REVIEW OF LITERATURE
2. REVIEW OF LITERATURE

2.1 History and Nomenclature

Giardia lamblia, the intestinal protozoan with a long and venerable history of about 300 years, remains even today, a biological enigma. The great Dutch microscopist Anton Van Leeuwenhoek, who recovered it from his own stool in 1681, is credited for its discovery (Dobell, 1920). The first report on the morphology of Giardia are those of Lambl (1859 and 1860) and Cunningham (1881). Subsequent to these early investigations, there was a flurry of activity in the light microscopic descriptions of Giardia resulting in the classic illustrations of trophozoites by Simon (1921), Hegner (1922), Kofoid and Swezy (1922) and Filice (1952). A lack of agreement regarding nomenclature in this group has existed from the time this organism was first described and continues to result in confusion. The nomenclatural problems involve both genus and species. Two generic names, Giardia and Lamblia are presently used to describe these organisms. The genus name Giardia was established by Kunstler (1882). However, Blanchard (1888) suggested that the genus be named Lamblia to commemorate Lambl (1859). It was Stiles (1915) who named this parasite as Giardia lamblia.

During the 19th Century, a number of different Giardia species were described in a wide variety of animals. The criteria that have been used to identify Giardia species include host specificity and morphological features such as body dimensions, variations in median body structure and morphology (Meyer and Radulescu, 1979; Filice, 1952). Based on those criteria Giardia are grouped into: Giardia agilis, G.muris and G.duodenalis found in amphibians, rodents and humans, respectively. While this scheme is simple, it does not provide a means for further
characterizing isolates within the three morphological groups. Within the
_**G.duodenalis**_ complex, organisms have been further speciated on the basis
of presumed host specificity and morphometrics. These criteria have resul-
ted in the description of more than 40 species (Kulda and Nohynkova,
1978).

The validity of _**Giardia**_ host-specificity has become increasingly
controversial. Recent evidences suggest that at least some _**Giardia**_ species
are capable of infecting more than one species of animals (Davies and
Hibler, 1978; Grant and Woo, 1978; Hewlett et al., 1982 and Thompson,
1988). Furthermore, circumstantial evidences suggest that human infections
may be acquired by ingestion of cysts from lower mammals (Dykes et al.,
1980). However, conflicting interpretations of cross-species transmission
of _**Giardia**_ arises doubts regarding the validity of using host specificity
as a major criterion for speciation (Bemrick, 1984; Woo and Peterson,
1986). Once separated from the animal host, it is not possible to unequi-
vocally identify the source of _**G.duodenalis**_ trophozoites by morphometrics
analysis. Erlandsen and Bemrick (1987) isolated _**G.psittaci**_ from budgeri-
gars (parakeets) which were morphologically distinct from _**G.duodenalis**_
group in that they lacked the marginal groove bordering the ventral adhe-
sive disc. This might suggest an evolutionary divergence in the genus
_Giardia._

### 2.2 Morphology and life cycle

_**Giardia lamblia**_ exhibits two stages in its life cycle, one is the
trophic stage while the other is the cystic stage. The flattened tear
drop shaped trophozoites are 5.7 μm wide, 10-12 μm long and 1-2 μm thick.
The dorsal surface is round and usually smooth but can have pebbled appear-
ance, depending on the hydration and surface vesicular activity
(Owen, 1980). The ventral surface is concave with a prominent anterior adhesive disc. It is not bilaterally symmetrical but is composed of a clockwise spiral of microtubules. Using SDS-gel electrophoresis, Feely et al. (1982) have presented evidence for the presence of actin, myosin and tropomyosin in the peripheral rim of the ventral disc of trophozoites. Holberton and Ward (1981) have shown that Triton-X-100 insoluble fractions of isolated disc consists of two major proteins—one tubulin and another a 30,000 dalton protein. The distribution of these contractile proteins in the ventral disc suggests their involvement in controlling the diameter of the disc. There are eight posteriorly directed flagella which emerge in pairs. The fine structure of the axonemes of the eight flagella is the typical 9+2 arrangement of microtubules common to most eukaryotic flagella. Electron microscope study of trophozoites reveals two ovoid nuclei, a pair of median bodies which is unique to this genus and has been used as a taxonomic tool. In G. lamblia, the median body is described as "hammer claw" in shape and lies transversely in the mid-portion of the cell body.

The cyst form is ellipsoidal, 8-12 μm long and 7-10 μm wide. The highly refractile cyst wall is approximately 0.3 μm thick (Sheffield and Bjcrvatn, 1977) and is remarkably flexible and resistant to environmental changes (Luchtel et al., 1980). Mature cysts have four nuclei with basal bodies and axial filaments located between the nuclei (Chatterjee, 1980). Infection with G. lamblia occurs as a result of ingestion of cysts. Excystation is initiated in the stomach at an optimal pH of 2.0 (Bingham and Meyer, 1979). The flagellated binucleate trophozoites colonize the distal duodenum and proximal jejunum (Owen et al., 1979) and maintain their position in the unstirred layer of mucus at the base of villi by adhesion to the microvillous brush border.
2.3 **Epidemiology**

Giardiasis is a disease of worldwide importance. Asymptomatic *G. lamblia* infections in industrialized countries is estimated to vary from 1 to 7 percent. The corresponding rate in developing countries is even higher, ranging from 5 to 50 percent (DuPont and Sullivan, 1986). Important loci of the parasite in the developed world are day care centres or residential areas of the mentally retarded persons where hygiene practices are unsatisfactory (Pickering et al, 1981 and 1984 and Keystone et al, 1984).

According to a survey conducted by the Center for Disease Control (CDC), USA, giardiasis was regarded as the number one intestinal parasitic infection in USA (Visvesvara, 1981). In recent years, waterborne outbreaks of giardiasis have been reported in North America from New York State (Shaw et al, 1977), New Hampshire (Lippy, 1978), Utah (Barbour et al, 1976) and Washington (Dykes et al, 1980). Other outbreaks have been reported from Leningrad (Fiumara, 1973 and Brodsky et al, 1974) and Island of Madeira (Lopez et al, 1978).

