REVIEW
OF
LITERATURE
2.1 Historical overview of amoebiasis

*Entamoeba histolytica* was discovered in late 19th century by British surgeon, Timothy Richard Lewis (1870) in India from the faecal sample of a cholera patient. In 1875, a Russian physician, F. Losch first observed active amoebae containing red blood cells in stool sample of a relapsing dysentery patient from St. Peterburg, Russia. He named this amoeba as *Amoeba coli* and showed successful transmission of the disease using patient's dysenteric menstruum in one of the four dogs inoculated. In 1883, Koch described liver abscess in some complicated dysentery cases and observed identical amoebae as described by Losch earlier. Kartulis (1885-1891) in Egypt first established the link between amoebae and pathological processes by demonstrating amoebae in tissues beneath ulcers, in the pus of liver and in brain abscess. He could also transmit the disease in kitten using fecal material from a patient. Strong (1900) from Philippines showed that amoebae from intestine of man could be pathogenic or non-pathogenic depending on the ulceration produced in the animals. Fritz Schaudinn (1903) showed that the pathogenic and non-pathogenic amoebae were present in human intestine. He assigned the names of two human-infecting amoebae to genus *Entamoeba* Casagrandi and Barbagallo 1895, and named pathogenic amoeba as *Entamoeba histolytica* and the non-pathogenic as *Entamoeba coli*. The classical
experiment of Walker and Sellard (1893) on human volunteers who were fed with cysts of *E. histolytica* developed amoebic dysentery while others when fed with cysts of *E. coli* remained symptomless. Dobell (1919) showed that only *E. histolytica* was pathogenic and other intestinal amoebae (*E. coli, E. nana, I. butschlli* and *D. fragilis*) live as commensals. Councilman and Lafleur (1891) introduced the terms "amoebic dysentery" and "amoebic abscess of liver". They carried out detailed pathological studies which confirmed the presence of amoeba in the lesions in intestine and liver.

2.2 Epidemiology

Amoebiasis has been reported from almost all parts of the world including the industrially developed countries. According to a WHO estimate 10% of the world's population harbour this parasite in their bowel but only 1% develop symptoms of amoebiasis (Walsh, 1986). Majority of the patients develop diarrhoea and some present symptoms of dysentery. Hepatic involvement is in much less percentage of the infected population. Asian countries, some of the African countries and Mexico are endemic for amoebiasis. In Mexico, about 25% of the population were found to be infected with *E. histolytica* and 10% of this population showed symptoms of amoebiasis (1981). About 5% of the total population had antibodies to *E. histolytica*. Fatality rates in hepatic involvement cases was 2-13% annually

<table>
<thead>
<tr>
<th>Continent</th>
<th>No. of infections $\times 10^6$</th>
<th>No. of cases of disease $\times 10^6$</th>
<th>No. of deaths $\times 10^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>North and South America</td>
<td>90</td>
<td>10</td>
<td>10-30</td>
</tr>
<tr>
<td>Asia</td>
<td>290</td>
<td>15-30</td>
<td>20-50</td>
</tr>
<tr>
<td>Africa</td>
<td>80</td>
<td>10</td>
<td>10-30</td>
</tr>
<tr>
<td>Europe</td>
<td>20</td>
<td>0</td>
<td>none</td>
</tr>
<tr>
<td>Total</td>
<td>480</td>
<td>34-50</td>
<td>40-110</td>
</tr>
</tbody>
</table>
(Walsh, 1986). In Africa (sub-Saharan and tropical areas) the infection rate is as high as in Mexico (Walsh, 1986). In developed countries the problem of amoebiasis is less common and mainly found amongst immigrants, recent travelers and homosexuals. Approximately 100,000 cases of clinical illness are registered in USA each year.

Reports during the last 10-15 years from different parts of India indicate a 5-50% prevalence rate of amoebiasis. According to Cole (1976) the population of Indian carriers is about 30%.

It is difficult to carry out comparative evaluation of epidemiological data obtained from different parts of the world because 1) the sample size in most of the studies is inadequate and 2) methods employed for stool examination and assays for measuring serum anti- E. histolytica antibodies were different in terms of different sensitivity and measure different types of antibodies.

