

diarrhea and intestinal malabsorption particularly in young persons (Craft 1982).

*Giardia* organisms are widely disseminated in the environment mainly in surface water and mammalian reservoir (Woo 1984). Developing new ways of controlling infection thus constitutes a major challenge for epidemiologists, clinicians and scientists (Gilman *et al.* 1985).

Giardiasis occurs in all parts of the world, not only in warm climates. The highest levels of endemicity occur in areas where sanitation and sanitary practices are poor and fecal contamination of the environment is common, these conditions prevail in many countries in the tropics and subtropics (Gilman *et al.* 1985 and Farthing *et al.* 1986b).

In the present work, a community-based prospective study was conducted among randomly selected 600 persons aging from one up to 50 years old. The study was planned to determine the prevalence of giardiasis and age relation factor in that community. It was also aimed to show the relation between prevalence of giardiasis and other parasitic diseases and relation between giardiasis and clinical manifestations.

The prevalence of giardiasis in the present study was **22.3%**; it is a high percentage in this community.

Among different parts of the world, several studies were done to show the prevalence of giardiasis, in Spain the

among 862 children under 5 years, giardiasis was found in 7.3% of them (Saidi *et al.* 1997).

In a study done in Lucknow- North India on 1061 children under 5 years age, *Giardia* infection was (32.9%) of them (Awasthi and Pande 1997).

Among the residents of four Italian psychiatric institutions, stools examination of 238 residents showed that *Giardia* infection was 9.2% (Gia Committi *et al.* 1997).

Comparing between the prevalence of giardiasis in the present work with those recently recorded from different parts of the world, it was much higher. This may be due to better sanitary conditions in these countries, but generally infections are usually more common in developing countries particularly in the young (Gupta and Urrutia 1982, Gilman *et al.* 1985).

As regards prevalence of giardiasis in Egypt several studies were conducted.

A prevalence among adult inhabitants in Alexandria was 7.5% (Morcos 1958).

Also another study in Alexandria showed that giardiasis was 2.1% among school children (Sherif *et al.* 1961).

Infection rate was reported as 2.7% among 800 Nubians (El-Zawahry 1964).

children and adults (Omar 1973).

In Assiut, the incidence of giardiasis was 10% on the top of the list of intestinal protozoa (Mandour *et al.* 1978).

In Banha and Tanta, the infection rates among preschool and school children were **26.2%** and **20.7%** respectively (Rizk and Antonios 1983).

In Assiut, a study showed that the most common parasitic infection was by *Giardia lamblia* as prevalence rate **26.9%** (El-Nazer 1983).

In Beni-Sweif Governorate, a survey on 444 inhabitants showed that giardiasis was **19.4%** (Boghdadi 1986).

In Assiut, a study showed that giardiasis was **24.4%** (Shaheen 1992).

A survey in Alexandria on 500 children from primary schools and from the student's hospitals, the infection rate of *Giardia lamblia* was **11.4%** (El-Sahn *et al.* 1992).

Parasitological examination was performed on stools samples from 200 children aging between 6-12 years attending a primary school in Shebin-Elkom city revealed that *Giardia* infection was detected in **4.5%** (Sharaf 1995)

recorded from previous studies done in Egypt, it showed that *Giardia* infection is relatively high in the presently studied population and this may be due to bad sanitation.

The high rate of infection may be due to several methods of transmission, as giardiasis is transmitted through contaminated water or food.

The importance of water borne transmission became apparent in North America during the mid 1960 when a large number of skiers in Colorado became infected (Craun 1984). Also person to person spread is particularly important in schools, day care centers and other residential institutions, this almost certainly relates to observation that on inoculation of only 10 to 100 cysts is required to initiate infection (Kean *et al.* 1979).

There is increasing evidence to suggest that giardiasis may be a zoonosis (Woo 1984, Thompson *et al.* 1988, Bermick and Erlandsen 1988).

Primary school children represent a very important sector in any community and the most vulnerable group (Ramses *et al.* 1985). In the present study the rate of giardiasis in children aging between 5-15 years was 14.6%, this is nearly similar to the results obtained in Alexandria on 500 primary school children and student's hospital as giardiasis was **11.4%** (El-Sahn *et al.* 1992).

reinfection (Karrar and Rahim 1995). In the present work prevalence of giardiasis in this age group was 10% and this is better than results recorded from a study conducted among randomly selected 300 children of the same age group in Khartoum where prevalence was **21.1%** (Karrar and Rahim 1995). This high rate may be due to low social level in that community.

Generally, Children have a higher incidence than adult, one possible explanation is that children have more intimate contact with family dogs which can serve as a reservoir host (Craft 1982).

