INTRODUCTION
1.1 Introduction

Amoebiasis is caused by a unicellular protozoan parasite, *Entamoeba histolytica*. It belongs to the order Amoebida of the class Rhizopoda in the phylum Protozoa. According to WHO technical report series (1969) "amoebiasis can be defined as a state of harbouring amoeba". WHO estimated that about 10% of the world population harbours this parasite and that about 10% of this infected population developing symptoms of amoebiasis. Symptomatic amoebiasis can be intestinal and/or extraintestinal. Amoebiasis accounts for about 48,000 deaths annually (WHO, 1985). Trophozoites of *E. histolytica* reside in the large intestine and multiply by binary fission. Some of these stop dividing and develop chitinous wall to form cysts. These cysts, when passed out in faeces, can infect other healthy individuals by feco-oral route. Cysts resist the acidic pH in stomach and in the terminal part of the ileum they excyst and a four nucleated amoeba is liberated which divides into four uninucleated trophozoites. These trophozoites can establish in the colon. Generally, trophozoites are discharged with the stool in the acute phase of diarrhoea or dysentery; while on the other hand, cysts are discharged in non-diarrhoeal cases. Symptomatic intestinal amoebiasis can be mild diarrhoea, dysentery, colitis, amoeboma or ulcerative post dysenteric colitis.
Extraintestinal amoebiasis leads most commonly to liver abscess. If the treatment is not provided, the infection may spread to other organs such as the lungs or even the brain. Amoebiasis can be cured effectively using amoebicides such as metronidazole and tinidazole provided, correct and timely diagnosis is made.

1.2 Diagnosis
Diagnosis of intestinal amoebiasis largely depends on demonstration of trophozoites or cysts in the stool samples. In case of amoebic liver abscess, clinical symptoms such as pain in the right hypochondrium, fever, liver abscess (ultrasonography), needle aspirate and serological tests are taken into account.

General clinical symptoms of amoebiasis are shared by many other diseases for which etiology and treatment is entirely different. Diarrhoea due to *Entamoeba 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, hence differential diagnosis of amoebiasis is very important. At present, diagnosis of amoebiasis largely depends on clinical symptoms supported by laboratory investigations e.g. demonstration of the parasite in the stool or liver pus and *Entamoeba histolytica*-specific antibodies in serum.
1.2.1 **Stool examination**

Demonstration of parasite in the stool sample is the most reliable proof of infection. Fresh fecal sample should be examined for demonstration of trophozoites because these do not survive beyond half an hour after passage of the stool. A direct wet mount in physiological saline is examined for trophozoites. Examination of three to four samples on separate days may result in 80-90% sensitivity. Staining and concentration methods for detection of cysts may improve the sensitivity of the test. Formalin-ether technique is used for concentration of cysts which has now been replaced with formalin ethyl acetate. For preservation, the stool sample is fixed using 5-10% formalin after appropriate staining. In the wet mount, rounded *Entamoeba coli* and *Endolimax nana* trophozoites may be mistaken for *E. histolytica* but the latter can be readily identified if ingested red blood cells are seen within the organism. If microscopic examination does not give unequivocal results, stool culture is recommended.

1.2.2 **Serology**

More than 95% of amoebic liver abscess (ALA) patients develop high levels of anti-*E. histolytica* antibodies. Detection of these antibodies in serum of patients suffering from invasive amoebiasis is of high diagnostic value. Various serological methods have been developed e.g. Indirect haemagglutination (IHA), Latex
agglutination [LA], Immuno-electrophoresis [IEP], Countercurrent immunoelectrophoresis [CIEP], Agar gel diffusion (AGD), Indirect immunofluorescence and Enzyme linked immunosorbent assay [ELISA]. In these assays crude sonicate of *E. histolytica* trophozoites is used and the antigen antibody reaction is detected either by agglutination of sheep red blood cells (IHA) or latex particles (LA) whereas in IEP, CIEP and AGD precipitation of antigen and antibody complex is observed. In ELISA and IIF revealing antibody is tagged with enzyme and fluorescent dye respectively to put into evidence the antigen and antibody reaction. AGD and IIF are less sensitive compared to IHA and CIEP. ELISA being most sensitive is chosen in the present study. All these methods are laboratory based requiring long incubation period, sophisticated instruments and expert personnel.

In order to develop simple, rapid, highly sensitive and specific immunodiagnostic tests for amoebiasis the present study was undertaken.

1.3 **Aims and objectives**

1. Development of a simple and rapid dot-enzyme linked immunosorbant assay (dot-ELISA) for detection of anti-amoebic antibodies in patients suffering from amoebic liver abscess. Development of a finger-prick assay for dot-ELISA.
2. Study of titres of IgG, IgM and IgA classes of antibodies in present and past infections.

3. Development of specific murine monoclonal antibodies against *Entamoeba histolytica*.

4. Development of enzyme immunoassay for the detection of antigen(s) in stool samples (for intestinal amoebiasis) using monoclonal and/or polyclonal antibodies.