2.3 Biology and life cycle of Entamoeba histolytica

It exists in two forms, either as trophozoite (growing or feeding stage) or as cyst (infecting stage). The trophozoites are facultative, anaerobic, uninucleate organisms and measure 20-60 μm when observed in tissues in contrast to 7-30 μm when seen in stools of infected individuals.
Until 1968, the trophozoites were grown, *in vitro*, with bacteria but Diamond (1968) described a complex liquid growth medium, TPS-1 and later in 1978 another modified medium called TYI-S-33 (tryp ticase, Yeast extract, Iron and Serum) was used for axenic growth of *E. histolytica* trophozoites at low pH (6.0-6.8). Diamond and Cunnick (1991) published a method for yet another partially defined medium called PDM-805, which is serum free, for axenic cultivation of *Entamoeba* species.

In *In vitro* the trophozoites are grown in glass tubes or tissue culture flasks, where pseudopodial movement of the parasite can be observed. The cytoplasm can be divided into clear translucent ectoplasm and granular endoplasm. Trophozoites contain glycogen, digestive vacuoles, helical rods of ribosomes, microfilament like structure and actin, but do not contain cytoplasmic microtubule, mitochondria, rough endoplasmic reticulum, cytoplasmic tubulin, or golgi apparatus. The trophozoites of have rough surface and many phagocytic and acid phosphatase staining lysosomal vacuoles. In the lumen of the intestine, the trophozoites undergo encystation. They develop a chitinuous wall and the nucleus divides and redivides into four daughter nuclei to form cysts. These cysts are discharged in large numbers with the stool and infect fresh host through feco-oral route via contaminated food and water or by direct contact. Cysts after reaching the intestine of
the new host undergo another division of nuclei to form eight uninucleated trophozoites which emerge to complete the life cycle. Liver is the most common site of metastasis for this parasite. Owen (1987) showed liver involvement in inbred CBA mice when infected intraintestinally with *E. histolytica* strain SA 408 (zymodeme II), after six months all mice had amoeba infected liver although two of them had extensive typical lesions in the liver. Infected individuals form the reservoir of infection and pass up to 45 million cysts per day, which can survive in moist conditions for weeks or months.

2.4 Hosts

*E. histolytica* primarily infects humans but asymptomatic natural infection in macaque monkeys has been observed. Invasive amoebiasis in naturally infected New World and Old World monkeys with or without clinical disease have been demonstrated by Beaver *et al.* (1989). Experimental animals like hamsters, rats, cats, dogs, guinea pigs and rabbits can be infected with *E. histolytica* to develop symptoms of amoebiasis. Bhol *et al.* (1989) published a model of hepatic amoebiasis in random bred mice. Hamsters are susceptible to liver abscess formation but are resistant to colonic invasion unlike guinea pigs and rats which are more susceptible to colonic disease.

2.5 Pathogenic and non-pathogenic strains

In epidemiological surveys, the population is screened for the presence of parasite in the stool samples or
specific antibodies in circulation. It has been very commonly observed that only 10-20% of the infected population develop symptomatic amoebiasis. Possible mechanisms by which majority of the population escape the invasion by the parasite, needs to be explored. Whether it is the immune system of the individual or the non-pathogenicity of the *E. histolytica* strains, is yet to be ascertained. There is no obvious morphological distinction between pathogenic (Ps) and nonpathogenic (NPs) strains. In vivo inoculation of *E. histolytica*, in experimental animals, remains the oldest technique for demonstration of the virulence of the parasite. This is tedious, time consuming and expensive, thus may not always be feasible. Therefore, comparatively easier and rapid methods of correct identification of Ps and NPs are being developed. Sargeaunt *et al.* (1978) described isoenzyme electrophoresis for distinguishing Ps from NPs. Briefly, *in vitro* cultured trophozoites are harvested and the lysate is subjected to thin-layer starch gel electrophoresis. Banding pattern of glucose phosphate isomerase (GPI), L-malate:NADP⁺ oxido-reductase (ME), phosphoglucomutase (PGM), hexokinase (HK) is noted using specific substrates. The bands of GPI to PGM are labelled alphabetically from α - δ. *E. histolytica* grown in culture from clinical cases of invasive amoebiasis show the presence of a β band in the absence of an α in PGM.
This is confirmed by fast running bands in HK. The one exception to this rule is zymodeme XIII, which does not have advanced bands in HK. *E. histolytica* are grown in culture from asymptomatic subjects passing cysts that average 12.5 μm in diameter with 1-4 vesiculated nuclei and contain smooth edged chromatoids. These show an a band in PGM and have slow running band in HK. This technique is currently used for strain identification but retains the drawbacks of being tedious, time consuming and requiring sophisticated instruments.

