THE EPIDEMIOLOGY OF GIARDIA INTESTINALIS, IN WADIHALOO VILLAGE, UNITED ARAB EMIRATES USING A FAECAL ANTIGEN ELISA

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by

IBRAHIM ALI ELKADI

Department of Tropical Medicine
and Infectious Disease
Liverpool School of Tropical Medicine
Liverpool L3 5QA, U.K.

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DEDICATION

I wish to dedicate this thesis to my wife, children, parents and the whole of my family.

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ABSTRACT

Enzyme-linked immunosorbent assay (ELISA) test was developed. Anti-Giardia sera were produced by immunisation of rabbits with trophozoites of Giardia intestinalis from axenic culture and with Giardia cysts isolated from infected human faeces on a 0.85M sucrose gradient and on Sephadex G.50 column. Purified fractions of these sera were prepared by affinity purification against trophozoite antigens (in antigen-Sepharose affinity column). Horseradish peroxidase (HRP) was conjugated to the affinity purified rabbit-anti-Giardia Ig.

3918 stool samples were examined with three different diagnostic methods to compare their results in detection of Giardia (Table 8.1). We found that 7.6%, 11.5% and 17.5% of total stool samples were positive for Giardia with direct microscopy, formal-ether concentration test and ELISA test to detect Giardia antigens respectively.

ELISA test was found detecting Giardia earlier than microscopical and concentration test. It was also shown to be more reliable and faster in the epidemiological investigation of a large number of stool samples, with sensitivity of 96% and specificity of 95%.

Three epidemiological survey rounds were done in the Wadihaloo village (UAE) during the study period. 982 stool samples were collected during these survey rounds. Giardia intestinalis was found in the village with a prevalence of 22%. The prevalence of Giardia was found to be more common amongst the females (25.5%) than amongst the males (22%).

The highest prevalence rate was found amongst the children aged between 0-6 years. The prevalence of Giardia was found more common amongst the local people (25.3%) than amongst the expatriates (19.5%) in the village. Also Giardia was found amongst all occupational groups. No significant relationship was found between the housing, source of drinking water and sanitation system and the prevalence of Giardia. We think most of the Giardia transmission in the village was caused by the direct method (with contaminated hand to the mouth), as no Giardia cysts were found in the water or in the stool samples of the animals.

1159 stool samples from the newly arrived (within 7 days) expatriates to UAE were examined in the labour clinic. UAE was found to be suffering mainly from the indigenous transmission of Giardia, and from a lesser extent from imported Giardia which was brought in through the expatriates (with a prevalence rate of 9.1%).

The effect of Giardia infection on the physical growth of a group of children (68) in the village at preschool age (below 6 years) were followed for 18 months.

1719 stool samples were collected from the group of children. 331 (19.3% of the total stool samples) were found positive for Giardia. 29 (42.7% of the total children) were found to be infected with Giardia. A significant change was found in the weight and the height amongst the children before and after Giardia infection.

Breastfeeding may play a role in protection from Giardia infection, as in the village most of the children up to 2 years old were breastfed and the prevalence of Giardia in this age group was found to be less than other age groups.

Giardia infection was found not to be the main cause of

diarrhoea in the village, but it could share with other organisms in causing diarrhoea.

Treatment of Giardia positive cases may play a role in controlling the disease.

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CHAPTER 1

INTRODUCTION

1.1. The Parasite and its Life Cycle

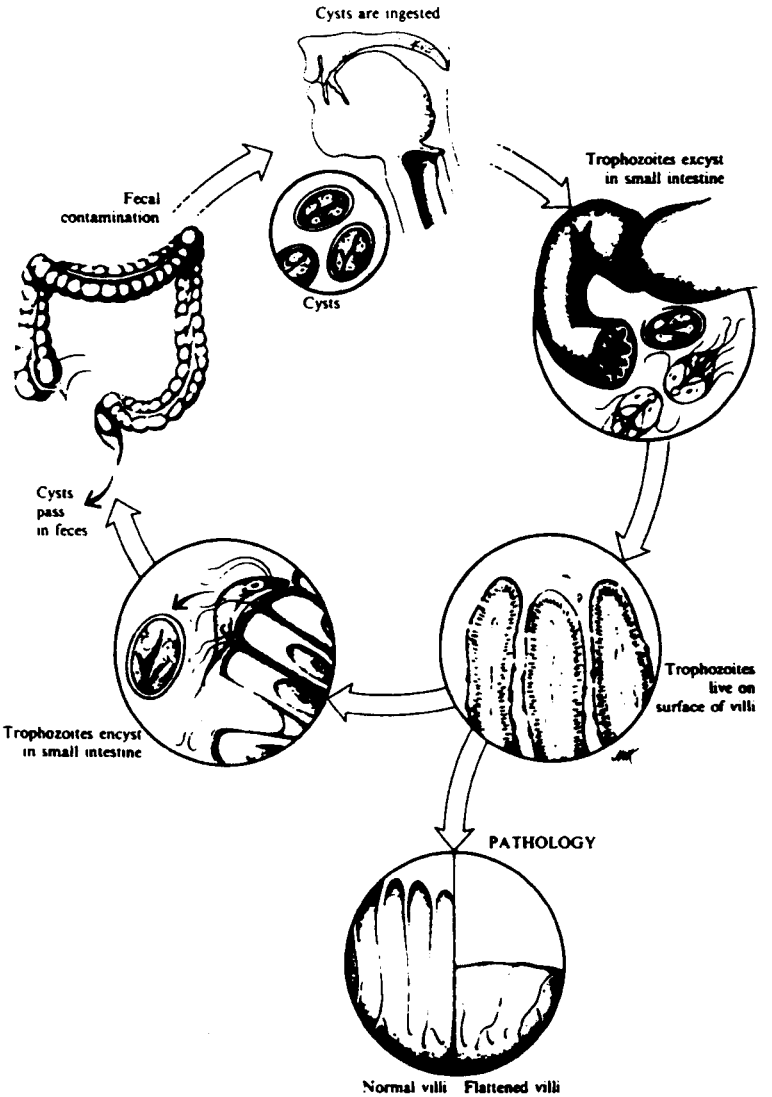
Giardia is the only flagellated protozoan parasite found in the human small intestine. It was considered to be a non-pathogenic commensal and was first described as early as 1681 by Anton Van Leeuwenhoek (Dobell, 1920), and subsequently by Lambl (1859). The formative classification of Giardia according to Levine et al, 1980 is shown in Table No. 1.1.

Giardiasis has more recently been recognised as a common cause of diarrhoea and malnutrition worldwide (Babb et al, 1971). However, most infected individuals are asymptomatic. Infection normally occurs after the ingestion of Giardia cysts and then excystation occurs in the acid environment of the stomach (Bingham and Meyer, 1979). Once the trophozoites are released they will multiply in the lumen of the duodenum and then will become attached to the mucosal epithelium. The life cycle is complete once the trophozoites encyst during their passage through the distal ileum and colon with subsequent excretion with the faeces (Figure No. 1.1). Maximum encystation takes place at pH 7.8 and small intestinal factors (bile salts and fatty acids) may influence encystation 'in vivo' as well 'in vitro' (Gillin et al, 1988).

Phylum: :	**SARCOMASTIGOPHORA**
Subphylum:	**MASTIGOPHORA**
Class :	**ZOOMASTIGOPHOREA**
Order :	**DIPLOMONADIDA**
Suborder :	**DIPLOMONADINA**
	Giardia

Table No. 1.1 A Newly Revised Classification of Giardia (Levine et al, 1980).

Figure 1.1 Life cycle of *Giardia intestinalis* (Karapelou, 1988)



1.2 Parasite structure and mobility

The Giardia trophozoite has a pyriform shape which is 12-15μm in length and 6-8μm in width and is binucleate. Four pairs of flagella extend from basal bodies anteriorly to emerge at anterior posterolateral, caudal and ventral positions. The median body of this parasite is quite unique to this genus and has been used as a taxonomic tool. This structure varies slightly for each of the three species i.e. G. agillis, G. duodenalis and G. muris. For example, the first species has a median body which can be described as teardrop in shape and is aligned parallel to the long axis of the cell. On the other hand, in the G. duodenalis group, this structure has a 'hammer claw' shape and lies transversely in the mid-portion of the cell body; whilst two small round centrally placed median bodies have been identified in the third species.

Other organelles found in the cytoplasm of the trophozoite include the rough endoplasmic reticulum, lysosomes, free ribosomes and glycogen particles; however, mitochondria and golgi bodies have not yet been identified. The ribosomes are smaller than counterparts found in other protozoa and comparisons to rRNA of archebacteria suggest that Giardia may have evolved from an early branch in eukaryotic development (Edlind and Chakraborty, 1987).

Bacterial endosymbionts have occasionally been observed in Giardia trophozoites (Radulescu et al. 1982; Feely et al, 1984) as well as virus-like particles (Feely et al, 1984). The cyst stage of the organism is oval in shape and measures 8-12μm by 7-10um and contains 2-4 nuclei basal bodies, axonemes of flagella and median bodies. Electron microscopy

reveals segments of rough endoplasmic reticulum and fragmented portions of the ventral disc. As for the trophozoite, the portions of ventral disc have been found to contain microtubules of tubulin and microribbon of giardin (Holberton and Ward, 1981; Crossley and Holberton, 1983).

Bingham and Meyer (1979) have observed that excystation of Giardia, from humans and animals, can be induced on exposure to hydrochloric acid at 37°C. In fact, excystation has been used as an index of viability for cysts stored at 8°C for a minimum period of seventy seven days; however, those stored at 37°C do not usually survive longer than four days. Rice and Schaefer (1981) have described a more complex but efficient method of excystation involving induction by sequential incubation in reagents which included HCl at pH2 and trypsin.

Trophozoite mobility is characterised by an oscillatory forward motion combined with rotation about the long axis. This is thought to be due to not only flagellar activity but also to the presence of contractile proteins within this parasitic organism (Feely et al, 1982). Attachment of the trophozoite to the epithelial surface has long been thought of as a function of the ventral disc; several mechanisms for this have been postulated including contractile activity of the ventrolateral flange (Friend, 1966), microtubular coiling-uncoiling (Mueller et al, 1974) and the activity of the ventral flagella create a negative pressure beneath the ventral disc (Holberton, 1973). Recently, lectin mediated attachment has been postulated as an additional mechanism of trophozoite attachment to mucosal epithelium. In fact, the surface membrane of G. intestinalis has been shown to contain mannose-binding lectin (Farthing et al, 1986a), also it has been found in the form of an intracellular prolectin which can be activated by trypsin (Lev et al, 1986).

1.3 Parasite isolation, culture and metabolism

Several methods of isolation and purification of Giardia trophozoites from intestinal washing have been described, including density gradient centrifugation, elution through nylon columns (Andrews et al, 1980) vibration and sedimentation (Roberts-Thomson and Mitchell, 1979) and also attachment to warm surfaces (Feely and Erlandsen, 1981). Cysts have been separated from faeces by sedimentation (Rendtorff, 1954), gradient centrifugation techniques (Roberts-Thomson et al, 1976; Moody, 1976; Bingham et al, 1979) and more recently a method employing a Sephadex-G50 column (Douglas et al, 1987).

Early reports of the culture of G. intestinalis 'in vitro' have been reviewed extensively by Meyer and Radulescu (1984). The isolation and axenic cultivation of Giardia from a human duodenal aspirate in HSP-2 medium was first reported by Meyer in 1976. Visvesvara (1980) adapted Giardia trophozoites from HSP-1 medium to grow in filter sterilised Diamond's TPS-1 medium containing inactivated bovine serum (Diamond, 1968). Gillin and Diamond (1979) achieved axenic culture of trophozoites in Diamond's TPS-1 and TYI-S-33 media and later (1980) reported clonal growth from TPS-1 medium made semi-solid by the addition of agarose. They also examined the roles of reducing agents such as L-cysteine and ascorbic acid, which are present in TSP-1 and TYI-S-33, in the survival, growth and attachment of the organism (Gillin, 1984). Mammalian bile has been found to promote growth of trophozoites in axenic cultures (Farthing et al, 1983; Keister, 1983). Farthing et al, (1982) found that a roller bottle culture system with increased surface

area to volume ratio was more efficient, giving more than double the trophozoite yield from stationary culture. Wieder et al (1983) have reported the successful growth of Giardia in a serum-free medium, a modification of TY1-S-33 with added bovine bile supplemented with bovine serum albumin (BSA) and lipoprotein cholesterol solution.

The development of these methods for the isolation of trophozoites and cysts excystation and axenic cultivation has allowed sufficient quantities of trophozoites to become available for detailed study of metabolism of this organism. Giardia intestinalis is an aerotolerant anaerobe which lacks mitochondria and oxidises both endogenous and exogenous glucose to organic end products (mainly ethanol and acetate) and CO_2 (Lindmark, 1980; Weinbanch et al, 1980; Jarroll et al, 1981). Evidence for a functional Krebs' cycle and of cytochrome-mediated oxidative phosphorylation is apparently lacking. However, there is evidence for a glycolytic pathway and the involvement of flavins and iron-sulphur proteins in the electron transport chain (Weinback et al, 1980). Lindmark (1980) has demonstrated and characterised a total of 13 glycolytic and electron transport enzymes.

Studies on lipid metabolism have revealed that Giardia trophozoites incorporate fatty acid, including arachidonic acid and cholesterol from the growth medium but are unable to synthesise phospholipids and sterols from precursors (Jarroll et al, 1981; Blair and Weller, 1987). Although Giardia is incapable of 'de novo' synthesis of purines and pyrimidines, salvage pathways have been demonstrated for both these bases (Wang and Aldritt, 1983; Aldritt et al, 1985).

1.4 Clinical Features of Giardiasis

The prepatent period (from infection to first detection of the parasite in the stool) averages nine days (Rendtoff, 1954) with an approximate incubation period (from infection to the first onset of symptoms) ranging between twelve to fifteen days (Walzer et al, 1971; Brodsky et al, 1974). The patient with giardiasis will be either in the acute, chronic or asymptomatic stage (passage of cysts).

The acute stage is characterised by anorexia, nausea, epigastric discomfort, belching and watery foul smelling diarrhoea with flatulence and abdominal distension. There may be tenderness in the abdomen on palpation in the right upper quadrant. Mucus and blood are absent from the stool in virtually all cases of giardiasis (Wolfe, 1975). This acute phase can last a few days but may continue for many months. In a case study by Brodsky et al (1974) the mean duration of symptoms was found to be 6.2 weeks.

The chronic phase of giardiasis may include features of malabsorption, steatorrhoea, debility and weight loss, with anaemia due to folic acid and vitamin B_{12} deficiency (Hoskins et al, 1967; Buckley, 1986; Cowen and Campbell, 1973; Wright et al, 1977a; Cordingley and Crawford, 1986) and also lactose intolerance (Jennings et al, 1968).

Giardiasis was found to be related to other diseases such as cholecystitis (Soto and Dreiling, 1977; Goldstein et al, 1978), granulomatous hepatitis and cholangitis (Roberts-Thomson et al, 1982), pancreatitis, urticaria (Webster, 1958), erythema nodosum (Harries and Taylor, 1986), reactive arthritis (Barton et al, 1986; Woo and Panayi, 1984; Shaw and Stevens, 1987), and peripheral neuritis (Bassett et al, 1978). Giardiasis

has been recognised as an unsuspected cause of malnutrition and diarrhoea in the elderly (Beaumont and James, 1986) and recent evidence has implicated giardiasis as an important cause of childhood growth retardation in the tropics (Cole and Parkin, 1977; Farthing et al, 1986b). It has been observed that spontaneous disappearance of parasites among adults is the general rule (Rendtorff, 1954; McMurray and Cayer, 1951) may be due to poor sensitivity of microscopical methods (Farthing et al. 1987; Sawitz and Faust, 1942), and variable excretion rates of the parasite in patients and from one day to another (Daneiger and Lopez, 1975), or may be due the development of immunity.

1.5 Pathology and Pathogenesis of Giardiasis

1.5.1 Pathology: Morphological changes in the small bowel

The small intestine, in most people with G. intestinalis infections, is normal or shows only mild non-specific changes (Morecki and Parker, 1967; Brandborg, 1971). However, some specimens which are normal on examination by light microscopy do show loss of microvilli when examined in the electron microscope (Barieri et al, 1970). In a study using scanning and transmission electron microscopy of biopsies, Erlandsen and Chase (1974) have shown abundant trophozoites covering large sections of intestinal villi, with normal epithelial cells apart from minor changes in microvilli at the site of attachments. A minority, particularly those from patients with more severe symptoms of disease, have histological changes of villus shortening, crypt elongation and an inflammatory cell infiltrate in the lamina propria (Wright et al, 1977a). Villus atrophy is a

rare event and is indistinguishable from that observed in coeliac disease, apart from the presence of Giardia trophozoites (Zamchek et al, 1963; Levinson and Nastro, 1978).

The increased numbers of intraepithelial lymphocytes observed in many patients with giardiasis may reflect an immune response to the parasite (Wright and Tomkins, 1977). It has been shown that chronic infection of mice with Giardia or Hexamita leads to a doubling of cell production in the crypts and an acceleration in the movements of the enterocytes along the wall of the villi (Macdonald and Ferguson, 1978). This histological picture has reverted to normal in almost all patients who have a second biopsy after eradication of the parasite.

1.5.2 Pathogenesis

Different hypotheses on the pathogenesis of diarrhoea and malabsorption, or both, in giardiasis have been proposed by several different authors (Figure No. 1.2) -:

(1) Irritation of intestinal mucosa; this leads to:

(a) Mucosal irritation with the parasite which results in an outpouring of mucus and fluid (Yardley et al, (1964).

(b) Inflammatory changes in the small intestine have been linked to the major histological changes found particularly in patients with malabsorption (Duncombe et al, 1978; MacDonald and Ferguson, 1978). Also in experimental infections of mice with G. muris, the villus abnormalities are less severe in hypothymic mice than in immunocompetent animals despite prolonged infection (Roberts-Thomson and Mitchell,

1979). Mast cell and local anaphylaxis may contribute to the intestinal inflammatory response and epithelial damage in giardiasis (Mitchell et al, 1982), possibly after sensitization to Giardia antigens analogous to the proposed pathogenesis in coeliac disease, in which similar histological features are observed (Farthing, 1987).

(2) Mechanical barrier of absorption: it has been frequently suggested that massive numbers of trophozoites, on the small intestinal mucosa, may act as a physical barrier to the passage of fluids and nutrients into the cell (Veghelyi, 1938),

(3) Damage to microvilli: The damage to microvilli observed on electron microscopy (Barbieri et al, 1970) may account for observed lactase deficiency; if, on the other hand, other enzyme deficiencies and transport mechanisms are observed then this may account for features such as Vitamin B_{12} deficiency, steatorrhoea and malabsorption of a test dose of xylose.

(4) Invasion of the lamina propria: Mucosal dysfunction secondary to trophozoite invasion of the lamina propria does occur; penetration of mucosa has been described by Morechi and Parker (1967), and Brandborg et al (1967) and confirmed by Saha and Ghosh (1977).

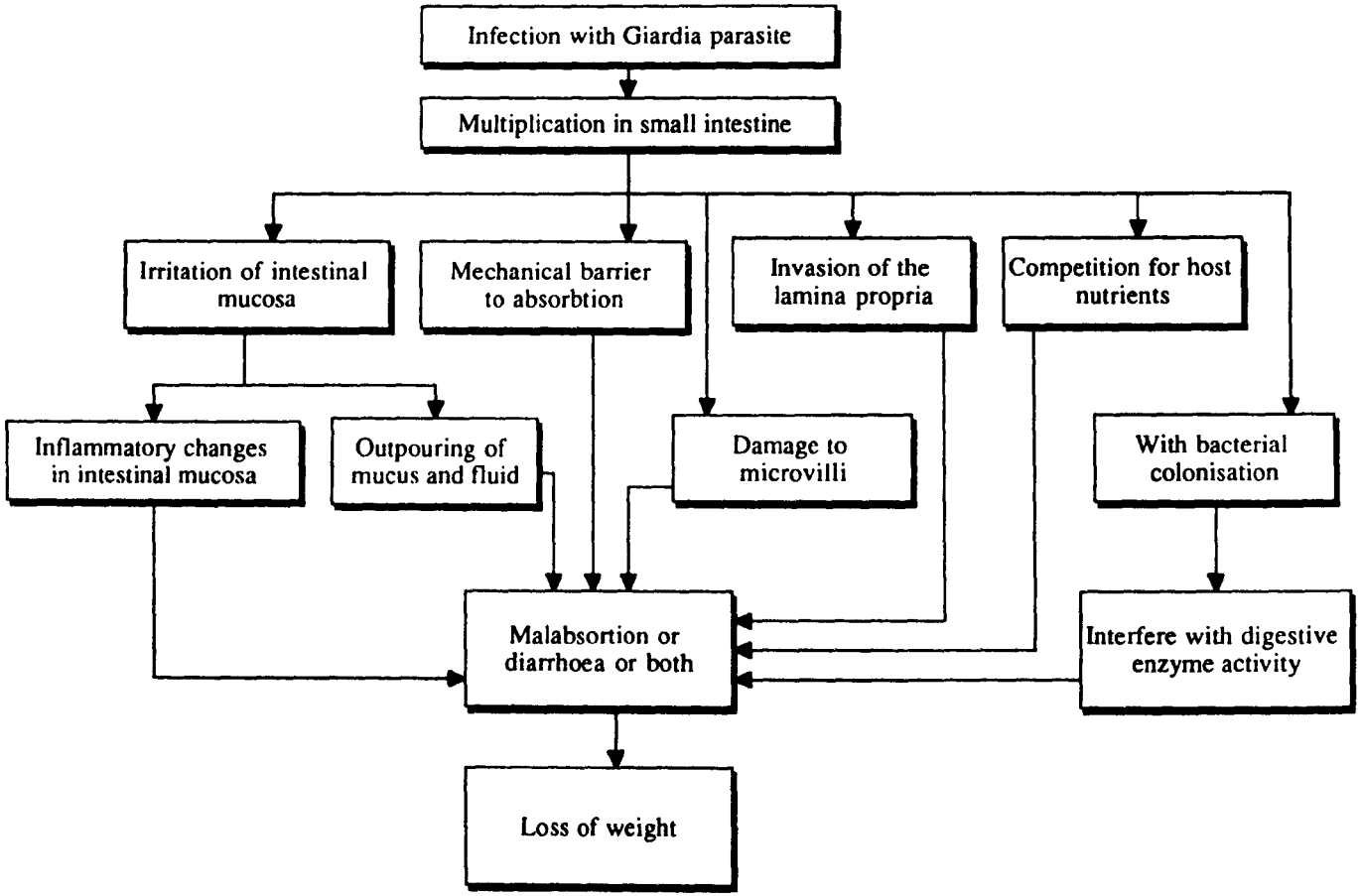


Figure 1.2 Pathogenesis of diarrhoea and malabsorption in giardiasis

(5) Competition of host nutrients: Competition exists between the parasite and the host for nutrients (Zamcheck, et al, 1963).

