INTRODUCTION
1. INTRODUCTION

Global estimates reveal that diarrhoeal disease causes nearly 5 million deaths every year in children under 5 years of age (Snyder and Merson, 1982). In India, the annual diarrhoea related death rate in children is around 1.5 million. After Rotavirus, *Escherichia coli* and *Shigella*, *Giardia* is the fourth largest group which causes high morbidity and infant mortality. According to a survey conducted by Mata *et al* (1972), *Giardia lamblia* is responsible for about 5% of acute and perhaps 20% of chronic cases of diarrhoeal illness.

*Giardia lamblia*, an intestinal protozoan parasite, is a frequent cause of both epidemic (Shaw *et al*, 1977; Lippy, 1978) and endemic (Islam *et al*, 1983) diarrhoeal illness in developed and developing countries. Infection is usually self-limiting (Wolfe, 1984) though some infected individuals may suffer from chronic or fulminating diarrhoea, if untreated. The potential pathogenicity of the flagellate protozoan *G. lamblia*, earlier considered to be an inconsequential commensal, has been increasingly appreciated in the last decade. Nevertheless, the mechanism of disease caused by this protozoan resident in the human host remains an enigma (Meyer and Radulescu, 1979).

The convention has prevailed that each host species has its own named *Giardia* species (Ansari, 1952) which has resulted in over 50 so named *Giardia* species. Little good evidence exists that each *Giardia* is, indeed, different from the other, either biologically or antigenically (Meloni *et al*, 1987; Nash, 1989). However, reports of transmission of human *Giardia* to specific pathogen free beagles does lend support to the
Giardia may be transmitted across the species barrier (Davies and Hibler, 1978).

Giardiasis in humans present a broad clinical spectrum. The majority of the infected persons are probably asymptomatic. Illness ranges from mild disease to severe malabsorption, diarrhoea and weight loss. Electron microscope studies have demonstrated alteration of enzyme-rich micro-villi of the epithelial cells with blunting and deformation of these structures, under the overlying sucking disc of trophozoites (Erlandsen & Chase, 1974). The dominant unanswered question which currently prevails relates to the relative contributing factors which manifests the variable clinical profiles of the disease.

Epidemiological observations on several giardiasis outbreaks (Moore et al., 1969; Istre et al., 1984) suggest that individuals repeatedly exposed to G. lamblia have a lower incidence of infection and symptoms than the newly exposed persons. This suggests that prior exposure imparts partial resistance to reinfection. The high frequency of giardiasis in patients with hypogammaglobulinemia (Ament et al., 1973) and the demonstration of spontaneous resolution of infection in man and experimental animals (Roberts-Thompson et al., 1976) has strongly suggested the existence of a protective immune response to Giardia. However, analysis of the immune response has only become possible in recent years with the development of methods for the culture of Giardia species in synthetic medium (Meyer, 1976). Investigators seeking to demonstrate circulating humoral immune response to Giardia in humans have established that measurable circulating antibodies develop after infection. The biological role of these antibodies remain conjectural. Presence of circulating antibody was shown by different serological tests by various groups of workers.
using standard *G. lamblia* antigens (Visvesvara et al., 1980; Smith et al., 1981). Cell mediated immune response in *giardiasis* is poorly understood although a role of T-lymphocyte has been established in hypothymic mice (Roberts-Thompson and Mitchell, 1978).

Assessment of the importance of cellular and humoral immunity in controlling *G. lamblia* infection ultimately involves identification of relevant parasite components responsible for it. Moore *et al* (1982) have fractionated the soluble antigen on high performance liquid chromatography (HPLC) and demonstrated that the immunological activity lies with the high molecular weight first fraction. However, report on the expression or association of this antigen on the surface of the parasite are yet to be ascertained. It is now believed that the surface of the parasite is a vital component in the interactions with the host to bring about successful parasitism (Chang and Fong, 1983). In a series of labelling and immunoprecipitation of surface antigen(s) with *giardiasis* patients sera different workers detected a single major polypeptide or series of major polypeptides (Einfeld and Stibbs, 1984; Edson *et al*, 1986; Clark and Holberton, 1986 and Wenmann and Taylor, 1987). However, the proteins iodinated by these iodination techniques do not represent the whole spectrum of membrane proteins which are antigenic in nature as the chemicals used (lactoperoxidase and IODOGEN), iodinate mainly the tyrosine residues of protein. Therefore, surface proteins having a low content of tyrosine, histidine, tryptophan and sulphydryl group will not be iodinated by surface labelling but may still be an abundant surface antigen (Upcroft *et al*, 1988). Studies are still needed to find out the particular proteins on surface of *G. lamblia* which are immunologically important in human *Giardia* infection. The glycoprotein nature of the *Giardia* membrane antigen was ascertained by biochemical analysis (Einfeld and Stibbs,
1984). The protein part was linked with N-acetyl-D-glucosamine sugar which serve as the specific receptor for wheat germ agglutinin (WGA) lectin (Ward et al, 1988). However, this requires further confirmation with other plant lectins. Killing of live G. lamblia by antisera has been reported (Hill et al, 1984). However, it needs further evaluation with antisera raised against biologically active antigens.

The existence of zoonotic reservoir (Meloni et al, 1988) and confusion of taxonomy of the genus Giardia (Bertram et al, 1983) has made it impossible to ascertain whether the human G. lamblia species is homogeneous or consisting of multiple strains with differences in antigenic and biological activity. Since strain differences contribute to the variation in pathogenicity, clinical manifestation and immune response (Nash et al, 1987), it will be of immense importance to detect biochemical and immunological differences among the isolates of G. lamblia infecting humans of same and different geographic areas.

In view of the above, the present study was designed with the following major Objectives:

a) To analyse the antigenic components of G. lamblia; identification and purification of immunologically active fraction and its subsequent localization on the parasite.

b) To isolate and purify the plasma membrane antigens of G. lamblia; analysis of its biochemical nature and immunological relevance in the host immune response.

c) To identify and characterize the immunodominant antigen(s) of plasma membrane and its possible use in future diagnosis and prophylaxis of giardiasis cases.
d) To investigate the role of circulating antibodies in different categories of giardiasis cases and Giardia free controls and development of a test system for routine serodiagnosis of current Giardia infection.

e) Lastly, to isolate and axenize the local strains of G.lamblia from different categories of giardiasis patients and to delineate the biochemical and immunological differences, if any, among the strains of human G.lamblia isolated from same or diverse geographic areas.