunochromatographic assays, conventional PCR, real-time PCR and loop-mediated isothermal amplification (LAMP).

INTRODUCTION

The genus *Entamoeba* contains many species, six of which *Entamoeba histolytica*, *Entamoeba dispar*, *Entamoeba moshkovskii*, *Entamoeba polecki*, *Entamoeba coli*, and *Entamoeba hartmanni* reside in the human intestinal lumen. *Entamoeba histolytica* is the only species definitely associated with pathological sequelae in humans; the others are considered non-pathogenic and rarely cause intestinal disease in humans (Clark & Diamond, 1991; Garcia & Bruckner, 1997). During the 1990s, enough evidence had accumulated to support the formal separation of two morphologically identical species of amoeba: the non-pathogenic *E. dispar* from the potentially pathogenic *E. histolytica* (Abd-Alla *et al.*, 1993; WHO, 1997; PAHO, 1997).

Entamoeba histolytica infections are commonly observed in tropical and subtropical regions of the world including Malaysia. In developed countries *E. histolytica* infections are commonly seen in travelers, recent immigrants, homosexual men and inmates of institutions. Humans are the primary host and the main source of infection is the cyst passing chronic patient or asymptomatic carrier. The infection is mainly transmitted via ingestion of water or food contaminated by faeces containing *E. histolytica* cysts (Leber & Novak, 1999; Stanley, 2003). Within the last few decades, many studies have reported the occurrence of this infection in homosexual men usually as the result of oral-anal and oral-genital sexual contact (Yi Chen *et al.*, 2007; Stark *et al.*, 2008). The infection of *E. histolytica* is known as amoebiasis and is globally considered a leading parasitic cause of human mortality (WHO, 1997; Haque *et al.*, 2003; Haque & Petri, 2006). It infects hundreds of millions of people per year. Analysis based on published data by Walsh (1986) indicated that 10% of the world's population was infected by *E. histolytica* and only 1% of the infected individuals developed invasive form of the disease, whilst 9% were asymptomatic.

Humans are the host of infection and there would not appear to be other meaningful animal reservoirs of *E. histolytica*. *Entamoeba histolytica* can be present in sewage and contaminated water. Cysts may remain viable in suitable aquatic environments for several months at low temperature. The potential for waterborne transmission is greater in the tropics, where the carrier rate sometimes exceed 50%, compared with more temperate regions, where the prevalence in the general population may be less than 10%. Person-to-person contact and contamination of food by infected food handlers appear to be the most significant means of transmission, although contaminated water also plays a substantial role. Ingestion of faecally contaminated water and consumption of food crops irrigated with contaminated water can both lead to transmission of amoebiasis. The transmission of *E. histolytica* by contaminated drinking water has been confirmed (Marshal *et al.*, 1997). The cysts are relatively resistant to disinfection and may not be inactivated by chlorination practices generally applied in the production of drinking water. Within a Water Safety Plan (WHO, 2004), control measures that can be applied to manage potential risk from *E. histolytica* include prevention of source water contamination by human waste, followed by adequate treatment and protection of water during distribution. Owing to the resistance of the cysts to disinfectants, *E. coli* (or, alternatively thermotolerant coliforms) cannot be relied upon as an index of the presence/absence of *E. histolytica* in drinking water supplies.

Morbidity and mortality data which existed prior to this time pertaining to cases of invasive disease were not greatly affected because all invasive disease was known to be caused by *E. histolytica*. However, because most prevalence data previously collected were associated with asymptomatic individuals, and a majority of asymptomatic individuals with cysts detected in their stool were actually infected with non-pathogenic *E. dispar*, the definite prevalence of *E. histolytica* has became a matter of speculation. On the other hand, the role of *E. moshkovskii*, a free living amoeba that is indistinguishable in its cyst and trophozoite forms from *E. histolytica* and *E. dispar*, plays in human infections is yet to be adequately defined. *Entamoeba moshkovskii* was first isolated from sewage effluents and recognized as an ubiquitous free-living organism in 1941 (Tshalaia, 1941). A recent study reported a high prevalence and association of *E. moshkovskii* with *E. histolytica* and *E. dispar* infections in young children in Bangladesh (Ali *et al.*, 2003). To date, human isolate of this species has been reported from many studies all over the world (Clark *et al.*, 1991; Haque *et al.*, 1998; Parija & Khairnar, 2005; Fotedar *et al.*, 2007; Tanyuksel *et al.*, 2007; Beck *et al.*, 2008; Ben Ayed *et al.*, 2008; Nazemalhosseini Mojarad *et al.*, 2010; Hamzah *et al.*, 2010).

Some of the original epidemiologic descriptions distinguishing *E. histolytica* from *E. dispar* infections originated from study conducted among asymptomatic semi

rural population in South Africa. Culture and zymodeme analysis were used for the identification of *Entamoeba* sp. and the findings reported an overall prevalence of *E. histolytica-E. dispar* complex of approximately 10%. On further analysis it was revealed that 90% of the asymptomatic individuals were infected with the non-pathogenic *E. dispar* and 10% asymptomatically harboured *E. histolytica* (Gathiram & Jackson, 1985). It was subsequently noted that approximately 10% of asymptomatic carriers of *E. histolytica* would develop invasive disease while a majority of others spontaneously cleared their infection by 1 year (Blessmann *et al.*, 2002a; Haque *et al.*, 2002).

In developing countries, waterborne gastrointestinal parasite pathogens such as *Cryptosporidium parvum*, *Giardia lamblia*, and *E. histolytica* are frequently associated with morbidity, particularly in children. In developed nations, outbreaks of *E. histolytica* infections have been caused by sewage contaminated water supplies (Barwick *et al.*, 1999). Many studies of *Entamoeba* species have used specimens from stool and and liver abscess samples. Investigations of the occurrence of *Entamoeba* species in surface and waste water in Thailand was carried out by Sirilak Sukprasert *et al.* (2008). In this study, DNA of 137 surface and waste water samples collected from Pathum Thani Province, Thailand were examined for *Entamoeba* spp. using PCR and genus-specific primers that amplify DNA of *E. polecki*, *Entamoeba chattoni*, *E. dispar*, *E. histolytica*, *E. hartmanni*, *E. coli*, and *E. moshkovskii*. The results showed that 27% of the samples were positive for *Entamoeba* species. When the positive samples were further examined by a single-round PCR assay specific for *E. histolytica*, *E. dispar* and *E. moshkovskii*, all were negative. In contrast of their study, two out of six water samples (32%) collected from Ankara river in Turkey were positive for *E. histolytica* by PCR (Bakir *et al.*, 2003). These results extend our knowledge on *E. histolytica* as waterborne parasite and such occurrence information on waterborne pathogens assists the management and treatment of municipal water.

Following this, many studies all over the world reported the true prevalence of *E. histolytica*, *E. dispar* and *E. moshkovskii* as summarized in Table 1. PCR, ELISA - antigen detection and isoenzymes analysis were the common techniques used for the specification of all three species of *Entamoeba*. Studies had shown that all the three techniques for species identification of *E. histolytica* in fresh stool showed excellent correlation with TechLab *E. histolytica* antigen detection test; TechLab *E. histolytica* antigen detection test was also found to be both rapid and technically simple (Haque *et al.*, 1998). In exception studies by Al-Hindi *et al.* (2005) in Gaza and Noor Azian *et al.* (2006) in Malaysia, all of the studies reported high prevalence of *E. dispar* as compared to *E. histolytica*. For example, the earliest community study in South Africa by Gathiram & Jackson (1985) reported a high prevalence of *E. dispar* as compared to *E. histolytica*. Subsequent community and hospital studies carried out a decade later confirmed the findings (Samie *et al.*, 2006; Fotedar *et al.*, 2007; Saeed *et al.*, 2007; Beck *et al.*, 2008; Nazemalhosseini Mojarad *et al.*, 2010).

A study to determine the prevalence and species distribution of *E. histolytica* and *E. dispar* was carried out in South Africa by examining stool samples collected from public hospitals and primary schools using ELISA and nested polymerase chain reaction (PCR). The results showed that *E. histolytica* was detected in 18.8% and 2.1% samples of patients from public hospitals and primary school children respectively, whereas 25.3% and 8.5% had *E. dispar* respectively (Samie *et al.*, 2006). A similar clinical setting study was also carried out in Sweden using established PCR and the analysis showed that 79.7% were positive for *E. dispar* and only 4.8% were positive *E. histolytica* (Lebbad *et al.*, 2005). A latest study in Iran reported that out of 3,825 stool samples examined using single-round PCR assay 3.5%, 91.4% and 3.5% were reported positive for *E. histolytica*, *E. dispar* and *E. moshkovskii* respectively (Nazemalhosseini Mojarad *et al.*, 2010).

Table 1. Prevalence of intestinal *E. histolytica*, *E. dispar* and *E. moshkovskii* infections

Method of diagnosis	Population studied	Prevalence (%) *Eh*	*Ed*	*Em*	Author
Isoenzyme analysis	Ecuador; community study; school children	*Ed* is 3.7 times higher than *Eh*			Gatti *et al.*, 2002
	UK; male homosexual	0.0	20.0	ND	Allason-Jones *et al.*, 1986
	South Africa; community study; all ages	1.0	9.0	ND	Gathiram & Jackson, 1985
ELISA: Antibody detection	Malaysia; community study; all ages Male Female	33.4 24.6 8.8			Zurainee Mohamed Nor, 2003
	Mexico; community study; all ages	8.4	ND	ND	Caballero-Salcedo *et al.*, 1994
	Malaysia; community study; adults Orang Asli Malay Indian Chinese	 9.7 7.2 5.4 3.6			Thomas & Yap, 1986
	Malaysia; community study – Orang Asli; children adult	 79.0 87.0			Gilman *et al.*, 1976
ELISA: Antigen detection	Nigeria; hospital study; all ages	ND	0.67	ND	Fadeyi *et al.*, 2009
	South Africa; HIV patients	4.0	ND	ND	Beck *et al.*, 2008
	Saudi Arabia; hospital study; all ages	2.7	ND	ND	Barnawi *et al.*, 2007
	Saudi Arabia; hospital study; all ages	4.3	95.7	ND	Saeed *et al.*, 2007
	South Africa; hospital study (all ages) school children	18.8 2.1	25.3 8.5	ND ND	Samie *et al.*, 2006
	Bangladesh; hospital study; children community study; urban slum; children community study; rural village; children	 4.2 4.3 1.0	 ND ND ND	 ND ND ND	Haque *et al.*, 2006
PCR	Iran; hospital study; all ages	3.45 1.7 *Ed* + *Em*	91.4	3.45	Nazemalhosseini Mojarad *et al.*, 2010
	South Africa; HIV patients	ND	5.0	13.0	Beck *et al.*, 2008
	Australia; hospital study; all ages	3.4 36.0 *Ed* + *Em* 1.1 *Eh* + *Ed* 1.1 *Eh* + *Em*	33.7	24.7	Fotedar *et al.*, 2007
	India; hospital study; all ages	3.5	9.3	1.9	Khairnar *et al.*, 2007
	Thailand; hospital study; all ages	*Eh*:*Ed*=1:6		ND	Hamzah *et al.*, 2006
	Malaysia; community study – Orang Asli; all ages	13.2	5.6	ND	Noor Azian *et al.*, 2006
	Brazil; community study; all ages	ND	74.2	ND	Pinheiro *et al.*, 2004
	Gaza Strip, Palestine; Hospital study; children	69.6 7.6 *Eh* + *Ed*	22.8	ND	Al-Hindi *et al.*, 2005
	Sweeden; hospital study; all ages	4.8	79.7	ND	Lebbad & Svard, 2005
	India; hospital study; all ages	1.7	8.8	2.2	Parija & Khairnar, 2005
	Vietnam; community study; adults	11.2	ND	ND	Blessmann *et al.*, 2002
	Ghana; community study; all ages		82.8	ND	Jaco *et al.*, 2003
	Philippines; community study; all ages	1.0	7.1	ND	Rivera *et al.*, 1998

Outbreaks of amoebiasis have been reported in several countries. Lalla *et al.* (1992) reported an outbreak of *E. histolytica* and *G. lamblia* infections in travellers returning from the tropics. Of 160 travellers from various regions in Italy who had taken part in a five-day organized trip to Phuket, Thailand, and been accommodated in the same luxury hotel, 17 showed either amoebic abscess or colitis. A pretested questionnaire that focused on the consumption of foods and beverages well known to be a source of intestinal infection in endemic areas was available from these 17 patients as well as from 41 out of 74 asymptomatic travellers. Stool samples for parasitological examination were also available. In patients affected with amoebic abscess, antibodies to *E. histolytica* were also determined. Overall, parasitological examinations were negative in eight (13.8%) patients, and 50 out of 58 (86.2%) were found to be positive. The prevalence of *E. histolytica* was 72.4% and 28 subjects (48.3%) were stool positive for both protozoa. No other intestinal parasites were found. The consumption of drinks with ice, ice cream and raw fruit in ice was significantly associated with *E. histolytica* infections. Vreden *et al.* (2000) reported an outbreak of amoebiasis in a family in The Netherlands. The index case was a 5 year old girl who presented with a 6 week history of abdominal pain and bloody diarrheal stools without fever. Stool cultures for bacterial pathogens remained negative. Because the girl had never been abroad, the possibility of amoebiasis was not considered by the physician until her mother gave a history of amoebic dysentery and persistent cyst passage after return from India 13 years back. In addition to the index case, the household contacts of the mother were her husband, a 4 year old son, a 2 year old son, and a nanny. None of the household contacts had traveled outside Western Europe or had developed signs or symptoms that were consistent with dysentery or extraintestinal invasive disease. However, the oldest son had an episode of abdominal cramps and nonbloody diarrhoea a few weeks after his sister had developed amoebic dysentery. Trophozoites and/or cysts of *E. histolytica/E. dispar* were found in stool specimens from the index case, her mother, and other household contacts. This outbreak of amoebiasis demonstrates that even with Western standards of hygiene, persistent cyst passage may result in transmission of *E. histolytica* to household contacts many years later. In Japan, Niichiro *et al.* (1999) reported an amoebiasis outbreak among the residents of two institutions for the mentally retarded in Osaka City. In February 1996, a liver abscess due to *E. histolytica* was found in a resident of institution A. In June, several residents in institution B complained of diarrhoea. Their stool contained *E. histolytica*. The stool specimens from the residents, their parents and the staff of the two institutions were examined for *Entamoeba*. Thirteen among 79 residents in institutions A (16.5%) and 29 among 69 residents in institution B (42.0%) carried *E. histolytica* cysts. The relationship of the outbreaks in the two institutions remains obscure but they could be related since four of the residents in institution A had once been residents of institution B and since one short stay individual in institution B acquired the parasite.