(6) Interference with digestive enzyme activity: Tomkins et al, (1978) believe that bacterial colonisation of the jejunum as seen in tropical sprue potentiates the damage observed in giardiasis cases, and may be responsible for the development of malabsorption. Bile salt deconjugation caused by bacterial overgrowth, may contribute to fat malabsorption. Giardia itself is unable to deconjugate bile salts, but has been found to take up conjugated bile salts (Halliday et al, 1986) which theoretically can lead to the depletion of the bile salt pool in those with chronic infections. A low concentration of bile salts in the intestinal lumen may also decrease the activity of pancreatic lipase (Keele and Neil, 1969). None of the different proposals described above appear to give a concrete and definitive explanation of the pathogenesis of diarrhoea or malabsorption in giardiasis.

1.6. Host Susceptibility and Immune Response

1.6.1 Host Susceptibility

Achlorhydria may predispose to symptomatic Giardia infections (Meyer and Radulescu, 1979), but, the evidence for this is poorly documented (Knight, 1978). An excess of patients of blood group A in this group over that in the general population had been reported (Barnes and Kay, 1977; Paulsen, 1977), but

this association has not been confirmed in a study by Jokipii and Jokipii (1980).

Relative deficiency in secretory IgA in patients with giardiasis was initially reported by Zinneman and Kaplan (1972) and Popovic et al, (1974). This, however, was not confirmed by Jones and Brown (1974). More recently, Gillon et al, (1982) found no significant difference in IgA and IgM levels in lamina propria plasma cells from control samples but an increase in IgA levels in samples from patients after treatment.

A higher frequency of giardiasis has been noted in patients with hypogammaglobinaemia (Ament and Rubin,1972; Ament et al, 1973), and more severe symptoms and histological changes (total villus atrophy) tent to occur in these people (Hartong et al, 1979; Webster, 1980).

1.6.2 Humoral Immunity

Anti-Giardia antibodies have been detected by using the IFAT and ELISA assays in approximately 80% of individuals with symptomatic giardiasis (Visvesvera et al, 1980; Smith et al, 1981). Cross-reactivity with Escherichia coli, Trichomonas vaginalis and Entamoeba histolytica was minimal, and high titres were present in some patients with recurrent Giardia intestinalis infections, suggesting that the humoral responses are not protective. Immunoblotting techniques have allowed the antibody response to individual Giardia antigens to be characterised. Taylor and Wenman (1987) have found that antibodies in the sera from giardiasis patients, acquired from diverse locations, recognised major antigens of molecular weight 31000M_r (probably the Giardin protein described by Crossley and Holberton,1983), and lesser antigens of 27000,

28000 and 56000 M_r; the latter might represent tubulin. In another study a Giardia surface protein of 88,000 M_r has been found to be recognised by sera from patients (Edson et al. 1986).

Specific anti-Giardia IgM antibody is produced early in human infections and declines after several weeks. This has been utilised in an ELISA for improved sero-diagnosis of giardiasis (Goka et al, 1986).

Raised levels of Giardia specific serum IgA antibodies, identified by Roberts-Thomson and Anders (1981), remain elevated for a substantial period after the infection has been eradicated. Additionally, increased levels of total (non-specific) IgE have been reported in association with urticaria and arthralgia in patients with giardiasis (Farthing et al, 1984), however, allergic responses in giardiasis patients are uncommon.

Giardia-specific secretory IgA and IgG antibodies have been detected on the surface of G. intestinalis trophozoites, in human jejunal biopsies and on the surface of G. muris trophozoites obtained from experimentally infected mice (Heyworth, 1986). These immunoglobulins may be associated with parasite clearance and protective immunity. In fact, both anti-Giardia IgA and IgG have been found in human and murine milk; the latter with protective effects towards suckling G. muris infected mice (Andrews and Hewlett, 1981; Miotti et al, 1985) and the former towards neonates (Farthing et al, 1986b). However, normal (non-immune) human milk has been found to kill trophozoites of Giardia intestinalis 'in vitro' (Gillin et al, 1983a; Hernell et al, 1986; Rohrer et al, 1986; Reiner et al, 1986; Gillin et al, 1983b). This effect is mediated by the fatty acids, monoglyceride and lysophosphatidyl-choline, and is produced by pre-incubation with sodium cholate (which

activates bile-salt stimulated lipase [BSL]) stimulates lipolysis (and killing) but only in the presence of sonicated milk. Zenian and Gillin (1987) have also observed that intestinal mucus protects Giardia from killing by milk.

1.6.3 The cellular immune response

The existence of a prominent cellular immune response during human and murine Giardia infections has been demonstrated by numerous histopathological studies (Roberts-Thomson et al, 1976; Wright et al, 1977; MacDonald and Ferguson, 1978). Intraepithelial lymphocytes and those in the lamina propria increase during G. muris infections in mice, and then decrease to a normal range following treatment and clearance of the parasite (Gillon et al, 1982). The appearance of intraepithelial lymphocytes in murine G. intestinalis infections coincides with a fall in parasite numbers within the intestinal lumen (Kanwar et al, 1984).

The intraepithelial cell population, although heterogeneous, (Mayrhofer, 1980; Strickland et al, 1975) appears to contain a predominance of T-cells (Ferguson, 1977). Kinetic studies, in G. intestinalis infected mice, have revealed an increase in the T-cell population of intraepithelial lymphocytes and also in the lamina propria (Upadhay et al, 1986). The number of Peyers patch leucocytes in BALB/c mice has been found to double during the course of G. muris infections with no significant change in percentages of Peyer's patch T helper (34%) or T suppressor (6%) lymphocytes; clearance of infection was associated with Th:Ts ratios of greater than 5:1 (Carlson et al, 1986).

Smith et al, (1982) found that lymphocytes from a

patient with chronic giardiasis but not from an acutely infected patient, showed a positive antigen-induced proliferative response 'in vitro', suggesting that there may be a T-cell response and that antigen-specific lymphocyte responsiveness might be helpful in distinguishing chronic from acute Giardia infections. Using hypothymic mice, Roberts-Thomson and Mitchell (1978) and Stevens et al (1978) observed that such animals sustained a prolonged and heavy experimental infection with G. muris.

Reconstitution with thymus, mesenteric lymph node and spleen cells from immunocompetent controls resulted in reduced villus-crypt ratios and a more rapid clearance of infection (Roberts-Thomson and Mitchell, 1978). On the other hand, attempts to transfer resistance to infection with single (Roberts-Thomson and Mitchell, 1978) or multiple (Underdown et al, 1981) injections of serum containing high levels of antibody were unsuccessful.

In a study using G. muris infected mice, Belosevic et al. 1986) found that treatment with cyclosporin A, prior to infection at weekly intervals, results in increased cyst output and delayed the elimination phase of infection. However, cyclosporin A treatment did not affect the ability of immune mice to resist infection.

Antibody dependent cellular cytotoxicity (ADCC) has been observed by Smith et al (1983), in a study using [H^3] TdR pulse labelled trophozoites of G. intestinalis. They observed ADCC for granulocytes (Polymorph nuclear leucocytes and to a lesser extent eosinophils) but not for lymphocytes, in the presence of either human or rabbit anti-Giardia serum. The effect was serum concentration-dependent and not complement-mediated; furthermore, IgG was the predominant active

immunoglobulin. Subsequently, both direct cytotoxicity and ADCC against G. intestinalis by splenic and intestinal lymphocytes from mice have been reported (Kanwar et al, 1986). The IgG and IgA isotypes from rabbit immune serum and immune mouse milk respectively, have been found to participate in ADCC in mice (Kaplan et al, 1985). Smith et al (1982) have documented spontaneous cytotoxicity of human peripheral blood monocytes for G. intestinalis trophozoites; however, their methodology and results have recently been questioned (Aggarwal and Nash, 1986). Hill and Pearson (1987) have since shown that the ingestion of trophozoites by human monocytic phagocytes, which is enhanced eight fold by the addition of twenty percent immune serum, is followed by the fusion of lysosomes and phagosomes, and later an oxidative burst with the ultimate killing of the invading parasites.

1.6.4 Complement

The role of complement in the killing of trophozoites of G. intestinalis has been investigated by Hill et al (1984) and Deguchi et al (1987). These workers found the only class of immunoglobulin which supported the lytic effect of complement was IgM, and that lysis could be accomplished in the absence of C9. Deguchi et al (1987) concluded from their study, that although activation of the classical pathway produced lysis of sensitised trophozoites, lysis may also proceed through a unique pathway of complement activation that requires C1 and factor B, but was independent of C4 and C2. Lysis of sensitised trophozoites can be accomplished by C_5b to C8 in the absence of C9.

1.7 DIAGNOSIS

1.7.1 Microscopical Examination of Stool Specimens

Although microscopy is easy (in the case of small numbers of stool samples), economical and is the traditional method for diagnosis, a single examination is believed to be successful in only about fifty percent of giardiasis cases (Kamath and Murugasu, 1974). It has been observed that in three or four different faecal examinations for the presence of Giardia trophozoites and cysts from patients the pattern of excretion may vary tremendously (Danciger and Lopez, 1975; Wright et al, 1977a). Wolfe (1979) reported cumulative diagnostic yields of 76%, 90% and 97% over three consecutive faecal examinations.

Motile trophozoites may be seen, occasionally, in an unconcentrated faecal wet preparation of diarrhoeal stool. However, a cyst concentration method using formol-ether (Allen and Ridly, 1970), or less commonly, zinc sulphate flotation (Faust et al, 1939) may be necessary in order to increase diagnostic sensitivity. Lugol's iodine is the most rapid and convenient stain for these wet preparations. If, on the other hand, staining and microscopy are to be delayed, then optimal staining procedures for the identification of cysts are trichrome staining following merthiolate-iodine-formalin concentration (MIFC) and preservation in polyvinyl alcohol or 10% formalin. In short, microscopy is time consuming, labour intensive (in the case of large numbers of stool samples), requires the expertise of an experienced technician and may not always be accurate.

1.7.2 Examination of duodenal or jejunal fluid

Duodenal aspiration, via a double lumen tube, is usually performed if clinical features of malabsorption or persistent diarrhoea are present and stool examination is unfruitful. This method of diagnosis allows both a large volume of intestinal fluid and a mucosal biopsy to be obtained simultaneously. Microscopic examination of this fluid should always be done immediately. In certain instances, this technique has proven to be even more productive than stool microscopy (Kamath and Murugascu, 1974). Conversely, Naik et al (1978) and Madanagoplan et al, (1975) have found that examination of fluid and biopsy may be negative when stools are parasite positive.

A smaller duodenal or jejunal fluid sample can be obtained by allowing the patient to swallow a brushed nylon thread, attached to a weighted rubber bag in a gelatin capsule (Enterotest) (Beal et al, 1970). After allowing at least four hours for the weighted end to reach the duodenum, the thread is withdrawn and then fluid and mucus from the bile-stained end are expressed on a slide; the sample is mixed with a drop of saline and is finally examined by microscopy for the presence of Giardia trophozoites. This method has been shown to be as equally effective as duodenal incubation (Bezjak, 1972; Rosenthall and Leibman, 1980).

1.7.3 Small bowel biopsy

Many fixed and stained biopsy sections may need to be searched before the trophozoites are seen attached to the microvillus border, especially in the crypts and rarely invading

the mucosa (Ament, 1972). On comparing stool examinations, mucosal impression smears and biopsies from five different giardiasis patients, three of these patients were found to be parasite positive using the latter methods. A comparative study of all four methods (i) stool examination (ii) duodenal aspiration (iii) mucosal smear and (iv) biopsy was undertaken by Kamath and Murugasu (1974) using twenty-one infected patients. Giardia parasites were found in twelve of these patients using the mucosal impression smear and biopsy, ten patients were positive using the duodenal aspirate technique whilst six were correctly diagnosed by stool examination.

Mucosal impression smears are quick and easy to prepare from the biopsy; furthermore, the trophozoites are more clearly visible in these samples than in the biopsy section (Ament, 1972). More recently, the histochemical peroxidase-anti-peroxidase technique has been used to identify Giardia trophozoites in biopsy sections (Fleck et al.1985).

1.7.4 Serology

The first report of the detection of human antibodies to Giardia intestinalis was by Ridley and Ridley (1976). They developed an indirect fluorescent antibody test (IFAT) using Giardia cysts as antigen. This assay detected 32 out of 36 cases of giardiasis with malabsorption, but failed to detect two cases without malabsorption. Wright et al. (1977b) re-evaluated this assay, and found that eighteen out of nineteen cases of giardiasis with malabsorption were correctly diagnosed whilst negative results were observed with twenty different controls which included cases of enteropathy, inflammatory bowel disease and tropical sprue. Although cross-reactivity with one

of the latter eleven samples was encountered in this test.

Visvesvara et al. (1980) developed an IFAT assay which detected twenty-nine from a total of thirty patients with symptomatic giardiasis using Giardia trophozoites from axenic culture. The titres obtained for the seropositive samples did not overlap with any of the negative controls. However, cross-reactivity with five hookworm patients and/or those with Entamoeba histolytica infections was observed in this immunofluorescence assay. The indirect enzyme-linked immunosorbent assay (ELISA) test developed by Smith et al. (1981), which uses intact trophozoites as the solid phase antigen, has been shown to cross-react with the test sera from 81% of patients with symptomatic giardiasis and 12% of seventeen different uninfected controls (i.e. reflecting an assay specificity of 88%). After monitoring the seropositive cases, antibodies were detected two weeks after infection up to a period of fifteen months.

Both the IFAT and ELISA techniques, using trophozoite antigen, were employed in a comparative study with one hundred and twenty-five patients (Wittner et al. 1983). The results from this study demonstrated that the ELISA was not as specific as the IFAT, which is contrary to the findings by Smith et al. (1981).

In summary, the IFAT and ELISA techniques described above are most sensitive in the diagnosis of cases with symptomatic giardiasis, especially those with malabsorption. However, these techniques are not reliable in detecting antibodies from patients who are asymptomatic cyst passers. Potential causes of serological diagnosis inaccuracy include the persistence of anti-Giardia IgG from past infections, and cross-reactivity with other parasites may account for the false

positive reading. On the other hand, delayed and unpredictable humoral immune responses could account for false negative results in these assays.

Goka et al. (1986) reported on an IgM specific ELISA, which excluded any persisting IgG titres from past infections. Fifty-two symptomatic patients were examined with thirty negative controls. The sensitivity and specificity of this assay were both found to be 96%. In two of out of the three patients studied longitudinally, IgM levels had fallen to the normal range two to three weeks after treatment. Vinayak et al (1978), in an immunodiffusion (precipitin) test using cyst antigen, demonstrated antibodies in 90.9% of patients with giardiasis, but not in the negative controls. However, this technique proved to be subjective in interpretation, and took five days to perform after a prolonged incubation period at 37^{o}C.

1.7.5 Antigen Detection

Giardia antigens were first detected by Craft and Nelson (1982) using a counter immunoelectrophoresis technique (CIEP), which was later re-evaluated by Vinayak et al. (1985). This method was highly sensitive but was technically difficult to perform and subjective in its interpretation, requiring careful matching of each sample result to the double precipitin line of the positive control. An IFAT method for detection of intact Giardia cysts in faeces was developed, but suffered from disadvantages of UV microscopy and also demonstrated significant cross-reactivity with Chilomastix mesnili.

Ungar et al. (1984) published the first use of an ELISA test for detection of Giardia antigen in faeces. This method used crude polyclonal reagents in the double-sandwich format with a

peroxidase-labelled antispecies conjugate as the final layer. In the evaluation of faecal samples, a high sensitivity and specificity of 92% and 98% respectively were achieved. However, the assay was not amenable to direct visual interpretation and required the subtraction of optical density values for individual negative controls. This was done, in order to compensate for false positive diagnostic reactions generated by non Giardia-specific antibody-binding components present in faeces.

A reliable ELISA method using affinity purified antisera to Giardia antigen has been described with sensitivity and specificity values of 98% and 100% respectively for cases of clinical giardiasis in the United Kingdom (Green et al. 1985). When the test was evaluated in the field study in Chile the sensitivity and specificity towards faecal Giardia antigens were 99% and 96% respectively (Goldin et al. 1990). This will be discussed in detail in Chapter 2.

Recently diagnostic kits have become commercially available. For example:

a. ProSpec T/Giardia diagnostic test (Enzyme immunoassay to detect Giardia-specific antigen 65), (Alexon, Inc., Mountain View, Calif, USA). This test was evaluated with stool samples collected from hospitalised adults and refugees attending a screening clinic, the sensitivity and specificity of the test were 96 and 100% respectively (Rosoff et al. 1989). The same test was re-evaluated with stool samples collected from children, the sensitivity and specificity of the test was 98 and 100%, respectively (Addiss et al. 1991).

b. Pro Well Giardia diagnostic test (Enzyme immunoassay for the qualitative determination of Giardia trophozoite and cyst antigen in faeces) (Zical Ltd., 3809 S Main St. Santa Ana, CA 92707, USA).

c. Giardia-Cel fluorescent antibody test (High-definition monoclonal diagnostic test, for the rapid detection of Giardia cysts in human faecal specimens as well as in water and sewage samples (Cellabs Diagnostics PTY LTD., Brookvale, N.S.W. 2100 Australia).

1.8 Epidemiology and Control

1.8.1 Host species and specificity

In the last twenty years there have been numerous reports which indicate that Giardia intestinalis may be a pathogen in man (Yardley et al. 1964; Doust et al, 1969; and Keystone et al. 1978). However, there is some evidence that man may acquire infection from other animals (Ruch, 1959; Reardon and Rininger, 1968; Myers and Kuntz, 1968; Davies and Hibler, 1979; and Dykes et al. 1980). Many mammalian species harbour their own Giardia species whose relationship to G. intestinalis has not yet been properly elucidated. Beavers have been incriminated as a reservoir of infection of hikers drinking stream water contaminated by wild animal faeces (Craun, 1984). G. intestinalis from man has been transmitted to other animals (Davies and Hibler, 1979; Meyer and Radulescu, 1980). Giardia cysts from beavers and deer have caused infection in human volunteers (Davies and Hibler, 1979).

Woo and Paterson (1986) concluded recently that dogs are not reservoirs of infection in humans, on the basis of negative cross-infection experiments. However, the fact that

dogs were not susceptible to infection with human Giardia. Isolates does not prove that humans are not refractory to infection with canine isolates. In attempts to excyst and culture Giardia isolates from humans and dogs, under identical conditions, Meloni and Thompson (1987) found that 44% of human isolates, but no canine isolates, were established in axenic culture.

Many early workers placed a great deal of reliance on minor morphological differences between trophozoites for species identification. Criteria used included (1) the slant of the nuclei in relation to the body axis (2) the points of emergence of the anterior flagella in relation to the position of the nuclei (3) the density of stained cytoplasm (4) the degree of nuclear chromatin and (5) the presence of granules at the point of exit of the flagella (Ansari, 1952). However, the present author agrees with the other studies (Grant and Woo, 1978) which indicated that these features were dependent on the position and stage of the trophozoite, and the preparation methods employed (the fixation, fixatives and stain).

Although Giardia has been divided into three species (Giardia intestinalis, G. muris and G. agillis) on the basis of the size and shape of trophozoites and their median bodies, morphometric studies of the intestinalis group from human and mammalian isolates have failed to be consistently useful as species criteria (Filice, 1952; Bertram et al. 1983).

The infection of human volunteers, with distinct human isolates of Giardia from axenic cultures has been studied by Nash et al (1987). This group has confirmed Koch's postulates for G. intestinalis and has also demonstrated strain variation in the pathogenicity of Giardia infection in humans. Antigenic differences have been used to distinguish different strains of

Giardia using polyclonal antisera, crossed immuno-electrophoresis (Smith et al. 1982; Nash and Keiseter, 1985) and monoclonal antibodies. Use of cloned isolates in future studies is imperative, as strains may be composed of several different genetic lineages.

The isoenzyme electrophoresis results in which six enzymes [hexokinase, malate dehydrogenase, malic enzyme (decarboxylase), glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and a-glycerophosphate dehydrogenase], were analyzed, has enabled the separation of six Giardia isolates into three different zymodemes (Bertram et al. 1983). These have been designated zymodeme I, from two humans and one cat isolate; zymodeme II, from a guinea pig; and zymodeme III from a human and rabbit isolate. Recent work by Korman et al. (1986) has resulted in further strain identification.