Imported giardiasis represents a considerable proportion of the reported cases, particularly travellers returning to the United States from Leningrad in Soviet Union (Brodsky et al, 1974). Giardiasis is also frequent in overland travellers across the Europe and Middle East to the Indian subcontinent. An outbreak has been reported on a Mediterranean cruise (Thompson et al, 1974). Islam et al (1983) have reported an incidence of as high as 82% in lactating mothers and 42% in their infants from Bangladesh. A seroepidemiological study by Gilman et al (1985) demonstrated that the disease was prevalent in 21% in normal (5–10 years old) and 51% in malnourished children (1–5 years of age).
G. lamblia infection has been reported from almost every part of India. The reported incidence has varied from 4.2 to 17 percent (Prakash and Tandon, 1966; Tewari and Tandon, 1974; Das et al, 1981 and Gupta and Kelkar, 1981) with greater frequency in children (Mir et al, 1979 and Walla et al, 1986). Sen et al (1985), reported an incidence of 12% G. lamblia infection among children (2-5 years) in hospitalized acute diarrhoea cases in Calcutta.

The common source of transmission is faecal-oral route (Owen, 1984). However, person to person transmission in household or institutional crowding combined with poor hygienic practices have been reported by various group of workers (Black et al, 1977; Keystone et al, 1978; Knight, 1978; Pickering et al, 1981 and Keystone et al, 1984). Bryan (1975) and Osterholm et al (1981) have demonstrated transmission via uncooked food contaminated with night soil. Giardia infected beavers and muskrats in infected water sheds had probably served as an amplification reservoir (Dykes et al, 1980). The high incidence of Giardia among homosexual males (12-13%) provides additional evidence for person to person transmission (William et al, 1978; Keystone et al, 1980 and McMillan, 1980). Campers in North America have become infected from drinking untreated stream water (Wolfe, 1989).

2.4 In vitro cultivation of the parasite

A search of the early literature yields three reports of successful cultivation of Giardia from humans, a half century or more ago. Chatterjee (1927) inoculated a portion of human stool containing motile trichomonads and Giardia cysts into normal saline in a test tube, which was sealed. By transferring a portion of the deposit to another tube in the same way, motile Giardia and trichomonads were maintained for 5 weeks.
Poindexter (1931) was able to induce excystation of Giardia cysts from humans and kept the resultant trophozoites alive for 19 days on Boeck-Drbohlav's medium and for 11 days on liver infusion agar. The number of organisms observed in successive subcultures led Poindexter (1931) to believe that the organism genuinely multiplied instead of mere excystation and trophozoite survival. Five media, all modifications of a pig liver-water bouillon, were initially used for Trichomonas culture. The most favourable medium tested contained bouillon at 15% concentration, an initial pH of 6.4 and penicillin and colimycin. The result of these workers suggested that heavy bacterial growth was responsible for the ultimate death of this protozoa. In 1960, Kerapetyan reported the culturing of Giardia trophozoites isolated from human duodenal contents, for 7 months in a complex medium, which contained yeast like fungi and chick fibroblasts.

By substituting chick fibroblast with human, horse or bovine serum, Karapetyan (1962) successfully cultivated Giardia isolated from rabbits. Soloviev (1971) observed that a low concentration (10%) of serum gave much better results in the isolation of Giardia strains than the 25% serum concentration employed by Karapetyan (1962). Meyer and Pope (1965) reported failure to culture rabbit Giardia using the method of Karapetyan (1962) but by modifying the above procedure, were successful in cultivating G.duodenalis from the rabbit and chinchilla. The modification involved the daily addition of fresh viable yeast, in addition to fresh medium, to their cultures. Trophozoites apparently can be cultured indefinitely in this monoxenic system.

Axenic culture of G.duodenalis was reported by Meyer (1970) from rabbit, chinchilla and cat. Giardia were first established in culture with yeast in Medium No. 1 described by Karapetyan (1962) (which contains
inactivated human serum) and then transferred to a yeast extract containing medium designated M-3. The isolation and axenic cultivation of *Giardia* from the duodenal aspirate of a human with giardiasis was demonstrated by Meyer (1976). The medium designated as HSP-2, contained Hank's solution, inactivated human serum, phytone peptone, glucose, medium NCTC 135 and reducing agents. Visvesvara later in 1980 described a detailed method of cultivation of *Giardia* trophozoites in a modified Diamond's TPS-1 medium (Diamond, 1968). In this medium, inactivated bovine serum was substituted with horse serum, broth sterilization was accomplished by filtration rather than autoclaving, and NCTC 109 was employed instead of the vitamin mixture used by Diamond (Diamond et al, 1978). Smith et al (1981) reported the successful isolation and axenic cultivation of *Giardia* in a modified filter sterilized Diamond's TYI-S-33 medium (Diamond et al, 1978) from duodenal aspirate of patients. A comparative growth study in both autoclaved and filtered Diamond's TYI-S-33 medium indicated that the growth of the organism was significantly higher in filtered TYI-S-33 medium (Belosevic et al, 1982). A modification of TPS-1 medium (Diamond, 1968) supplemented with fetal calf serum, NCTC 135 solution, L-glutamine and antibiotics was found useful in isolation of *G. lamblia* from human duodenal aspirates (Gordts et al, 1984).

Mammalian bile when added to modified TYI-S-33 medium, promotes the growth of *Giardia* (Farthing et al, 1983 and Keister, 1983). Mass cultivation of *G. lamblia* in a serum free medium was reported by Weider et al (1983). Serum free medium for *G. lamblia* cultivation, was described by Gillin et al (1986) contained an artificial biliary lipid dispersion consisting of six bile salts, phosphatidyl choline and cholesterol, in the ratios characteristic of human bile. Gault et al (1987) reported that
the co-culture of trophozoites with human intestinal epithelium cells also promoted growth, in a serum free medium especially in the presence of mucous and/or biliary lipids. An in vitro encystation medium for *G. lamblia* trophozoites which is a modification of the original TY1-S-33 medium by the addition of sodium glycocholate, myristic acid and oleic acid has very recently been described (Reiner et al, 1989).

2.5 **Diagnosis**

Infection by *Giardia* can result in an array of symptoms, none of them pathognomonic; thus the diagnosis of giardiasis cannot be made on the basis of the patient's symptoms. Jennings *et al* (1976) suggested that the disease should be suspected in patients with lowered levels of jejunal disaccharidase.