Pathogenesis

Entamoeba histolytica was first described by Lambl in 1859 and the pathogenesis of this infection was first described in St Petersburg by Fedor Losch in 1875 in the stool of a Russian suffering from dysentery. Infection of *E. histolytica* develops following ingestion of the quadrinucleate cysts of *E. histolytica*; encystation of the cysts in the intestinal lumen produces trophozoites and thereby colonizes the large intestine. The trophozoite may remain confined to the intestinal lumen feeding on bacteria and cellular debris. Galactose and N-acetyl-D-galactosamine (Gal/GalNAc)-specific lectin are used by the trophozoites to adhere to colonic mucins and colonize the large intestine (Petri *et al.*, 2002); aggregation of the trophozoites in the mucin layer will trigger encystations; cysts are then excreted in stool.

Colitis results when the trophozoites penetrate the intestinal epithelium. The ability of trophozoite to invade intestinal mucosa depends on their genetic,

immunoenzymatic profile, their ability to produce proteolytic enzymes and enabling resistance to complement mediated lyses. Trophozoites invasion are initiated by killing of epithelial cells and inflammatory cells (neutrophils and lymphocytes); interaction of the amoeba with intestinal epithelium causes an inflammatory response marked by the activation of nuclear factor and secretion of lymphokines (Eckmann *et al.*, 1995; Seydel *et al.*, 1998). The development of epithelial response may depend on trophozoite virulence factors and this will lead to intestinal abnormalities through neutrophil-mediated damage. In early invasive lesion with superficial ulceration, three main consecutive events occur and there are focal superficial epithelium erosion followed by small glandular foci of microinvasion and mild to moderate neutrophil infiltration of lamina propria.

The mechanism involves in superficial epithelium erosion is complex; once protective mucus barrier has been broken down, trophozoites of *E. histolytica* will adhere and make a contact-dependent damage and lyses of epithelium cells. Lectins, amebapores and proteases are the three molecules involve in this event (Martinez-Palomo *et al.*, 1985). The second event is characterized by continuing lyses of cells with the aid of proteolytic enzymes (i.e. cysteine proteinase, phospholipase and haemolysin) that degrade elastin, collagen and fibrinogen. Tissues penetration of trophozoites is also assisted through its locomotion activities and proteolytic degradation of extracellular matrix components of the colonic mucosa (Espinosa-Cantellano & Martinez-Paloma, 2000). In the final event, infiltration of neutrophil and other inflammatory cells around trophozoites leads to rapid lyses of inflammatory cells and tissue necrosis (Espinosa-Cantellano & Martinez-Paloma, 2000). Peptide-mediated lysosome enzymes released by the lysed inflammatory cells contribute to the destruction of host tissue and extend of the lesion. Neutrophil representing over 90% of the circulating granulocytes, respond to a variety of cytokines and soluble factors during the inflammatory process. The first sign of colonic aggression visualized by sigmoidoscopy is nonspecific thickening of the mucosa or pin head-size micronodules.

The late invasive lesion is characterized by extension of mucosal ulcer deep into a larger area of the submucosa. Once the interglandular epithelium has been invaded by trophozoite, the underlying tissue offers little resistance, allowing extension of the ulcer laterally, creating the classical flask-shaped amoebic ulcer (Stanley, 2003). Histopathology shows necrotic areas and vascular congestion with minimal inflammation in contrast with the extension of the lesion. Trophozoites may be found in the surface layer of the ulcers; bacterial infection causing deficiency of blood supply leads to necrosis, haemorrhage and gangrene and subsequent perforation of the intestinal wall (Haque *et al.*, 2003). Bowel complications occur in 1-4% of patients and invasive extraintestinal complication is very uncommon and may be present in 0.1 to 1% of symptomatic patients.

Clinical features

Clinical features of amoebiasis range from asymptomatic colonization to amoebic dysentery and invasive extraintestinal amoebiasis, which is manifested most commonly in the form of abscesses in liver and lungs. It has been recognized that disease expression of amoebiasis varies geographically. For example, invasive disease in Egypt is predominantly amoebic colitis (Abd-Alla *et al.*, 2002) whereas in South Africa there is an excessive rate of amoebic liver abscess (ALA). In fact, in Hue City, Vietnam, an overall estimated frequency of ALA was recently reported to be as high as 21 cases per 100,000 inhabitants (Blessmann *et al.*, 2002b). Although 90% of *E. histolytica* infections remain asymptomatic, approximately 50 million people have invasive disease, resulting in 100,000 deaths per year (WHO, 1997), placing amoebiasis as the second leading cause of death from parasitic diseases worldwide (Stanley, 2003).

Individuals harbouring *E. histolytica* (asymptomatic carriers) can develop antibody titers in the absence of invasive

disease (Jackson *et al.*, 1985; Gathiram & Jackson, 1987; Ravdin *et al.*, 1990). Asymptomatic colonization with *E. histolytica*, if left untreated can lead to amoebic dysentery and a wide range of other invasive diseases, but more often the infection resolves spontaneously without the development of diseases (Gathiram & Jackson, 1987; Haque *et al.*, 2001; Blessmann *et al.*, 2002b). Cohort studies reported that when asymptomatic individuals were followed up for 1 year, 4 to 10% of them developed colitis or extraintestinal diseases (Gathiram & Jackson, 1987; Haque *et al.*, 2001).

Natural history of acute amoebic colitis has a gradual onset, with a 1-2 weeks history of mild-to-moderate abdominal pain and tenderness, tenesmus and watery diarrhoea with five to seven episodes per day with scare amounts of faeces, abundant mucus with or without blood. About 80% of patients complain of localized abdominal pain; some patients may have only intermittent diarrhoea alternating with constipation. Fever is unusual, occurring in <40% of patients (Adams & MacLeod, 1977). Other associated symptoms are weight loss and anorexia. Microscopically, trophozoites can be detected in submucosal tissue or faecal samples using normal saline temporary staining and the finding can be confirmed by permanent staining technique i.e. trichrome staining. Since *E. histolytica* invades the colonic musoca, faeces are almost universally positive for occult blood.

This syndrome resolves within a few days following appropriate anti-amoebic treatment. Severe cases of amoebic colitis are characterized by dysenteric stools, diffuse abdominal pain, high fever and severe dehydration; patient usually appears very ill. Differential diagnoses include infection with bacteria such as *Shigella*, *Salmonella*, *Campylobacte*r and enterohaemorrhagic *Escherichia coli* and noninfectious causes which include inflammatory bowel disease, ischaemic colitis and diverticulitis. Other spectrums of acute intestinal amoebiasis include extensive fulminant necrotizing colitis, toxic megacolon and perianal ulceration. Patient with fulminant amoebic colitis usually presents with profuse bloody diarrhoea, fever, pronounced leukocytosis, and widespread abdominal pain, often with peritoneal signs and extensive involvement of the colon (Takahashi *et al.*, 1997). Although fulminant necrotizing colitis and toxic megacolon are very rare, they are usually associated with a high mortality rate.

Another clinical spectrum of intestinal amoebiasis is chronic intestinal amoebiasis and patient with this condition present with intermittent abdominal pain, diarrhoea and weight loss. Patients who are at increased risk of severe disease include those who are very young, very old, malnourished, pregnant and those who are receiving corticosteroid. Recent studies in non endemic areas of amoebiasis i.e. Japan, Taiwan, Republic of Korea and Australia have reported *E. histolytica* as an emerging pathogen in men who have sex with men (MSM) (Ohnishi *et al.*, 2004; Hung *et al.*, 2005; Tsai *et al.*, 2006; Park *et al.*, 2007; Stark *et al.*, 2008). MSM are also reported to be in the higher risk to develop invasive amoebiasis (Hung *et al.*, 2005).

Acute complications of intestinal disease include bleeding, perforation, peritonitis, perianal skin ulceration and rectovaginal fistulas. Deep ulcer may heal with stricture and adhesion and these will lead to intestinal obstruction. Ameboma results from the formation of annular colonic granulation tissues in the caecum and ascending colon may mimic carcinoma of colon is a rare late complication of intestinal amoebiasis (Adams & MacLeod, 1977). On rare occasions, *E. histolytica* trophozoites enter the bloodstream and disseminate to other body sites. Amoebic liver abscess (ALA) is the most frequent manifestation of extraintestinal amoebiasis (Bruckner, 1992). Abscess complicates amoebiasis in 3 to 9% of patients (Frey *et al.*, 1989). The clinical presentation is highly variable, ranging from weight loss, weakness, and low-grade fever on an acute to febrile illness. Pain may include vague right-upper-quadrant discomfort, point tenderness between ribs on palpation, or pleuritic discomfort, spreading to the right shoulder. Patients, who present acutely with symptoms of less than two weeks of duration,

have more prominent abdominal pain with fevers and rigors (Katzenstein *et al.*, 1982).

Anemia, leukocytosis and elevated alkaline phosphatase are often noted, but jaundice, striking transaminase elevation, and eosinophilia are unusual (Guerrant, 1986). Older patients tend to present with chronic illness, lasting longer than two weeks. They have less fever (only 30%), and may have a wasting disease with significant weight loss (Katzenstein *et al.*, 1982). The number of patients presenting with acute ALA seems to be increasing, possibly reflecting earlier diagnosis and better access to medical care. ALA is characterized by a significant male preponderance and it is a disease seen most commonly in patients who reside in or have emigrated from an endemic area (Hughes & Petri, 2000). Cutaneous amoebiasis is a rare extraintestinal manifestation of *E. histolytica* infection (Mhlanga *et al.*, 1992). In children it always occurs in the anogenital or perineal region as a result of direct inoculation of trophozoites from prolonged contact with infected stools in a child's diaper. Direct inoculation of skin has also been reported from scratching, anal or vaginal intercourse and following surgical drainage or spontaneous rupture of an abscess at a colostomy site or laporatomy incision (Magana-Garcia & Arista-Viveros, 1993a).

Diagnosis

Microscopic techniques employed in a diagnostic clinical laboratory include wet preparation, concentration, and permanently stained smears for the identification of *E. histolytica/E. dispar/E. moshkovskii* in faeces. Microscopic examination of a direct saline (wet) mount is a very insensitive method (<10%) which is performed on a fresh specimen (Huston *et al.*, 1999). The sample should be examined within 1 hour of collection to search for motile trophozoites which may contain RBCs. However, in patients who do not present with acute dysentery, trophozoites will not contain RBCs. Patients with asymptomatic carriage generally have only cysts in the faecal sample. Although the concentration technique is helpful in demonstrating cysts, the use of permanently stained smears (trichrome or iron hematoxylin) is an important method for recovery and identification of *Entamoeba* species. Microscopy is a less reliable method of identifying *Entamoeba* species than either culture or antigen detection tests (Krogstad *et al.*, 1978; Haque *et al.*, 1995). The sensitivity of microscopy can be poor (60%) and confounded with false-positive due to misidentification of macrophages as trophozoites, PMNs as cysts (especially when lobed nuclei of PMNs break apart), and other *Entamoeba* species (Gonzalez-Ruiz *et al.*, 1994; Haque *et al.*, 1997; Haque *et al.*, 1998; Tanyuksel & Petri, 2003). As *Entamoeba* trophozoites generally degenerate rapidly in unfixed faecal specimens (Proctor, 1991) and refrigeration is not recommended, specimens should be preserved with a fixative which prevents the degradation of the morphology of the parasite and allows concentration and permanent smears to be performed. Fixatives used for the concentration procedure include Schaudinn's fluid, merthiolate iodine-formalin, sodium acetate-acetic acid formalin (SAF), or 5% or 10% formalin. The fixatives for the permanently stained smears include trichrome, iron hematoxylin, Ziehl-Neelsen stains, modified polyvinyl alcohol (PVA) and SAF. Examination for ova and parasites in a minimum of three stool samples over no more than 10 days is recommended, as these organisms may be excreted intermittently or may be unevenly distributed in the stool. This improved the detection rate to 85 to 95% (Li & Stanley, 1996).

Culture techniques for the isolation of *Entamoeba* species have been available for over 80 years. Culture media include xenic (diphasic and monophasic) and axenic systems. Xenic cultivation is defined as the growth of the parasite in the presence of an undefined flora (Clark & Diamond, 2002). The xenic culture of *E. histolytica* was first introduced by Boeck and Drbohlave in 1925 in a diphasic egg slant medium, and a modification of this medium (Locke-egg) is still used today. Different monophasic media that were developed for *E. histolytica* are the egg yolk infusion medium of Balamuth

(Balamuth, 1946), Jones's medium (Jones, 1946), and TYSGM-9 (Diamond, 1982). Of the different media developed for the xenic cultivation of *E. histolytica*, only three media, diphasic Locke-egg, Robinson's medium (Robinson, 1968), and the monophasic TYSGM-9 (Diamond, 1982) are in common use. Axenic cultivation involves the cultivation of parasites in the absence of any other metabolizing cells (Clark & Diamond, 2002). The axenic cultivation of *E. histolytica* was first achieved by Diamond (1961). The monophasic medium TP-S-1 was developed and used widely for culture of *E. histolytica* in different research laboratories (Diamond, 1968; Clark & Diamond, 2002). Currently, TYI-S-33 (Diamond, Harlow & Cunnick, 1978) and YI-S (Diamond, Clark & Cunnick, 1995) are the most widely used media for axenic cultivation of *E. histolytica* (Clark & Diamond, 2002).