Finally restriction endonuclease analysis of DNA has allowed the separation of Giardia isolates from human and lower animals (Nash et al. 1985). The two techniques used for this were (i) agarose gel electrophoresis followed by ethidium staining and (ii) Southern blotting with recombinant plasmids using Giardia DNA as probes. The latter method yielded a greater variety of patterns amongst i.e nine amongst fifteen different isolates. Both cDNA and genomic DNA of G. intestinalis have been produced; however, the products have yet to be fully characterised with respect to diagnostic or epidemiological potential (Upcroft et al. 1987).

1.8.2 Transmission

The number of G. intestinalis cysts excreted in stools

may reach a maximum of 900 million during one day (Feacham et al. 1983). The median infective dose for man is between 25 and 100 cysts (Rendtorff, 1954; Rendtorff, 1979; Rendtorff and Holt, 1954). Infections in adults, demonstrable by the presence of cysts in faeces, and therefore communicable, have been experimentally observed to last up to forty-one days. In children, the course of infection may be much longer even up to several years (Wright, 1980). The patient with marked symptoms of intestinal giardiasis is a potent source of infective cysts. This may be a reason why outbreaks of giardiasis are more common than those of amoebiasis. In developing countries, giardiasis is more prevalent in poor communities with inadequate sanitation, and where the pattern of infection is typically endemic. Recorded infection rates, amongst children between one and five years of age, have been the highest in various communities and ranged from one to over twenty percent (Feacham et al. 1983).

Transmission to human occurs by :-

i. Faecal-oral transmission

Most of these infections are probably spread by the hand-to-mouth method. Host factors facilitating this transmission include:-

a. Diarrhoeal stools aiding dispersal in the environment, particularly by infants and mentally retarded people, accounting for outbreaks of giardiasis in child day-care centres (Black et al. 1977; Keystone et al. 1978; Pickering et al. 1984) and mental institutions (Yoeli et al. 1972).

b. Occurrence of asymptomatic infection leading to contamination by people unaware of their infectivity.

c. Reinfection of people with normal immune response may

occur with a sufficiently large infective dose (Rendtorff, 1954).

d. Sexual activity, particularly between homosexual males, has been described as a factor facilitating Giardia transmission (Meyers et al. 1977; Schmerin et al. 1978 and Levine,1991).

e. Overcrowding and lack of sanitation.

ii. Waterborne spread of giardiasis:

Another means of spread is via drinking water, particularly surface water that has been either untreated or insufficiently treated. The best documented cases have been in developed countries, particularly in the USA and USSR. For example, during an outbreak in this disease at a ski resort at Aspen, Colorado in 1965 (Moore et al. 1969) fluorescent and detergent tracers placed in the sewers were detected in two of the three water sources. Other endemics, in the United States between 1965 and 1981, have been reviewed by Craun (1984). During this period, fifty-three waterborne outbreaks of infections were documented involving more than 20,000 individuals (Craun, 1984).

Waterborne giardiasis has also afflicted travellers into Leningrad, USSR (Jokipii and Jokipii, 1974) and a waterborne outbreak has recently been reported in the United Kingdom following repairs to a water mains supply (Jephcott et al. 1986). As discussed earlier, it is strongly suspected that animal reservoirs both domestic and wild, may be an additional source of human infection (Davies and Hibler, 1979). In fact, the beaver is the most probable cause for the contamination of water sources in North America (Davies and Hibler, 1979; Dykes et al. 1980).

iii. Transmission via food

Food contamination with Giardia may occur either from contaminated hands or from contaminated insects (mechanically). However, transmission via food has been rarely reported (Osterholm et al. 1981). This may be due to the difficulty of being able to provide proof.

1.8.3 Control measures

Areas for consideration in the control of Giardia infection in the community include:-

1.8.3.1 Individual control

This should include :-

(a) Public health education programmes about Giardia, its life cycle, methods of its transmission, and ways of protection from this parasite.

(b) Treatment of the positive cases reduce the reservoir. Several drugs were found to have an effect on Giardia intestinalis 'in vitro' (Boreham et al. 1984; Boreham et al. 1987, Boreham, 1988; Farthing and Inge, 1986; Inge and Farthing, 1987; Meloni et al. 1990) and 'in vivo' with different side effects (Bassily et al. 1970; Bassily, 1987 and Al Waili, et al. 1988).

The effect of the drugs on the Giardia parasite were different from one drug to another, either by effecting the growth or both growth and adherence (Jokipii and Jokippi, 1980;

Gillin and Diamond, 1981; Smith et al. 1982; Boreham et al. 1984). Although several drugs are available for effective treatment of positive cases, therapeutic failure was occasionally seen (Levi et al. 1977; Kavonsi, 1979; Mendelson, 1980; Craft et al. 1981; Upcroft et al. 1990). Even though no agent was recommended for mass chemotherapy, due to their side effects and expense, Buelna (1989) found that furazolidone (furoxone) and metronidazole (Flagyl) were equally safe and effective in treating children with giardiasis.

1.8.3.2 Environmental control

This should include:-

(a) Removal of Giardia cysts from the environment. Tests for G. intestinalis cysts in water and other samples are currently quite inadequate. The presence of cysts (at concentrations below 400 per litre) may be missed altogether (Feacham et al. 1983). The sensitivity of detection and specific identification of cysts may be improved by fixing and staining material 'in situ' on a filter membrane (Spaulding et al. 1983) or the application of an IFAT technique (Sauch, 1985).

Establishing cyst viability is complex and controversial. Traditionally eosin exclusion and excystation 'in vitro' have been reviewed by Jarroll et al. (1983). However, fluorescence in diacetate and propidium iodide staining (Schupp and Erlandsen, 1987a) and differential interference contrast or brightfield microscopy (Schupp and Erlandsen, 1987b) have all given more reliable results. Giardia cysts are resistant to chlorination under conditions

often found in water treatment plants and particularly at low temperatures (Feachem et al. 1983). Resin disinfectants (strongly basic quaternary ammonium anion exchange resin thiodide or penta iodide) are effective in the inactivation of Giardia cysts (Marchin et al. 1983) and may find use in individual portable water treatment devices.

Filtration experiments have indicated that Giardia cysts can be removed from drinking water supplies by coagulation plus granular medium filtration, or by diatomaceous earth filtration but only with a high quality filter plant operation (Logsdon et al. 1979).

(b) Sanitation control by proper sewage disposal. These expensive measures can be justified only as part of a comprehensive effort to control all waterborne enteric diseases (Stevens, 1985).

CHAPTER 2

DEVELOPMENT OF ENZYME-LINKED IMMUNOSORBENT ASSAY FOR DETECTION OF GIARDIA ANTIGEN IN FAECES

2.1. Introduction

Diagnosis of giardiasis both in industrialised and developing countries relies largely on the microscopic detection of stages of the parasite (trophozoites and cysts) in faeces, duodenal fluid or small intestinal mucosal impression smears and biopsies (Kamath and Murugasu, 1974). Faecal examination for trophozoites and cysts remain the most widely used conventional test (Sawitz and Faust, 1942), but it is time consuming, labour intensive and depends heavily on the skill of an experienced observer. It was found that even after examination of three faecal specimens from patients with known giardiasis up to 15% of these individuals have negative stool examination (Farthing et al. 1987; Sawitz and Faust, 1942). False negatives are due to the poor sensitivity of microscopical methods, but also to the fact that cyst excretion rates are highly variable in patients and from one day to another (Daneiger and Lopez, 1975). For these reasons diagnosis by microscopy is not entirely reliable (Wolfe, 1984). The alternative methods of small bowel biopsy or duodenal aspiration are invasive and impractical, especially for children.

An indirect fluorescent antibody test (IFAT) using Giardia cysts as antigen was developed by Ridley and Ridley (1976), and re-evaluated by Wright et al. (1977). Using

Giardia trophozoites from axenic culture as antigen, Visvesvara et al. (1980) developed an IFAT, which was compared by Wittner et al. (1983) with an indirect ELISA test developed by Smith et al. (1981), employing intact trophozoites as a solid phase antigen. These serodiagnostic tests were most sensitive in diagnosis of patients with symptomatic giardiasis especially with malabsorption, but did not reliably detect asymptomatic cysts passers. Recently Goka et al. (1986) developed an ELISA test to demonstrate specific anti-Giardia IgM antibody in adults, with both a sensitivity and a specificity of 96%, while Sullivan et al. (1991) used the same test in children, with a sensitivity and a specificity of 63% and 93%, respectively.

Because of the erratic nature of Giardia cyst excretion, the time consuming nature of microscopy and the unreliability of serology for diagnosis, a simple method for detection of free Giardia antigens in faeces which does not necessarily rely on the presence of intact parasites has long been needed.

The first radioimmunoassays (RIA) developed for the detection of antigens in faeces were used in the diagnosis of rotavirus and Hepatitis A infections (Hollinger et al. 1975; Kalica et al. 1977 and Hall et al. 1977). The disadvantages of using radioisotopes, which include short shelf life, health hazards and the need for complex detection equipment, have led to the adaptation of non-isotopic assays for field use in the diagnosis of parasitic disease (Voller et al. 1976; Voller and De Savigny, 1981). An ELISA test was developed to detect rotavirus antigens in faeces (Yolken et al. 1977).

In the development of these and subsequent assays, many problems peculiar to the faecal environment were

recognised. These included the destructive properties of faecal components causing solid phase disorption or lysis (Hanvanich et al. 1985), and copro antibodies and other factors linking successive immunoreagent layers in the absence of antigen to generate false positive results (Yolken and Stopa, 1979; Viscidi et al. 1984; Krause et al. 1983).

Counter immuno-electrophoresis was used for the detection of Giardia antigens in faeces (Craft and Nelson, 1982; Vinayak et al. 1985). This method was highly sensitive but was technically difficult to perform and subjective in its interpretation, requiring careful matching of each sample result to the double preciptin line of the positive control. The ELISA test was first used for detection of Giardia antigen in faeces by Ungar et al. (1984), and re-evaluated by Vidal et al. (1991). This method used crude polyclonal reagents in the double-sandwich format with a peroxidase labelled antispecies conjugate as the final layer. The sensitivity of this test was 92%, and when re-evaluated it was 84%. The assay was amenable to direct visual interpretation, but required the subtraction of optical density values for individual negative controls, which were included to compensate for false positive diagnostic reactions generated by non Giardia-specific antibody-binding components of faeces. A reliable ELISA test using affinity purified antisera to Giardia antigen has been described with a degree of separation of positive from negative optical density readings sufficient to allow direct visual interpretation, and with sensitivity and specificity of 98% and 100% respectively in cases of clinical giardiasis in the United Kingdom (Green et al. 1985). When the test was evaluated in a field study in Chile the sensitivity and specificity to detect faecal Giardia

antigens were 99% and 96% respectively (Goldin et al. 1990). Recently the test was re-evaluated to compare the same stool samples preserved in phosphate-buffered saline (PBS) (pH7.2) or in 2% formalin/PBS (Goldin et al. 1992).

Recently an ELISA test employing monospecific antibodies against Giardia specific 66KDa antigen present in stool was developed (Dutt and Vinayak, 1990; Dutt et al. 1991). Even this test was highly sensitive and specific, but was faced with the same problems as the ELISA test of Ungar et al. (1984). More recently a dot-ELISA test employing monospecific antibodies to a Giardia specific copro antigen with a molecular mass of 66KDa was developed (Vinyak et al. 1991). This test was found to have less sensitivity than micro ELISA (Vinayak et al. 1991). In this study we used the ELISA test developed by Green et al. (1985), with some minor technical alterations, for:

a. Evaluating the test in the field study.
b. Studying the epidemiology of Giardia in Wadihaloo village.
c. Studying the role of expatriates in importing Giardia to the UAE.
d. Studying the impact of Giardia on the physical growth of children in Wadihaloo village.
e. Studying the source of Giardia infection in Wadihaloo village.
f. Studying the role of treatment in Giardia control.

2.2. Materials and Methods

2.2.1. Antigen Production

2.2.1.1. *Giardia* trophozoites from axenic culture

Axenic culture of the organisms was performed using a minor modification of the TYI-S-33 culture medium described by Keister (Keister 1983). (For medium recipe and preparation, see Appendix).

Parasites were cultured as follows:

One screw-capped ampoule of cryopreserved *G.intestinalis* trophozoites (Portland 1 human strain, provided by Dr. D. C. Warhurst, London School of Hygiene and Tropical Medicine) was taken up from liquid nitrogen storage, thawed quickly to 37°C in a water bath, and the contents inoculated into two flat sided 13ml plastic culture tubes (Nunc) filled with 10ml of culture medium.

These were tightly capped and incubated at 37°C. The medium was changed on the second day by briskly decanting the warm tube and refilling immediately with fresh medium. Cultures were checked on an inverted microscope, the trophozoites form an adherent monolayer on the flat surface of the tubes. To expand the culture, trophozoites were dislodged by repeated gentle inversion of the culture tube, after chilling on ice for 30 minutes.

Subculturing was done by two methods:

a. An old culture tube was centrifuged at 200xg for 10 minutes and the pellet resuspended in 1ml of medium, 100µl of a heavy cell suspension (usually 2 x 10^4 to 2 10^5 cells) sufficient to generate maximum growth was added to the new culture tube containining fresh medium.

b. The contents of the old culture tube were added to new culture tubes containing fresh medium (1ml in each tube).

The culture tubes were incubated at 37°C in a slightly inclined position and were subcultured at intervals of 72 and 96 hours.

The final harvest from culture was achieved by chilling tubes on ice for 30 minutes to detach the organisms, centrifuging at 200 xg for 10 minutes and washing the pellet 3 times in cold phosphate buffered saline (PBS pH 7.2). The parasites were counted in a haemocytometer.

The preparation of antigen by disruption was performed as follows:

1. The harvested washed pellet of parasites was resuspended in a small volume of PBS and freeze-thawed by submerging in liquid nitrogen and allowing to thaw in running tap water. This process was repeated twice.
2. The preparation was then sonicated at a wavelength of 12U for 30 seconds on ice. This was repeated three times with a one minute interval between bursts (to avoid over-heating).
3. Protein concentration was determined by the method of protein-dye binding as described by Bradford (1976).

2.2.1.2. Isolation of *Giardia* cysts from faeces

Only faecal samples known to contain high concentrations of cysts (as detected by microscopy of iodine-stained formol-ether concentrates, Allen and Ridly, 1970)

were processed.

a. By a modification of the method of Roberts-Thomson: (Roberts-Thomson et al. 1976; Moody, 1976; Bingham et al. 1979).

Approximately 5gm of fresh stool was emulsified with cold distilled water with a mortar and pestle, made up to 100ml with distilled water, then filtered through a wire sieve, (mesh size 450μm).

The faecal suspension was then allowed to stand in an ice-water bath for 30 minutes to allow large particles to settle out. 10ml of supernatant was carefully layered onto 10ml of cold 0.85M sucrose in a 20ml plastic universal container, which was then centrifuged at 200 xg for 15 minutes at 4°C. Material from the water-sucrose interface of several tubes was carefully aspirated with a pipette, then washed by 1:10 dilution in distilled water followed by centrifugation at 600 xg for 10 minutes. The pellet was then resuspended and the sucrose density gradient procedure repeated. Material at successive stages of the process was monitored for cyst content by microscopy. Finally, lipid soluble debris was removed by resuspending the final pellet in 7ml of distilled water in a large screw-topped glass tube, adding 5ml of diethylether, vortexing the tightly capped tube for 3 minutes, followed by centrifugation at 600 xg for 5 minutes. Material at the ether-water interface was loosened with a stick and the supernatant removed by brisk decantation, retaining the Giardia cyst pellet at the bottom of the tube.

b. By Sephadex G.50 (Douglas et al 1987).

Sephadex G.50 (Sigma -G.50.-80) was suspended in PBS (30gm/litre) and allowed to swell for 3 to 5 days at room temperature before being used.

Approximately 1-1.5gm of fresh stool was emulsified (dilution 1:20) with PBS or cold distilled water with a mortar and pestle, then filtered through a wire sieve (mesh size 450µm). The faecal suspension was then allowed to stand in an ice water bath for 30 minutes to allow large particles to settle out. The supernatant was carefully layered onto the packed column. The parasites were washed through the column with 5 volumes of cold distilled water or PBS, then collected into tubes. (The separation of Giardia from faeces is probably due to the configuration of the Sephadex, which retains most faecal material, allowing the parasites, and some bacteria to pass through). Parasites collected were washed 4-5 times in cold distilled water or PBS by centrifugation at 200 xg for 5 minutes at 4°C (to remove most of the remaining bacteria). The cysts were counted in a haemocytometer chamber.

The trophozoites and the cysts were stored at -20°C.

2.2.1.3. Cryopreservation of Giardia trophozoites

The cryopreservation procedure employed (Warhurst and Wright, 1979) was as follows:

A tube of G.intestinalis culture was chilled and centrifuged at 200 xg for 10 minutes at 4°C. Most of the supernatant was decanted and the pellet resuspended on a concentration of approximately 10gm/ml. This suspension was

distributed in 0.25ml amounts into plastic screw capped ampoules, and an equal volume of 15% dimethyl sulphoxide (Sigma D-5879) in MRC Ringer was added and mixed. The suspension was allowed to equilibrate at room temperature (20°C) for 5 minutes.

The ampoules were then placed in an insulated box of crushed ice and transferred to a -70°C freezer. After 6 hours the ampoules were transferred to storage in liquid nitrogen. Recovery of frozen sample was as described above.

2.2.2. Purification of Antibodies

2.2.2.1. Immunization protocols (Green et al 1985).

Three Dutch white rabbits (Cheshire Rabbits Ltd, Tarporley, Cheshire) were used.

1. Intact Giardia trophozoites in saline (for immunization of rabbit number one).

   Trophozoites of G.intestinalis (Portland I human strain) were harvested from axenic culture and washed 3 times in PBS (as described above).

   Approximately 5 x 10^6 trophozoites were resuspended in 1.0ml of PBS and the rabbit was immunized by intraperitoneal (IP) injection of the intact parasites. The procedure was repeated on days 14, 30 and 40. The animal was bled on day 40, (venous blood obtained from the ear vein was allowed to stand in a test tube at room temperature until it clotted). The clot was loosened from the wall of the tube with a stick and after incubation at 4°C for two hours to allow clot retraction, the tube was centrifuged at 400xg for 15 minutes, and the serum retained. The bleeding was

repeated at 10 weekly intervals after booster doses of antigen.

2. Disrupted Giardia trophozoites in Freund's adjuvant (for immunization of rabbit number two). 0.5ml of a disrupted trophozoite preparation in PBS at a concentration of 10^7/ml (as described above), was emulsified in 1.0ml of Freund's complete adjuvant (Sigma F5881) (FCA) by rapid passage between two 2ml glass syringes via a dual needle for 5 minutes. The state of the emulsion was then tested by ejection of a droplet onto water. The rabbit was immunised by intramuscular (IM) injection of 0.75ml into each of two sites. The procedure was repeated, substituting Freund's incomplete adjuvant (Sigma F5506) (FIA) for complete on days 14, 30 and 40. The animal was bled on day 40 and at 10 weekly intervals after booster doses of antigen.

3. Disrupted Giardia cysts in Freund's adjuvant (for immunization of rabbit number three).

   Giardia cysts were isolated from infected human faeces (as described above) disrupted by freeze/thaw and sonication cycles, then 5 x 10^6 were emulsified in FCA as in (2). The rabbit was immunised by IM injection on the days specified above, and using FIA on the 2nd injection and subsequent boosters.

2.2.2.2. Affinity Purification (isolation) of anti-Giardia antibodies

Affinity chromatography is a simple technique for the

purification of even minor components from complex mixtures. It involves the irreversible (covalent) immobilization of a ligand (here, Giardia antigen) to a matrix such as agarose (here Sepharose 4B). When serum containing a complex antibody mixture is added, only specific antibody binds to the system. Other non specific components are washed away through the column. Elution of specific antibody can then be achieved by disruption of the antigen-antibody interactions by changing environmental conditions (e.g. such as ionic strength or pH).

A. Preparation of a Giardia antigen-Sepharose affinity column.

1. A Giardia trophozoite pellet of approximately 5 x 10^8 parasites was harvested from axenic culture. Washed in PBS, and the total protein content estimated by the method of protein-dye binding as described by Bradford (1976).
2. A soluble extract of total proteins including membrane components was prepared by resuspending the freeze/thawed parasites in 5ml of lysis buffer (see Appendix), containing the detergent Nonidet P-40 (Sigma N 6507) 0.5% (v/v), and gently stirring at 4°C for 30 minutes.
3. The mixture was then centrifuged at 200xg for 15 min. at 4°C and dialysed against PBS, followed by two 2 litre changes of coupling buffer (0.1 M $NaHCO_3$ pH 8.3 containing 0.5M NaCl, appendix). The final protein concentration was adjusted to 2.5mg/ml with coupling buffer.
4. Approximately 1.7g (dry wt) of Cyanogen-bromide

actived (CNBr) Sepharose-4B (Pharmacia 17-0430-01) was swollen and then washed with 5 x 200ml changes of 1 mM HCL in a glass beaker, decanting the "beads" at each change.

5. The gel was then transferred to a 20ml glass universal container on ice and washed quickly (by centrifugation at 200 x g for one min.) with 15 ml of coupling buffer at 4°C.

6. The supernatant was quickly aspirated and the 10ml of Nonidet P-40 extract of Giardia protein was added immediately. The glass container was then incubated at 4°C for 16 hours on a rotating wheel.