2.5.1 **Stool examination**

Like many other parasitic infections, giardiasis is also diagnosed by demonstrating the causative organism in the host or in the host's excretion. Traditionally, *Giardia* infections have been diagnosed by detecting the trophozoites or cysts in the host's faeces (Paine and Gluck, 1976; Steele and McDermott, 1977). Motile trophozoites are more likely to be present in diarrhoeic stools while the cysts are more likely to be present in formed stools. No relation exists between the number of organisms excreted and the patient's symptoms, or faecal consistency (Meyer and Radulescu, 1979). An important point in many *Giardia* infections is that neither cysts nor trophozoites may occur in the faeces; hence a negative stool does not rule out *Giardia* infection. Jokipii and Jokipii (1977) have demonstrated the existence of a prepatent period, that is, the interval between acquisition of the parasite and its detectability in the
new host in Giardia infection. Danciger and Lopez (1975) identified high, low and mixed excretors; attempts to increase parasite excretion with purgatives failed.

2.5.2 Examination of intestinal fluid

Duodenal intubation is a common way of obtaining intestinal fluid for examination (Townley et al, 1971; Levinson and Nastro, 1978). Opinion on the value of intestinal fluid examination is divided, some workers opine that it is more reliable than stool examination in making the diagnosis (Kamath and Kurugasu, 1974) while others have shown that stool examination is better than duodenal fluid and biopsy examination (Naik et al, 1978; Madangopalon et al, 1975). Whatever the controversies may be, it has now been proved that duodenal aspirates are very helpful in the correct diagnosis of giardiasis. Rojas et al (1989) have demonstrated a hundred percent correlation between detection of Giardia infection by duodenal intubation and a positive anti Giardia antibody response by Indirect immunofluorescent (IIF) test among children (1-5 years old).

2.5.3 Entero-test

Some of the unpleasantness of duodenal intubation can be avoided by the use of a commercially marketed device (Beal et al, 1970), the Entero-test capsule which consists of a nylon string about 1 meter long attached to a weight embedded in a gelatin capsule. The free end of the string is retained and the capsule swallowed; as the gelatin dissolves, the string unwinds and reaches the jejunum when fully extended. Giardia trophozoites attached to the string are gently retrieved and its adherent fluid expressed on a slide and examined for trophozoites. A number of workers have reported favourable results using the capsule technique.
2.5.4 **Small bowel biopsy**

Small intestinal biopsies may yield better diagnosis of *Giardia* infections where other methods fail; such specimens may be examined after sectioning and Giemsa staining or mucosal impression smears may be made (Carswell et al., 1973; Tomkins and James, 1974; Hortong, 1977). According to Klima et al. (1977) electron microscopic examination of the intestinal mucosa may demonstrate better understanding of *Giardia* infection.

2.5.5 **Gastrointestinal Radiology**

*Giardia* may result in non specific intestinal changes that, although not a diagnostic feature, may support disease diagnosis in about 20% of the patients with *giardiasis*. These abnormalities have been detected by barium examination (Marshak and Lindner, 1976; Menitove et al., 1978).

2.6 **Antigenic analysis**

Despite the elapse of 300 years since the discovery of *Giardia lamblia*, very little is known about its immunobiology. The principal reason was the inability to grow pure cultures of *G. lamblia* and therefore there was always a dearth of the organisms to conduct studies of this nature. Recent developments in the formulation of culture media (Meyer, 1976; Visvesvara, 1980) for the axenic cultivation of *G. lamblia* have facilitated studies on the antigenic analysis of the parasite.

2.6.1 **Soluble antigens**

Information available to date on the antigenic make up of *G. lamblia* or other species of *Giardia* is very limited. Osipova et al. (1974)
prepared immune serum in rabbits by subcutaneously injecting a mixture of extracts of *Lamblia duodenalis* and examined the serum by reacting it with antigenic extracts of *L. duodenalis* and *L.intestinalis*. *L. duodenalis* antigens reacted with its homologous serum and produced one precipitin line but did not react with the heterologous antigen (i.e., *L.intestinalis*). Visvesvara and Healy (1979) immunized rabbits with sonicated antigens of axenically grown *G. lamblia* trophozoite. The hyperimmune serum, upon reaction with the sonicated antigens in gel diffusion produced eight precipitin lines. As many as 22 precipitin arcs were produced when the antigens were electrophoretically fractionated in agarose gel and allowed to react with the hyperimmune serum (Visvesvara et al, 1977). Thus, the antigenic complexity of *G. lamblia* became clearly evident. Comparison of the antigenic profile of *G. lamblia* grown in several types of media supplemented with human, bovine or rabbit sera revealed that the basic antigenic structure of the organism remained the same irrespective of the medium in which the parasites were grown (Visvesvara et al, 1977). According to Visvesvara et al (1980), there appeared to be an antigenic identity among *Giardia* strains recovered from humans, cats and guineapigs in the gel diffusion experiment. The complexity of *G. lamblia* antigen was further illustrated in two dimensional crossed immunoelectrophoresis (2D-CIE), more than 30 precipitin arcs were observed in the test (Visvesvara, 1981).

Moore et al (1982) attempted to characterize *G. lamblia* antigens using Sodium dodecysulphate polyacrylamide gel electrophoresis (SDS-PAGE), High performance liquid chromatography (HPLC) and Enzyme-linked immunosorbent assay (ELISA). The SDS-PAGE analysis revealed a minimum of 20 distinct bands ranging in molecular weight from 125,000 to 14,000 dalton. Five major peaks were recovered after fractionating the antigens on HPLC.
Different fractions from these peaks were allowed to react with the anti-
*G. lamblia* antibodies produced in rabbits. Immunological reactivity was
found to be associated with the high molecular weight fractions. However,
antigens of diagnostic significance could not be demonstrated. In addition,
Smith *et al* (1982) carried out antigenic analysis of four strains of
*G. lamblia* originating from widely different geographic localities (in
Afghanistan, Puerto Rico, Ecuador and Oregon) using SDS-PAGE, 2d-CIE and
ELISA techniques. Demonstrable differences in the protein distribution
patterns of the four strains after SDS-PAGE were not evident. However,
distinct differences were detected between the Oregon strain that has
been the longest in (over 7 years) *in vitro* culture and the other three
strains using the 2d-CIE technique.