Use of specific monoclonal antibodies has been demonstrated by Strachan *et al.* (1988). They have described immunofluorescence assay system using specific monoclonal antibodies (MAbs) for identifying Ps and NPs. Petri *et al.* (1990) have recently published a radioimmunoassay (RIA) system employing MAbs to different epitopes of galactose and N-acetylgalactosamine (gal/GalNAc) inhibitable lectin, which could differentiate all 18 pathogenic strains from 32 non-pathogenic strains tested. Blakely *et al.* (1990) have identified a unique 30 kDa surface antigen by Western blots with human immune sera on all 15 isolates of *E. histolytica* from patients with invasive amoebiasis but not on the other 15 strains from asymptomatic patients.

Recently, some investigators reported genetic variations among different strains of *E. histolytica* and suggested
molecular basis of pathogenicity. Initial reports of using specific DNA probes for differentiating Ps from NPs have come from Garfinkel et al. (1989). They used randomly cloned DNA sequences as probes and identified two different restriction fragment length patterns (RFLPs) from Ps and NPs. Tannich et al. (1989) constructed cDNA libraries from Ps and NPs. They selected cDNA clones specific to Ps using serum samples from invasive amoebiasis patients after absorbing them with NPs. Using this clone they isolated a homologous clone from the cDNA library of non-pathogenic amoebae. Nucleotide sequence analysis and comparison of the predicted amino acid sequences for both clones disclosed 12% evolutionary divergence in structure. Hybridization of both cDNA clones revealed two distinct Southern blot patterns, each characteristic of Ps and NPs. They proposed that the detection of a pair of evolutionarily related but distinct genes in Ps and NPs and the consistency with which these genes are present in the isolates argues for genetically defined sub-species of E. histolytica. But they failed to explain the possibility of phenotypic conversion in some strains, which contributes to virulence. Some strains e.g. SAW 1734R, have been shown to convert zymedeme while axenizing (Mirelman et al., 1986). E. histolytica has extrachromosomal circular DNA molecules containing rRNA genes (Bhattacharya et al., 1989). Huber et al. (1989)
found clusters of tandemly repeated PvuI-specific sequences (in Ps) and BamHI sequences (in NPs) on extrachromosomal DNA molecule. Bracha et al. (1990) reported successful identification and differentiation of 81 different isolates of E. histolytica from different parts of the world using DNA probes described by Huber et al., 1989. When DNA from different Ps and NPs hybridized, only one probe gave positive result with the strain tested. They observed good correlation amongst the three different techniques used e.g. zymodeme pattern analysis, reactivity with monoclonal antibodies specific for Ps and Southern hybridization. Mirelman et al. (1990) reported few sequences of PvuI found in SAW 1734R c1AR (NP) but which remained undetected due to low copy number. The copy number got amplified when there was zymodeme conversion from NP to P during axenization.

These are the most recent techniques available for identification of different strains of E. histolytica but it remains to be seen whether Ps and NPs are two different strains, subspecies or species.