7. After allowing the gel to settle, the supernatant was aspirated and retained for analysis. The unreacted sites on the gel were blocked by addition of 15ml of 0.1M glycine HCl in coupling buffer followed by rotation at 4°C for 16 hours.

8. After allowing the gel to settle, the supernatant was removed, then the gel was washed in a glass beaker with 200ml of coupling buffer, followed by 0.1M sodium acetate buffer pH4 containing 0.5M NaCL (Appendix), then finally by 0.1M Tris HCl buffer pH 8.0 containing 0.5M NaCl.

9. The gel was transferred to a plastic column (initially a 10ml plastic syringe blocked with nylon wool, for subsequent columns, purpose-made columns (Econo-column, Bio-Rad 731-1550) were used.

10. The column was washed alternately with 5 column volumes (about 30ml) of Tris-HCl buffer pH 8 and with the sodium acetate buffer pH 4. The column was finally washed with PBS, and stored at 4°C after

equilibrating with PBS containing 0.02% (w/v) merthiolate (Thimerosal, Sigma T5125). A total of 6 columns were prepared subsequently in a similar manner.

11. The coupling efficiency of the method was assessed by analysis for residual protein (by the method of Protein-dye binding as described by Bradford 1976) at the pre and post coupling stages of preparation.

B. Affinity purification:

1. Each of the three pooled serum preparations was processed on the column. The approximate column capacity for each was determined by allowing serial doubling diluations of serum in PBS (0.5ml) to equilibrate, for 30 minutes at room temperature on a rotating wheel, with 1.0ml aliquots of 50% gel in PBS in Eppendorf tubes, followed by centrifugation at 10000 x g for 3 minutes. The appropriate volume of serum in relation to wet column volume was selected from the tube dilution yielding minimum specific antibody in the supernatant.
2. For affinity purification all reagents and the column were allowed to reach room temperature before use to avoid bubble formation disrupting the flow characteristics of the column.
3. The column was washed with 30ml of PBS (running buffer). 3ml of serum was loaded carefully onto the top of the column with a Pasteur pipette, and recirculated twice through the column.
4. The column was then washed with 50ml of PBS.

5. Bound antibody was eluted by addition of 0.1M glycine- HCl buffer pH 2.5.

6. 3ml fractions were collected on glass tubes, into the bottom of which had been placed 200µl of 2M tris-HCl pH8. (This volume had been determined by titration to alter the pH of 3ml of glycine buffer to 7.4). The eluate was neutralised immediately on collection by agitation of the relevant tube. Each fraction was transferred to ice immediately on collection.

7. Protein content of fractions was estimated by determining the absorbance at 280nm in UV spectrophotometer (Sp8-500, Philips). An extinction coefficient E (0.1%/280), of 1.41 for the optical density (OD) of a 1mg/ml solution of immunoglobulins (Ig) at 280nm was used for the calculation).

8. The first 6 positive fractions (2-7 inclusive) were pooled, dialysed against PBS at 4°C, then concentrated by vacuum dialysis, or, for pools from several columns concentrated in an Amicon protein concentrator using a Diaflo YM 10 membrane.

9. The final affinity isolated antibody solution was centrifuged at 10000 x g for 10min to remove particulate material, protein concentration was determined by absorbance at 280nm (as described above), and the final concentration adjusted to 10mg/ml and stored in 200µl aliquots at -20°C.

10. The column after use was washed alternately with 5 column volumes of HCl buffer pH8 and sodium acetate buffer pH 4, then washed and stored with PBS containing 0.02% methiolate at 4°C.

11. For more rapid production of larger batches of

antibody, all 6 columns were processed in parallel.

2.2.2.3.Conjugate preparation

Horseradish peroxidase (HRP) was conjugated to the affinity purified rabbit-anti-Giardia Ig by a two step glutaraldehyde method (Avrameas and Ternynck, 1971).

1. 17mg of HRP (Type VI, Sigma P8375) was dissolved in 0.34ml of 0.1M PBS, pH 6.8 containing 1.25% glutaraldehyde (grade 1, Sigma G5882), in the original HRP bottle. The mixture was rotated overnight at room temperature.
2. The glutaraldehyde-activated peroxidase was dialysed against two litres of 0.15M NaCl in a short length of boiled dialysis tubing (Visking size 1-8/32", Medicell Int. Ltd) secured by two dialysis clips at 4°C overnight.
3. The final volume was made up to 1.7ml with 0.15M NaCl saline.
4. Affinity purified rabbit-anti-Giardia Ig had been dialysed against and diluted to 5mg/ml in 0.15M NaCl.
5. 1.7ml of antibody solution was mixed with 1.7ml of the glutaraldehyde activated HRP solution and 0.17ml of 1M carbonate/bicarbonate buffer pH9.5 in a 5ml glass bottle and was rotated for 24 hours at 4°C.
6. 0.17ml of 0.2M lysine solution was added and the mixture was rotated at room temperature for two hours.
7. The mixture was dialysed against 2 x 2 litre changes of PBS pH7.4 overnight.

8. The enzyme labelled antibody was precipitated by the additon of an equal volume of saturated ammonium sulphate (SAS), and centrifugation at 600 x g for 30 minutes. The precipitate was similarly washed twice in 50% SAS, then suspended in 1.7ml of PBS.
9. The conjugate was dialysed against 3 x 2 litre changes of PBS overnight at 4°C then centrifuged at 10,000 x g for 30 minutes and the dark sediment discarded. Bovine serum albumin (BSA) (Sigma A 4503) was added to a final concentration of 1%.
10. The conjugate was stored in 50% glycerol at -20°C.
11. Products at each step were observed by absorption spectroscopy, using quartz cuvettes.

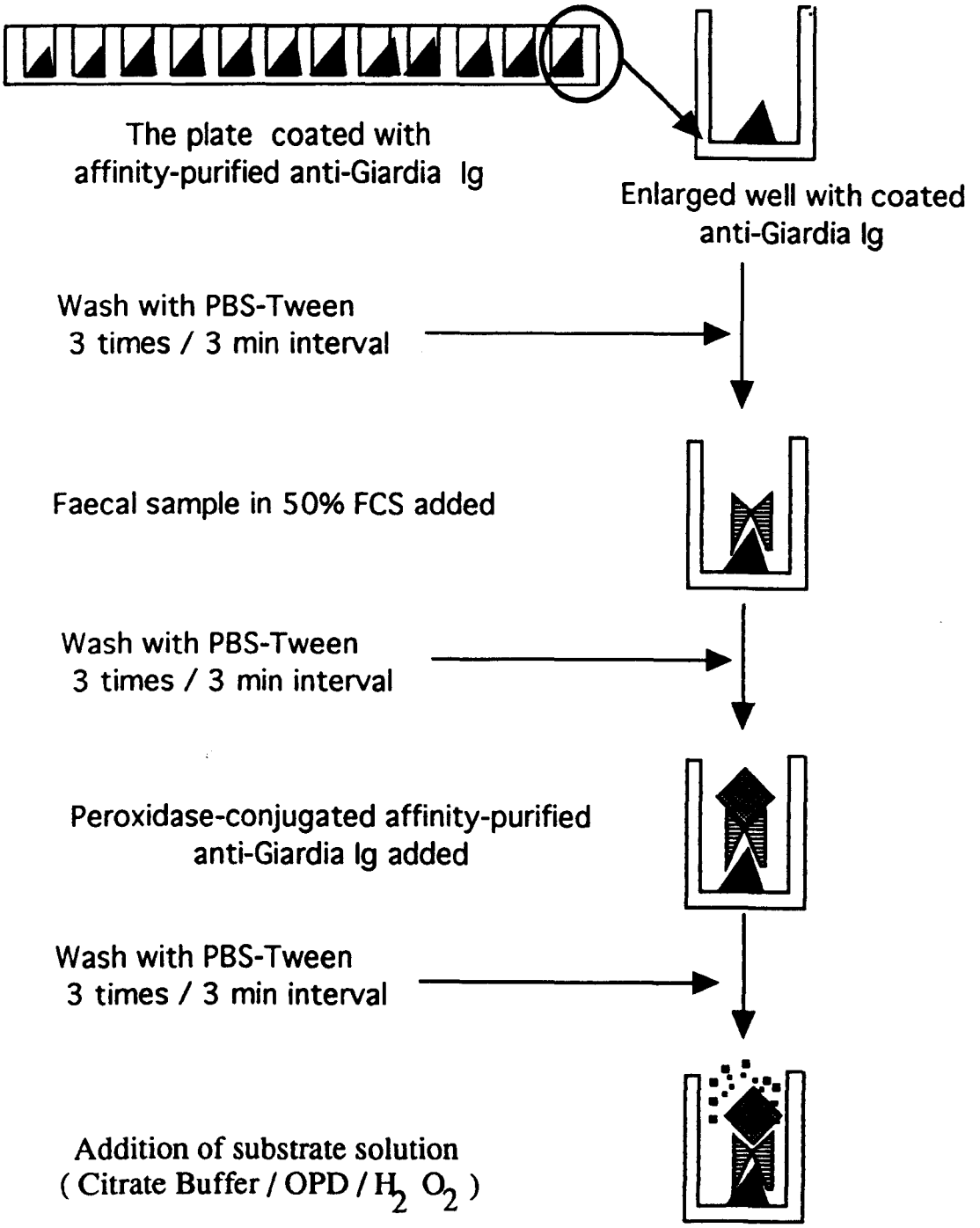
2.2.3. Development of the Sandwich ELISA (Figure 2.1.).

In the development of the test we will follow Green et al. (1985) protocol with some modification.

2.2.3.1. Protocol for the sandwich ELISA for detection of Giardia antigen in faeces (Green et al 1985).

1. To each well of a Dynatech ELISA plate (M129A, 655001) add 200µl of affinity purified pooled Giardia antisera at a concentration of 10µg/ml in carbonate coating buffer pH9.6 (see Appendix). Incubate the plate overnight at 4°C.
2. Wash the plate three times with PBS containing 0.05% "Tween 20" (PBS Tween) with a three minutes incubation after each time.

Figure 2.1 Two site sandwich ELISA for detection of Giardia antigen in faeces



3. To each well in duplicate add 200µl of a mixture of equal volumes of Foetal Calf Serum (FCS) and a 10gm% suspension of stool sample in PBS. Include a 50% FCS in PBS control in each assay. Incubate for one hour at 37°C.
4. Wash plate again as in step 2.
5. Add 200µl of affinity-purified rabbit-anti-Giardia-peroxidase conjugate at optimal dilution (1:1000) in 50% FCS in PBS-Tween, to each well. Incubate at room temperature for 90 minutes.
6. Wash plate again as in Step 2.
7. Add 200µl of substrate solution (see Appendix) to each well and incubate in the dark at room temperature for 30 minutes.
8. Stop the reaction by addition of 50µl of 2.5M H_2SO_4 to each well.
9. Read optical density in an ELISA reader at 492nm. (Titertec Multiskan) Micro-ELISA reader (Flow Laboratories).

2.2.4. Evaluation of the ELISA test

50 randomly selected microscopically positive for Giardia, faecal samples, and another 20 faecal samples (from known negative control) negative for Giardia by microscopy of formol-ether concentrates, were examined with ELISA test to assess the sensitivity and specificity of the test.

2.3. Results

2.3.1. Antigen Production

During subculturing of the Giardia trophozoites, we found that the method of directly adding the contents of the old culture tube (without centrifugation) to the new culture tubes containing fresh medium (1ml in each tube), after dislodging the trophozoites from the surface of the tube by chilling on ice for 30 minutes and repeated gentle inversion of the culture tube was easier, quicker and more economical than the other method which needs centrifugation.

For isolation of Giardia cysts from infected stool, Sephadex-G.50 method was found more effective in separation of the cysts from the debris, than sucrose method (Roberts-Thomson method), but the number of cysts recovered was less.

2.3.2. Purification of Antibodies

During the making of the affinity column, the binding efficiency of the Giardia trophozoite antigen to the CNB-r Sepharose 4B was estimated.

$$\text{Binding efficiency} = \frac{a-b}{a} \times 100\%$$

where a = the amount of Giardia protein added to the activated gel in mg, and b = the amount of residual (unbound) Giardia protein in the supernatant after completion of the reaction.

Fractions 2 to 7 inclusive from different columns were dialysed, pooled, concentrated and the protein content was determined, fractions were stored in aliquots at -20°C.

2.3.3. Modification in the ELISA assay

2.3.3.1. Determination of optimum capture antibody concentration (Figure 2.2)

1. Six rows of 6 wells of ELISA plate were coated, with 200µl per well of affinity purified rabbit-anti-Giardia Ig in coating buffer pH9.6 at concentration of 0.75µg, 1.5µg, 3µg, 4µg, 6µg and 9µg/well in the first, the second, the third, the fourth, the fifth and the sixth row respectively. The plate was incubated overnight at 4°C.
2. The plate was then washed three times with PBS-Tween with a three minute incubation after each time.
3. To each well in duplicate in each row, added 200µl of equal volumes of FCS and, 10gm% suspension of pooled positive stool in PBS, 10gm% suspension of pooled negative stool in PBS and PBS only respectively. The plate was incubated for one hour at 37°C.
4. The plate was washed as in step 2.
5. To each well was added 200µl of affinity-purified rabbit-anti-Giardia peroxidase conjugate at a dilution of 1 in 1000 in 50% FCS in PBS-Tween. The plate was incubated for 90 minutes at room temperature.
6. The plate was washed again as in step 2.
7. To each well 200µl of substrate solution was added. The plate was incubated in the dark at room temperature for 30 minutes.
8. The optical density was read in an ELISA reader at a wavelength of 490nm.

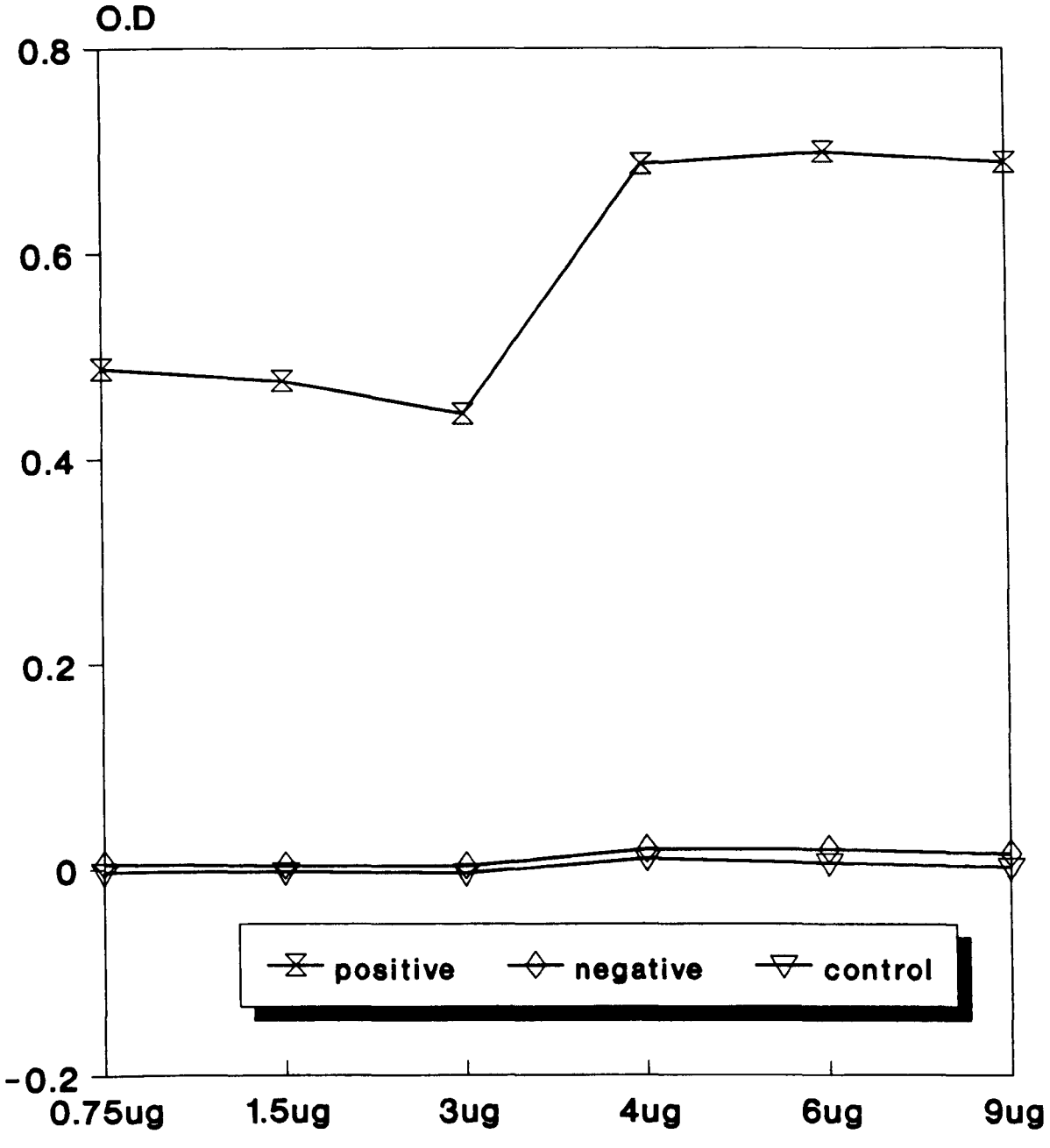


Fig.2.2 Titration of capture antibody in the ELISA assay .

The increase in the concentration of antibody in the ELISA assays, as well as leading to an increase in sensitivity it may also increase the non specific binding to which antibody-excess assays are prone, Figure 2.2 shows that the optimal concentration of capture antibody was 15µg/ml (3µg/well).

2.3.3.2. Comparison between centrifuged and non centrifuged stool samples (Figure 2.3)

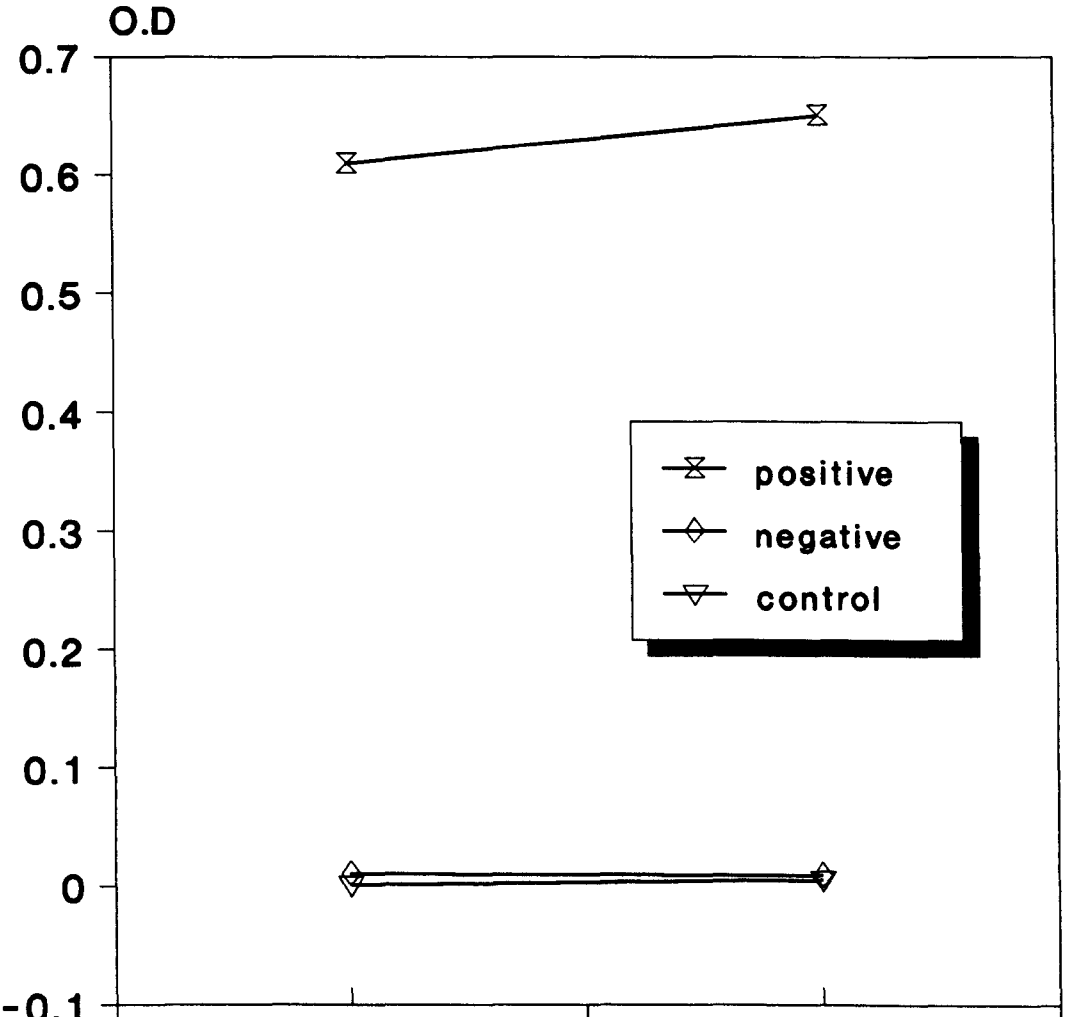
1. Two rows of 6 wells of an ELISA plate were coated with 200µl per well of affinity purified rabbit-anti-*Giardia* Ig with a concentration of 15µg/ml (3µg/well) in coating buffer. The plate was incubated overnight at 4°C.
2. The plate was then washed three times with PBS-Tween with a three minute incubation after each time.
3. To each well in duplicate in each row, 200µl of equal volumes of FCS and 10gm% suspension of pooled positive stool in PBS, 10gm% suspension of pooled negative stool in PBS and PBS only were added respectively. The suspension of the stool samples in the first row were left to stand for 30 minutes, while the samples in the second row were centrifuged at 200 xg for 10 minutes. The plate was incubated for one hour at 37°C.
4. The plate was washed as in step 2.
5. Subsequent steps in the assay were performed as in (2.3.3.1.).