2.6.2  Cyst and stool antigens

The first isolation of antigen from the stools of *G. lamblia*
positive patients was accomplished by Fosoff and Stibbs (1986). The mol.
wt. of the antigen identified on the cyst wall of *Giardia* was 65,000
dalton. This antigen did not cross react with any other parasitic protozoa
but the sera against the antigen reacted with the 65,000 polypeptide
constituting the surface protein of the *Giardia* trophozoite.

Gillin *et al* (1987), however, showed that the major cyst antigens
were in the molecular weight reign of 21, 28 and 49 kDa. In contrast to
the earlier studies, the trophozoites from control cultures did not react
with the cyst antiserum. Recent analysis of the surface carbohydrate
moieties of *Giardia* cyst wall showed the polysaccharide chitin as a major
structural component of the cyst wall (Ward *et al*, 1985).


2.6.3 **Surface and membrane antigens**

The exposed surface of the parasite is vital in their interactions with the host to precipitate successful parasitism. This is the very site involved in the extraction of nutrients to sustain their life as well as the site actively involved in stopping the parasiticidal action of the host (Chang and Fong, 1983). The surface properties and activities of *Leishmania* species generally conform with those of a typical eukaryotic cell. Dwyer (1980) first isolated the plasma membrane from *Leishmania*. Barden et al (1983) purified and characterized plasma membrane from *Physarum polycephalum* amoebae. Both alkaline phosphatase and 5'-nucleotidase activity was detected in the membrane fraction. Biochemical assays of plasma-membrane associated enzymes have just begun to receive attention. McLaughlin and Muller (1979) demonstrated calcium dependent ATPase in *E.histolytica*. Alley et al (1980) detected acid phosphatase activity in plasma membrane fractions while Gottlieb and Dwyer (1981) demonstrated acid phosphatase activity on the surface of *L.donovani* promastigotes. Konigk and Putferken (1980) detected membrane nucleotidases and found them to differ between two leishmanial stages.

Studies on the biochemical analysis of the plasma membrane of *G.lamblia* has been the event of much recent focus. The molecular basis of the specific adherence of *Giardia* to host cells is unknown. Membrane bound lectins are believed to mediate several specific cell-cell interactions; including those between parasite and host cells (Pereira, 1984).

Hill et al (1981) examined the surface carbohydrates of *G.lamblia* by using six plant lectins. Wheat germ agglutinin (WGA), agglutinated the highest percentage of live trophozoites and fluorescein labelled WGA bound to them. The remaining five lectins either bound non-specifically
or exhibited low percentages of binding. This study demonstrated the abundance of N-acetyl-D-glucosamine moieties on the surface of *G. lamblia* which might be important in the elicitation of host immune responses (Hill et al, 1981). Lev et al (1986) described the existence of a mannose-6-phosphate lectin on the surface of *Giardia* trophozoites which upon activation with protease agglutinated intestinal cells to which parasites adhere in vivo.

Farthing et al (1986) reported on the characterization of a surface lectin from *G. lamblia*. This surface lectin had the sugar specificities for D-glycosyl and D-mannosyl residues. In SDS-PAGE analysis this lectin produced four bands in between the molecular weight of 57,000 and 78,000. Since the luminal surface of human intestinal epithelium cells possess mannosyl residues, Farthing et al (1986) suggested that these might serve as the receptor for *Giardia* lectin at the site of colonization. However, in a more recent study, Ward et al (1988) analyzed the carbohydrate residues of the trophozoite surface membrane of *G. lamblia*. By using a panel of 13 lectins with varying sugar specificities they observed that β-linked N-acetyl-D-glucosamine was the only detectable saccharide moiety on the plasma membrane of this parasite.

Until recently, very few studies have been conducted on the surfaces of *G. lamblia*. The existence of trophozoite surface components that are readily iodinated on live cells and then gradually released into the medium over a 24-hr period was reported by Nash et al (1988). The surface antigens were polydisperse in their molecular weights in SDS-PAGE ranging from 94,000 to 225,000. Further, it was also documented that the major secretory product of WB strain differs from that of P-1 in antigenicity. Enfield and Stibbs (1984) characterized the trophozoite surface
antigens of four G. lamblia strains by immunoelectrophoresis, radiiodination and immunoprecipitation and demonstrated the presence of a major surface iodinated G. lamblia antigen, with a molecular weight of 82,000. This antigen was further characterized as a glycoprotein and was detected in all of the four strains of G. lamblia examined.

Murine monoclonal antibodies (MAbs) against live G. lamblia trophozoites specific for flagella and attachment disc have been developed (Torlan et al., 1984). The MAbs were found to react with 53,000 and 55,000 molecular weight antigens and also showed cross reactivity with bovine tubulin of similar molecular weight and probably Giardia tubulin. The first successful demonstration on the importance of surface proteins in the immune response of humans was reported by Edson et al. (1986) who identified an 88,000 mol. wt. surface polypeptide from radiiodinated trophozoites which was readily immunoprecipitated by human anti-G. lamblia sera. This surface antigen was found specific for G. lamblia as it did not cross react with other closely related parasitic protozoans including G. muris. Giardia duodenalis strains isolated from human, domestic and wild animals of a common geographic area were analysed for protein profile and antigenic samples (Wenman et al., 1986). A high degree of antigenic sharing among the strains was observed and the major antigenic proteins were found at 62, 52, 38 and 31 kDa, among which the 52 and 31 kDa proteins were the major surface exposed trophozoite components.

Currently, particular interest is being paid to identifying membrane antigens that may be important in inducing immunity to giardiasis. Results have been achieved by indirect means such as antibody labelling, immunoelectrophoresis and surface radiiodination. Clark and Holberton (1986) first isolated the plasma membranes directly from Giardia which
made research on the surface antigens more easier. The membrane was purified by centrifugation on either a sucrose step gradient or on a self generated percoll gradient. The major protein as revealed by SDS-PAGE were in the mol. wt. of 75 kDa, 58/54 kDa, 32-38 kDa and 15-22 kDa. However the minor components of 190 kDa, 160 kDa, 120 kDa and 28 kDa were also detected. Kum Kum et al (1988) isolated plasma membrane from axenically grown G. lamblia and found a significant enrichment of the membrane bound marker enzymes Ca\(^++\) ATPase/Mg\(^++\) ATPase. Additionally, the existence of two major antigens (82 kDa and 52 kDa) on the surface of G. lamblia trophozoites was documented.