2.6 Pathogenesis

The adherence of E. histolytica trophozoites with colonic epithelium seems to be a prerequisite for the invasion. It uses various mechanisms to destroy the host tissues e.g. proteolytic enzymes, cytotoxins, contact dependent cytolysis and phagocytosis. Numerous
proteolytic enzymes have been isolated from *E. histolytica* (Jarumilinta & Maegraith, 1989; Neal, 1960) but no correlation between enzymatic activity and pathogenicity was observed. There is no evidence to support that the secreted amoebic enzymes have cytolethal or cytolytic activity in tissue culture. The molecular mechanism of host cell lysis is not clear but it is obvious that the parasite uses the above mentioned mechanisms in one way or the other to bring about cell death of the host tissues. A number of cell lines, such as chinese hamster ovary cells (CHO), have been shown to adhere to *E. histolytica* trophozoites *in vitro* (Ravdin et al., 1980, Ravdin & Gurrent, 1981). They reported that millimolar concentrations of galactose or N-acetyl-D-galactosamine could inhibit the adherence. Furthermore asialooroso-mucoid (ASOR) and asialofetuin (ASF), were 1000 times more effective than galactose. Petri et al. (1989) isolated and purified gal/galNAc adherence lectin (205 kDa) of *E. histolytica*. This lectin consisted of two subunits viz. 170 & 35 kDa, identified in SDS-PAGE under reducing conditions. Petri et al. (1990) also developed monoclonal antibodies which recognized only 170 kDa subunit and could completely inhibit adherence. Similar results have been obtained using purified human and rat colonic mucins rich in galactose and GalNAc (Chadee et al., 1987). In addition, Kobiler and Mirelman (1980) have shown involvement of chitotriose (trimer or tetramer of N-
acetyle-D-glucosamine, 220 kDa). Ravdin et al. (1988) have demonstrated a rise in intracellular free calcium (Ca++) levels in target cells after adherence with amoeba. Elevated levels of (Ca++) preceded non-specific membrane permeability and death of the target cell. There was no significant increase in (Ca++) levels of amoeba. Galactose could abrogate this rise in (Ca++) and this effect could be reversed using purified amoebic gal/galNAc adherence lectin.

2.7 Clinical manifestations
Clinical manifestations of amoebiasis vary from asymptomatic infection to invasive fatal disease. Incubation period of the disease, before clinical symptoms appear, may vary from a few weeks to months or years. The three major categories of the disease are discussed below.

1. Asymptomatic infection: Majority of the infected individuals do not develop symptoms of amoebiasis and are classified as asymptomatic carriers which are cyst passers.

2. Intestinal amoebiasis: The milder form of the disease can be lower abdominal pain and diarrhoea. Bloody diarrhoea, dysentery and fever may develop in severe forms of the disease. In complicated intestinal amoebiasis cases, bowel perforation and peritonitis are very common but post-dysenteric ulcerative colitis is less common.
3. Extra-intestinal amoebiasis may or may not follow amoebic dysentery. Liver is the most common organ for E. histolytica to metastasize. The clinical symptoms may include weight loss, weakness and low grade fever or acute, febrile illness. Most of the patients complain of pain in the right hypochondrium region. If specific treatment is not provided, the liver may rupture and the infection may erode through the diaphragm into the thoracic cavity or into the pericardial sac and through the peritoneum into the peritoneal cavity. Embolic spread may result in abscess formation in other organs, sometimes in the brain. Cerebral abscess as a consequence of secondary amoebic infection of the lung, is very likely.

2.8 Chronic carriers
Acute amoebic infections can be treated with drugs yet some of the patients become chronic cyst passers or the so called "healthy carriers", become refractory to drugs and serve as reservoir of infection. They may develop the symptoms again, later in life depending on factors such as host microenvironment and local immunity of the gut and/or reinfection with the virulent strain of E. histolytica. Gathiram and Jackson (1987) have pointed out that about 1% of the asymptomatic carriers, in Durban area were harbouring pathogenic zymodemes and were positive for anti-E. histolytica antibodies. Only 10% of them developed amoebic colitis and the rest
remained asymptomatic and were spontaneously cured within one year. They proposed that the treatment of asymptomatic carriers containing pathogenic zymodemes is as important as treatment of patients suffering from invasive amoebiasis. Furthermore, the contacts of such patients should also be treated to ensure control of disease transmission. Baveja and her group (1990) have also laid emphasis on the treatment of such subjects. Majority of the infected population is constituted by these cyst passers therefore it is not practical and economically viable to treat all these patients. However, it has been suggested that those cyst passers involved in food handling should be effectively treated and subjected to regular stool examination for reinfection. A single course of drug treatment may not be effective, hence, special care and monitoring should be done while treating cyst passers along with routine hygienic practices to control the disease.