It was found that no difference in the ELISA assay could be detected if the suspension (stool in PBS) was

centrifuged or left to precipitate for 30 minutes.

2.3.3.3. Titration of conjugate on the ELISA assay (Figure 2.4)

1. Six rows of 6 wells of an ELISA plate were coated with 200µl per well with affinity purified rabbit-anti-Giardia Ig with a concentration of 15µg/ml (3µg/well) in coating buffer pH9.6. The plate was incubated overnight at 4°C.
2. The plate was then washed three times with PBS-Tween with three minutes incubation after each time.
3. To each well in duplicate in each row, 200µl of equal volumes of FCS and 10gm% suspension of pooled positive stool in PBS, 10gm% suspension of pooled negative stool in PBS and PBS only were added respectively.
   The plate was incubated for one hour at 37°C.
4. The plate was washed as in step 2.
5. 200µl of affinity-purified rabbit-anti-Giardia-peroxidase conjugate in 50% FCS in PBS-Tween was added to each well in the following dilutions: 1 in 200, 1 in 400, 1 in 600, 1 in 800, 1 in 1000 and 1 in 1200 in the first, the second, the third, the fourth, the fifth and the sixth row respectively. The plate was incubated for 90 minutes at room temperature.
6. The plate was washed as in step 2.
7. Subsequent steps in the assay were performed as in (2.3.3.1.).



	noncentrifuged	centrifuged
positive	0.609	0.65
negative	0.01	0.009
control	0.001	0.005

Fig. 2.3 Comparison between using noncentrifuged and centrifuged stool samples in the ELISA assay.

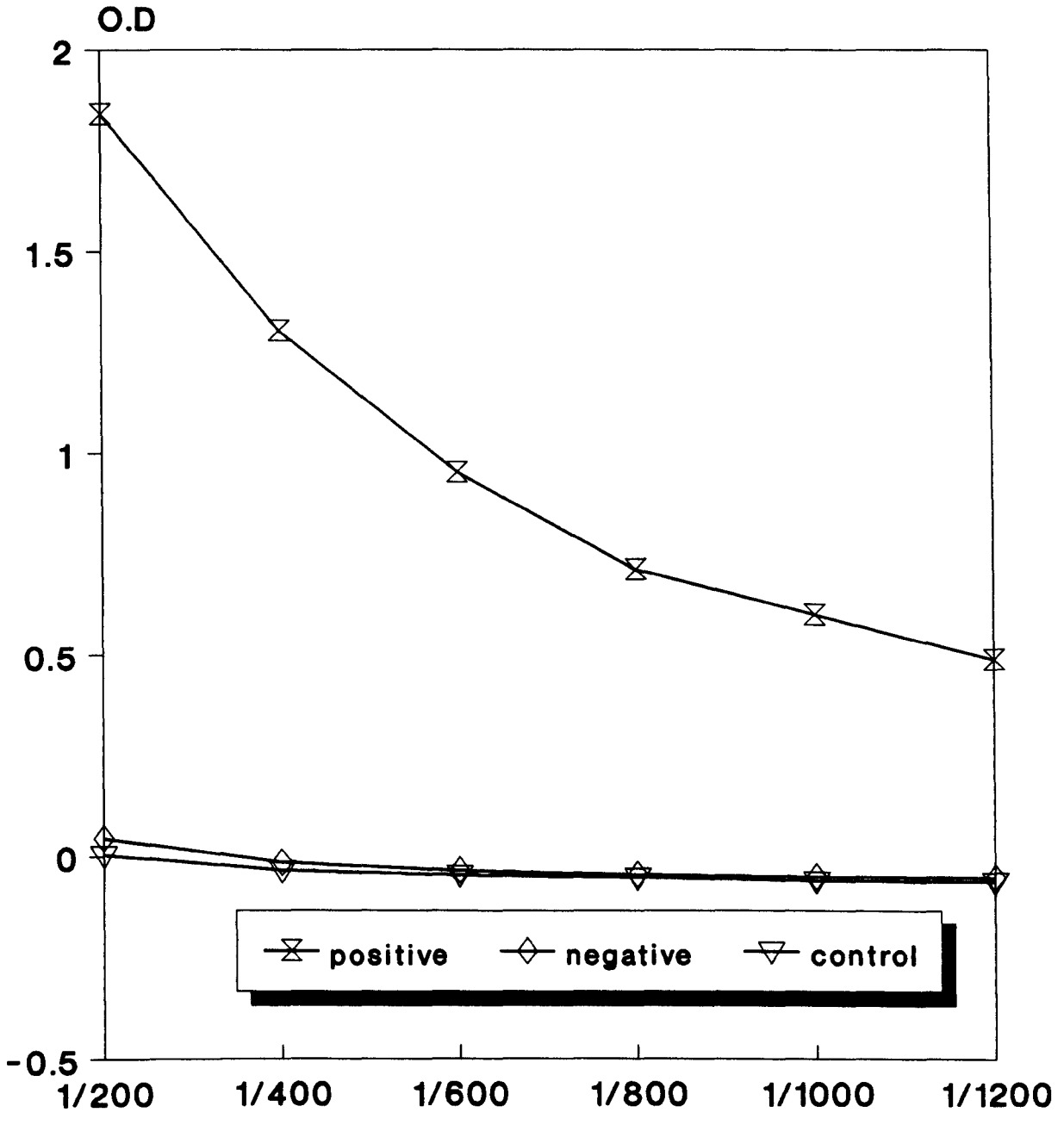


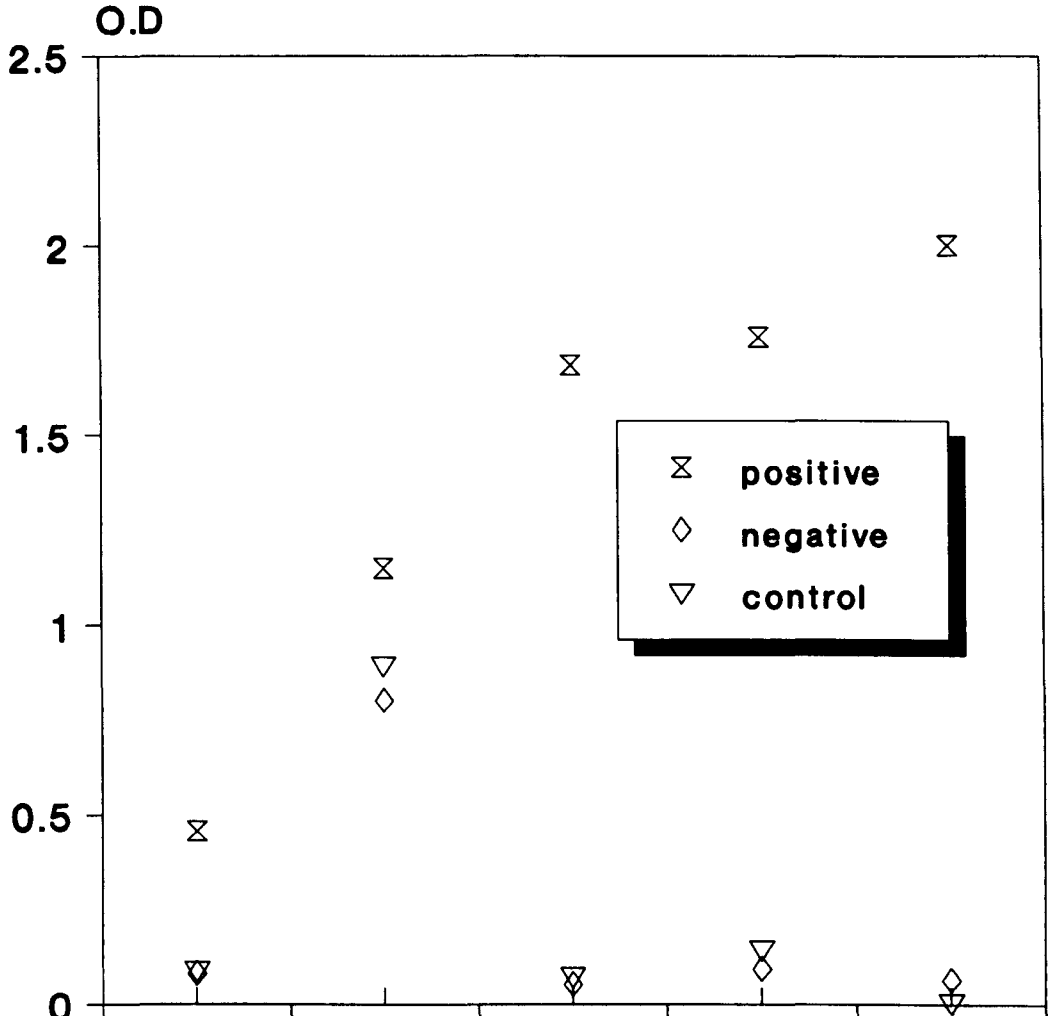
Fig.2.4 Titration of conjugate in the ELISA assay.

It was found the optimal concentration of the conjugate to be 1 in 800 dilution, because it was suitable and economical

2.3.3.4. Comparison of foetal calf serum, horse serum, bovine serum albumin (BSA) and cow serum as sample buffer constituents (Figure 2.5).

1. Five rows of 6 wells of an ELISA plate were coated with 200µl per well with affinity purified rabbit-anti-Giardia Ig with concentration of 15µg/ml in coating buffer pH9.6. The plate was incubated overnight at 4°C
2. The plate was then washed three times with PBS-Tween with a three minute incubation between each time.
3. To each well in duplicate in each row, added 200µl of 10gm% suspension of pooled positive stool samples in PBS, 10gm% suspension of pooled negative stool samples in PBS and PBS only respectively, mixed with equal volume of FCS, horse serum, BSA 1%, 50% cow serum or 50% BSA 2% in PBS-Tween in the first, the second, the third, the fourth and the fifth row respectively.

   The plate was incubated for one hour at 37°C.
4. The plate was washed as in step 2.
5. 200µl of affinity-purified rabbit-anti-Giardia peroxidase conjugate at dilution of 1 in 800 in 50% FCS, 50% horse serum, 50% BSA 1%, 50% cow serum or 50% BSA 2% in PBS-Tween in the first, the second, the third, the fourth and fifth row respectively.



	H.S	C.S	BSA 1%	BSA 2%	FCS
positive	0.456	1.148	1.685	1.758	2
negative	0.08	0.801	0.052	0.093	0.064
control	0.089	0.894	0.074	0.145	0.004

Fig.2.5 Comparison of foetal calf serum, horse serum, bovine serum albumin & cow serum as protectants in the ELISA assay.

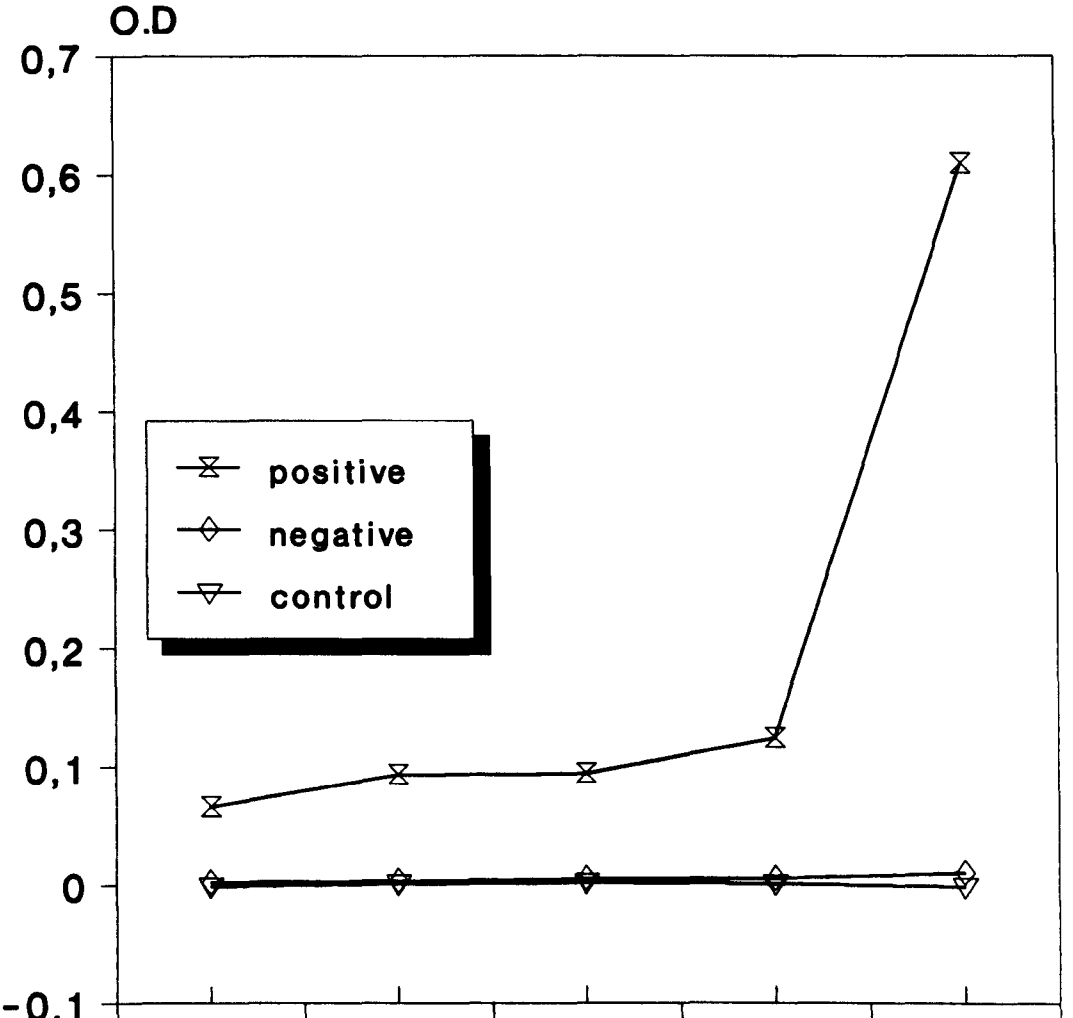
The plate was incubated for 90 minutes at room temperature.

6. The plate was washed as in step 2.
7. Subsequent steps in the assay were performed as in (2.3.3.1.).

It was found that foetal calf serum (FCS) was found to be marginally superior to bovine serum albumin (BSA), as a sample buffer protectant. The presumed mechanism of action is that blocking proteins act as competing substrates for faecal proteases, which interfere with immunoassays.

2.3.3.5. Determination of the optimal incubation time of substrate solution (Figure 2.6).

1. To one row of 6 wells of an ELISA plate was added 200µl of affinity purified pooled Giardia antisera at a concentration of 15µg/ml in coating buffer pH9.6, the plate was incubated overnight at 4°C.
2. The plate was then washed three times with PBS-Tween with a three minute incubation after each time.
3. To each well in duplicate in the row, added 200µl of equal volumes of FCS and, 10gm% suspension of pooled positive stool in PBS, 10gm% suspension of pooled negative stool in PBS and PBS only respectively.
   The plate was incubated for one hour at 37°C.
4. The plate was washed as in step 2.
5. To each well 200µl of affinity-purified rabbit-anti-Giardia peroxidase conjugate was added at dilution of 1 in 800 in 50% FCS in PBS-Tween. The plate was incubated for 90 minutes at room temperature.



	15 min.	30 min.	45 min.	60 min.	60 min.*
positive	0,066	0,093	0,094	0,124	0,609
negative	0,002	0,003	0,005	0,005	0,01
control	-0,001	0,001	0,002	0,001	-0,001

Fig.2.6 Determination the optimal incubation time of substrate solution (*=with acid)

6. The plate was washed again as in step 2.
7. To each well 200µl of substrate solution was added. The plate was incubated in the dark at room temperature for 15, 30, 45 and 60 minutes.
8. Subsequent steps in the assay were performed as in 2.3.3.1.

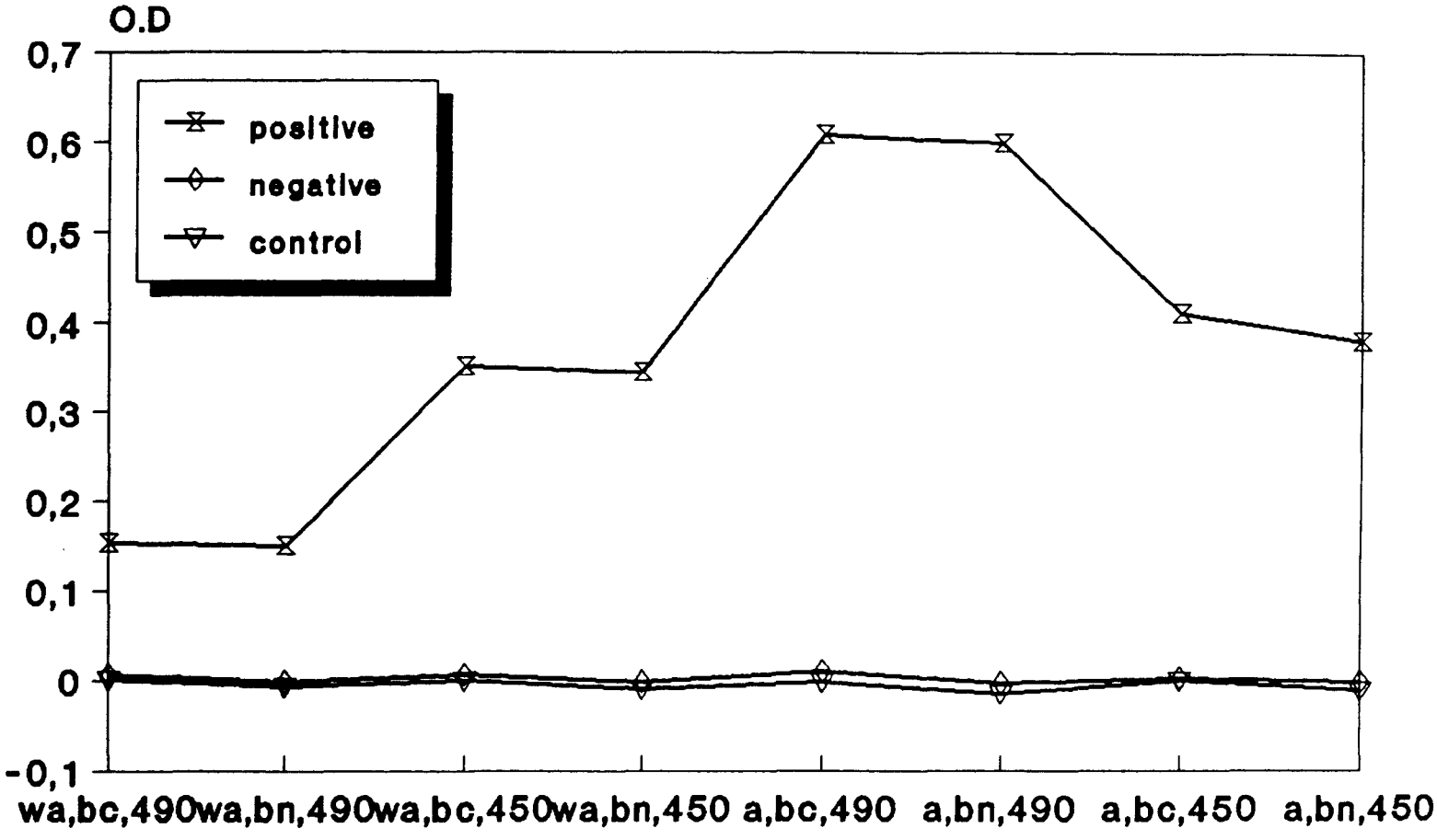
   The minimum substrate incubation time giving optimal absorbance reading was found to be 60 minutes.

2.3.3.6. Comparison of reading ELISA plate with and without adding acid, and at two different wavelengths (450nm and 490nm) (Figure 2.7).

1. To one row of 6 wells of an ELISA plate 200µl of affinity purified pooled Giardia antisera was added at a concentration of 15µg/ml in coating buffer pH9.6. The plate was incubated overnight at 4°C.
2. The plate was then washed three times with PBS-Tween with a three minute incubation after each time.
3. To each well in duplicate in the row, added 200µl of equal volumes of FCS and, 10gm% suspension of pooled positive stool in PBS, 10gm% suspension of pooled negative stool in PBS, and PBS only respectively.

   The plate was incubated for one hour at 37°C.
4. The plate was washed as in step 2.
5. To each well 200µl of affinity-purified rabbit-anti-Giardia peroxidase conjugate was added at dilution of 1 in 800 in 50% FCS in PBS-Tween. The plate was incubated for 90 minutes at room temperature.

Fig.2.7 Comparison of reading ELISA plate without and with acid,also with 450 and 490nm wave length.