Culture of *E. histolytica* can be performed from faecal specimens, rectal biopsy specimens or liver abscess aspirates. As the liver abscess aspirates of ALA patients are usually sterile (98% cases) (Blessmann *et al.*, 2002b), addition of a bacterium or a trypanosomatid is necessary before inoculation of amoebae into xenic culture (Freedman, Maddison & Elsdon-Dew, 1958; Wang, Jen & Cross, 1973; Clark & Diamond, 2002). The success rate for culture of *E. histolytica* is between 50 and 70% in reference laboratories (Clark & Diamond, 2002). As culture of *E. histolytica* from clinical samples such as faeces or liver abscess has a significant false-negative rate and is technically difficult, it has not been adopted as a routine clinical laboratory. *Entamoeba dispar* can be grown in xenic culture; however, most isolates grow poorly in monoxenic culture and the growth of only a few strains has been reported to be viable in axenic culture, suggesting that *E. dispar* may be less able than *E. histolytica* to obtain nutrients in a particle-free medium (Clark, 1995; Kobayashi *et al.*, 1998). The use of different media for the culture of *E. dispar* has been investigated and these studies indicate that YI-S may not be a suitable medium for the culture of *E. dispar* (Kobayashi *et al.*, 1998). For *E. moshkovskii* strains, culture media employed include TTY-SB-monophasic with the trypanosomatid, TP-S-1-GM monophasic for the axenic culture of amoebae (Diamond, 1968) and the TP-S-1-GM monophasic medium (Diamond & Bartgis, 1970). Other media containing bovine serum used for culture *E. moshkovskii* include axenic medium TYI-S-33 with 10% bovine serum at 24°C (Diamond, Harlow & Cunnick, 1978) or xenic medium TYSGM-9 with 5% bovine serum at either 24°C or 37°C (Diamond, 1982).

The pioneering work of Sargeaunt *et al.* (1978) demonstrated that isoenzyme analysis of cultured amoebae would enable the differentiation of *Entamoeba* species. A zymodeme is defined as a group of amoeba strains that share the same electrophoretic pattern and mobilities for several enzymes. Zymodemes consist of electrophoretic patterns of malic enzyme, hexokinase, glucose phosphate isomerase and phosphoglucomutase isoenzyme (Sargeaunt *et al.*, 1987). A total of 24 different zymodemes have been described, of which 21 are from human isolates (9 of *E. histolytica* and 12 of *E. dispar*). The presence of starch in the medium influences the most variable zymodeme patterns (Blanc & Sargeaunt, 1991) and many zymodemes "disappear" upon removal of bacterial floras, suggesting that at least some of the bands are of bacterial rather than amoebal origin (Jackson & Supersad, 1997). If the zymodemes defined by stable bands alone are counted, only three remain for *E. histolytica* (II, XIV and XIX) and one for *E. dispar* (I). Isoenzyme (zymodeme) analysis of cultured amoebae enables differentiation of *E. histolytica* from *E. dispar* and was considered the gold standard for diagnosis amoebic infection prior to development of newer DNA-based techniques.

Many different assays have been developed for the detection of antibodies, including indirect hemagglutination (IHA), latex agglutination, immunoelectrophoresis, counterimmunoelectrophoresis (CIE), the amoebic gel diffusion test, immunodiffusion, complement fixation, indirect immunofluorescence assay (IFA), and enzyme-linked immunosorbent assay (ELISA). A variety of antibody assays for detection of *E.*

histolytica antibodies in human serum are also commercially available (Table 2). Complement fixation tests appear to be less sensitive than others, cost more to perform and are not used by most laboratories. IHA is simple to perform and has been shown to be a highly specific (99.1%) diagnostic tool in human immunodeficiency virus-infected patients presenting with gastrointestinal symptoms (Hung *et al.*, 1999). However, the lower sensntivity may lead to false-negative results compared to ELISA. The latex agglutination test appears to detect the same antibody as IHA. Commercial kits are available and the test can be performed in 10 min. However, due to nonspecific reactions, the specificity of this test appears to be disappointing (Sanchez-Giillen *et al.*, 2000). Immunoelectrophoresis, CIE and immunodiffusion use the property of antibody and antigen precipitation in agar gel membrane. Sheehan *et al.* (1979) reported that detection of antibody to extraintestinal *E. histolytica* by CIE is time-consuming but has a high sensitivity (100%) in patients with invasive amoebiasis. Detection of antibodies using the IFA test was shown to be rapid, reliable and reproducible and helps to differentiate ALA from other nonamoebic etiologies. In addition to this, IFA tests has been shown to differentiate between past (treated) and present disease (Garcia *et al.*, 1982). A study conducted by Jackson *et al.* (1984) indicated that monitoring of immunoglobulin M (IgM) levels using the IFA can be of clinical value in a short period of time after infection, with more than half of the subjects having negative results at 6 months or 100% becoming negative by 46 weeks after treatment. In ALA the sensitivity of the IFA is reported to be 93.6%, with a specificity of 96.7%, making it more sensitive than the

Table 2. Commercially available antibody assays for diagnosis of amoebiasis

Antibody assay	Sensitivity (%) & Reference	Specificity (%) & Reference	Manufacturer
Cellognost-Amoebiasis (IHA)	100[a] (Pillai *et al.*, 1999) 99 (Hira *et al.*, 2001)	90.9-100[a] (Pillai *et al.*, 1999) 99.8 (Hira *et al.*, 2001)	Dade Behring Marburg GmbH, Marburg, Germany
Novagnost *Entamoeba* IgG	> 95[b]	> 95[b]	NovaTec Immunodiagnostica GmbH, Dietzenbach, Germany
Bichro-Latex Amibe	93.3 (Van Doorn *et al.*, 2005) 98.3 (Robert *et al.*, 1990)	95.5 (Van Doorn *et al.*, 2005) 96.1 (Robert *et al.*, 1990)	Fumoze Diagnostics, Levallois-Perret Cedex, France
I.H.A Amoebiasis	93.4 (Robert *et al.*, 1990)	97.5 (Robert *et al.*, 1990)	Fumoze Diagnostics, Levallois-Perret Cedex, France
Amoeba-Spot IF	NA[c] (Gatti *et al.*, 2002)	NA (Gatti *et al.*, 2002)	bioMerieux, Marcy-l'Etoile, France
Amoebiasis Serology microplate ELISA	95[b]	97[b]	Light Diagnostics
Amoebiasis Serology microwel EIA (HK-9 antigen, axenic)	97.9 (Hira *et al.*, 2001) 92.5 (Shenai *et al.*, 1996)	94.8 (Hira *et al.*, 2001) 91.3 (Shenai *et al.*, 1996)	LMD Laboratories, Inc., Carlsbad, CA
RIDASCREEN *Entamoeba* (IgG detection)	100[b] 97.7-100 (Knappik *et al.*, 2005)	95.6[b] 97.4 (Knappik *et al.*, 2005)	R-Biopharma AG, Darmstadt, Germany

[a] For the titer of ≥1:64, 100% sensitive and 90.9% specific: for the titer of ≥1:512, 100% sensitive and 100% specific.
[b] As recommended by the manufacturer.
[c] NA. not available.

ELISA (Shamsuzzaman *et al.*, 2000). A negative test therefore indicates that a patient never had invasive amoebiasis. However, this test requires skills in culture and subsequent antigen preparation, making it difficult to undertake in a routine clinical laboratory (Patterson & Schoppe, 1982). ELISA is the most popular assay in diagnostic laboratories throughout the world and has been used to study the epidemiology of asymptomatic disease (Gonzalez *et al.*, 1995) and the diagnosis of symptomatic amoebiasis after faecal examination. This method is widely thought to be sufficient for clinical purposes, particularly for diagnosis of patients with ALA and can be easily performed in a clinical laboratory. It may also be useful in the evaluation of intestinal and extraintestinal infections where amoebiasis is suspected but organisms cannot be detected in faeces (Rosenblatt, Sloane & Bestrom, 1995). A microtiter ELISA to detect antibodies to *E. histolytica* (LMD Laboratoriesm Inc., Carlsbad, CA) has been shown to be 97.9% sensitive and 94.8% specific for detection of *E. histolytica* antibodies in ALA patients (Hira *et al.*, 2001).

Several investigators have developed ELISAs for the detection of antigens in faecal samples. These antigen detection tests have a sensitivity approaching that of stool culture and are rapid to perform. Antigen-based ELISA kits that are specific for *E. histolytica* use monoclonal antibodies against the Gal/GalNAc-specific lectin of *E. histolytica* (*E. histolytica* II; TechLab, Blacksburg, VA) or monoclonal antibodies against serine-rich antigen of *E. histolytica* (Optimum S kit; Merlin Diagnostika, Bornheim-Hersel Germany). Other ELISA kits for antigen detection include the *Entamoeba* CELISA PATH kit (Cellabs, Brookvale, Australia), which uses a monoclonal antibody specific for lectin of *E. histolytica*, and the ProSpecT EIA (Remel Inc,; previously manufactured by Alexon-Trend, Inc., Sunnyvale, CA), which detects *E. histolytica*-specific antigen in faecal specimens (Table 3). In addition to the above mentioned clinical assays, research-based detection tests have included the use of monoclonal antibodies against a lectin-rich surface antigen (Petri & Singh, 1999), a lipophosphoglycan (Mirelman *et al.*, 1997), a 170-kDa-adherence lectin amoebic antigen detected in saliva (Abd-Alla *et al.*, 2000) and an uncharacterized antigen (Wonsit *et al.*, 1992). The *E. histolytica* TechLab kit was designed in 1993 to detect specifically *E. histolytica* in faeces (Haque *et al.*, 1997; Haque *et al.*, 1998). This antigen detection test captures and detects the parasite's Gal/GalNAc lectin in stool samples. The lectin is conserved and is highly immunogenic and because of the antigenic differences in the lectins of *E. histolytica* and *E. dispar*, the test enables specific identification of the disease-causing *E. histolytica*. The level of detection of amoebic antigens is quite high, requiring approximately 1,000 trophozoites per well (Haque *et al.*, 1993; Mirelman *et al.*, 1997). However, this test suffers from the disadvantage that the antigens detected are denatured by fixation of the stool sample, therefore limiting testing to fresh or frozen samples. Nevertheless, this test has demonstrated good sensitivity and specificity for detection of *E. histolytica* antigen in stool specimens of people suffering from amoebic colitis and asymptomatic intestinal infection (Haque *et al.*, 1995, 1997, 1998). The ProSpecT EIA (Remel Inc.) is a microplate EIA which detects both *E. histolytica* and *E. dispar*. However, this assay cannot differentiate between *E. histolytica* and *E. dispar*. The advantage of this test is that it can be performed on fresh, frozen or Cary-Blair specimens but not on formalin-fixed faecal samples. The sensitivity of the ProSpecT EIA was compared with that of conventional microscopy (using wet mounts and concentration methods) for the diagnosis of *E. histolytica/E. dispar*, and a sensitivity of 78% and specificity of 99% were reported (Ong *et al.*, 1996). In another study, by Gatii *et al.* (2002), the reported sensitivity and specificity of ProSpecT ELISA were 54.5% and 94%, respectively compared to culture and zymodeme identification for *E. histolytica/E. dispar*.

The Triage parasite panel (TPP) (Biosite Diagnostic Ins., San Diego, CA) is the first immunochromatographic assay for the simultaneous detection of antigens specific for *G. lamblia*, *E. histolytica/E. dispar* and

Table 3: Commercially available antigen assays for the diagnosis of amoebiasis

Test	Sensitivity (%) & Reference	Specificity (%) & Reference	Manufacturer	Detection limit
TechLab *E. histolytica* II[a]	96.9-100[b] 14.2[c] (Gatti *et al.*, 2002) 87.5[d] (Haque *et al.*, 1997) 86[e] (Haque *et al.*, 1997) 71 (Visser *et al.*, 2006) 95[f] (Haque *et al.*, 1995) 79[g] (Roy *et al.*, 2005)	94.7-100[b] 98.3[c] (Gatti *et al.*, 2002) 100[d] (Haque *et al.*, 98[e] (Haque *et al.*, 1997) 100 (Visser *et al.*, 2006) 93[f] (Haque *et al.*, 1995) 96[g] (Roy *et al.*, 2005)	TechLab, Blacksburg, VA	0.2-0.4 ng of adhesion per well
***Entamoeba* CELISA-PATH**[a]	95-100[b]	93.100[b]	Cellabs Pty Ltd., Brookvale, Australia	0.2-0.4 ng of adhesion per well
Optimum S *E. histolyitca* antigen ELISA[a]	100 (Pillai *et al.*, 1999)	NP[h]	Merlin Diagnostika, Berheim-Hersel, Germany	Not given
Triage parasite panel[i]	96[j] (Garcia *et al.*, 2000) 68.3[k] (Pillai & Kain, 1999) 100[l] (Sharp *et al.*, 2001)	99.1[j] (Garcia *et al.*, 2000) 100[k] (Pillai & Kain, 1999) 100[l] (Sharp *et al.*, 2001)	BIOSITE Diagnostics, San Diego, CA	Not given
ProSpecT *E. histolytica* microplate assay[i]	87[m] 54.5[c] (Gatti *et al.*, 2002) 78[n] (Ong *et al.*, 1996)	99[m] 94[c] (Gatti *et al.*, 2002) 99[n] (Ong *et al.*, 1996)	REMEL Inc., Lenexa, KS[o]	40 ng/ml of *E. histolytica*-specific antigen

[a]Specific for *E. histolytica*
[b]Sensitivity and specificity compared to culture/zymodeme, as cited by the manufacturer
[c]Sensitivity and specificity compared to culture and microscopy
[d]Compared to isoenzyme analysis
[e]Compared to culture
[f]Compared to culture and microscopy
[g]Compared to real-time PCR
[h]NP. not published
[i]Cannot distinguish between *E. histolytica* and *E. dispar*
[j]Compared to permanent staining with trichrome and modified acid-fast stains
[k]Compared to ProSpecT *Entamoeba histolytica* microplate assay
[l]Compared to ovum and parasite examination
[m]As mentioned by the manufacturer, related to ovum and parasite identifications
[n]Compared to microscopy (wet mounts and concentration)
[o]Previously manufactured by Alexon-Trend, Inc., Sunnyvale, CA

C. parvum. The immunochromatographic strip used in this assay is coated with monoclonal antibodies specific for the 29-kDa surface antigen (*E. histolytica/E. dispar*), alpha-1-giardin (*G. lamblia*), and protein disulfide isomerase (*C. parvum*). By using specific antibodies, antigen specific for these organisms from the stool samples are captured and immobilized on a membrane. A high sensitivity (96% to 100%) and specificity (99.1% to 100%) of the TPP kit compared to microscopy (stool ova and parasite examination) for *E. histolytica/E. dispar* were reported (Garcia, Shimizu & Bernard, 2000; Sharp *et al.*, 2001). In another study, although the specificity of the Triage kit was high (100%), the sensitivity was low (68.3%) compared to that of the ProSpecT test (Pillai & Kain, 1999). A recent study from Sweden has compared the TPP test with PCR and demonstrated a low sensitivity for TPP assay (Leiva *et al.*, 2006). The advantage of the TPP method is that it can be performed in approximately 15 min with fresh or frozen, unfixed human faecal specimens.