Studies on cDNA and genomic DNA of G. intestinalis have shown the expression of a 32 kDa protein in E. coli. The protein was found associated with the protein of flagella, axonemes, spiral part of ventral disc and surface of trophozoite (Upcroft et al, 1987).

In a more detailed study, Upcroft et al (1988) have characterized the 32 kDa immunodominant antigen of G. intestinalis. This antigen was purified and following immunofluorescence assay, the antigen was localized as a flagellar and trophozoite surface component. The synthesis of cDNA from the mRNA showed in vitro translation of this 32 kDa protein. Taylor and Weenman (1987) elucidated the major antigenic determinant of Giardia under conditions of natural human infection by immunoblot experiments. Although several proteins were found to be antigenic when reacted with sera from infected patients, the dominant antigen appeared to be a polypeptide with a molecular weight of 31 kDa. Immunogenicity of radiolabelled surface components of G. lamblia was tested against the sera from symptomatic and asymptomatic patients in their ability to precipitate the radiolabelled antigens (Ortega Pierres et al, 1988). The major precipitable
radiolabelled antigens identified were in 85, 63 and 55 kDa. However, some of the unlabelled components of the G.lamblia with the molecular weights between 35 and 200 kDa were also observed to be immunoreactive.

2.7 **Antigenic variation**

Differences among G.lamblia isolates have been recorded by a number of laboratories using a variety of biological and biochemical markers.

2.7.1 **Biological differences**

Biological differences include the ability to grow Giardia in culture, infectivity to animals and humans, the course of infection in animals and the ability of a Giardia RNA virus to infect some Giardia isolates.

Faubert et al (1983) conducted a comparative study on infection with Giardia spp. in gerbils and observed marked differences in cyst excretion in gerbils infected with different species of Giardia. Similarly, the ability of G.duodenalis from dogs and humans to grow in culture were compared and distinct differences among the isolates were discernible (Meloin et al, 1987). Aggarwal and Nash (1987a) studied the biological behaviour of G.lamblia isolates by infecting gerbils with two antigenically different strains of G.lamblia (namely WB and GS-E). Differences in the course of infection, resistance to reinfection and host humoral responses were observed between the two isolates. Further, it was documented that Giardia possessing different surface antigens have different patterns of infection and induce qualitatively and quantitatively different immune responses. Variable infectivity of Giardia cysts isolated from 10 humans,
in gerbil was also reported (Visvesvara et al, 1988). The rate of infectivity was 0–100% with cysts from different symptomatic and asymptomatic patients. Biological differences among the strains of Giardia in producing infection in humans have been demonstrated by Nash et al (1987). The fifteen human volunteers was divided in three groups (five each) of which two groups were inoculated with two strain of G.lamblia strains GS/M and Isr and the third group serve as control. All the volunteers inoculated with GS/M and none with Isr were found infected. This indicated the intrinsic differences between the isolates.

De Jonckheere and Gordts (1987) had investigated the occurrence of a RNA virus in 38 axenically grown Giardia strains from different geographic areas. Differences, both, in sensitivity of Giardia strains to transfection and in infectivity of the RNA virus from different Giardia strains were observed but no correlation was demonstrated between the presence of the RNA virus in Giardia isolates and their in vitro resistance to some anti-protozoal drugs nor with the fact that the strain originated from symptomatic or asymptomatic carrier. Miller et al (1988) differentiated G.lamblia isolates on the basis of the presence or absence of double stranded RNA virus. A survey of 38 axenic isolates of G.lamblia derived from humans and animals showed that 28 isolates lacked the RNA virus, 19 could be readily infected by the virus, and the remaining 9 isolates proved to be resistant to Giardia lamblia virus infection even when the ratio between virus to parasite reached as high as 106 to 1.

2.7.2 Biochemical differences

The biochemical differences include variation in isoenzyme patterns and restriction endonuclease patterns.
I) Isoenzymes

Enzyme differences have been utilised for clarifying differences among protozoan isolates. Comparisons of isoenzymes by starch gel electrophoresis (SGE) and polyacrylamide gel electrophoresis (PAGE) have been used in differentiation of large number of protozoan parasites like the invasive from non-invasive strains of *E. histolytica* (Sargeaunt et al., 1978) and *E. histolytica* from non-pathogenic intestinal amoebae (Sargeaunt and Williams, 1978). Enzymes of *Naegleria* spp. (Visvesvara and Healy, 1980), *Leishmania* spp. (Al. Taqi and Evans, 1978), the *Paramecium Aurelia* group (Tait, 1978) and trypanosomes (Gibson et al., 1980; Murray, 1982).

In *Giardia*, only a few reports on isoenzymes studies are available. Bertram et al. (1983) analysed six enzymes from the trophozoites of five axenically cultured isolates of *Giardia* from human, cat, and guineapig by SGE and PAGE. On the basis of zymogram patterns, the five *Giardia* isolates were grouped into 3 zymodemes with *Giardia* isolated from different mammalian hosts sharing multiple isoenzymes. Korman et al. (1986) compared the human isolates from Jerusalem and Bethesda by isoenzyme analysis using thin layer SGE. Among the five human isolates tested, four distinct zymodemes were observed and significant heterogeneity in *G. lamblia* isolates both from widely separated areas and within a single region was discernible. Similarly, the enzyme profile of three New Delhi strains of *G. lamblia* isolated from symptomatic cases and a Portland-1 isolate when compared in SGE revealed that the three New Delhi strains were heterogeneous while one New Delhi isolate was similar to the Portland-1 isolate (Baveja et al., 1986). Meloni et al. (1988) compared thirty isolates of *G. duodenalis* from humans and felines by isoenzyme electrophoresis, using 10 enzyme systems and observed 13 different zymodemes. Subsequently Meloni et al. (1989) characterized 47 isolates of *G. duodenalis* using a non-radio-
labelled DNA probe and isoenzyme electrophoresis and observed 15 different zymodemes. The notable observation was that the sensitivity of isoenzyme electrophoresis was found to be equal or more than DNA probe in detecting the heterogeneity among the isolates.