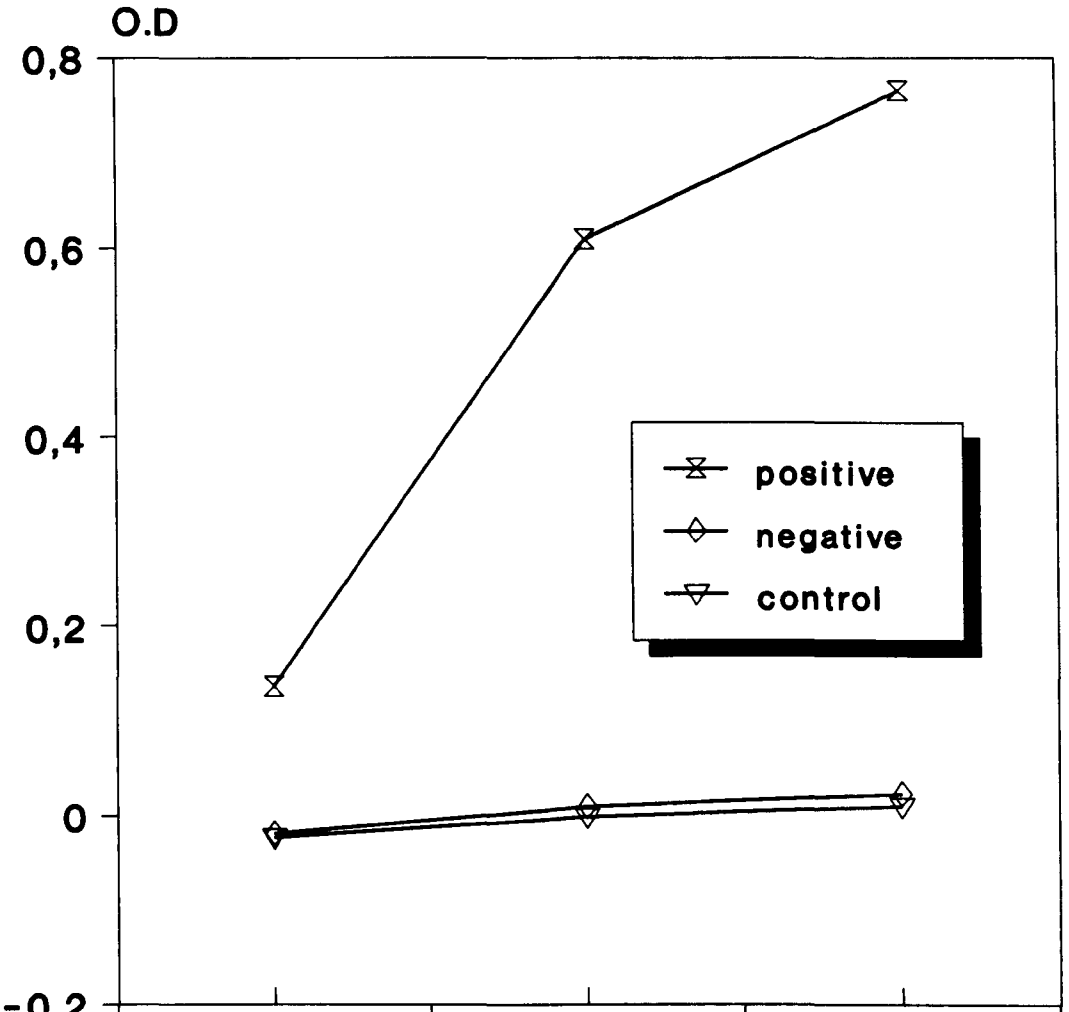


acid(a),without acid(wa),blank cotrol(b)
blank neg.(bn),wave length(450 or 490).

6. The plate was washed as in step 2.
7. To each well 200µl of substrate solution was added. The plate was incubated in the dark at room temperature for 60 minutes.
8. The plate was read with ELISA plate reader before and after adding acid and with two different wavelengths 450nm and 490nm.
   In the relation between the acid and the wavelength. The acid was found to give optimal absorbance readings with the wavelength 490nm. Without acid the optimal absorbance was with the wavelength 450nm.

2.3.3.7. Comparison between using two types of acid to stop reaction of substrate (Figure 2.8).

1. To three rows of 6 wells of an ELISA plate 200µl of affinity-purified pooled Giardia antisera was added at a concentration of 15µg/ml in coating buffer pH9.6. The plate was incubated overnight at 4°C.
2. The plate was then washed three times with PBS-Tween with a three minute incubation after each time.
3. To each well in duplicate in each row, added 200µl of equal volumes of FCS and, 10gm% suspension of pooled positive stool in PBS, 10gm% suspension of pooled negative stool in PBS, and PBS only respectively, the plate was incubated for one hour at 37°C.
4. The plate was washed as in step 2.



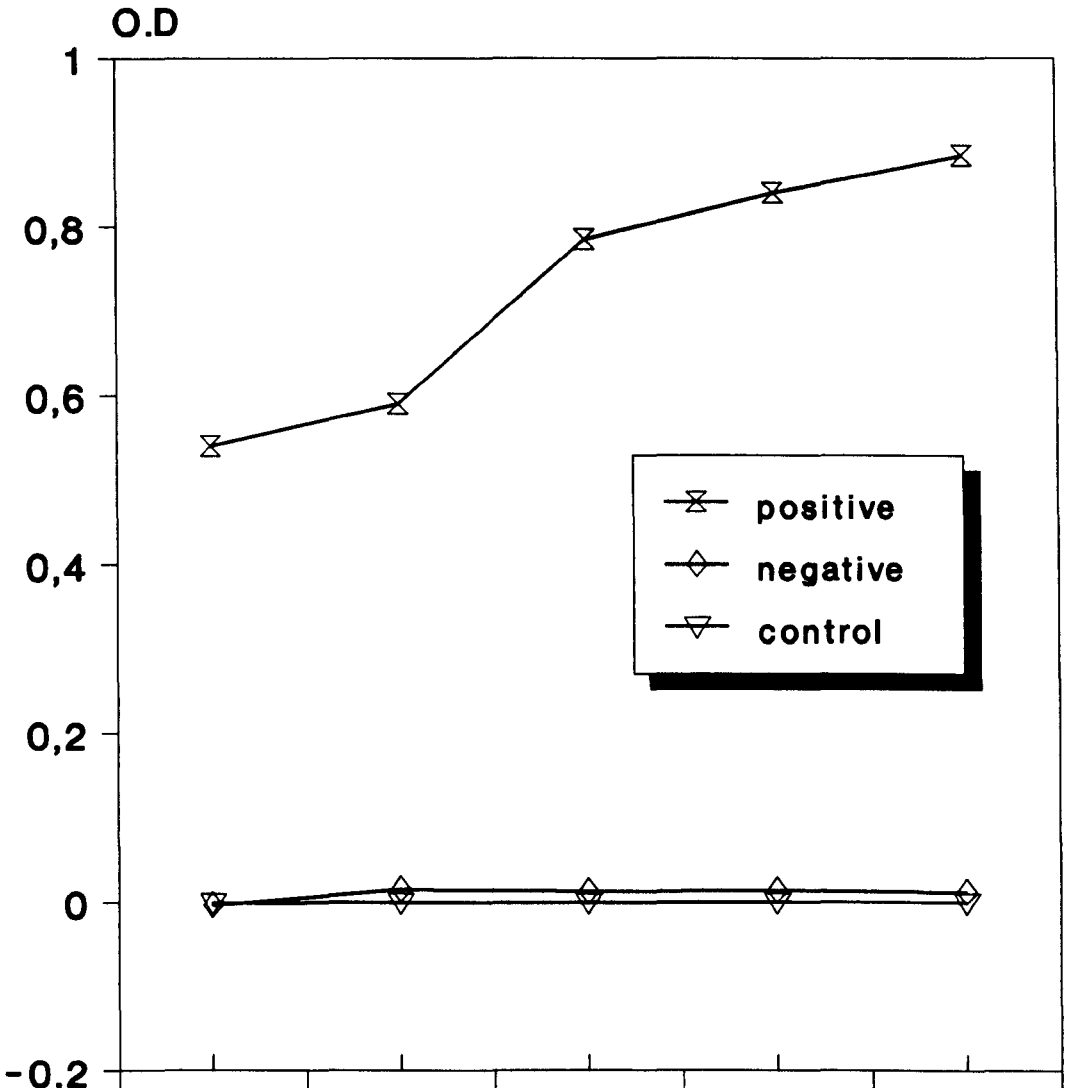
	without acid	with HCL	with H2SO4
positive	0,136	0,609	0,765
negative	-0,019	0,01	0,023
control	-0,023	-0,001	0,01

Fig. 2.8 Comparison between using HCL and H2so4 acids to stop substrate reaction in the ELISA assay.

5. To each well 200µl of affinity-purified rabbit-anti Giardia peroxidase conjugate was added at dilution of 1 in 800 in 50% FCS in PBS-Tween. The plate was incubated for 90 minutes at room temperature.
6. The plate was washed as in step 2.
7. To each well 200µl of substrate solution was added. The plate was incubated in the dark at room temperature for 60 minutes.
8. No acid was added to the first row, while to the second and the third rows, of an ELISA plate, a 50µl of 2.5M of H_2SO_4 and HCl acid were added respectively.
9. The OD was read at 490nm in an ELISA plate reader. There was no difference between the optimal absorbance reading for both acids (HCl and H_2 SO_4).

2.3.3.8. Comparison between five types of ELISA plates (Figure 2.9)

1. One row of six wells of five different ELISA plates, (i. Dynatech Immulon I No. 011-010-3350 Dynatech Laboratories Inc; ii. Costar No. 3590 Certified surface chemistry; iii. Nunc-Immuno plate Maxisorp F96 No. 4-39454 Inter Med; iv. Flow No.I Linbro/Titertek No. 0040493 Flow Laboratores; v. Flow No. II Linbro No. 0020035 Flow Laboratories) were coated with 200µl per well of affinity purified pooled Giardia antisera at a concentration of 15µg/ml in coating buffer pH9.6. The plates were incubated overnight at 4°C.



	Dynatech 1	Flow no.1	Flow no.2	Maxisorp	Costar
positive	0,54	0,59	0,785	0,84	0,885
negative	-0,004	0,015	0,013	0,014	0,012
control	-0,001	0	0	0	0

Fig. 2.9 Comparison between five types of ELISA plates

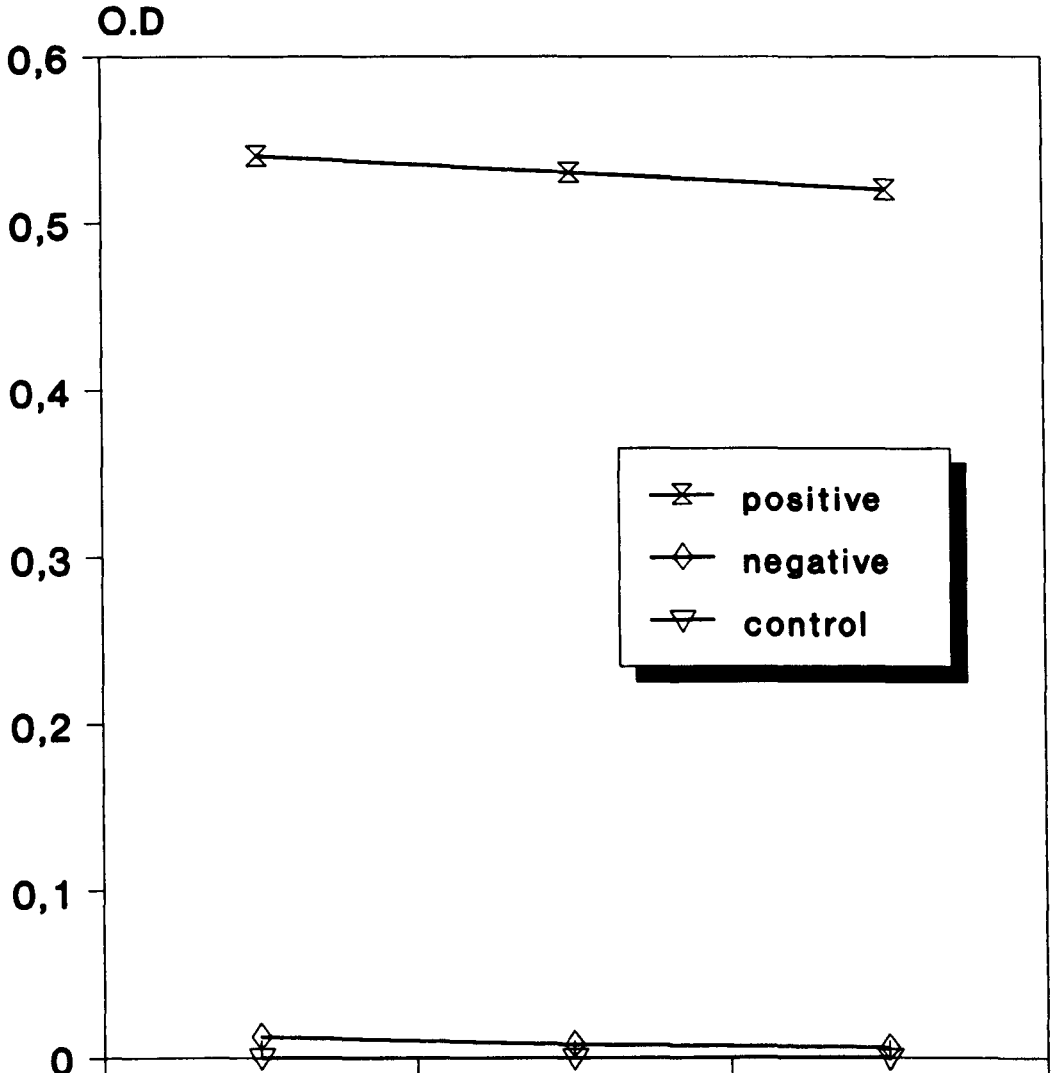
2. The plates were then washed three times with PBS-Tween with a three minutes incubation after each time.
3. To each well in duplicate in the row in each plate, added 200µl of equal volumes of FCS and, 10gm% suspension of pooled positive stool in PBS, 10gm% suspension of pooled negative stool in PBS, and PBS only respectively. The plates were incubated for one hour at 37°C.
4. The plates were washed as in step 2.
5. To each well in each plate 200µl of affinity-purified rabbit-anti Giardia peroxidase conjugate was added at dilution of 1 in 800 in 50% FCS in PBS-Tween. The plates were incubated for 90 minutes at room temperature.
6. The plates were washed as in step 2.
7. To each well in each plate, 200µl of substrate solution was added. The plates were incubated in the dark at room temperature for 60 minutes.
8. To each well in each plate, 50µl of 2.5 HCl was added.
9. The OD was read at 490nm in an ELISA reader.

Costar ELISA plate was found to give optimal absorbance reading, and its price was found to be reasonable compared to others (Table 2.1).

2.3.3.9. Comparison between three types of ELISA plate reader machines (Figure 2.10).

1. One row of six wells in an ELISA plate was coated with 200µl of affinity purified rabbit-anti-Giardia Ig with a concentration of 15µg/well in coating buffer pH9.6. The plate was incubated overnight at 4°C.
2. The plate was then washed three times with PBS-Tween with a three minutes incubation after each time.
3. To each well 200µl of stool samples suspension in 50% FCS was added. The plate was incubated for one hour at 37°C.
4. The plate was washed as in step 2.
5. To each well 200µl of affinity-purified rabbit-anti-Giardia peroxidase conjugate was added at dilution of 1 in 800 in 50% FCS in PBS-Tween. The plate was incubated for 90 minutes at room temperature.
6. The plate was washed again as in step 2.
7. To each well 200µl of substrate solution was added. The plate was incubated in the dark at room temperature for 60 minutes.
8. To each well 50µl of 2.5M HCl was added.
9. The optical density was read in on three different ELISA plate reading machines 1. Automatic Microplate reader. A Dynatech product MR600, 2. Automatic ELISA plate reader, Flow Labs. Multiskan Plus MKII. 3. Portable microtitre plate reader CLS962, Cambridge Life Sciences (CLS) at 490nm.

   The three ELISA plate reader machines were found to give the same absorbance readings.



	MR 600	MK.2	CLS 962
positive	0,54	0,53	0,52
negative	0,012	0,008	0,006
control	0	0	0

Fig.2.10 Comparison between three types of ELISA plate reader machines

Table 2.1. Difference in Price of Five Types of ELISA plates (1991)

Type of ELISA plate	Company Name	Price per plate (£)
Dynatech Immulon I No. 011-010-3350	Dynatech Laboratories	0.6945
Costar No. 3590	Costar	0.693
Maxisorp F96 Nunc-Immuno plate No. 4-39454	NUNC	0.95
Flow No. I Linbro/ Titertek No. 0040 493	Flow Laboratories	0.57
Flow No. II Linbro No. 002 0035	Flow Laboratories	0.86

2.3.4. Assessment of the sensitivity and specificity of the ELISA test (Figure 2.11)

The cut-off point value (for positive ELISA O.D. reading), was calculated by mean plus three standard deviations for the negative control faecal samples ELISA O.D. readings, (cut-off point = 0.097). The results of ELISA test for faecal samples were compared with microscopical results. Two of fifty positive faecal samples (with microscopy) was found negative (false negative) with ELISA test. The sensitivity of ELISA test was 96%. And one of twenty negative faecal samples (with microscopy) was found positive (false positive) with ELISA test. The specificity of ELISA test was 85%.

2.4. Discussion

2.4.1. Reagent Preparation

Rapid and easy methods of subculturing are required to produce large numbers of Giardia trophozoites required during the development the ELISA test. So large numbers of subculturing tubes are required, and the centrifugation method takes a longer time and it may lead to the loss of some of the trophozoites.

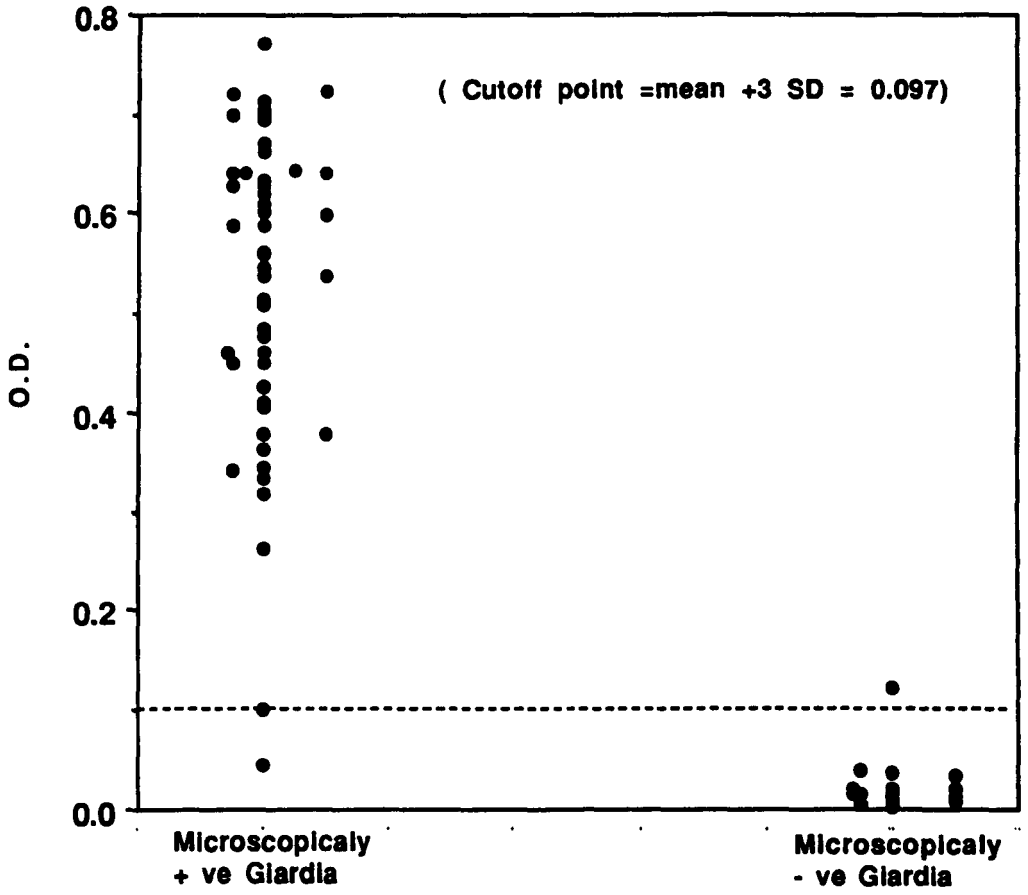


Fig. 2.11 Comparing the results of concentration test with ELISA O.D.for faecal samples to detect Giardia.

Only 20-40ml of immune serum can be extracted from the rabbit in each bleeding time. Large numbers of rabbits, or a larger animal may be required to produce a large amount of immune serum for developing a larger scale of the test. The prevalence of anti-rabbit copro antibodies in the population tested appears to be very low. Horses might be an acceptable alternative, but use of bovine antisera is more likely to generate false positive assay reaction from copro antibodies as a consequence of prior human exposure to immunoglobulins in milk and under cooked beef (Hunter and Budd, 1980).

The pooled sera undoubtedly contains antibodies to faecal micro organisms - particularly the cyst immune sera (due to the faecal origin of the cyst preparations) even the Sephadex-G.50 isolation method gives more pure Giardia cysts. Induction of encystation in axenic culture from trophozoites (Gillin et al. 1987) deserves evaluation, but also sera of normal rabbits. Affinity purification was therefore necessary with trophozoite antigen as the most appropriate for binding to sepharose in view of its purity and its (partial) success as the solid phase antigen.

The decision of how far out the pool fractions were involved a compromise between high avidity reagent and the technical difficulties of concentrating large volumes of eluate. Six small columns were produced instead of a single large column in order to minimise antibody transit time in the denaturing conditions of acid buffer through the column prior to neutralisation in the collecting tube. For preparation of large amounts of antibody, a batch method or alternative elution conditions could be tried. In recent years, gels have been used with spacer arms interposed between matrix and

ligand to reduce steric hindrance and increase column capacity (Pharmacia 1979, Dean et al 1985), which may increase the efficiency of production of specific immune reagents for use in assays.