There is now a wide variety of PCR methods, targeting different genes, which have been described for detection and differentiation of the three *Entamoeba* species. The consistent genetic diversity

detected between the 18S rDNAs of *E. histolytica* and *E. dispar* initiated the use of 18S rDNA as a target for differentiation of the two species (Clark & Diamond, 1991, 1992; Cruz-Reyes *et al.*, 1992). DNA extracted from laboratory-cultured trophozoites and DNA recovered directly from microscopy-positive fecal samples using the manual and automated methods were tested, and the PCR methods proved to be highly sensitive and specific for detecting *Entamoeba* DNA (Clark & Diamond, 1993, 1997; Troll, Marti & Weiss, 1997; Ramos *et al.*, 2000; Heckendorn *et al.*, 2002; Moran *et al.*, 2005). PCR assays targeting 18S rDNA are widely used for the detection and differentiation of *Entamoeba* species, as these targets are present multicopy, extrachromosomal plasmids in the amoeba (Bhattacharya *et al.*, 1989), making the 18S rDNA more easily detected than a DNA fragment of a single-copy gene. The successful use of PCR in studying the epidemiology of *Entamoeba* infection was first reported by Acuna-Soto *et al.* (1993). They used DNA extracted directly from faeces, avoiding the need to culture trophozoites and the primers were targeted to amplify the extrachromosomal circular DNA. This gene target was subsequently used by other researchers (Aguirre *et al.*, 1995; Britten *et al.*, 1997). This PCR target, with colorimetric detection of the product was also used with DNA extracted from faecal samples, using a modification of the QIAGEN kit (Aguirre *et al.*, 1995; Verweij *et al.*, 2000). Primers for the 29-kDa/30-kDa antigen gene have been used for distinguishing among pathogenic and non-pathogenic species of *Entamoeba* using conventional PCR (Tachibana *et al.*, 1991). In research laboratories, this target has been used for analyses of microscopy-positive faeces which have been cultured in the laboratory (Tachibana *et al.*, 2000; Pinheiro *et al.*, 2004) as well as formalin-fixed faecal samples (Rivera *et al.*, 1996, 1998, 2006).

Other gene targets for PCR include two protein-encoding genes which have been shown to exhibit polymorphism in the coding region. These are the serine-rich *E. histolytica* protein (SREPH) gene (Stanley *et al.*, 1990) and the chitinase gene (De la Vega *et al.*, 1997). SREPH as a target was reported for the amplification of DNA recovered from laboratory cultures and microscopy-positive faeces concentrated by the zinc-sulfate gradient floatation techniques (Ramos *et al.*, 2005). A nested SREPH PCR approach was recently used to investigate *E. histolytica* diversity in a single human population, using DNA extracted from microscopy-positive faeces (Ayeh-Kumi *et al.*, 2001). PCR using the cysteine proteinase gene and actin genes as targets was also used to study the epidemiology of amoebiasis (Freitas *et al.*, 2004). In addition, a novel PCR assay based on the *E. histolytica* hemolysin gene HLY6 (hemo PCR) was developed for the detection of *E. histolytica* DNA with faecal and ALA samples and was shown to have 100% sensitivity and specificity (Zindrou *et al.*, 2001). PCR for the detection of *E. histolytica* DNA from liver abscess samples was first employed using the gene encoding the 30-kDa antigen and 100% sensitivity was reported. In another study, PCR performed on liver samples demonstrated only 33% sensitivity for the presence of *E. histolytica* using primers specific for 18S rDNA of *E. histolytica*, whereas the second pair, specific for the 30-kDa antigen gene (Tachibana *et al.*, 1992), showed a sensitivity of 100% (Zengzhu *et al.*, 1999). Direct amplification for detection of *E. histolytica* DNA (without the extraction of DNA) from ALA pus was reported using 10 different previously published primer pairs (used for amplification of *E. histolytica* from liver and stool samples) (Zaman *et al.*, 2000). Of the 10 different primer pairs tested, two pairs i.e., P1-P2 targeting extrachromosomal circular DNA of *E. histolytica* (Acuna-Soto *et al.*, 1993) and P11-P12 targeting the 30-kDa antigen gene (Tachibana *et al.*, 1992) gave 100% sensitivity. Another PCR assay (hemo PCR), based on the novel hemolysin gene HLY6 of *E. histolytica* was analyzed for the liver abscess samples. The hemo-PCR gave a positive result for 89% of ALA samples compared to 77% and 28% for the 30-kDa antigen gene and 18S rDNA, respectively (Zindrou *et al.*, 2001). The hemo-PCR was found to be a valuable diagnostic tool for identification of *E. histolytica* in liver and

faecal samples. For the identification of *E. moshkovskii* in faecal specimens, a ribiprinting method was first reported by Haque *et al.* (1998). Subsequently, a PCR for the identification of *E. moshkovskii* in faecal samples was developed as a nested 18S rDNA PCR followed by restriction endonuclease digestion (Ali *et al.*, 2003). This method has a high sensitivity and specificity (100%) with DNA extracted directly from stool samples using the QIAGEN stool extraction kit (Fotedar *et al.*, 2007).

Real-time PCR is a new and a very attractive methodology for laboratory diagnosis of infectious diseases because of its characteristics that eliminate post-PCR analysis, leading to shorter turnaround times, a reduction in the risk of amplicon contamination of laboratory environments and reduced reagents costs (Klein, 2002). This approach allows specific detection of the amplicon by binding the one or two fluorescence-labeled probes during PCR, thereby enabling continuous monitoring of amplicon (PCR product) formation throughout the reaction. An important aspect of real-time PCR is enhanced sensitivity compared to conventional PCR, with an ability to detect 0.1 cell per gram of faeces (Blessmann *et al.*, 2002a). In addition, real-time PCR is a quantitative method and allows the determination of the number of parasites in various samples. Distinct real-time PCR protocols have recently been published for identification and differentiation of *E. histolytica* from *E. dispar*. These include a Light Cycler assay utilizing hybridization probes to detect amplification of the 18S rDNA from faecal samples (Blessmann *et al.*, 2002a; Calderaro *et al.*, 2006) and two TaqMan assays, one targeting the 18S rDNA (Verweij *et al.*, 2003, 2004; Kebede *et al.*, 2004) and another targeting the episomal repeats using DNA extracted from faecal samples collected from primates and humans (Verweij *et al.*, 2003). A molecular beacon-based real-time PCR targeting 18S rDNA of *E. histolytica* for use on faecal and ALA specimens was described (Roy *et al.*, 2005). A SYBR green real-time assay targeting the 18S rDNA was described by Qvarnstrom *et al.* (2005). The sequences selected in the majority of these real-time studies have included rDNA as the target for PCR. A recent evaluation of three real-time PCR assays, focusing on the weakness and strengths of each assay and their usefulness for clinical laboratory diagnosis, was published by Qvarnstrom *et al.* (2005). This study highlighted major differences in detection limits and assay performance that were observed among the evaluated tests. Two of the assays in this study could not reliably distinguish *E. histolytica* from *E. dispar*, including the Light Cycler assay (Blessmann *et al.*, 2003) and the TaqMan assay targeting episomal repeats (Verweij *et al.*, 2003). A multiplex real-time assay was subsequently developed for detection of different intestinal parasites with 100% sensitivity and specificity (Verweij *et al.*, 2004). This assay allows detection of *E. histolytica*, *G. lamblia* and *C. parvum* and offers the possibility of introducing DNA detection in the routine diagnosis of intestinal parasitic infections. The implementation of such multiplex assays and the development of automated DNA isolation procedures could have a tremendous impact on routine parasitology practice. Accurate diagnosis necessitates that the same reaction conditions are used for a standard and for the sample. Duplex or multiplex approaches with internal standardization provide a solution for this problem. A real-time PCR for detection of *E. moshkovskii* in clinical samples has not yet been reported. Further research is therefore required to develop these methods for the detection of *E. moshkovskii*.

Recently, a novel nucleic acid amplification method termed loop-mediated isothermal amplification (LAMP) has been developed (Liang *et al.*, 2009). The loop-mediated isothermal amplification (LAMP) assay was originally developed by Notomi *et al.* (2000), Mori *et al.* (2001) and Nagamine *et al.* (2002) (Eiken Chemical Co., Ltd., Japan). LAMP employs a DNA polymerase with strand displacement activity and four primers that recognize six sequences on the target DNA. This method amplifies DNA with high specificity, sensitivity, and rapidity under isothermal conditions. Since this reaction is performed under isothermal conditions (60

to 65°C), simple incubators such as a water bath or heat block are adequate for the DNA amplification (Notomi *et al.*, 2000). Moreover, a large amount of white precipitate of magnesium pyrophosphate is produced as a byproduct, which enables the visual judgment of amplification by the naked eye (Mori *et al.*, 2001). Considering these advantages, the LAMP assay could become a valuable diagnostic tool in developing countries or hospital laboratories. With the LAMP assay, *E. histolytica* in faecal samples can be detected rapidly via the naked eye under UV light without any other sophicticated equipment (Mori *et al.*, 2001) and LAMP outperforms microscopy in its ability to discriminate *E. histolytica* from *E. dispar* and *E. moshkovskii*. Since commercial kits such as the TechLab II kit detect the antigens of *E. histolytica*, they were not compared with the LAMP assay which is based on DNA detection. The LAMP assay developed by Liang *et al.* (2009) has levels of sensitivity and specificity similar to those of nested PCR and can be useful for clinical detection and active surveillance of *E. histolytica* parasites in countries where amoebiasis is endemic. The LAMP assay requires minimal laboratory facilities and can differentiate DNA samples from *E. histolytica* and *E. dispar*. Morever, compared to the requirements for performing nested PCR, the simplicity and affordability of the LAMP assay allow easy identification of *E. histolytica*.

Epidemiology of amoebiasis in Malaysia

Entamoeba histolytica is an enteric anaerobic protozoan parasite that causes about 50 million infections with a death rate of over 100,000 worldwide annually. Although the parasite has a worldwide distribution, high prevalence of more than 10% of the population have been reported varies with the population of individuals affected, different between countries and areas with different socioeconomic conditions. Areas of highest incidence (due to inadequate sanitation and crowding) include most developing countries in the tropics, particularly Mexico, Indian and nations of Central and South America, tropical Asia, and Africa. In Malaysia, foodborne and waterborne diseases which are closely associated with environmental and personal hygiene practices are still among the major health problems in Malaysia; intestinal protozoan infections for example are still a public health concern in Malaysia, although are well controlled and only sporadically limited to specific areas or within certain population groups i.e. the aboriginal settlements and amongst people living in remote area (Ministry of Health Malaysia, 2008).

The prevalence of *E. histolytica/E. dispar* in Malaysia has been reported by many researchers, from way back in the sixties (Table 4); the prevalence ranged from 1% to 83%. The prevalence varied with the population studied, whether preservative were used for faeces collection, frequency of faeces collected for examination and technique used in the detection of the protozoa. Desowitz *et al.* (1961) reported a prevalence rate of 5-10% in the earliest study carried out among population living in various villages in Singapore and medical students of Singapore University. Following that, many studies reported on the prevalence of *E. histolytica/E. dispar* especially among Orang Asli, estate and rural village communities (Heyneman *et al.*, 1967; Balasingam *et al.*, 1969; Bisseru & Aziz, 1970). Except the study by Bolton (1968), all studies in the sixties and seventies were community studies. In the early eighties Noor Hayati *et al.* (1981) and Hamimah *et al.* (1982) reported 3.4% and 2.3% of children admitted with gastroenteritis symptoms excreted cysts of *E. histolytica/E. dispar* respectively. The prevalences were in agreement with finding reported earlier by Bolton (1968). A recent study carried out among population living in tropical highland and mountainous area in Sabah showed a high prevalence of *E. histolytica/E. dispar* (21.0%) (Nor Aza *et al.*, 2003). Only one faecal specimen was collected from each subject and the faeces were not fixed. If faeces were collected more than once and fixed in preservative a higher prevalence of *E. histolytica/E. dispar* would be expected in their study. A later study carried out among school children in Sabah confirmed that *E.*

Table 4. Intestinal *E. histolytica/E. dispar* in Malaysia

Infection & method of diagnosis	Population studied	Prevalence (%)	Author & Year
Intestinal Infection – Microscopy	Community – village and medical students	5-10	Desowitz *et al.* (1961)
	Hospital – Orang Asli; all age	3.1-10.3	Bolton (1968)
	Community – Orang Asli; children	1.5	Bisseru & Aziz (1970)
	Community – Orang Asli; all age	5.1	Dunn (1972)
	Community – Orang Asli; all age	8.7	Dissanaike *et al.* (1977)
	Community – estate workers; adult	1.3	Sinniah *et al.* (1978)
	Community – village people; all age	1.2	Nawalinski & Roundy (1978)
	Hospital – children	3.4	Noor Hayati *et al.* (1981)
	Hospital – children	2.3	Hamimah *et al.* (1982)
	Community – school children	4.4	Sinniah (1984)
	Community – Orang Asli and medical students	14.4	Che Ghani *et al.* (1987)
	Community – Orang Asli; all age	8.6	Lai (1992)
	Community – Orang Asli children	61.5	Karim *et al.* (1995)
	Community – Orang Asli; children	9.0	Rahmah *et al.* (1997)
	Community – Orang Asli; all age	11.5	Noor Hayati *et al.* (1998)
	Community – Orang Asli; all age	6.9	Kamel *et al.* (2002)
	Community – Interior population; all age	21.0	Nor Aza *et al.*(2003)
	Community – Urban population; all age	0.4	Jamaiah & Rohela (2005)
	Community – Orang Asli; children	8.9	Norhayati *et al.* (2006)
	Community – Orang Asli; all age	18.5	Noor Azian *et al.* (2007)
	Community & hospitalized – Orang Asli; all age	9.4	Lokman *et al.* (2007)
	Community – school children	83.9	Mahsol *et al.* (2008)
	Community – Orang Asli; children	22.5	Hartini & Mohamed Kamel (2009)
Intestinal Infection-PCR	Community – Orang Asli; all age	13.2 Eh 5.6 Ed	Noor Azian *et al.* (2006)

histolytica/E. dispar infection was common with a prevalence of 83.8%. In this study, formal-ether concentration method was performed to enhance the detection of cysts (Mahsol *et al.*, 2008).