2.9 Immune response during the disease

E. histolytica induces both humoral and cellular immune responses in humans (Romia et al., 1988).

2.9.1 Humoral immunity

In majority of the invasive infections high levels of anti-E. histolytica antibodies are generated (Krupp, 1970; Juniper et al., 1972; Patterson et al., 1980), which does not seem to provide protection to the host but
serve as a diagnostic marker. These antibodies persist for few years in patients. Generally, asymptomatic infected individuals develop antibodies less frequently. A high serum titre could be suggestive of recent infection but there are no evidences in support. There is no correlation between severity of infection and antibody titres. Circulating levels of IgG class of antibodies are highest followed by IgM and IgA.

Maddison (1965) showed that the precipitation pattern (in gel diffusion) at the time of relapse in one patient with amoebic liver abscess and in two with amoebic dysentery were the same as found in initial examination, thereby suggesting that the antibodies were not protective. In in vitro studies, it has been shown that trophozoites either shed off or ingest human antibodies to E. histolytica (Calderon and Avila, 1986). Local immune response in the gut is not very well understood but coproantibodies of IgA class have been reported which do not persist for long (Mahajan et al., 1972). Circulating immune complexes have been reported in invasive cases of amoebiasis (Koster et al., 1981). Reed et al. (1983) demonstrated that majority of the pathogenic strains of E. histolytica are resistant to complement mediated lysis while non-pathogenic strains are susceptible.

2.9.2 Cellular immunity

Cell mediated immunity has been shown to be important in
limiting the invasive amoebiasis. Trophozoites of virulent strains of *E. histolytica* have been shown to kill neutrophils in vitro, in contrast to killing of the trophozoites of low virulence by the neutrophils. Guerrero *et al.* (1976) have shown the killing of trophozoites by lymphocytes obtained from ALA patient immediately after recovery. A soluble factor secreted from the lymphocytes in in vitro culture could kill the trophozoites as compared to the controls that could not secrete such factor where the trophozoites could kill the lymphocytes. An antibody-independent, contact-dependent mechanism of the trophozoites killing by gamma interferon activated macrophages has been shown (Ghadirian & Bout, 1988). Salata *et al.* (1984) have shown in vitro killing of virulent *E. histolytica* trophozoites using cytotoxic T lymphocytes. OKT8 antibodies could abrogate contact-dependent killing mechanism which has been demonstrated under the microscope (Salata *et al.*, 1984). Ghadirian *et al.* (1987) have demonstrated the importance of both macrophages and T lymphocytes in nude mice, in limiting the multiplication of *E. histolytica* trophozoites. Similarly Owen DG (1987) has shown the persistence of trophozoites in rnu rnu athymic rats even after 12 months, when fed orally with cultured trophozoites of two different strains of pathogenic zymodeme II. In contrast, in control Wistar rats remained infected but with no
apparant disease for six months; fecal samples from them became negative after one week. All the athymic rats were still infected at 12 months and amoebae could be cultured from their stools. A state of transient immunodepression facilitating amoeba to evade the defence mechanisms of the host was demonstrated by Gandhi et al. (1986), which could be removed by treatment and clinical recovery. Salata et al. (1986) have clearly demonstrated the decrease in T4 to T8 ratio in ALA patients compared to controls. DeLeon (1970) published an uncontrolled five year epidemiological follow-up study of 1029 ALA patients in Mexico city resulted in only 0.29% relapse. Salata et al. (1986) have reported that after recovery from ALA, specific cell mediated immune mechanisms develop that are also effective against the parasite in vitro.

2.10 Diagnosis

General clinical symptoms of amoebiasis are common to many other diseases for which etiology and treatment is entirely different, making differential diagnosis of amoebiasis very important. Diarrhoea due to E. histolytica can be confused with giardiasis and salmonellosis. Amoebic dysentery is most commonly confused with bacterial dysentery. Amoebic liver abscess must be distinguished from hydatid cyst and pyogenic liver abscess. Currently, the diagnosis of amoebiasis largely depends on clinical symptoms
supported by laboratory tests e.g. demonstration of the parasite in the stool or liver pus and *E. histolytica* specific antibodies in the serum (Healy, 1986).