11) DNA sequence

The differences in the sequences of DNA result in a change in the sites of cleavage by restriction endonucleases that may result in a change in size or in number of resulting DNA fragments or in both. Nash et al (1985) compared the DNA banding pattern of 11 human and 4 animal isolates of Giardia by agarose gel electrophoresis (AGE) after restrict digestion of DNA. Two major groups were distinguished by AGE. In Southern blot analysis using recombinant plasmids containing Giardia DNA as probes, nine different patterns among the 15 isolates emerged. One common banding pattern in six isolates (two animal and four human) was observed while the remainder of the isolates exhibited unique patterns, with the exception of two identical isolates from sisters. Three isolates (one from a beaver and two from human) were markedly different from Giardia with the common banding patterns whereas the other six unique isolates varied moderately. Uji et al (1988) compared the genetic similarity between Giardia isolates obtained from various mammalian species found in one specific geographic area. Analysis of the Giardia DNA by restriction endonuclease digestion and by Southern blot technique using $^{32}$P labelled whole DNA as probes failed to demonstrate any significant differences among the isolates. However, in Southern blot DNA hybridization Uji et al (1985) detected a minor DNA fragment in beaver, muskrat and sheep isolates which was absent in DNA from the dog and 3 human isolates. The restriction endonuclease pattern of 49 isolates from different animals and geographic
areas with Hind III and subsequent hybridization to non-radiolabelled probe (pBM9) showed seven Schizodemes (Meloni et al., 1989).

2.7.3 **Differences in surface antigen(s)**

The biological and biochemical differences are relatively stable over time and presumably reflect basic differences in the isolates. However, perhaps the greatest degree of diversity was noticed among residues in the surface antigens of *G. lamblia* isolates (Nash, T.E., 1989). Acrylamide electrophoresis of surface-labelled *Giardia* has revealed a wide variety of proteins (Nash et al., 1983). Antisera raised to specific isolates reacted with the surface components and excretory-secretory product of heterologous isolates to varying degrees. Ungar and Nash (1987) showed that some antisera failed to react with the surface of heterologous isolates, although, very strong reaction with the homologous isolates was evident. Kasprzak et al. (1987) have demonstrated the antigenic differences among 20 *G. lamblia* isolates from symptomatic and asymptomatic patients. Antisera against *G. lamblia* (Portland-1), *G. Cat1* (Cat-1) and *G. cavae* (GP-1) was allowed to react with all strains and further studied by immunofluorescence assay. A wide range of cross-reactivity among strains isolated from the same area was observed.

The ability of single trophozoite to generate organisms with varying surface antigens was tested by exposing the clones of the WB isolate which possess a 170 kDa surface antigen to a cytotoxic MAb against 170 kDa antigen (Nash et al., 1988; Miller et al., 1988). On the same aspect which resulted immobility and death within minutes of the trophozoites possessing this antigen against the same MAb (Nash and Aggarwal, 1986). Further investigations along the same line indicated that although almost all the trophozoites were killed, a few survived and significantly the
MAb 6E7 failed to bind to the surfaces of the surviving progeny as revealed by the lack of cytotoxicity and by indirect immunofluorescence (Adams et al, 1988). Additionally, Western blots using MAb 6E7 or polyclonal antiserum failed to detect the 170 kDa antigen; surface radiolabelling showed the loss of the 170 kDa antigen and the replacement by a series of new antigens; Northern blots using a portion of the 170 kDa antigen as probe failed to detect transcripts in the progeny. One of the cloned progeny possessed a 64 kDa, major surface antigen which reacted specifically with a newly produced cytotoxic MAb, 5Cl (Nash et al, 1988). As before, after exposure to MAb 5Cl almost all the trophozoites were killed but the surviving 5CLR no longer possessed the 64 kDa antigen which was replaced by a new series of surface antigens. In subsequent experiments, Aggarwal et al (1989) have shown that these variations exist in almost all the isolates tested with either polyclonal sera or with MAbs.

The nature and composition of the varying surface antigens appear to be unique. A 1 Kb fragment (M 2-1) in λ gt-11 expressed a peptide which reacted with MAb 6E7 (Adam et al, 1988). The putative amino acid sequence revealed 12% cysteine and contained two and one half tandem repeats of 65 and 37 amino acids in length, respectively. The confirmation of high cysteine rich content was obtained by (35S) cysteine incorporation studies using a parent - progeny pair. Cysteine was incorporated predominantly into the 170 kDa antigen of a 6E7 clone and into the 64 kDa major surface antigen of the immediate 6E7, 5ClS cloned progeny (Adam et al, 1988).

Aggarwal et al (1989) studied the cysteine incorporation using other isolates with different varying surface antigens revealed similar results which suggested that increased cysteine content in a common
requirement for *Giardia* varying surface antigens. Little is known about the mechanisms involved in antigenic variation but these variations are likely to involve movement of genes or gene fragments encoding varying surface antigens (Nash, 1989).

Experimental infections in gerbils and humans have documented antigenic variation *in vitro* as well. Aggarwal and Nash (1988) inoculated gerbils with either 6E7S or 6E7R clones ofWB and the surface of the reactivated trophozoites were studied. The initial surface antigen was lost within the first week of infection in every infection and was replaced by new antigens which remained unchanged until self-cure 5 weeks later. Antigenic variation also occurred in two clones of isolate GS which were infected in human volunteers (Nash, 1989). After inoculation with defined clones, the surface antigens of trophozoites recovered from cysts, intestinal contents and directly on trophozoites obtained from the small intestines revealed a gradual loss of the initial surface antigen after day 14 and practically a complete loss by day 22.

### 2.8 Immunodiagnosis

The high frequency of giardiasis in patients with hypogammaglobulinemia (Ament & Rubin, 1972) and demonstration of spontaneous resolution of infection or resistance to reinfection in man and experimental animals (Roberts-Thompson et al, 1976) have 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 *in vitro* culture of axenic *Giardia* (Mayer, 1976). Cell mediated immune mechanisms in giardiasis are poorly understood. A high prevalence of G.lamblia

2.8.1 Immunoglobulin levels in giardiasis

Elevated levels of IgA have been reported by Roberts-Thompson et al (1982). However, highly depressed level of serum IgG has also been documented (Roberts-Thompson & Anders, 1984). Minor changes in serum IgA and IgG concentrations have been demonstrated by (Roberts-Thompson & Anders, 1981). However, these changes were not significant when correlated between total serum IgA and IgA anti-Giardia levels or between total serum IgG and IgG anti-Giardia antibody levels in patients infected with Giardia. Immunoglobulin concentrations in the intestinal secretions from patients with giardiasis were first studied by Zinneman and Kaplan (1972) who showed reduced levels of secretory IgA in patients with giardiasis. According to Mayer and Radulescu (1979) secretory IgA may, however, ultimately play a role in preventing giardiasis. No differences in IgE levels have been observed between patients with giardiasis and controls (Brown et al, 1973; Geller et al, 1978) and IgE levels were unrelated to giardiasis in patients with hypogammaglobulinemia (McLaughlan et al, 1974).