In peninsular Malaysia, many recent studies reported high prevalence of *E. histolytica/E. dispar* (Karim *et al.*, 1995; Rahmah *et al.*, 1997; Noor Hayati *et al.*, 1998; Noor Azian *et al.*, 2007; Lokman *et al.*, 2007; Hartini & Mohamed Kamel, 2009). A study carried out among Orang Asli communities in Pos Piah reported 11.5% of population studied was infected with *E. histolytica/E. dispar* (Noor Hayati *et al.*, 1998). In their study, faeces were collected once and fixed in polyvinyl-alcohol. A study by Noor Azian *et al.* (2007) among aborigines population also reported high prevalence of *E. histolytica/E. dispar* (18.5%). In their study faeces were collected in three consecutive days and fixed in polyvinyl-alcohol upon examination. Cysts were excreted intermittently, thus collection of faeces of more than one and use of polyvinyl-alcohol to fix the faeces contributed to the high prevalence of *E. histolytica/E. dispar* infection detected in this study. High prevalence was also reported by Lokman *et al.* (2007). A recent study by Hartini & Mohamed Kamel (2009) reported 22.5% of Orang Asli children studied harbored *E. histolytica/E. dispar* cysts. On the other hand, the prevalence of *E. histolytica/E. dispar* was very low in the urban community of Malaysia. A study by Jamaiah & Rohela (2005) among the public in Kuala Lumpur reported a very low prevalence (0.4%).

As almost all surveys previously done relied on stool analysis by microscopy, there is no reliable data on the epidemiology of *E. histolytica* in Malaysia. It is not only in Malaysia, but also in the other parts of the world in general that the current epidemiology of amoebiasis is confusing, mainly because of the recently appreciated distinction between *E. histolytica*, *E. dispar* and *E. moshkovskii*. Until relatively recently, *E. histolytica* and *E. dispar* were not differentiated, and infection with either

of the two species was referred to as 'amoebiasis', resulting in an overestimation of the true prevalence. In Malaysia, no attempt study is made to differentiate cysts and trophozoites of morphologically identical *E. histolytica*, *E. dispar* and *E. moshkovskii*. This is because all community and hospital studies are based entirely on microscopical examination of fresh faeces specimens for parasite identification. Very little information is available on the prevalence of the two or three *Entamoeba* species. To the best of our knowledge and up to this date, only one community study that has been carried out in Malaysia to differentiate the two species (Nor Azian *et al.*, 2006). In this study the true prevalence of *E. histolytica* and *E. dispar* was determined using Nested PCR and Restriction Enzyme (RE) digestion. The findings showed that out of 31 specimens that were positive for *E. histolytica/E. dispar* microscopically, 13.2% and 5.6% were positive for *E. histolytica* and *E. dispar* respectively. This finding contradicts most studies as reports usually documented higher prevalence of *E. dispar* as compared to *E. histolytica*.

The earliest reports on invasive amoebiasis in Malaysia were by Chellapa & Rangabasham (1977), Vijendran (1977), Balasengaram (1981) and Goh *et al.*, (1987). A case report by Manukaran *et al.* (1983) reported a rare clinical presentation of intestinal amoebiasis with multiple colonic perforations and ruptured liver abscess in a 43-year-old Indian labourer. Goh *et al.* (1987) in their study had reviewed 204 cases of liver abscess seen between 1970 and 1985 in University Hospital, Kuala Lumpur; the findings showed 44.1%, 11.8%, and 0.5% of liver abscess were classified as amoebic, pyogenic and tuberculous. The cause of liver abscess in the remaining of 43.6% was not established. This study also reported fever with chills and rigors, right hypochondrial pain and tender hepatomegaly were the most common clinical presentations of amoebic liver abscess (ALA). Almost 87% of ALA seen in this study is a single abscess and mostly located in the right lobe. The patients were predominantly males, Indians and in the 30-60 age group.

A 10-year retrospective study on amoebiasis was also carried out in University Hospital Kuala Lumpur between 1984 to 1994 by Jamaiah & Shekar (1999); of 51 amoebiasis cases traced, 30 (59%), 20 (39%) and 1(2%) were amoebic dysentery, ALA and combination of amoebic dysentery and ALA respectively. Most of the cases were reported in Malays, majority in males and unemployed. The most common clinical presentations were diarrhoea and dysentery. Trophozoites of *E. histolytica* were only identified in 13 (43%) and 9 (30%) of faeces and intestinal biopsy of amoebic dysentery patients respectively. Only one out of 20 ALA cases showed trophozoites in the faeces and biopsy (Jamaiah & Shekar, 1999). These findings showed the difficulty in isolating trophozoites from clinical specimens; thus antigen or antibody test and PCR are very useful to confirm the diseases. A latest 10-year retrospective study in the same hospital showed a decreasing trend of invasive amoebiasis as compared to studies by Goh *et al.* (1987) and Jamaiah & Shekar (1999); only 34 cases were traced and analysis showed ALA was the commonest presentation with 22 (65%) cases. Amoebic dysentery was seen in 12 (35%) of patients (Farhana *et al.*, 2009). The clinical presentations were almost similar with study carried out by Goh *et al.* (1987).

Magnitude of invasive amoebiasis can also be in the community by assessing the antibody titers. Gilman *et al.* (1976) reported 44% of asymptomatic family members of Orang Asli patients diagnosed with acute amoebic dysentery were seroresponders. This study also reported that Orang Asli who lived near towns had significantly more seroresponders (32%) than Orang Asli who lived in deep jungle village (4%). Sero-epidemiological studies of specific antibodies to *E. histolytica* using Indirect Immunofluorescent test among different races in Malaysia by Thomas & Yap (1986), demonstrated low prevalence of antibody in Chinese (3.6%) as compared to Orang Asli (9.7%), Malay (7.2%) and Indians (5.4%).

In conclusion amoebiasis is still a public health and of clinical importance in Malaysia. Since 90% of *E. histolytica* infection is

asymptomatic, determination of the true prevalence of *E. histolytica* infection in the community is very crucial to predict the clinical burden of ameobiasis. Thus, future epidemiological study should give priority to the determination of true prevalence of *E. histolytica* and *E. dispar*.

REFERENCES

Abd-Alla, M.D., Jackson, T.F., Gathiram, V., el-Hawey, A.M. & Ravdin, J.I. (1993). Differentiation of pathogenic *Entamoeba histolytica* infections from non-pathogenic infections by detection of galactose-inhibitable adherence protein antigen in sera and faeces. *Journal of Clinical Microbiology* **31:** 2845-2850.

Abd-Alla, M.D., Jackson, T.F., Reddy, S. & Ravdin, J.I. (2000). Diagnosis of invasive amoebiasis by enzyme-linked immunosorbent assay of saliva to detect amebic lectin antigen and anti-lectin immunoglobulin G antibodies. *Journal of Clinical Microbiology* **38:** 2344-2347.

Abd-Alla, M.D., Wahib, A. & Ravdin, J.I. (2002). Diagnosis of amoebic colitis by antigen capture ELISA in patients presenting with acute diarrhoea in Cairo. *Egyptian Tropical Medcine and International Health* **7:** 1-6.

Acuna-Soto, R., Samuelson, J., De Girolami, P., Zarate, L., Millan-Velasco, F., Schoolnick, G. & Wirth, D. (1993). Application of the polymerase chain reaction to the epidemiology of pathogenic and nonpathogenic *Entamoeba histolytica. American Journal of Tropical Medicine and Hygiene* **48:** 58-70.

Adams, E.B. & MacLeod, I.N. (1977). Invasive amoebiasis. I. Amoebic dysentery and its complication. *Medicine* **56:** 315-323.

Aguirre, A., Warhurst, D.C., Guhl, F. & Frame, I.A. (1995). Polymerase chain reaction-solution hybridization enzyme-linked immunoassay (PCR-SHELA) for the differential diagnosis of pathogenic and non-pathogenic *Entamoeba histolytica. Transaction of the Royal Society of Tropical Medicine and Hygiene* **89:** 187-188.

Ali, I.K., Hossain, M.B., Roy, S., Ayeh-Kumi, P.F., Petri Jr., W.A., Haque, R. & Clark, C.G. (2003). *Entamoeba moshkovskii* infection in children, Bangladesh. *Emerging of Infectious Disease* **9:** 580-584.

Al-Hindi, A., Shubair, M.E., Marshall, I., Ashford, R.W., Sharif, F.A., Abed, A.A. & Kamel, E.G. (2005). *Entamoeba histolytica* and *Entamoeba dispar* among children in Gaza, Gaza Strip? *Journal of Egyptian Society of Parasitology* **35:** 59-68.

Allason-Jones, E., Mindel, A., Sargeaunt, P. & Williams, P. (1986). *Entamoeba histolytica* as a commensal intestinal parasite in homosexual men. *New England Journal of Medicine* **315:** 353-356.

Ayeh-Kumi, P.F., Ali, I.M., Lockhart, L.A., Gilchrist, C.A., Petri Jr., W.A. & Haque, R. (2001). *Entamoeba histolytica*: genetic diversity of clinical isolates from Bangladesh as demonstrated by polymorphisms in the serine-rich gene. *Experimental Parasitology* **99:** 80-88.

Bakir, B., Tanyuksel, M., Saylam, F., Sultan, T., R. Engin, A., Ali Kasim, H. & Metin, H. (2003). Investigation of waterborne parasites in drinking wáter sources of Ankara, Turkey. *The Journal of Microbiology* **41:** 148-151.

Balamuth, W. (1946). Improved egg yolk medium for cultivation of *Entamoeba histolytica* and other intestinal protozoa. *American Journal of Clinical Pathology* **16:** 380-384.

Balasingam, E., Lim, B.L. & Ramachandran, C.P. (1969). A parasitological study of Pulau Pinang and Perhentian Kechil, off Trengganu, West Malaysia. *Medical Journal of Malaya* **23:** 300.

Balasengaram, M. (1981). Management of hepatic abscess. *Current Problems in Surgery* **18:** 282-340.

Barnawi, A.B., Tonkal, A.M., Fouad, M.A. & Al-Braiken, F.A. (2007). Detection of *Entamoeba histolytica/dispar* in stool specimens by using enzyme-linked immunosorbent assay in the population of Jeddah City, Saudi Arabia. *Journal of Egyptian Society of Parasitology* **37:** 143-150.

Barwick, R.S., Uzicanin, A., Lareau, S.M., Malakmadze, N., Imnadze, P., Iosaza, M., Ninasvili, N., Wilson, M., Bishop, H., Hightower, A., Petri Jr., W.A. & Juranek, D.D. (1999). Outbreak of amoebiasis in Tblisi, Republic of Georgia. p. 234. Abstract 48th Annual Meeting of American Society of Tropical Medicine & Hygiene.

Beck, D.L., Dogan, N., Maro, V., Sam, N.E., Shao, J. & Houpt, E.R. (2008). High prevalence of *Entamoeba moshkovskii* in a Tanzanian HIV population. *Acta Tropica* **107:** 48-49.

Ben Ayed, S., Aoun, K., Maamouri, N., Ben Abdallah, R. & Bouratbine, A. (2008). Short Report: First molecular identification of *Entamoeba moshkovskii* in human stool samples in Tunisia. *American Journal of Tropical Medicine and Hygiene* **79:** 706-707.

Bhattacharya, S., Bhattacharya, A., Diamond, L.S. & Soldo, A.T. (1989). Circular DNA of *Entamoeba histolytica* encodes ribosomal RNA. *Journal of Protozoology* **36:** 455-458.

Bisseru, B. & Aziz, A.A. (1970). Intestinal parasites, eosinophilia, haemoglobin and gamma globulin of Malay, Chinese and Indian schools. *Medical Journal of Malaya* **25:** 29-33.

Blanc, D. & Sargeaunt, P.G. (1991). *Entamoeba histolytica* zymodemes: exhibition of gamma and delta bands only of glucose phosphate isomerase and phosphiglucomutase may be influenced by starch content in the médium. *Experimental Parasitology* **72:** 87-90.

Blessmann, J., Buss, H., Un, P.A., Dinh, B.T., Ngo, Q.T., Van, A.L., Alla, M.D., Jackson, T.F., Ravdin, J.I. & Tannich, E. (2002a). Real-time PCR for detection and differentiation of *Entamoeba histolytica* and *Entameba dispar* in faecal samples. *Journal of Clinical Microbiology* **40:** 4413-4417.

Blessmann, J., Van Linh, P., Nu, P.A., Thi, H.D., Muller-Myhsok, B., Buss, H. & Tannich, E. (2002b). Epidemiology of amoebiasis in a región of high incidence of amoebic liver abscess in central Vietnam. *American Journal of Tropical Medicine Hygiene* **66:** 578-583.

Blessmann, J., Ali, I.K., Un, P.A., Dinh, B.T., Viet, T.Q., Van, A.L., Clark, C.G. & Tannich, E. (2003). Longitudinal study of intestinal *Entamoeba histolytica* infections in asymptomatic adult carriers. *Journal of Clinical Microbiology* **41:** 4745-4750.

Bolton, J.M. (1968). Medical services to the aborigines in West Malaysia. *British Medical Journal* **2:** 818-823.

Britten, D., Wilson, S.M., McNerney, R., Moody, A.H., Chiodini, P.L. & Ackers, J.P. (1997). An improved colorimetric PCR-based method for detection and differentiation of *Entamoeba histolytica* and *Entamoeba dispar*. *Journal of Clinical Microbiology* **35:** 3044-3045.

Bruckner, D.A. (1992). Amebiasis. *Clinical Microbiology Reviews* **5:** 356-369.

Caballero-Salcedo, A., Viveros-Rogel, M., Salvatierra, B., Tapia-Conyer, R., Sepulveda-Amor, J., Gutierrez, G. & Ortiz-Ortiz, L. (1994). Seroepidemiology of amebiasis in Mexico. *American Journal of Tropical Medicine and Hygiene* **50:** 412-419.

Calderaro, A., Gorrini, C., Bommezzadri, S., Piccolo, G., Dettori, G. & Chezzi, C. (2006). *Entamoeba histolytica* and *Entamoeba dispar*: comparison of two PCR assays for diagnosis in a non-endemic setting. *Transaction of the Royal Society of Tropical Medicine and Hygiene* **100:** 450-457.

Che Ghani, M., Mohamed, A.M. & Oothuman, P. (1987). Infection with *Entamoeba histolytica* and *Giardia lamblia* in an urban slum, rural villages and medical students in peninsular Malaysia. *Tropical Biomedicine* **4:** 150-154.

Chelaapa, M. & Rangabashyam, N. (1977). Amebic liver abscess in a review and study in 167 cases. *Medical Journal of Malaysia* **31:** 192-196.