*Giardia lamblia* is considered one of major causative agents of diarrhea in children under 5 years of age (Aihara 1997). Other serious complications may occur as retinal changes (Corsi *et al.* 1998), decrease in blood platelets with increased phagocytic platelets (Matowicha *et al.* 1996) and decrease in iron absorption (DE-Morais *et al.* 1996).

As regards the relation between giardiasis and clinical manifestation. Symptoms of giardiasis ranged from asymptomatic carriage to diarrhea, nausea, vomiting and malabsorption (Wolf 1984). The infection may be transient or chronic, sometimes lasting for years (Chester *et al.* 1985).

In the present study 50% of infected adult group showed clinical manifestations prevalence of diarrhea among clinical manifestation is the least one. These results are approximately

techniques were conducted in the present study which aimed to show the difference between the two isolates.

The first technique is animal inoculation, both large and small cysts were used separately in animal infection.

It was planned to use rats in present work as the rat model of infection with *Giardia lamblia* has many similarities to giardiasis in human (Craft 1982). This will give more accurate evaluation of experimental infection by *Giardia* cyst. Also the infection was carried out in weanling rats about one month old and 30 gram in weight; as young rats were found to be more susceptible to infection than older ones, moreover at this age rats were considered to be parasite-free (Sehgal *et al.* 1976).

The infection of both groups of rats was carried out using large doses of freshly voided cysts about  $10^5$  cysts per ml.

Inspite of these precautions, only the group which was inoculated by the large *Giardia* cysts contracted the infection as the cysts appeared in their stools after about one week post inoculation with histopathological changes in the villi of their small intestine while the rats inoculated by the small cysts did not take the infection as no cysts in their stools or histopathological changes in the villi of the small intestine occurred.

The prepatent period was found to be about one week. This is in agreement with findings of previous work, which

infective human cysts. Those observations may indicate that infection with *Giardia lamblia* is not restricted to human being and that giardiasis may occur among domestic rats, which are widely spread. So that it can be considered as a reservoir hosts for human giardiasis (Anand *et al.* 1980).

The group of rats which were inoculated with *Giardia lamblia* trophozoites did not contract the infection as no cysts or trophozoites appeared in their stools and no histopathological changes occurred in their intestinal villi. This may agree with fact that the infective stage of giardiasis is the cyst and for infection by trophozoites it must be lavaged directly into the small intestine (Feely *et al.* 1984).

In the present work, small segments from the stomach antrum of infected rat groups were taken one week post inoculation for histopathological and electron microscopic studies. It showed that giardia trophozoites were present adherent to gastric mucosa with shedding of some mucosal areas only in the rat group infected with large sized *Giardia* cysts. This may be an evidence of *Giardia* colonization in the stomach. This coincides with other reports suggesting giardiasis of the stomach (Quincey *et al.* 1992, Doglioni *et al.* 1992, Grooms and Schanable 1993, Berney *et al.* 1994).

These results were attentive because in humans trophozoites seem to be found principally in the duodenum and jejunum (OberHuber and Stolte 1990, Abbas *et al.* 1994) these sites are sites of predilection for *Giardia* colonization due to

their gastric mucosa this also agrees with Nash *et al.* (1987) who reported that different strains of *Giardia lamblia* have been shown to have different pathogenic effects on the host.

Giardiasis is usually diagnosed by microscopic stools examination for cysts or trophozoites and in spite of using concentration techniques most studies indicated that even after examination of three consecutive samples on different days only about **80%** of infections are detected (Kamath and Murugasu 1974, Wolfe 1978, Meyer and Radulescu 1979), also duodenal aspirate or jejunal sampling are often impractical (Sun 1980). Also testing for the presence of serum antibodies remains unsatisfactory for diagnosis of giardiasis (Lungstrom and Castor 1992). The availability of an immune diagnostic assay which can detect small amounts of antigen in feces will have the potential to improve the diagnosis, the sensitivity and specificity of assays for the detection of *Giardia* antigens in stools have been evaluated by several laboratories (Rosoff and Stibbs 1986, Golden 1993). Also when the assay was compared to the microscopic examination it had a sensitivity and a specificity of **98%** and **100%** respectively (Addis 1991).

In the present study DIALAB giardiasis antigen test was used for detection of *Giardia* antigen in the stools the study showed that 6 samples which were negative by microscopic examination for *Giardia* showed positive ELISA test this indicates a high sensitivity of ELISA technique to detect *Giardia* antigen even when present in small amounts this has

the samples which contained small sized cysts was positive. This result agrees with the results obtained from study based on experimental infection of human by two different isolates (GSM and IS) (Nash *et al.* 1987). It showed that non-of the IS inoculated volunteers became infected and non of their stools contained *Giardia* antigen; in contrast, all GSM inoculated volunteers became infected and *Giardia* antigen was present one or more times in the stools of each (Nash *et al.* 1987).