The population of immunoglobulin bearing plasma cells of the lamina propria of small bowel of infected patients have been evaluated by Ridley and Ridley (1976). An early immune response in the lamina propria is restricted to IgM production followed by IgA and IgG. A similar early
IgM synthesis in jejunal mucosa was observed by Popovic et al (1974). The same investigators observed a low percentage of cells containing IgA and secretory IgA; these levels were elevated after parasite eradication. Gillon et al (1982) demonstrated the rise in IgE and IgD bearing cells in the jejunal mucosa of giardia patients which became reversible following successful treatment.

Using an enzyme-linked immunosorbent assay Smith et al (1981) demonstrated that IgG antibodies could be detected in 81% of patients with symptomatic giardiasis and in 12% of the control subjects. The IgG antibody persisted in most patients for up to 15 months after cure. The usefulness of IgM antibody responses to Giardia in infected humans has been demonstrated (Goka et al, 1986). Nash et al (1987) also detected the IgM antibody response to Giardia in all experimentally infected humans but this response was low in chronically infected and rechallenged individuals.

2.8.2 Humoral antibody response

To date many tests have been reported for the detection of humoral antibody response in the sera of giardiasis patients. The tests that have been employed are listed below:

1) Immunodiffusion (ID) test

An ID test for demonstrating _G. lamblia_ antibodies using a soluble antigen obtained by sonication of cysts isolated by zinc sulphate floatation was used by Vinayak et al (1978). Ten of the eleven serum samples from giardiasis patients reacted with the soluble antigens and produced one or two precipitin lines, whereas none of the 16 serum samples from healthy controls and none of 15 serum samples from patients with other parasitic infections gave positive results.
Jokipii and Jokipii (1982) used the same test to measure the serum concentrations of IgG, IgA, IgM and IgD in 105 unselected patients with confirmed symptomatic giardiasis and in 43 control blood donors. No correlation was found between either immunoglobulin levels susceptibility to giardiasis, or severity of symptoms or incubation time. IgD level were reported to be lower in giardiasis patients who experienced weight loss than in other giardiasis patients or control subjects. IgM levels also were slightly elevated in those patients with recently acquired giardiasis.

11) Counter immuno-electrophoresis (CIE) test

Craft and Nelson (1982) developed the CIE test for detection of Giardia antigen in faeces and duodenal fluid. Direct microscopic examination of faecal samples revealed 66 cases of giardiasis out of the 276 patients with acute and chronic diarrhoea. CIE test for G.lamblia faecal antigen was positive in 65 of the 66 patients with giardiasis. Using anti G.lamblia (Portland-1) trophozoite antibodies in CIE test, Vinayak et al (1985) had detected stool antigens from 94% of the symptomatic and asymptomatic giardiasis patients. They did not find any false positive results in children with other infections.

111) Indirect immunofluorescence (IIF) test

Ridley and Ridley (1976) developed an IIF test using G.lamblia precysts from patients as antigen for the diagnosis of the three main immunoglobulins, IgA, IgG and IgM. Of the 89 patients and controls examined, one third or more of the patients were positive for G.lamblia antibodies with the positive sera showing titres 50 or above. The anti-G.lamblia activity was found to reside in the IgG class. Using cysts as
antigen, 18 of the 19 giardiasis patients with malabsorption were IIF positive with titres ranging from 20 to 320. Twenty control subjects, 8 patients with gluten enteropathy, 12 patients with inflammatory bowel disease, and 10 of the 11 patients with acute tropical sprue were IIF negative (Wright et al, 1977). Using the same test and the same type of antigen Lopez-Brea et al (1979) obtained positive serologic results with a titre of 320 in a 17-month old child with a history of chronic diarrhoea. The child was treated with metronidazole, and an IIF test on a follow up serum sample 24 days later revealed a drop in the titre to 40.

Visvesvara and Healy (1979) examined serum specimens collected from symptomatic and asymptomatic patients residing in different geographic localities using the IIF test. Axenically grown, 2 mercaptoethanol-treated and formalin-fixed G. lamblia trophozoites were used as antigens. The serum titres of giardiasis patients (both asymptomatic and symptomatic) ranged from 2 to 1024. At a lower dilution of the positive control serum, the organisms fluoresced brightly and uniformly over the entire surface including the flagella while at higher dilutions, the fluorescence was confined to the surface membrane. In a more carefully controlled study, the IIF test for G. lamblia antibodies was shown to be specific and reproducible (Visvesvara et al, 1980b). The serum anti-G. lamblia antibody titres ranged from 16 to 1024 in 29 of the 30 giardiasis patients, whereas titres of other patients and controls were 16 or below. The specificity of the IIF test was also ascertained by absorbing a positive sera with homologous (G. lamblia) and heterologous (E. histolytica, I. vaginalis and E. coli) antigens. Riggs et al (1983) used an IIF test for the detection of Giardia cysts. High titre rabbit antisera directed against G. lamblia cysts proved effective in the detection of cysts in patients stool;
however, cross reactivity with *Chilomastix mesnili* was noted. Wittner et al. (1983) measured and compared the antibody response in giardiasis by indirect fluorescent antibody method (IFA) and by the enzyme-linked immunosorbent assay (ELISA) and found ELISA to be less sensitive than IFA test. Patients with symptomatic giardiasis showed higher IFA titres compared with patients who did not have giardiasis and patients who had asymptomatic giardiasis. Similarly, using the IFA test, Wimiecka et al. (1984) observed higher positivity in giardiasis patients, however, 7% false positive results in healthy control and patients infected with other parasites was also observed. Sauch (1985) developed a method employing immunofluorescence and phase-contrast microscopy to detect *Giardia* cysts in raw and treated water supplies. By using anti-*Giardia* cyst antiserum and fluorescein conjugate, giardiasis cysts fluoresced bright green when illuminated in UV light.