Clark, C.G. (1995). Axenic cultivation of *Entamoeba dispar* Brumpt 1925, *Entamoeba insolita* Geiman and Wichterman 1937 and *Entamoeba ranarum* Grassi 1879. *Journal of Eukaryotic Microbiology* **42:** 590-593.

Clark, C.G. & Diamond, L.S. (1991). Ribosomal RNA genes of 'pathogenic' and 'non-pathogenic' *Entamoeba histolytica* are distinct. *Molecular Biochemistry and Parasitology* **49:** 297-302.

Clark, C.G. & Diamond, L.S. (1992). Differentiation of pathogenic *Entamoeba histolytica* from other intestinal protozoa by riboprinting. *Archives of Medical Research* **23:** 15-16.

Clark, C.G & Diamond, L.S. (1993). *Entamoeba histolytica*: a method for isolate identification. *Experimental Parasitology* **77:** 450-455.

Clark, C.G. & Diamond, L.S. (1997). Intraspecific variation and phylogenetic relationships in the genus *Entamoeba* as revealed by riboprinting. *Journal of Eukaryotic Microbiology* **44:** 142-154.

Clark, C.G. & Diamond, L.S. (2002). Methods for cultivation of luminal parasitic protists of clinical importance. *Clinical Microbiology Reviews* **15:** 329-341.

Cruz-Reyes, J.A., Spice, W.M., Rehman, T., Gisborne, E. & Ackers, J.P. (1992). Ribosomal DNA sequences in the differentiation of pathogenic and non-pathogenic isolates of *Entamoeba histolytica*. *Parasitology* **104:** 239-246.

De la Vega, H., Specht, C.A., Semino, C.E., Robbins, P.W., Eichinger, D., Caplivski, D., Ghosh, S. & Samuelson, J. (1997). Cloning and expression of chitinases of *Entamoeba*. *Molecular and Biochemical Parasitology* **85:** 139-147.

Desowitz, R.S., Zaman, V. & Ng, K.W. (1961). The incidence of intestinal parasites in various communities of Singapore Island. *Singapore Medical Journal* **2:** 91.

Diamond, L.S. (1961). Axenic cultivation of *Entamoeba histolytica*. *Science* **134:** 336-337.

Diamond, L.S. (1968). Improved method for the monoxenic cultivation of *Entamoeba histolytica* Schaudinn, 1903 and *E. histolytica*-like amoebae with trypanosomatids. *Journal of Parasitology* **54:** 715-719.

Diamond, L.S. (1982). A new liquid médium for xenic cultivation of *Entamoeba histolytica* and other lumen dwelling protozoa. *Journal of Parasitology* **68:** 958-959.

Diamond, L.S. & Bartgis, I.L. (1970). *Entamoeba moshkovskii*: axenic cultivation. *Experimental Parasitology* **28:** 171-175.

Diamond, L.S., Clark, C.G. & Cunnick, C.C. (1995). YI-S, a casein free medium for axenic cultivation of *Entamoeba histolytica*, related *Entamoeba*, *Giardia intestinalis* and *Trichomonas vaginalis*. *Journal of Eukaryotic Microbiology* **42:** 277-278.

Diamond, L.S., Harlow, D.R. & Cunnick, C.C. (1978). A new médium for the axenic cultivation of *Entamoeba histolytica* and other *Entamoeba*. *Transaction of the Royal Society of Tropical Medicine and Hygiene* **72:** 431-432.

Dissanaike, A.S., Kan, S.P., Thomas, V. & Ong, H.T. (1977). Studies of parasitic infections in Orang Asli (Aborigines) in Peninsular Malaysia. *Medical Journal of Malaysia* **32:** 48-55.

Dunn, F.L. (1972). Intestinal parasitism in Malayan aborígenes (Orang Asli). *Bulletin of World Health Organization* **46:** 99-113.

Eckmann, L., Reed, S.L., Smith, J.R. & Kagnoff M.F. (1995). *Entamoeba histolytica* trophozoites induces an inflammatory cytokines response by culured human cells through the paracrine action of cytolytically released interleuken-1 alpha. *Journal of Clinical Investigation* **96:** 1269-1279.

Espinosa-Cantellano, M. & Martinez-Paloma, A. (2000). Pathogenesis of intestinal amebiasis: from molecules to disease. *Clinical Microbiology Reviews* **13:** 318-331.

Fadeyi, A., Nwabuisi, C., Adegboro, B., Akanbi II, A.A., Fowotade, A. & Odimayo, M.S. (2009). Apparent rarity of *Entamoeba histolytica* and other intestinal parasites in acute and persistant diarrhoea patients attending Ilorin Hospitals: Time for ELISA antigen based amoebiasis diagnosis. *European Journal of Scientific Research* **3:** 388-397.

Farhana, F., Jamaiah, I., Rohela, M., Abdul-Aziz, N.M. & Nissapatorn, V. (2009). A ten year (1999-2008) retrospective study of amoebiasis in University Malaya Medical Centre (UMMC), Kuala Lumpur, Malaysia. *Tropical Biomedicine* **26:** 262-266.

Fotedar, R., Stark, D., Beebe, N., Marriott, D., Ellis, J. & Harkness, J. (2007). PCR detection of *Entamoeba histolytica*, *Entamoeba dispar*, and *Entamoeba moshkovskii* in stool samples from Sydney, Australia. *Journal of Clinical Microbiology* **45:** 1035-1037.

Frey, C.F., Zhu, Y., Suzuki, M. & Isaji, S. (1989). Liver abscess. *Surgical Clinics of North America* **69:** 259-271.

Freitas, M.A., Vianna, E.N., Martins, A.S., Silva, E.F., Pesquero, J.L. & Gomes, M.A. (2004). A single step dúplex PCR to distinguish *Entamoeba histolytica* from *Entamoeba dispar*. *Parasitology* **128:** 625-628.

Freedman, L., Maddison, S.E. & Elsdon-Dew, R. (1958). Monoxenic culture of *Entamoeba histolytica* derived from human liver abscesses. *South Africa Journal of Medical Science* **23:** 9-12.

Garcia, L.S. & Bruckner, D.A. (1997). Diagnostic Medical Parasitology. ASM Press, Washington, DC.

Garcia, L.S., Bruckner, D.A., Brewer, T.C. & Shimizu, R.Y. (1982). Comparison of indirect fluorescent-antibody amoebic serology with counter immunoelectrophoresis and indirect hemagglutination amoebic serologies. *Journal of Clinical Microbiology* **5:** 603-605.

Garcia, L.S., Shimizu, R.Y. & Bernard, C.N. (2000). Detection of *Giardia lamblia*, *Entamoeba histolytica/Entamoeba dispar* and *Cryptosporidium parvum* antigens in human faecal specimens using the Triage parasite panel enzyme immunoassay. *Journal of Clinical Microbiology* **38:** 3337-3340.

Gathiram, V. & Jackson, T.F.H.G. (1985). Frequency distribution of *Entamoeba histolytica* zymodemes in a rural South African population. *The Lancet* **8431:** 719-721.

Gathiram, V. & Jackson, T.F.H.G. (1987). A longitudinal study of asymptomatic carriers of pathogenic zymodemes of *Entamoeba histolytica*. *South African Medical Journal* **72:** 669-672.

Gatti, S., Swierczynski, G., Robinson, F., Anselmi, M., Corrales, J., Moreira, J., Montalvo, G., Bruno, A., Maserati, R., Bisoffi, Z. & Scaglia, M. (2002). Amebic infections due to *Entamoeba histolytica-E. dispar* complex: a study of the incidence in a remote rural area of Ecuador. *American Journal of Tropical Medicine and Hygiene* **67:** 123-127.

Gilman, R.H., Davis, C., Gan, E. & Bolton, M. (1976). Seroepidemiology of amoebiasis in the Orang Asli (Western Malaysian aborígenes) and other Malaysians. *American Journal of Tropical Medicine and Hygiene* **25:** 663-666.

Goh, K.L., Wong, N.W., Paramsothy, M., Nojeg, M. & Somasundaram, K. (1987). Liver abscess in the tropics: experience in the University Hospital, Kuala Lumpur. *Postgraduate Medical Journal* **63:** 551-554.

Gonzalez, C.R., Isibasi, A., Ortiz-Navarrete, V., Paniagua, J., Garcia, J.A., Ramirez, A., Salvatierra, B., Tapia, R., Sepulveda, J., Gutierrez, G. & Kumate, J. (1995). Prevalence of antibodies against *Entamoeba histolytica* in Mexico measured by ELISA. *Epidemiology and Infection* **115:** 535-543.

Gonzalez-Ruiz, A., Haque, R., Aguire, A., Castanon, G., Hall, A., Guhl, F., Ruiz-Palacios, G., Miles, M.A. & Warhurst, D.C. (1994). Value of microscopy in the diagnosis of dysentery associated with invasive *Entamoeba histolytica*. *Journal of Clinical Pathology* **47:** 236-239.

Guerrant, R.L. (1986). The global problems of amoebiasis: current status, research needs, and opportunities for progress. *Review of Infectious Disease* **8:** 218-227.

Hamimah, I., Zahedi, M. & Ainiyah, A.J. (1982). The prevalence of intestinal parasites among children at General Hospital, Kuala Lumpur, Malaysia. *Medical Journal of Malaysia* **37:** 373-377.

Hamzah, Z., Petmitr, S., Mungthin, M., Leeyayoova, S. & Chavalitshewinkoon-Petmitr, P. (2010). Development of multiplex real-time polymerase chain reaction for detection of *Entamoeba histolytica*, *Entamoeba dispar*, and *Entamoeba moshkovskii* in clinical specimen. *American Journal of Tropical Medicine and Hygiene* **83:** 909-913.

Haque, R., Faruque, A.S.G., Hahn, P., Lyerly, D. & Petri Jr., W.A. (1997). *Entamoeba histolytica* and *Entamoeba dispar* infection in children in Bangladesh. *Journal of Infectious Diseases* **175:** 734-736.

Haque, R., Ali, I.K.M., Akther, S. & Petri Jr., W.A. (1998). Comparison of PCR, isoenzyme analysis and antigen detection for diagnosis of *Entamoeba histolytica* infection. *Journal of Clinical Microbiology* **36:** 449-452.

Haque, R., Kress, K., Wood, S., Jackson, T.F.G.H., Lyerly, D., Wilkins, T. & Petri Jr., W.A. (1993). Diagnosis of pathogenic *Entamoeba histolytica* infection using a stool ELISA base don monoclonal antibodies to the galactose specific adhesin. *Journal of Infectious Diseases* **167:** 247-249.

Haque, R., Neville, L.M., Hahn, P. & Petri Jr., W.A. (1995). Rapid diagnosis of *Entamoeba* infection by using *Entamoeba* and *Entamoeba histolytica* stool antigen detection kits. *Journal of Clinical Microbiology* **33:** 2558-2561.

Haque, R., Ali, I.K.M., Sack, R.B., Farr, B.M., Ramakrishnan, G. & Petri Jr., W.A. (2001). Amoebiasis and mucosal IgA antibody against the *Entamoeba histolytica* adherence lectin in Bangladeshi children. *Journal of Infectious Disease* **183:** 1787-1793.

Haque, R., Duggal, P., Ali, I.M., Hossain, M.B., Mondal, D., Sack, R.B., Farr, B.M., Beaty, T.H. & Petri Jr., W.A. (2002). Innate and acquired resistance to amoebiasis in Bangladeshi children. *Journal of Infectious Disease* **86:** 547-552.

Haque, R., Huston, C.D., Hughes, M., Houpt, E. & Petri Jr., W.A. (2003). Current concepts: Amoebiasis. *New England Journal of Medicine* **348:** 1565-1573.

Hartini, Y. & Mohamed Kamel, A.G. (2009). *Entamoeba histolytica/E. dispar* infection among Aborigines at Pos Lenjang, Pahang. *Jurnal Sains Kesihatan Malaysia* **7:** 59-64.

Heckendorn, F., N'Goran, E.K., Felgar, I., Vounatsou, P., Yapi, A., Oettli, A., Marti, H.P., Dobler, M., Traore, M., Lohourignon, K.L. & Lengeler, C. (2002). Species-specific field-testing of *Entamoeba* sp. in an area of high endemicity. *Transaction of Royal Society of Tropical Medicine and Hygiene* **96:** 521-528.

Heyneman, D., Ramachandran, C.P., Balasingam, E. & Umathevy, T. (1967). Pulau Tioman: A combined parasitological survey. III. Preliminary observations of intestinal parasitism in the island population. *Medical Journal of Malaya* **21:** 265-268.

Hira, P.R., Iqbal, J., Al-Ali, F., Philip, R., Grover, S., D'Almeida, E. & Al-Eneizi, A.A. (2001). Invasive amoebiasis: challenges in diagnosis in a non-endemic country (Kuwait). *American Journal of Tropical Medicine and Hygiene* **65:** 341-345.

Hughes, M.A. & Petri Jr., W.A. (2000). Amoebic liver abscess. *Infectious Disease Clinics of North America* **14:** 565-582.

Hung, C.C., Chen, P.J., Hsieh, S.M., Wong, J.M., Fang, C.T., Chang, S.C. & Chen, M.Y. (1999). Invasive amoebiasis: an emerging parasitic disease in patients infected with HIV in an area endemic for amoebic infection. *AIDS* **13:** 2421-2428.

Hung, C.C., Deng, H.Y., Hsiao, W.H., Hsieh, S.M., Hsiao, C.F. & Chen, M.Y. (2005). Invasive amebiasis as an emerging parasitic disease in patients with human immunodeficiency virus type 1 infection in Taiwan. *Archive of Internal Medicine* **165:** 409-419.

Huston, C.D., Haque, R. & Petri Jr., W.A. (1999). Molecular-based diagnosis of *Entamoeba histolytica* infection. *Expert Reviews in Molecular Medicine* **22:** 1-11.

Jackson, T.F., Anderson, C.B. & Simjee, A.E. (1984). Serological differentiation between past and present infections in hepatic amoebiasis. *Transaction of*

Royal Society of Tropical Medicine and Hygiene **78:** 342-345.

Jackson, T.F., Gathiram, V. & Simjee, A.E. (1985). Seroepidemiological study of antibody responses to the zymodemes of *Entamoeba histolytica*. *The Lancet* **1:** 716-719.

Jackson, T.F. & Suparsad, S. (1997). Zymodeme stability of *Entamoeba histolytica* and *E. dispar*. *Archives of Medical Research* **28:** 304-305.