### 2.10.1 Stool examination

Demonstration of the parasite in the stool sample is the most reliable proof of infection. Fresh stool sample should be examined for demonstration of trophozoites because they do not survive for more than thirty minutes, after passage of the stool. A direct wet mount in physiological saline is examined for trophozoites, three to four samples on separate days may result in 80-90% sensitivity (Sawitz and Faust, 1942). Staining and concentration methods for detection of cysts may improve the sensitivity of the test (Shetty and Prabhu, 1988). Formalin-ether technique used for concentration of cysts later replaced with formalin ethyl acetate (Young *et al.*, 1979). For preservation, the stool sample is fixed using 5-10% formalin after appropriate staining. In wet mount, the rounded trophozoites may be confused with trophozoites of *Entamoeba coli* or *Endolimax nana*. Trophozoites of *E. histolytica* with ingested red blood cells can be readily identified. Stool culture is done for confirmation of ambiguous results.

### 2.10.2 Serology

Detection of anti-*E. histolytica* antibodies in serum may help provide supportive evidence to clinical symptoms.
in the diagnosis of amoebiasis. Serology is routine procedure done for the diagnosis of amoebiasis. Various serological methods have been developed e.g. Indirect haemagglutination assay (IHA), Latex agglutination (LA), Immuno-electrophoresis (IEP), Countercurrent immunoelectrophoresis (CIEP), Agar gel diffusion (AGD), Indirect immunofluorescence (IIF), and Enzyme linked immunosorbent assay [ELISA].

Indirect haemagglutination is the most commonly used technique in which sheep red blood cells (SRBCs) coated with amoebic antigen are incubated with different dilutions of patients' serum. In a positive reaction SRBCs agglutinate and make a carpet of cells at the bottom of the well and in a negative reaction the SRBCs settle in the middle of the well giving a botton like appearance. A cut off titre is decided depending on the controls for discriminating positive reaction from negative. Krupp (1974) comparatively evaluated CIEP with IHA and immunodiffusion (ID) in the serodiagnosis of amoebiasis and found CIEP to be as sensitive as IHA although the former was more sensitive than ID. Similarly Katzenstein et al. (1982) showed CIEP to be more sensitive than agar gel diffusion (AGD). Goldman (1966) and Agarwal (1981) demonstrated complexity and low sensitivity and specificity of IIF as compared to IHA. Knobloch and Mannweiter (1983) found ELISA to be 100% sensitive within a week of onset of infection.
compared to IHA (72.7%), CFT (72.9%) and LA (60%).

Robert et al. (1990) have reported a new bicoloured latex agglutination (BLA) test for the detection of antibodies against *E. histolytica*, which is comparable to any other assay in terms of sensitivity and specificity. The five minute BLA shows promise as a good assay system for routine diagnosis and epidemiological surveys. Some investigators have reported that IgG is the major class of antibodies produced in response to invasive amoebic infection (Hock et al., 1989; Sather et al., 1988). Amongst different isotypes of IgGs, IgG4 is predominant compared to IgG 1, IgG 2, and IgG 3 (Kollaritsch et al., 1990). Aceti et al. (1989) suggested that *E. histolytica* can induce detectable level of circulating specific IgE without change in total serum IgE. Salivary anti-*E. histolytica* IgA class of antibodies for the diagnosis of intestinal amoebiasis was reported by del Muro et al. (1990). They obtained 85% sensitivity and 98% specificity was the best when correlated with stool examination reports. This assay could become a good candidate for immunoepidemiologic survey of intestinal amoebiasis in children. Detection of all isotypes of anti-amoebic antibodies results in higher sensitivity. Till recently most of the immunodiagnostic tests reported were complex, time consuming and laboratory based. Now efforts are on to develop field-based assays which
should be rapid, simple, highly sensitive and specific. One such development in rapid serology of amoebiasis is the immunoblot assay reported by Kumar et al. 1985. Followed by dipstick version developed by our group (Sharma et al., 1988). Dipstick dot-ELISA, in principle, is similar to the conventional plate ELISA but utilizes nitrocellulose as solid support for immobilization of antigen. The dipstick dot-ELISA can be performed in field conditions without any sophisticated instruments. The test takes 35 minutes to complete and the reagents can be packaged in a small handy box. Such technology can improve the diagnosis of amoebiasis in small and remont areas that do not have access to well equipped laboratories. Furthermore, to simplify collection storage and isolation of serum, many investigators (Mathews et al., 1980; Ganju et al., 1988), including our group have investigated the use of filter papers. These can later be eluted and used in the assays. The stability of such filter papers have been found to be three months at room temperature, which makes them ideal for transportation without a cold chain.