Jokipii et al. (1988) evaluated the serum antibodies to cysts of *G.lambila* by IIF test and demonstrated that the titres of antibody increased with the duration of infection and declined after successful treatment. Recently, Rojas et al. (1989) investigated serum antibodies to *G. lambila* in 50 of the 100 children with clinical giardiasis and for the presence of demonstratable parasite by IIF test using *G.lambila* trophozoites as antigen. Children with positive duodenal aspirate had positive serology and good correlation between parasitological and serological results were obtained in the 1 to 5 years age group suggesting an age dependent increase of the antibody titre in giardiasis.

iv) **Enzyme-linked immunosorbent assay (ELISA)**

Smith et al. (1981) developed an ELISA test using axenically grown, unfixed intact *G.lambila* trophozoites as antigen. Results of the ELISA
test revealed that 81% of the serum samples from symptomatic patients were positive for anti-Giardia IgG-antibody at a titre of 64, whereas 12% of the normal controls and 14% of the unselected sera were also positive at the same titre. G. lamblia antibody persisted anywhere from 4 weeks to 15 months following therapy.

Ungar et al (1984) developed an ELISA for the detection of G. lamblia in human faeces. Stool specimens from 92% and from 2% of the patients without demonstrable Giardia organisms in the stool were found positive by this test.

In a more critical investigation, Haralabidis (1984) measured the anti-Giardia antibodies in asymptomatic giardiasis patients using Giardia cyst as antigen in the ELISA test. Out of 92, a total of 91 asymptomatic giardiasis patients were found positive and all the controls were negative. However, cross reactivity between the anti-G. lamblia antibodies and antigens of Toxoplasma gondii, Sarcocystis spp., Leishmania donovani, Fasciola hepatica, Echinococcus granulosus, Cysticercus tenuicollis, Taenia saginata, Dipylidium caninum, Trichuris suis, Trichinella spiralis and Litomosoides carinii was observed in this study. In 1985 Mohimen et al developed an ELISA system to detect anti-G. lamblia antibodies by using antigens extracted by the detergent Triton X-100.

An immunodiagnostic system for detection of Giardia antigen in faeces by a rapid visual ELISA test was developed by Green et al (1985). This ELISA was extremely sensitive and 100% specific and the entire test could be performed in 3 hours. A comparative analysis of four methods namely complement fixation (CF) test, indirect haemagglutination (IHA) test, enzyme-linked immunosorbent assay (ELISA) and lectin immuno test (LIT) for detecting antibodies in asymptomatic giardiasis revealed that
the ELISA was the most sensitive followed by LIF, CF and IHA (Nacapunchi et al., 1986). However, when specificity was analysed, CF was found to be highly specific and was, thus, recommended for use in serodiagnosis.

Goka et al. (1986) demonstrated the role of anti-Giardia IgM antibodies in the diagnosis of acute giardiasis cases by ELISA test. Efficiency of ELISA test with direct stool microscopy was evaluated by Allison et al. (1988). Microscopically, _G. lamblia_ cyst was detected in 2 out of 46 patients with acute diarrhoea and one out of the 37 patients with dyspepsia. However, when all the stool samples were subjected to ELISA, five stools were found positive including these three.

Stibbs et al. (1988) described an ELISA test employing rabbit and mouse antisera to _G. lamblia_ cyst to detect antigen in formalin fixed and unfixed human stool, with a sensitivity of about 91.5%. According to them the storage of stool eluates for more than 6 months at 4°C or as formalinized eluates did not affect the ability of the assay to detect the giardial antigen.

2.8.3 Cell mediated response

1) Lymphocyte proliferation

An increase in intraepithelial lymphocytes has been found in small bowel biopsies from patients with giardiasis (Wright and Tomkins, 1977; Ferguson et al., 1976). Although this cell population is heterogeneous and may contain several T-cell subpopulations, B-cells, and precursors of mast cells (Strickland et al., 1975; Mayrhofer, 1980) but T-lymphocytes appear to be the predominant cell (Ferguson, 1977). Smith (1985) examined the capacity of lymphocytes to respond to an extract of solubilized _G. lamblia_ in an in vitro proliferation assay. The lymphocytes from
a patients with chronic giardiasis showed a positive antigen-induced proliferative response. These observations suggest that there may be a T-cell response to G.lamblia and that antigen specific lymphocyte responsiveness may be helpful in distinguishing chronic from acute giardiasis patients (Smith, 1984).

11) **Spontaneous cell-mediated cytotoxicity**

   Studies of cellular effector mechanisms emphasize the capacity of monocytes-macrophages and granulocytes to participate in the killing of parasites. In this regard, mouse (Owen et al, 1981), rabbit (Radulescu and Meyer, 1981) and human (Hill and Pearson, 1987) macrophages were shown to be capable of phagocytosing G.lamblia trophozoites. Investigation of this process revealed that human monocytes exhibit spontaneous cytotoxicity for G.lamblia trophozoites, which suggests that macrophage cytotoxic mechanism may be important in the host response to Giardia (Smith et al, 1982).

   In contrast, Aggarwal and Nash (1986) demonstrated that the mononuclear leucocytes do not have any cytotoxic effect on Giardia and surprisingly it survived significantly better when the MNL were present. Heyworth (1988) attempted to quantify the leucocytes from the intestinal lumen of Giardia infected immunocompetent mice and nude mice and he observed no appreciable differences in the number of macrophages in both the cases.

111) **Antibody dependent cellular cytotoxicity (ADCC)**

   Although lymphocytes and granulocytes do not exhibit spontaneous cytotoxicity for G.lamblia, however, granulocytes are cytotoxic for trophozoites in the presence of serum containing anti-G.lamblia antibodies
(Smith et al, 1982). The sensitizing antibodies which appear to be of the IgG isotype, were not cytotoxic for *Giardia* in the absence of effector cells, even in the presence of complement (Smith, 1985). This observation is in contrast to the report of Hill et al (1984) who showed that sera from donors with and without a history of giardiasis were capable of killing *G. lamblia* trophozoites and pointed out that *G. lamblia* could activate complement via the classical pathway.