Jaco, J.V., Fieke, O., Eric, A.T., Alexis, N., Juventus, Z. & Anton, M.P. (2003). Prevalence of *Entamoeba histolytica* and *Entamoeba dispar* in northern Ghana. *Tropical Medicine and International Health* **8:** 1153-1156.

Jamaiah, I. & Rohela, M. (2005). Prevalence of intestinal parasites among members of the public in Kuala Lumpur, Malaysia. *Southeast Asian Journal of Tropical Medicine and Public Health* **36:** 68-71.

Jamaiah, I. & Shekar, K.C. (1999). Amoebiasis: A 10 year retrospective study at the University Hospital, Kuala Lumpur. *Medical Journal of Malaysia* **54:** 296-302.

Jones, W.R. (1946). The experimental infection of rats with *Entamoeba histolytica*; with a method for evaluating the anti-amoebic properties of new compounds. *Annals of Tropical Medicine and Parasitology* **40:** 130-140.

Kamel, A.G.M., Sham, K., Karen, L. & Norazah, A. (2002). Protozoan infection amongst Orang Asli (aborigines) community in Pangsoon, Malaysia. *International Medical Journal* **9:** 7-10.

Karim, R., Rahmah, N., Khairul, A., Mehdi, R. & Abdullah, B. (1995). Parasitic infections in the aboriginal community at Temengor, Hulu Perak, Malaysia. *Malayan Nature Journal* **48:** 425-432.

Katzenstein, D., Rickerson, V. & Braude, A. (1982). New concepts of amebic liver abscess derived from hepatic imaging, serodiagnosis and hepatic enzymesin 67 consecutive cases in San Diego. *Medicine (Baltimore)* **61:** 237-246.

Kebede, A., Verweij, J.J., Endeshaw, T., Messele, T., Tasew, G., Petros, B. & Polderman, A.M. (2004). The use of real-time PCR to identify *Entamoeba histolytica* and *E. dispar* infections in prisoners and primary-school children in Ethiopia. *Annals of Tropical Medicine and Parasitology* **98:** 43-48.

Khainar, K., Parija, S.C. & Palaniappan, R. (2007). Diagnosis of intestinal amoebiasis by using nested polymerase chain reaction-restriction fragment length polymorphism assay. *Journal of Gastroenterology* **59:** 49-58.

Klein, D. (2002). Quantification using real-time PCR technology: applications and limitations. *Trends in Molecular Medicine* **8:** 257-260.

Knappik, M., Borner, U. & Jelinek, T. (2005). Sensitivity and specificity of a new commercial enzyme-linked immuno-assay kit for detecting *Entamoeba histolytica* IgG antibodies in serum samples. *European Journal of Clinical Microbiology and Infectious Diseases* **24:** 701-703.

Kobayashi, S., Imai, E., Tachibana, H., Fujiwara, T. & Takeuchi, T. (1998). *Entamoeba dispar*: cultivation with sterilized *Crithidia fasciculate*. *Journal of Eukaryotic Microbiology* **45:** 3S-8S.

Krogstad, D.J., Spencer Jr., H.C., Healy, G.R., Gleason, N.N., Sexton, D.J. & Herron, C.A. (1978). Amoebiasis: epidemiologic studies in the United States, 1971-1974. *Annals of Internal Medicine* **88:** 89-97.

Lai, K.F.P. (1992). Intestinal protozoan infections in Malaysia. *Southeast Asian Journal of Tropical Medicine and Public Health* **23:** 578-576.

Lalla, F.D., Rinaldi, E., Santoro, D., Nicolin, R. & Tramarin, A. (1992). Outbreak of *E. histolytica* & *G. lamblia* infections in travellers returning from the tropics. *Infection* **20:** 78-82

Lebbad, M. & Svard, S.G. (2005). PCR differentiation of *Entamoeba histolytica* and *Entamoeba dispar* from patients with amoeba infection initially diagnosed by microscopy. *Scandinavian Journal of Infectious Disease* **37:** 680-685.

Leber, A.L. & Novak, S.M. (1999). Intestinal and urogenital amoeba, flagellates, and ciliates. In *Manual of Clinical Microbiology*, 7th ed., P.R., Murray, E.J.

Baron, M.A. Pfaller, F.C., Tenover, & Yolken, R.H. (eds.). American Society of Microbiology Press, Washington D.C., pg.1391-1405.

Leiva, B., Lebbad, M., Winiecka-Krusnell, J., Altamirano, I., Tellez, A. & Linder, E. (2006). Overdiagnosis of *Entamoeba histolytica* and *Entamoeba dispar* in Nicaragua: a microscopic, Triage parasite panel and PCR study. *Archives of Medical Research* **37:** 529-534.

Li, E. & Stanley Jr, S.L. (1996). Protozoa: Amoebiasis. *Gastroenterology Clinics of North America* **25:** 471-492.

Liang, S.Y., Chan, Y.H., Hsia, K.T., Lee, J.L., Kuo, M.C., Hwa, K.Y., Chan, C.W., Chiang, T.Y., Chen, J.S., Wu, F.T. & Ji, D.D. (2009). Development of loop-mediated isothermal amplification assay for detection of *Entamoeba histolytica*. *Clinical Microbiology* **47:** 1892-1895.

Lokman Hakim, S., Gan, C.C., Malkit, K., Noor Azian, M.M., Chong, C.K., Shaari, N., Zainuddin, W., Chin, C.N., Sara, Y. & Lye, M.S. (2007). Parasitic infections among Orang Asli (Aborigine) in the Cameron Highlands, Malaysia. *Southeast Asian Journal of Tropical Medicine and Public Health* **38:** 415-419.

Magana-Garcia, M. & Arista-Viveros, A. (1993). Cutaneous amoebiasis in children. *Pediatric Dermatology* **10:** 352-355.

Mahsol, H.H., Desa, Z.A.M., Jalil, M.F. & Ahmad, A.H. (2008). Gastrointestinal protozoan pararasites amongst school children in Inaman Sabah. *Borneo Science* **23:** 39-45.

Manukaran, M.N., Ahmad, H. & Abdullah, I. (1983). Amoebiasis with multiple colonic perforation and ruptured liver abscess – A case report. *Medical Journal of Malaysia* **38:** 71-73.

Martinez-Palomo, A.I., Gonzalez-Robles, B., Chavez, E., Orozco, S., Fernandez-Castelo, S. & Cervantes, A. (1985). Structural bases of the cytolytic mechanism of *Entamoeba histolytica*. *Journal of Parasitology* **32:** 166-175.

Mhlanga, B.R., Lanoie, L.O., Norris, H.J., Lack, E.E. & Connor, D.H. (1992). Amoebiasis complicating carcinomas - a diagnostic dilemma. *American Journal of Tropical Medicine and Hygiene* **46:** 759-764.

Ministry of Health Malaysia. (2008). WPR Memorandum from the Office of the WHO Representative for Brunei Darussalam, Malaysia, and Singapore. Ref V2/27/1.

Mirelman, D., Nuchamowitz, Y. & 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*. *Journal of Clinical Microbiology* **35:** 2405-2407.

Moran, P., Ramos, F., Ramiro, M., Curiel, O., Gonzalez, E., Valadez, A., Gomez, A., Garcia, G., Melendro, E.I. & Ximenez, C. (2005). *Entamoeba histolytica* and/or *Entamoeba dispar*: infection frequency in HIV+/AIDS patients in Mexico City. *Experimental Parasitology* **110:** 331-334.

Mori, Y., Nagamine, K., Tomita, N. & Notomi, T. (2001). Detection of loop-mediated isothermal amplification reaction by turbidity derived from magnesium pyrophosphate formation. *Biochemical and Biophysical Research Communications* **289:** 150-154.

Nagamine, K., Hase, T. & Notomi, T. (2002). Accelerated reaction by loop-mediated isothermal amplification using loop primers. *Molecular and Cellular Probes* **16:** 223-229.

Nawalinski, T. & Roundy, L.M. (1978). Intestinal parasitism in a Kampong on Pulau Pangkor, West Malaysia. *Southeast Asian Journal of Tropical Medicine and Public Health* **9:** 440-441.

Nazemalhosseini Mojarad, E., Nochi, Z., Sahebekhtiari, N., Rostami Nejad, M., Dabirri, H., Reza Zali, M., Kazemi, B. & Haghighi, A. (2010). Discrimination of *Entamoeba moshkovskii* in patients with gastrointestinal disorders by single-rounded PCR. *Japanese Journal of Infectious Disease* **63:** 136-138.

Niichiro, A., Nishikawa, Y., Yasukawa, A. & Haruki, K. (1999). *Entamoeba histolytica* outbreaks in institutions for the mentally retarded. *Japan Journal of Infectious Diseases* **52:** 135-136.

Nor Aza, A., Ashley, S. & Albert, J. (2003). Parasitic infections in human communities living on the fringes of the Crocker Range Park Sabah, Malaysia. ASEAN *Review of Biodiversity and Environmental Conservation (ARBEC)*. January-March 2003.

Noor Azian, M.Y., Lokman Hakim, S. & Maslawaty, M.N. (2006). Use of molecular tools to distinguish *Entamoeba histolytica* and *Entamoeba dispar* infection among the aborigines in Cameron Highlands. *Tropical Biomedicine* **23:** 31-36.

Noor Azian, M.Y., San, Y.M., Gan, C.C., Yusri, M.Y., Nurulsyamzawaty, Y., Zuhaizan, A.H., Maslawaty, M.N., Norparina, I. & Vythilingam, I. (2007). Prevalence of intestinal protozoa in an aborigine community in Pahang, Malaysia. *Tropical Biomedicine* **24:** 55-62.

Noor Hayati, M.I., Ali Azman, M. & Oothuman, P. (1981). Prevalens parasit usus pada kanak-kanak yang mengalami diarea di Hospital Besar, Kuala Lumpur. *Jurnal Perubatan Universiti Kebangsaan Malaysia* **3:** 28-37.

Noor Hayati, M.I., Sano, M., Mohammod, C.G., Norhayati, M., Rohani, A.K. & Halimah, A.S. (1998). Infeksi parasit usus pada masyarakat Orang Asli di Pos Piah, Sungai Siput (U) Perak. *Pascasidang Simposium Sains Kesihatan Ke 2*, *Universiti Kebangsaan Malaysia*, 24-25 April 1998 Puri Pujangga.

Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N. & Hase, T. (2000). Loop-mediated isothermal amplification of DNA. *Nucleic Acids Research* **28:** E63.

Ohnishi, K., Kato, Y., Imamura, A., Fukayama, M., Tsuunnoda, T. & Sakaue , Y. (2004). Present characteristics of symptomatic *Entamoeba histolytica* infection in the big cities of Japan. *Epidemiology of Infection* **132:** 57-60.

Ong, S.J., Cheng, M.Y., Liu, K.H. & Horng, C.B. (1996). Use of the ProSpecT microplate enzyme immunoassay for the detection of pathogenic and non-pathogenic *Entamoeba histolytica* in faecal specimens. *Transaction of Royal Society of Tropical Medicine and Hygiene* **90:** 248-249.

Pan American Health Organization (PAHO). (1997). *Epidemiology Bulliten* **18:** 13-14.

Parija, S.C. & Khairnar, K. (2005). *Entamoeba moshkovskii* and *Entamoeba dispar* associated infection in Pondicherry, India. *Journal of Health Polpulation and Nutrition* **23:** 292-295.

Park, W.B., Choe, P.G., Jo, J.H., Kim, S.H., Bang, J.H. & Kim, H.B. (2007). Amebic liver abscess in HIV-infected patients, Republic of Korea. *Emergency of Infectious Disease* **13:** 516-517.

Patterson, M. & Schoppe, L.E. (1982). The presentation of amoebiasis. *Medical Clinics of North America* **66:** 689-705.

Petri Jr., W.A. & Singh, U. (1999). Diagnosis and management of amoebiasis. *Clinical Infectious Disease* **29:** 1117-1125.

Petri Jr., W.A., Haque, R. & Mann, B.J. (2002). The bittersweet interface of parasite and host: Lectin-carbohydrate interactions during human invasión by the parasite *Entamoeba histolytica*. *Annual Review of Microbiology* **56:** 39-64.

Pillai, D.R. & Kain, K.C. (1999). Immunochromatographic strip-based detection of *Entamoeba histolytica-E. dispar* and *Giardia lamblia* coproantigen. *Journal of Clinical Microbiology* **37:** 3017-3019.

Pillai, D.R., Keystone, J.S., Sheppard, D.C., MacLean, J.D., MacPherson, D.W. & Kain, K.C. (1999). *Entamoeba histolytica* & *Entamoeba dispar*: epidemiology and comparison of diagnostic methods in a setting of non endemicity. *Clinical Infectious Diseases* **29:** 1315-1318.

Pinheiro, S.M.B., Carneiro, R.M., Aca, I.S., Irmao, J.I., Morais, M.A., Coimbra, M.R.M. & Carvalho, L.B. (2004). Determination of the prevalence of *Entamoeba histolytica* and *Entamoeba dispar* in the Pernambuco State of Northeastern

Brazil by a polymerase chain reaction. *American Journal of Tropical Medicine and Hygiene* **70:** 221-224.

Proctor, E.M. (1991). Laboratory diagnosis of amoebiasis. *Clinical Laboratory Medicine* **11:** 829-859.

Qvarnstrom, Y., James, C., Xayavong, M., Holloway, B.P., Visvesvara, G.S., Sriram, R. & Da Silva, A.J. (2005). Comparison of real-time PCR protocols for differential laboratory diagnosis of amoebiasis. *Journal of Clinical Microbiology* **43:** 5491-5497.

Rahmah, N., Ariff, R.H., Abdullah, B., Shariman, M.S., Nazli, M.Z. & Rizal, M.Z. (1997). Parasitic infections among aborigine children at Post Brooke, Kelantan, Malaysia. *Medical Journal of Malaysia* **52:** 412-415.

Ramos, F., Moran, P., Gonzalez, E., Garcia, G., Ramiro, M., Gomez, A., de Leon Mdel, C., Melendro, E.I., Valadez, A. & Ximenez, C. (2005). *Entamoeba histolytica* and *Entamoeba dispar*: prevalence infection in a rural Mexican community. *Experimental Parasitology* **110:** 327-330.

Ramos, F., Valdez, E., Moran, P., Gonzalez, E., Padilla, G., Gomez, A., Ramiro, M., Melendro, E.I., Munoz, O., Clark, C.G. & Ximenez, C. (2000). Prevalence of *Entamoeba histolytica* & *Entamoeba dispar* in a highly endemic rural population. *Archives of Medical Research* **31:** 34-35.