Detection of antibodies is an indirect way of diagnosis therefore one should look for the presence of antigen(s). Antibody detection tests have some inherent drawbacks e.g. (1) not all invasive amoebiasis patients develop high levels of antibodies, (2) antibodies persist in circulation for a long period of time, hence
positivity may indicate past exposure to the parasite, (3) only 40-60% of intestinal amoebiasis patients develop antibodies, (4) the level of antibodies cannot be correlated with the severity of the disease.

2.10.3 Detection of antigen(s)
Sandwich ELISA using polyclonal and/or monoclonal antibodies for detection of *E. histolytica* antigen(s) have been reported. Pillai and Mohimen (1982) have reported a solid phase sandwich RIA for the detection of immune complexes in circulation using polyclonal anti-*E. histolytica* sera from rabbit and patients of invasive amoebiasis in various combinations. Although they report 100% sensitivity and specificity, the assay is complex and involves extensive purification of the polyclonal antibodies. Mahajan *et al.* (1974) used CIEP technique to detect antigens in liver pus of ALA cases using rabbit polyclonal serum. They found 92% sensitivity and 100% specificity. Irshad *et al.* (1985) have described the use of immunofluorescence technique to detect antigen in liver pus of ALA patients using antibodies from ALA patients. They found 85.7% sensitivity and 100% specificity. All the tests described above are not field based and requires well equipped labs. The emphasis now is on development of simple field based assays.

Sandwich ELISA for detection of *E. histolytica* specific
antigen was first reported by Root et al. 1978. Initially, polyclonal antibodies were used in the assays (Grundy et al., 1987) but due to the problems of batch to batch variation, limited availability and the extensive purification procedures required for the assay, led investigators to use specific monoclonal antibodies (Ungar et al., 1985). The utility of ELISA is evident from the data of Merino et al. 1990. Of the 150 patients with gastrointestinal symptoms, they found 116 were ELISA positive for antigen in feces. Of these only 52 showed E. histolytica by rectosigmoido-scopy of rectal smear and a triple stool search for ova-bacteria-parasite (OBP). All the ELISA positive patients recovered after antiamoebic treatment and the symptoms disappeared. The reason for the low accuracy obtained with the standard diagnostic methods, OBP and rectal profile may be attributed to are inadequate sample collection, and deficient preservation. Antiparasitic or antibacterial treatment prior to sample collection together with inexperience and deficient lab skill and limited time available for performing the examinations also contribute to error. These problems can be overcome by ELISA, which detects specific antigen rather than the whole parasite.

2.11 Vaccine

Vaccination in an endemic area may be of use in limiting the morbidity and mortality due to amoebiasis. Most
vaccine trials, using crude whole or fractionated sonicate of axenic *E. histolytica* trophozoites, have been conducted on experimental animals. There is no data available of such studies on humans. Initially, crude sonicate of whole amoeba was used for vaccination followed by intracecal challenge with amoeba. Swartzwelden and Muller (1950) reported 37% protection in rats. Krupp (1974) fractionated *E. histolytica* sonicate and showed complete protection in guinea pigs immunized intra-muscularly with the highest molecular weight fraction and partial protection with lower molecular weight fraction. Ghadirian et al. (1978, 1980) showed complete protection from ALA in hamsters when intradermal immunization with axenic *E. histolytica* trophozoites or crude sonicate was followed by intrahepatic inoculation with amoeba. Immunity can be transferred from vaccinated hamsters to normal by transferring peritoneal or spleen cells (Ghadirian and Meerovitch., 1983) thereby preventing normal hamsters from hepatic amoebiasis. Complete protection from amoebiasis in experimental models have been demonstrated by many investigators. However, due to lack of such data from human subjects, it is very difficult to predict the usefulness of these protective antigens in man.