The two step glutaraldehyde method (Avrameas and Ternynck, 1971) used for the conjugation of horseradish peroxidase (HRP) to rabbit anti-Giardia affinity-purified immunoglobulin (Ig) was found to be reliable and simple to perform. At the final stage, the separation of the conjugate (Ig-HRP) from free glutaraldehyde - activated HRP by 50% saturated ammonium sulphate precipitation was found to yield a conjugate with satisfactory assay performance.

2.4.2. Assay optimisation

Greater sensitivity is achieved by the use of excess antibody reagent, ensuring that all the free antigen takes part in the generation of the positive response. On the other hand, increasing the concentration of antibody as well as leading to an increase in sensitivity may increase the non-specific binding to which antibody-excess assays are prone. In our assay, the problem of cross reactivity has been largely eliminated by affinity purification of reagents. As shown in Figure 2.2 a suitable concentration of affinity purified antibodies was 15mg/ml (3µg/well).

One of the advantages of this assay is that it can be performed with less equipment, (Figure 2.3) as there is no difference between centrifuged and non centrifuged stool suspensions. The peroxidase (HRP) enzyme label gave very satisfactory performances in this assay. This enzyme has the advantage of being a relatively inexpensive plant product

(approx £24 per 5000 units (Sigma-P8375)), with a substrate producing a dark, easily visible product. Alternative enzymes worthy of evaluation include urease, alkaline phosphatase, β-galactosidase and glucose oxidase. The carcinogenic nature of many of the substrates for HRP may make it a less appropriate choice as a label for use by diagnostic laboratories.

An enzyme lable was considered more appropriate than a radio-isotope because of the need for a visually interpretable assay and the disadvantages of radio-immuno assays (biological hazards, need for complex and expensive counting equipment, limited shelf-life of radio-active labelled reagents etc).

Different dilutions of the conjugate in foetal calf serum were tested (Figure 2.4), 1 in 800 dilution was found both suitable and economical.

The inhibitory activity of stool suspension was corrected by 50% FCS (more effective than the other protein solutions as Casein, BSA and gelatin) The presumed mechanism of action is that blocking proteins act as competing substrates for faecal proteases. FCS is unique in the group tested as it also contains several naturally occurring protease inhibitors (Viscidi et al 1984). Protease activity in stool would be expected to be both of human and microbial origin. The effectiveness of specific trypsin inhibitors suggests that human pancreatic trypsin may be exerting a major role in interfering with immunoassays.

Preliminary results (Figure 2.5) indicate that BSA (approx. £64 per 100gm (Sigma A8022)) may be the second choice after FCS (approx £60 per 500ml [Flow 29-101-54]) as a sample buffer protectant. Assays utilising bovine reagents however may be at a disadvantage in some countries such as

India for religious and cultural reasons.

During the ELISA procedure, if the substrate incubated in the dark for a longer time (Figure 2.6.) the reaction becomes more visible and it gives higher readings and this could be useful in places where there is no acid (which stop the substrate reaction with the enzyme).

Blanking the ELISA plate on the well with control sample (FCS with PBS) or on the well with negative sample (known negative stool suspension with FCS) gives no difference in O.D. readings (Figure 2.7) whether with acid or without acid, even with different wavelengths.

Reading the ELISA plate with wavelength 450nm gives higher O.D. reading than using wavelength 490nm if there was no acid added to the plate (Figure 2.7), but the O.D. reading becomes higher if the plate is read with wavelength 490nm after adding acid.

Adding sulphuric acid (H_2 SO_4) to the ELISA plate gives a dark yellow colour with relatively higher O.D. reading than adding hydrochloric acid (HCl) which gives an orange colour (Figure 2.8.). HCl however is safer and therefore more suitable for use in remote areas. Costar ELISA plate gave higher O.D. reading compared with other types of ELISA plates (Dynatech Immulon I, Flow No. I, Flow No. II and Maxisorp-Nunc) (Figure 2.9.). Costar ELISA plates compared reasonably in price with other ELISA plates (Table 2.1.).

The cheaper, rechargable and lightweight portable microtitre plate reader gave the same O.D. reading as the other two ELISA plate reader machines (Figure 2.10, Table 2.2.) The portable microtitre plate reader (CLS 962) was considered more cost effective for use in the remote areas.

From the results of optimization of the assay, the

following protocol has been designed for field work.

1. To each well of a Costar ELISA plate No. 3590 (Certified Surface Chemistry) add 200µl of affinity purified pooled Giardia antisera at a concentration of 15µg/ml (3µg/well) in carbonate coating buffer pH9.6. Incubate the plate overnight at 4°C.
2. Wash the plate three times with PBS-Tween 0.05% (PBST) with a three minute incubation after each time.
3. To each well in duplicate add 200µl of a mixture of equal volumes of FCS and a 10g% suspension of stool sample in BPS. Incubate for two hours at room temperature, or for one hour at 37°C. Include a 50% FCS in PBS control in each assay.
4. Wash plate again as in step 2.
5. Add 200µl of affinity-purified rabbit-anti-Giardia-peroxidase conjugate at dilution 1: 800 in 50% FCS in PBST, to each well. Incubate at room temperature for 90 minutes.
6. Wash plate again as in step 2.
7. Add 200µl of substrate solution to each well and incubate in the dark at room temperature for 60 minutes.
8. Stop the reaction by addition of 50µl of 2.5M HCl to each well.
9. Read optical density in an ELISA reader (Portable Microtitre Plate reader CLS962) at 490nm.

Table 2.2. Difference in price of three types of ELISA Plate Reader Machines (1991)

Types of ELISA plate reader machine		Price (£)
Automatic Miroplate reader Product MR 600	A Dynatech	5249.00
Automatic ELISA plate reader Multiskan plus MKII	Flow Labs	5495.00
Portable Microtitre Plate Reader 1600.00 Cambridge Life Sciences, CLS 962		1600.00

2.4.3. Conclusion

Because the same polyclonal antibody mixture formed both sides of the assay, the sample and conjugate steps could not be combined in order to further shorten the assay time. This could readily be achieved if different monoclonal antibodies were substituted. Although in this assay the problem of reactivity has been largely eliminated by affinity purification of reagents, other less demanding strategies have been adopted to circumvent this. Multilayer two-site ELISAs for faecal antigen detection have been developed in which crude (or only partially purified) antisera of different species were used as capture and detecting antibody with demonstration of the latter in turn by an antispecies conjugate. These methods have generally used one or more of the following manoeuvres to reduce cross reactivity with non specific binding.

a. Removal of antispecies cross reactivity between the first two antibody layers by inclusion of non-immune serum of the capture species in the second antibody step.

b. Immuno absorption of contaminating antibodies reacting with unwanted faecal antigens (especially bacterial) (Makino et al 1983).

c. Use of assay controls for each sample in wells coated with non-immune immunoglobulin with subsequent subtraction of control from test O.D. values to eliminate the effect of linking of capture and conjugate antibodies in the absence of specific antigen (Yolken and Stopa 1979).

Greater sensitivity is achieved by the use of excess antibody reagent, ensuring that all antigen takes part in the generation of the positive signal, sensitivity in this assay depends mainly:-

a. On the specific activity of the labelled antibody (type of label-isotope or enzyme and number of signal units generated per antibody molecule).

b. On the degree of non-specific binding.

c. Much less on the antibody affinity and experimental error.

Attempts to improve assay sensitivity have been made in recent years, such as the amplified ELISA, utilising an enzyme-substrate cascade traditionally employed in isoenzyme electrophoresis (Siddle, 1985), and new chemiluminescent and bioluminescent systems (Kohen et al 1985). These however require relatively complex equipment for interpretation and although the positive signal may be improved greatly due to concurrent amplification of background, specificity may remain the same or even deteriorate unless simultaneous steps are taken to further reduce non-specific binding. A single sandwich format reduces some unacceptable background activity 'noise' but requires serum purification and conjugate preparation. A ELISA assay for Giardia antigen detection in faeces, with a minimum number of steps, using serum of only one animal species, and with specificity (96%) and sensitivity (95%) has been developed. This assay has a clear visual end point and should fulfill a need in busy clinics in remote areas or during epidemiological surveys where colorimetric equipment may not be available. With further development it may serve as a basis for the production of a diagnostic kit and for adaptation to a plate-free (e.g. dipstick) form.

CHAPTER 3

EPIDEMIOLOGY OF G.INTESTINALIS IN WADIHALOO VILLAGE

3.1. Introduction

3.1.1. General introduction

Giardia intestinalis is a common human intestinal protozoan parasite found in both industrialised and less developed countries (Harter et al. 1982; Boreham et al. 1981; Chavalittamrong et al. 1978). Infections are also found in technologically sophisticated societies, as well as in the traditional and transitional communities of the developing world (Oyerinde et al. 1977). A large proportion of infected individuals are asymptomatic (Lopez et al. 1980; Jokipii, 1971), and some of them have severe diarrhoea, intestinal malabsorption and weight loss (Dupont and Sullivan, 1986).

The median infective dose for a person is between 25 and 100 cysts (Rendtorff, 1954; Rendtorff and Holt, 1954). Transmission of G.intestinalis infection to humans occurs either from person to person by hand-to-mouth or via drinking contaminated water (Hopkins and Juranek, 1991). Some factors enhance the spread of infection such as diarrhoeal stools particularly by infants and mentally retarded people (Black et al. 1977; Keystone et al. 1978; Pickering et al. 1984; Cohen, 1991). The occurrence of asymptomatic infection leading to contamination by people unaware of their infectivity and transmission via food has been rarely reported (Osterholm et al. 1981).

The reservoir of G.intestinalis is man, but there is some evidence that man may acquire infections from other animals.

In developing countries, giardiasis is more prevalent in poor communities with inadequate sanitation and the pattern of infection is typically endemic. Recorded prevalence in various communities range from 1 to over 20%, the children between 1 and 5 years old having the highest infection rates (Feachem et al 1983; Develoux et al. 1990; Janoff et al. 1990; Omar et al. 1991).

3.1.2. United Arab Emirates

3.1.2.1.Geography:

The United Arab Emirates is situated on the Eastern Coast of the Arabian peninsula at the Southern end of the Arabian Gulf which it shares with the State of Qatar, Kingdom of Saudi Arabia and Sultanate of Oman. The country covers an area of about 80,000 sq.km., the mountain range of Hajar which stretches north-south for 90km, is furrowed by many steep arid valleys. It is separated on the east from the Gulf of Oman by a narrow coastal plain and the mountains are separated from the Arabian Gulf by a sandy and gravelled plain (where the majority of the human settlements are located). The plain progressively narrows in a northerly direction until it joins the coast line in the northern half of Ras Al Khaimah. Along the Gulf there are about 200 islands which are part of the UAE (Figure 3.1) and (Figure 3.2).

Figure 3.1

UNITED ARAB EMIRATES

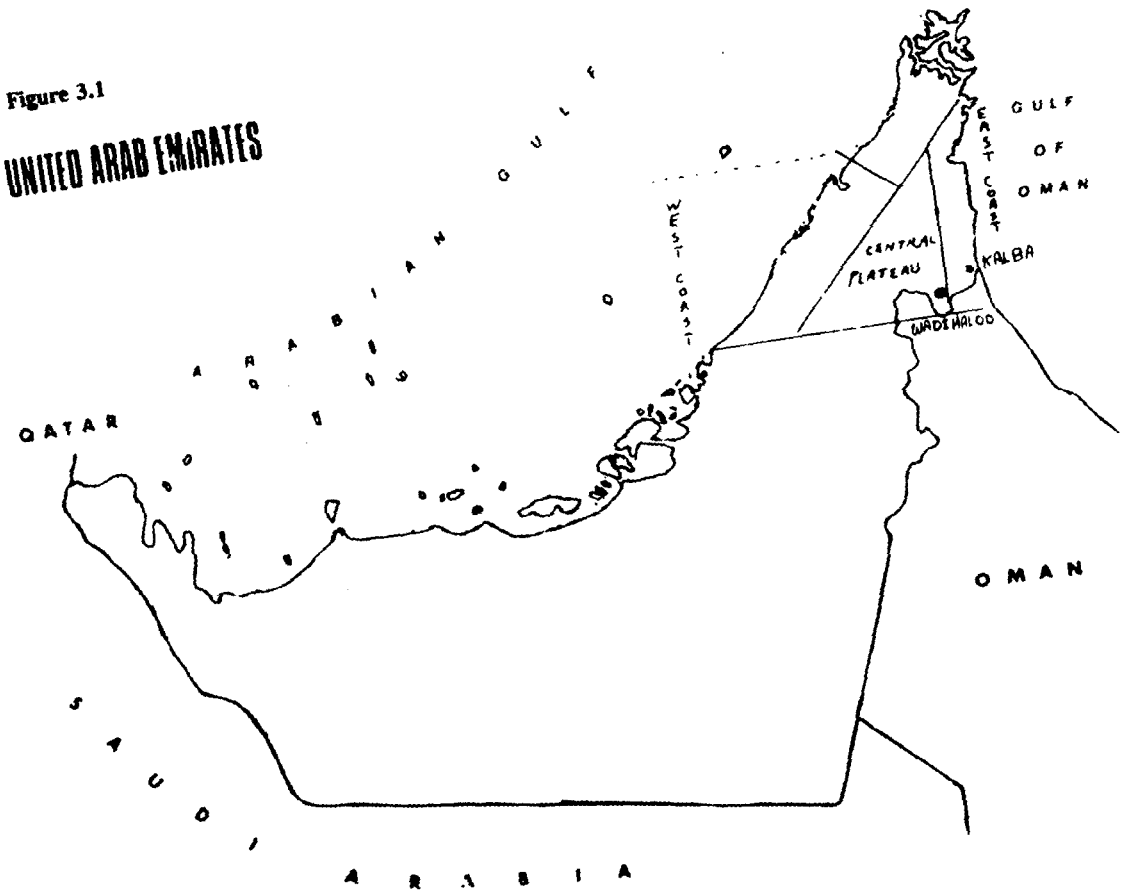
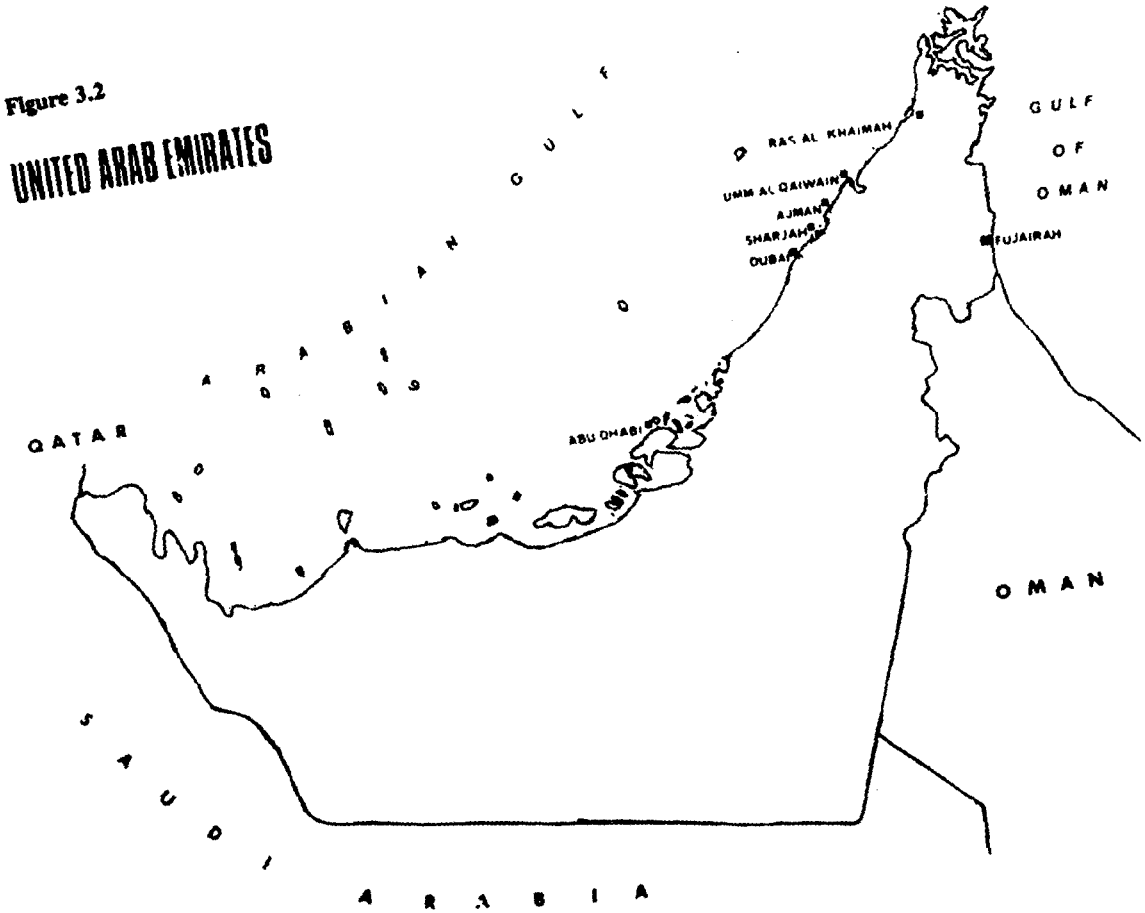


Figure 3.2

UNITED ARAB EMIRATES



3.1.2.2. Administrative Divisions:

The UAE is a federal state with seven Emirates which make up the federation, six of them (Abu Dhabi, Dubai, Sharjah, Ajman, Umm Al Qiwain and Ras Al Khaimah) are situated on the western coast along the Arabian Gulf, whilst the seventh Emirate (Fujarah) lies on the Eastern coast on the Gulf of Oman. (Figure 3.2).

3.1.2.3. Demography

The recent socio-economic development which has taken place in the UAE has been reflected in the demographic structure of the community. Demographic changes were mainly characterised and influenced by:-

a. An influx of expatriates who come from many countries and cover various social strata.

b. Mobility of the population both internally, between different emirates and externally, as a result of the regular and frequent turnover of expatriate population groups.

c. Improving economic ability of the local population which has facilitated a further rise in birth rate. The Crude Birth Rate (CBR) reached 32/1000 by the year 1989.

d. The positive impact of socio economic development on health status which has also resulted in declining mortality among all age groups and a longer life expectancy currently estimated at 73 years for females and 70 for males.

The estimated population of the UAE for the year 1989 was 1.63 million (based on the 1985 census). The child population below <5 years of age constituted 14.1% and children below 15 years about 33.5% of the total population for the year 1989. The male: female ratio among adult population (between age of 15-44) is approximately 2.2 to 1 due mainly to the disproportionate representation of single adult expatriate males (Figure 3.3) (M.O.H. Annual Report 1989a).

3.1.2.4.Climate

The UAE southern desert part is crossed by the Tropic of Cancer. The country lies two thirds in the subtropical zone and for one third in the tropical zone. The climate is generally hot with a short and mild winter and varies between the coastal and inland regions. During the winter there is erratic rainfall, sometimes a few showers occur in May and October to November. The average rainfall ranges between 0.2-100.4mm in the coastal area and from 0.1-108.6mm in the central areas. During winter the maximum temperature ranges between 21-34°C whilst the minimum ranges between 3-13°C. During summer the maximum temperature ranges between 37-47°C and the minimum varies between 11-30°C. The relative humidity is usually between 60-100% throughout the year in the coastal strip, whilst it is 25-57% in the central hill and plain area.

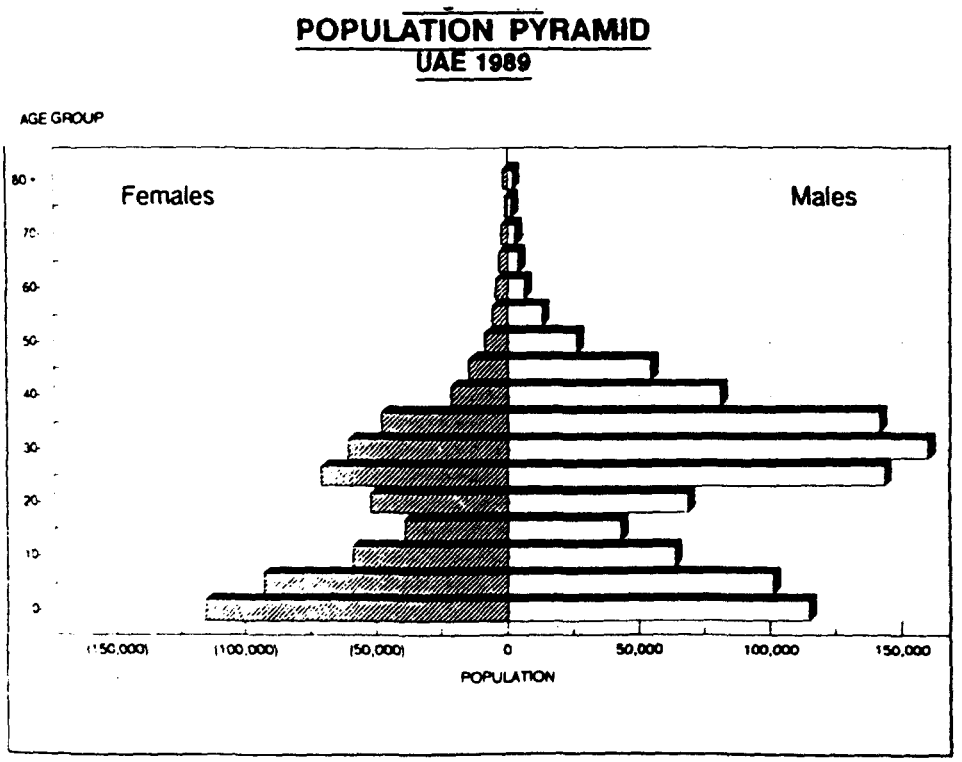


Fig.3.3 Showing the population pyramid in UAE (1989),related to age groups in years and sex(MOH annual report 1989).