Ravdin, J.L., Jackson, T.F., Petri Jr., W.A., Murphy, C.F., Ungar, B.L., Gathiram, V., Skilogiannis, J. & Simjee, A.E. (1990). Association of serum antibodies to adherence lectin with invasive amoebiasis and asymptomatic infection with pathogenic *Entamoeba histolytica*. *Journal of Infectious Disease* **162:** 768-772.

Rivera, W.L., Santos, S.R. & Kanbara, H. (2006). Prevalence & genetic diversity of *Entamoeba histolytica* in an institution for the mentally retarded in the Philippines. *Parasitology Research* **98:** 106-110.

Rivera, W.L., Tachibana, H. & Kanbara, H. (1998). Field study on the distribution of *Entamoeba histolytica* and *Entamoeba dispar* in the noorthern Philippines as detected by the polymerase chain reaction. *American Journal of Tropical Medicine and Hygiene* **59:** 916-921.

Rivera, W.L., Tachibana, H., Silva-Tahat, M.R., Uemura, H. & Kanbara, H. (1996). Differentiation of *Entamoeba histolytica* & *Entamoeba dispar* DNA from cysts present in stool specimens by polymerase chain reaction: its field application in the Philippines. *Parasitology Research* **82:** 585-589.

Robert, R., Mahaza, C., Bernard, C., Buffard, C. & Senet, J.M. (1990). Evaluation of a new bicolored latex agglutination test for immunological diagnosis of hepatic amoebiasis. *Journal of Clinical Microbiology* **28:** 1422-1424.

Robinson, G.L. (1968). The laboratory diagnosis of human parasitic amoebae. *Transaction of Royal Society of Tropical Medicine and Hygiene* **62:** 285-294.

Rosenblatt, J.E., Sloan, L.M. & Bestrom, J.E. (1995). Evaluation of enzyme-linked immunoassay for the detection in serum of antibodies to *Entamoeba histolytica*. *Diagnostic Microbiology & Infectious Disease* **22:** 275-278.

Roy, S., Kabir, M., Mondal, D., Ali, I.K., Petri Jr., W.A. & Haque, R. (2005). Real-time PCR assay for diagnosis of *Entamoeba histolytica* infection. *Journal of Clinical Microbiology* **43:** 2168-2172.

Saeed, A., Al-Harithi & Manal, B. Jamjoom. (2007). Diagnosis and differentiation of *Entamoeba* infection in Makkah Al Mukarramah using microscopy and stool antigen detection test. *World Journal of Medical Science* **2:** 15-20.

Samie, A., Larry, C., Obi, Pascal O Bessong, Suzanne Stroup, Eric Houpt, Richard L Guerrant. (2006). Prevalence and species distribution of *Entamoeba histolytica* and *Entamoeba dispar* in the Venda Region, Limpopo, South Africa. *American Journal of Tropical Medicine and Hygiene* **75:** 565-571.

Sanchez-Giillen, M.C., Velazquez-Rojas, M., Salgado-Rosas, H., Torres-Rasgado, E., Perez Fuentes, R., Martinez-Munguis, J. & Talamas-Rohana, P. (2000). Sero-prevalence of anti-*Entamoeba histolytica* antibodies by IHA and ELISA assay in blood donors from Puebla. Mexico. *Archives of Medical Research* **31:** S53-S54.

Sargeaunt, P.G., Williams, J.E. & Grene, J.D. (1978). The differentiation of invasive and non-invasive *Entamoeba histolytica* by isoenzyme electrophoresis. *Transaction of Royal Society of Tropical Medicine and Hygiene* **72:** 519-521.

Sargeaunt, P.G., Jackson, T.F., Wiffen, S., Bhojnani, R., Williams, J.E., Felmingham, D., Goldmeir, D., Allason-Jones, E., Mindel, A. & Phillips, E. (1987). The reliability of *Entamoeba histolytica* zymodemes in clinical laboratory diagnosis. *Archives of Investigation Medical* **18:** 69-75.

Seydel, K.B., Li, E., Zhang, Z. & Stanley Jr, S.L. (1998). Epithelial cell-mediated inflammation plays a crucial role in early tissue damage in amebic infection of human intestine. *Gastroenterology* **115:** 1446-1453.

Shamsuzzaman, S.M., Haque, R., Hasin, S.K. & Hashiguchi, Y. (2000). Evaluation of indirect fluorescent antibody test and enzyme-linked immunosorbent assay for diagnosis of hepatic amoebiasis in Bangladesh. *Journal of Parasitology* **86:** 611-615.

Sharp, S.E., Suarez, C.A., Duran, Y. & Poppiti, R.J. (2001). Evaluation of the Triage Micro Parasite Panel for detection of *Giardia lamblia*, *Entamoeba histolytica/ Entamoeba dispar* and *Cryptosporidium parvum* in patient stool specimens. *Journal of Clinical Microbiology* **39:** 332-334.

Sheehan, D.J., Bottone, E.J., Pavletich, K. & Heath, M.C. (1979). *Entamoeba histolytica*: efficacy of microscopic, cultural and serological techniques for laboratory diagnosis. *Journal of Clinical Microbiology* **10:** 128-133.

Shenai, B.R., Komalam, B.L., Arvind, A.S., Krishnaswamy, P.R. & Rao, P.V. (1996). Recombinant antigen-based avidin-biotin microtiter enzyme-linked immuno-sorbent assay for serodiagnosis of invasive amoebiasis. *Journal of Clinical Microbiology* **34:** 828-833.

Sinniah, B., Sinniah, D., Singh, M. & Hussein, H. (1978). Parasitic infections among school children of Pulau Ketam. *Southeast Asian Journal of Tropical Medicine and Public Health* **9:** 272-276.

Sinniah, B. (1984). Intestinal protozoan and helminth infectins and control of soil transmitted helminths in Malay school children. *Public Health* **98:** 152-156.

Sirilak Sukprasert, Pongruj Rattaprasert, Zulhainan Hamzah, Oleg V. Shipin & Porntip Chavalitshewinkoon-Petmitr. (2008). PCR detection of *Entamoeba* sp. from surface and waste wáter samples using genus-specific primers. *The Southeast Asian Journal of Tropical Medicine and Public Health* **39:** 6-9.

Stanley, S.L., Jr. (2003). Amoebiasis. *Lancet* **361**: 1025-1034.

Stanley, S.L., Jr., Becker, A., Kunz-Jenkins, C., Foster, L. & Li, E. (1990). Cloning and expression of a membrane antigen of *Entamoeba histolytica* possessing multiple tandem repeats. *Proceedings of the National Academy of Sciences of the United States of America* **87:** 4976-498.

Stark, D., Sebastian, J., van Hal, S., Matthews, G., Harkness, J. & Deborah, M. (2008). Invasive amebiasis in men who have sex with men, Australia. Emerging Infectious Stanley Jr, S. L. Amoebiasis. *Lancet* **361:** 1025-1034.

Tachibana, H., Kobayashi, S., Nagakura, K., Kaneda, Y. & Takeuchi, T. (2000). Asymptomatic cyst passers of *Entamoeba histolytica* but not *Entamoeba dispar* in institutions for the mentally retarded in Japan. *Parasitology International* **49:** 31-35.

Tachibana, H., Kobayashi, S., Okuzawa, E. & Masuda, G. (1992). Detection of pathogenic *Entamoeba histolytica* DNA in liver abscess fluid by polymerase chain reaction. *International Journal of Parasitology* **22:** 1193-1196.

Tachibana, H., Kobayashi, S., Takekoshi, M. & Ihara, S. (1991). Distinguishing pathogenic isolates of *Entamoeba histolytica* by polymerase chain reaction. *Journal of Infectious Diseases* **164:** 825-826.

Takahashi, T., Gamboa-Dominguez, A., Gomez-Mendex, T.J., Remes, J.M., Martinez-Gonzalez, D., Gutierrez-Saldivar, J., Morales, J.C., Granados, J. & Sierra-Madero, J. (1997). Fulminant amoebic colitis: analysis of 55 cases. *Disease of Colon and Rectum* **40:** 1362-1367.

Tanyuksel, M. & Petri Jr., W.A. (2003). Laboratory diagnosis of amoebiasis. *Clinical Microbiology Review* **16:** 713-729.

Tanyuksel, M., Ulukanligil, M., Guclu, Z., Araz, E., Koru, O., Petri Jr., W.A. (2007). Two cases of rarely recognized infection with *Entamoeba moshkovskii. American Journal of Tropical Medicine and Hygiene* **76:** 723-724.

Thomas, V. & Yap, P.L. (1986). Immunodiagnosis and seroepidemiology of amoebiasis in Selangor, Malaysia. *Philippine Journal of Microbiology and Infectious Diseases* **15:** 48-52.

Troll, H., Marti, H. & 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. *Journal of Clinical Microbiology* **35:** 1701-1705.

Tshalaia, L.E. On a species of *Entamoeba* detected in sewage effluents. (1941) *Medical Parazitology (Moscow)* **10**: 244-252.

Tsai, J.J., Sun, H.Y., Ke, L.Y., Tsai, K.S., Chang, S.Y., Hsieh, S.M., Hsiao, C.F., Ye., J.H., Hung, C.C. & Chang, S.C. (2006). Higher seroprevalence of *Entamoeba histolytica* infection is associated with human immunodeficiency virus type 1 infection in Taiwan. *American Journal of Tropical Medicine and Hygiene* **74:** 1016-1019.

Van Doorn, H.R., Hofwegen, H., Koelewijn, R., Gilis, H., Peek, R., Wetsteyn, J.C., Van Genderen, P.J., Vervoort, T. & Van Gool, T. (2005). Use of rapid dipstick and latex agglutination tests and enzyme-linked immunosorbent assay for serodiagnosis of amoebic liver abscess, amoebic colitis and *Entamoeba histolytica* cyst passage. *Journal of Clinical Microbiology* **43:** 4801-4806.

Verweij, J.J., Blange, R.A., Templeton, K., Schinkel, J., Brienen, E.A., Van Rooyen, M.A., Van Lieshout, L. & Polderman, A.M. (2004). Silmultaneous detection of *Entamoeba histolytica*, *Giardia lamblia* and *Cryptosporidium parvum* in fecal samples by using multiplex real-time PCR. *Journal of Clinical Microbiology* **42:** 1220-1223.

Verweij, J.J., Blotkamp, J., Brienen, E.A., Aguirre, A. & Polderman, A.M. (2000). Differentiation of *Entamoeba histolytica* and *Entamoeba dispar* cysts using polymerase chain reaction on DNA isolated from faeces with spin columns. *European Journal of Clinical Microbiology and Infectious Diseases* **19:** 358-361.

Verweij, J.J., Oostvogel, F., Brienen, E.A., Nang-Beifubah, A., Ziem, J. & Polderman, A.M. (2003). Prevalence of *Entamoeba histolytica* and *Entamoeba dispar* in northern Ghana. *Tropical Medicine and International Health* **8:** 1153-1156.

Verweij, J.J., Vermeer, J., Brienen, E.A., Blotkamp, C., Laeijendecker, D., Van Lieshout, L. & Polderman, A.M. (2003). *Entamoeba histolytica* infections in captive primates. Parasitology Research **90:** 100-103.

Vijendran, M. (1977). The diagnosis and current treatment of liver abscess. *Medical Journal of Malaysia* **32:** 133-138.

Visser, L.G., Verweij, J.J., Van Esbroeck, M., Edeling, W.M., Clerinx, J. & Polderman, A.M. (2006). Diagnostic methods for differentiation of *Entamoeba histolytica* and *Entamoeba dispar* in carriers: performance and clinical implictions in a non-endemic setting. *International Journal of Medical Microbiology* **296:** 397-403.

Vreden, S.G.S., Visser, L.G., Verweij, J.J., Blotkamp, J., Stuiver, P.C., Aguirre, A. & Polderman, A.M. (2000). Outbreak of amoebiasis in a family in The Netherlands. *Clinical Infectious Diseases* **4:** 1101-1104.

Walsh, J.A. (1986). Problems in recognition and diagnosis of amoebiasis: estimation of the global magnitude of morbidity and mortality. *Review of Infectious Disease* **8:** 228-238.

Wang, L.T., Jen, G. & Cross, J.H. (1973). Establishment of *Entamoeba histolytica* from liver abscess in monoxenic cultures with hemoflagellates. *American Journal of Tropical Medicine and Hygiene* **22:** 30-32.

Wonsit, R., Thammapalerd, N., Tharavanij, S., Radomyos, P. & Bunnag, D. (1992). Enzyme-linked immunosorbent assay based on monoclonal and polyclonal antibodies for the detection of *Entamoeba histolytica* antigens in fecal specimens. *Transaction of Royal Society of Tropical Medicine and Hygiene* **86:** 166-169.

World Health Organization. (1997). World Health Organization/Pan American Health Organization/UNESCO report of a consultation of experts on amoebiasis. *Weekly Epidemiology Record* **72:** 97-99.

World Health Organization. (2004).Guidelines for Drinking-wáter Quality. Vol 1: 3rd ed.

Yi Chen, Yunzhi, Z., Bin, Y., Tangkai, Q., Hongzhou, L., Xunjia, C. & Hiroshi, T. (2007). Seroprevalence of *Entamoeba histolytica* infection in HIV-infected patients in China. *American Journal of Tropical Medicine and Hygiene* **77:** 825-828.

Zaman, S., Khoo, J., Ng, S.W., Ahmed, R., Khan, M.A., Hussain, R. & Zaman, V. (2000). Direct amplification of *Entamoeba histolytica* DNA from amoebic liver abscess pus using polymerase chain reaction. *Parasitology Research* **86:** 724-728.

Zengzhu, G., Bracha, R., Nuchamowitz, Y., Cheng, W. & Mirelman, D. (1999). Analysis by enzyme-linked immunosorbent assay and PCR of human liver abscess aspirates from patients in China for *Entamoeba histolytica. Journal of Clinical Microbiology* **37:** 3034-3036.

Zindrou, S., Orozco, E., Linder, E., Tellez, A. & Bjorkman, A. (2001). Specific detection of *Entamoeba histolytica* DNA by hemolysin gene targeted PCR. *Acta Tropica* **78:** 117-125.

Zurainee Mohamed Nor. (2003). Amebiasis ekstraintestinal di kalangan pesakit di Pusat Perubatan Universiti Malaysia. Prosiding Seminar Penyelidikan Jangka Pendek, Universiti Malaya, 11-12 Mac, 2003.