The present results showed that samples containing trophozoites only were negative by ELISA and this coincides with results obtained from a study, which demonstrated that *Giardia* trophozoites possessing different surface antigens which have different patterns of infection, and induce qualitatively and quantitatively different immune response (Aggarwal and Nash 1987).

Concerning the results of the present study, it showed that the cysts give copro-antigen positive results more than the trophozoites and this may be explained by the results of Boone *et al.* (1999). This study concluded that ELISA can detect cyst wall protein (CWPI) which is a useful diagnostic marker because it is highly stable and secreted in large amount by encysting trophozoites. It is resistant to degradation by protease and is unaffected by oxidation with sodium peroxide. Thus ELISA which appeared to be more sensitive for *Giardia lamblia* detection than did microscopic stools examination which should be useful as an epidemiological tool, particularly

Protein electrophoresis of large cysts, small cysts and trophozoites showed that they had different protein separation patterns.

In the present work, only proteins, which have molecular weight more than 180, 170 KDa, are in common between the three isolates. Those proteins were detected by immunoprecipitations and immunoblotting techniques using polyclonal non-specific and mono clonal antibodies (Farthing *et al.* 1996).

There is a marked difference in protein distribution between large and small cysts. This may suggest the presence of two different strains of *Giardia lamblia* as these results may be supported by a study which demonstrated strain variations in the pathogenicity of *Giardia* infection in humans conducted by Nash and Herrington (1987) also there is a distinct genetic difference between some *Giardia* isolates (Andrews and Mayrhofer 1993.)

*Giardia* isolates from humans have been compared using a variety of techniques including endonuclease restriction banding patterns (Nash *et al.* 1985), pulse-field gradient electrophoresis (Adam *et al.* 1988). Isoenzymes (Bertram *et al.* 1983, Melonyet *et al.* 1983, Beveja *et al.* 1986), ability to grow *in vitro*, sensitivity to chemotherapeutic agents (Boreham *et al.* 1988).

mice (Davies and Hiller 1979) and gerbils (Visvesvara *et al.* 1988) with cysts from humans. They suggested that the differences observed in cyst excretion were due to inherent differences among *Giardia* spp. In the present study protein electrophoretic analysis of large cysts demonstrated the presence of 88 KDa antigen which was described by more than one group of investigators (Edson *et al.* 1986). This antigen is known to be a target for immune response in human giardiasis (Farthing *et al.* 1996). The present work also demonstrated an antigen of 50 KDa, which is said to be a stimulus for serum antibody reactivity by Muller *et al.* (1996). These data may confirm that the large cysts are more pathogenic than the small cysts, which do not contain the 50&88 KDa antigens. Another confirmation is that large cysts contain an 83 KDa antigen (83H7), which is resistant to intestinal protease representing an immuno-evasive strategy which extends the survival time of the parasite (Stager *et al.* 1997a). This antigen also is not present in the small cysts

SDS PAGE showed that both large sized cysts and trophozoites having a 50, 55, 83 KDa in common.

The humoral immune responses to *Giardia* antigens were detected 14-22 days post infection (Nash *et al.* 1990, Muller *et al.* 1996, Arguello and Ortega 1997). Serum antibodies were preferentially directed against 83 KDa antigen (VSPH7). Which are of IgM and IgG class (Granat *et al.* 1998). And the parasitocidal effect of these antibodies has been assessed (Nash *et al.* 1988, Stager *et al.* 1997). Also the IgM antibody which is

Salmonella vaccine (Stager *et al.* 1997b). They demonstrated that per-oral administration of the vaccine stimulates synthesis of serum IgG and intestinal IgA antibodies directed against VSPH 7.

As regards trials for *Giardia* culture, inoculation of both trophozoites and cysts into CPLM media did not show either multiplication of trophozoites or excystation of cysts and this may be due to that this media is specific only for *Trichomonas* cultivation. (Diamond 1957).

Inspite that *Giardia* can be initiated directly with cysts discharged in feces into TPS-1 media (Kasprzak and Majewska 1983). In the present study inoculation of cysts into that media resulted in failure of excystation and this may be explained by a result of a study which reported that excystation entails detection of environmental stimuli across the tough extracellular cyst wall leading to highly coordinated physiological, structural and molecular responses. These findings show that excystation of *Giardia lamblia* is a highly complex and active process and provides important insights into its cellular and molecular components. (Hetsko *et al.* 1998).

As regards to trial to culture of *Giardia* in experimental animals, inoculation of *Giardia* cysts into mice has establish the infection since stools examination post inoculation for several weeks did not show cysts or trophozoites. But rat inoculation by *Giardia* cysts especially the large sized cysts