3.1.2.5. Agriculture

The areas under cultivation have increased and rapidly developed, the country is planning self sufficiency in food during the present decade and each year there is a great increase in the field of agriculture.

The hydrological resources in UAE are classified as follows:

a. Artesian Wells scattered all over the area (water is pumped from the wells to cement reservoirs for the irrigation of the cultivated lands).

b. a few springs.

c. pools in the bed of the valleys.

d. Channels: fresh water comes out from the mountains and is carried from higher areas to the lower areas through man made underground channels.

3.1.3. Wadihaloo Village

Wadihaloo village is one of several villages in the U.A.E., it is a small village, related to Kalba (small city on the east coast) and both belong to Sharjah Emirate. The village is located between the mountains in the central plateau, (Figure 3.1). A four wheel drive car was directed to the west from Kalba hospital (in Kalba city) on a rocky road between the mountains. and reached the Wadihaloo village within an hour.

The distance between Kalba hospital and the village was 35km. On both sides of the road there were some small villages, some water pockets in the valleys (especially in the winter), and some remnants of old houses (Figure 3.4 and Figure 3.5).

In the village there was a small clinic, (Figure 3.6.), small police station, small school, two small shops, two small mosques and two separated groups of houses.

We did three separate demographic survey rounds in the village, to find out the following:

1. The total population in the village in relation to age groups, sex, nationality, occupation and place of work.

2. Type of houses in relation to water supply and sewage system.

3. Number of farms and their role in food supply to the village.

4. The prevalence of G. intestinalis in the village, in relation to age group, sex, nationality, occupation, place of work, type of houses, place of living, sources of drinking water and type of latrines.

3.2. Material and Methods

3.2.1. General Measures

Permission was obtained from the Ministry of Health before we started the study in the village, and we explained the aim of the study to the Chief and some people in the village.

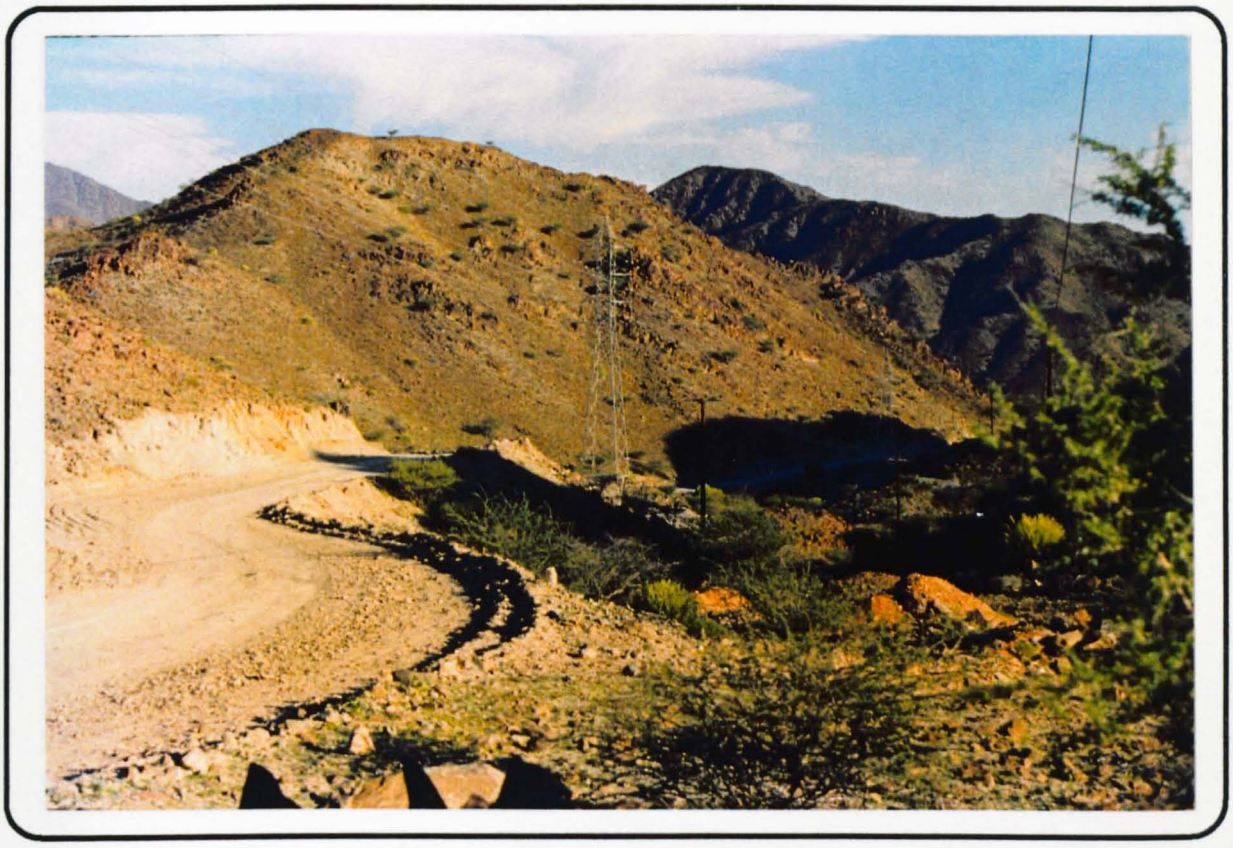


Figure 3.4 Showing the road between Kalba city and Wadihaloo village

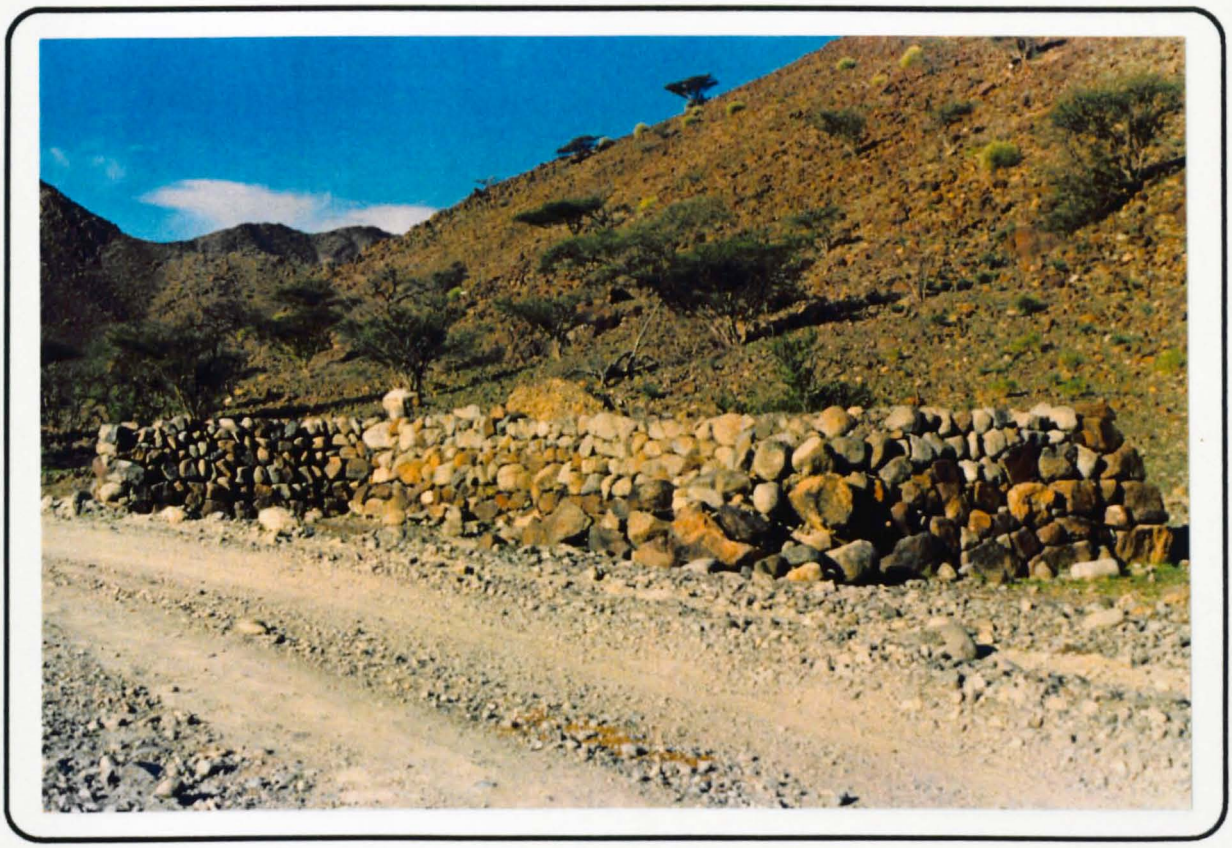


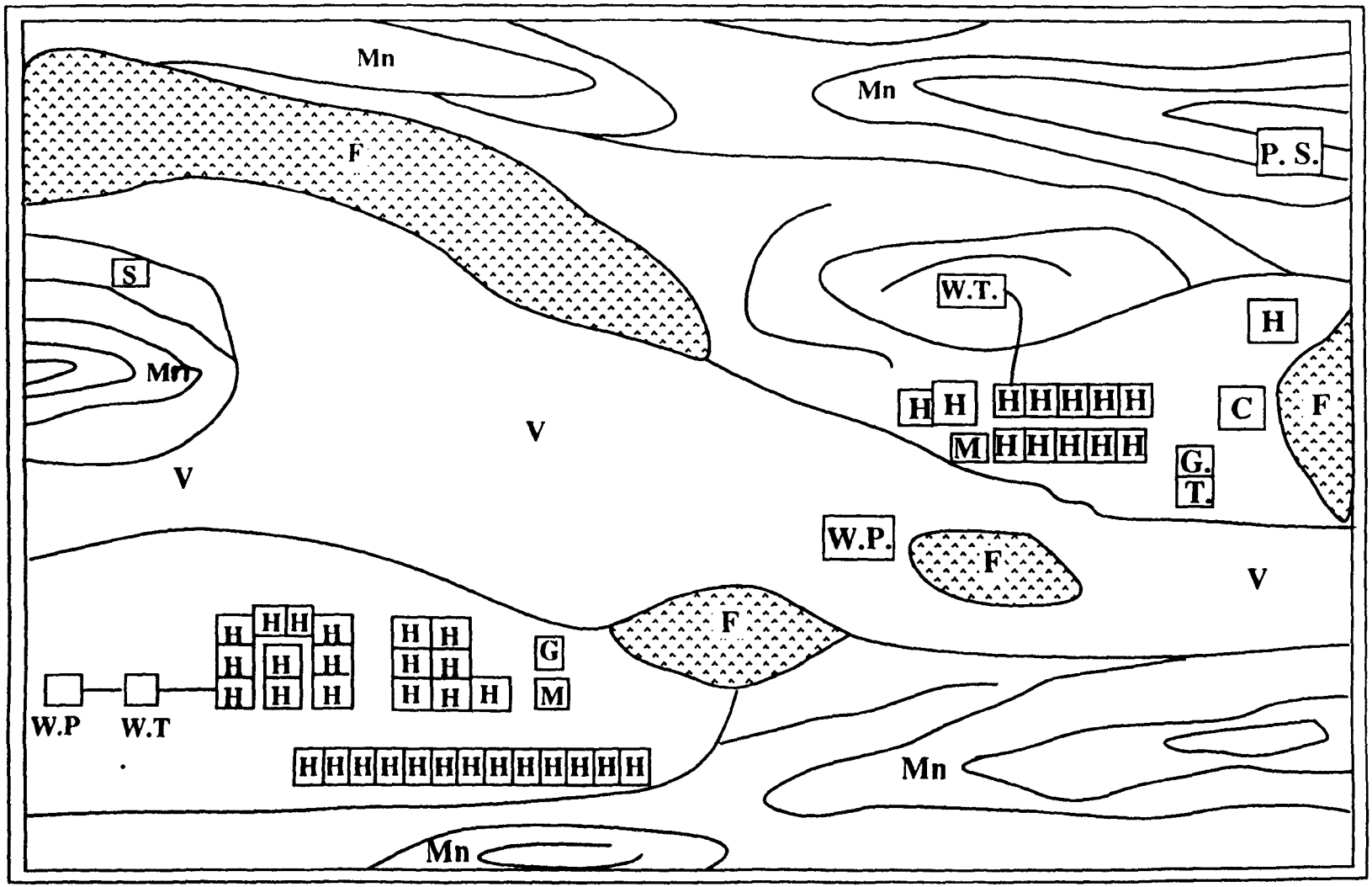
Figure 3.5 Showing the remains of the old houses on the road between Kalba city and Wadihaloo village

Figure 3.6 Showing ministry car in front of the clinic in Wadihaloo village

Fig. 3.7 Map of Wadihaloo village.

KEY

H = Houses
C = Clinic
P.S = Police Station
G = Grocery
T= Tailor
M = Mosque
W.P = Water Pump
W.T = Water tank
S = School
Mn = Mountains
F = Farms
V = Valley



Maps were drawn of the village to localise the place of the houses, the clinic, the police station, the school, the source of drinking water and some farms, (Figure 3.7). Questionnaires were prepared including personal code number, family code number, sex, date of birth, nationality, number of educational years, occupation, place of work, number of times leaving the village, number of days spent outside the village, place of living, type of house, source of drinking water and type of latrine for each person, and number of farms belonging to each family.

3.2.2. The Survey rounds

Three survey rounds were done. The first one was done in August 1988, the second one in October 1989 and the third one in October 1990. During each round the questionnaire (see appendix) was filled in for each person in the village. Each person was also asked about diarrhoea and the number of loose motions per day and duration in days during the last two weeks. Each person in the village was asked to provide a stool sample in each survey round. The collected stool samples were coded with personal numbers, family number and round number. And they were then taken to Kalba hospital to be examined by laboratory technicians with direct smear microscopically for Giardia and other intestinal parasites. The stool samples were then stored at 4°C overnight. On the next day, part of each negative sample was re-examined with direct microscopy. Part of each faecal sample was mixed properly with 10% formol saline. The mixture was filtered through wire mesh (size 250μm). Ether was added and the sample shaken

vigorously for a few minutes, left to stand for 30 minutes and the fatty plug was loosened from the wall of the tube with a stick. The supernatant fluid was poured into chloros. And the deposit was examined by microscopy with Lugols iodine. The negative concentrated stool samples were re-examined by the Khorfakkan hospital laboratory technician.

For ELISA examination part of the faecal samples were suspended in phosphate buffer saline (PBS pH7.2) at a concentration of 10gm% mixed properly. Large particulate matter was removed by filtration through wire mesh. The suspension was left to stand for 30 minutes. The supernatant was collected and stored at -20°C.

The ELISA procedure was that outlined in Chapter 2. The positive-negative cut-off value was calculated as the arithmetic mean of negative samples + 3SD.

The data was arranged on spreadsheets using Integrated 7 software (Version 1.5, 1987), and Lotus 1-2-3 software (release 2.01, 1987). Then the data was analysed using Minitab software (release 7.2, 1989), and Epi Info software (version 5, 1990).

3.3. Results

3.3.1. Location of the village

The Wadihaloo village was a small isolated village about 35Km to the west between the mountains from Kalba hospital.

3.3.2. The population in the Wadihaloo village

3.3.2.1. Number of the population and sex

The total number of population in the Wadihaloo village increased in each survey round. It was 292, 341 and 364 in the first, second and third survey rounds respectively. Due to influx of expatriates to the village, the percentage of the males from the total population was also found to increase in each survey round. It was 55.1%, 56.3% and 57.7% in the first, the second and the third survey round respectively.

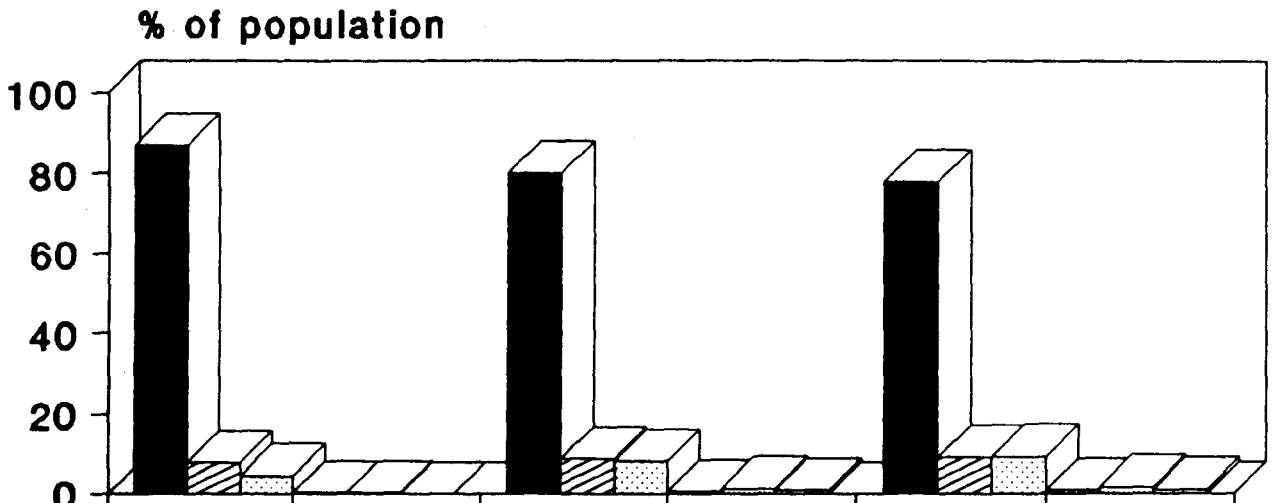
90 children in the village were found to be between 0-6years, at the end of the first survey round. The percentage of people from the total population at age below 13 and above 50 years were found to decrease from the previous survey round. The number of males and females were equal in most of the age groups, but at the age of the expatriates the number of males was double or more. This may be due to the influx of male expatriates to the village (Table 3.1).

3.3.2.2. Nationality of the population

Most of the people in the Wadihaloo village were local (from U.A.E.) and they represented 87% of the total population at the end of the first survey round. Also there were people from other nationalities, their percentage of the total population increased in each survey round (Figure 3.8).

Table 3.1 Population in Wadihaloo village in age groups related to sex in three demographic survey rounds

Age groups in years	1st Round Male	1st Round Female	1st Round Total	2nd Round Male	2nd Round Female	2nd Round Total	3rd Round Male	3rd Round Female	3rd Round Total
0-6	45	45	90 (30.8%)	45	46	91 (26.7%)	47	77	91 (25.0%)
7-12	23	27	50 (17.0%)	26	28	54 (15.8%)	29	29	58 (15.9%)
13-18	11	11	22 (7.5%)	11	19	30 (8.8%)	12	22	34 (9.3%)
19-24	16	11	27 (9.3%)	21	14	35 (10.3%)	16	13	29 (8.0%)
25-30	16	7	23 (7.9%)	21	9	30 (8.8%)	27	13	40 (11.0%)
31-40	22	11	33 (11.3%)	38	10	48 (14.1%)	44	10	54 (14.8%)
41-50	8	5	13 (4.5%)	9	7	16 (4.7%)	14	7	21 (5.8%)
51-60	13	10	23 (7.9%)	11	11	22 (6.4%)	11	11	22 (6.1%)
60+	7	4	11 (3.8%)	10	5	15 (4.4%)	10	5	15 (4.1%)
Total	161	131	292 (100.0%)	192	149	341 (100.0%)	210	154	364 (100.0%)



	1st round	2nd round	3rd round
UAE %	87	80,1	78
Pak.%	7,9	8,8	9,1
Bang.%	4,5	8,2	9,3
Indian %	0,3	0,6	0,8
Sril.%	0,3	1,4	1,7
Eygpt.%	0	0,9	1,1

Fig.3.8 Percentage of population related to nationality in Wadihaloo village in three demographic survey rounds.

Table 3.2. Population in Wadihaloo village in age groups related to nationality in three demographic survey rounds

Nationalitites	Age Groupings (years) 0-6	7-12	13-18	19-24	25-30	31-40	41-50	51-60	60+	Total
1st Round										
U.A.E.	90	50	22	23	10	16	10	22	11	254
Pakistan	0	0	0	3	5	11	3	1	0	23
Bangladesh	0	0	0	1	7	5	0	0	0	13
India	0	0	0	0	0	1	0	0	0	1
Sri Lanka	0	0	0	0	1	0	0	0	0	1
Egypt	0	0	0	0	0	0	0	0	0	0
Total	90	50	22	27	23	33	13	23	11	292
2nd Round										
U.A.E.	91	54	30	25	13	13	11	21	15	273
Pakistan	0	0	0	1	2	22	4	1	0	30
Bangladesh	0	0	0	6	13	9	0	0	0	28
India	0	0	0	0	0	2	0	0	0	2
Sri Lanka	0	0	0	3	1	1	0	0	0	5
Egypt	0	0	0	0	1	1	1	0	0	3
Total	91	54	20	35	30	48	16	22	15	341
3rd Round										
U.A.E.	91	58	34	22	18	13	12	21	15	284
Pakistan	0	0	0	0	2	23	7	1	0	33
Bangladesh	0	0	0	4	17	13	0	0	0	34
India	0	0	0	0	0	2	1	0	0	3
Sri Lanka	0	0	0	3	2	1	0	0	0	6
Egypt	0	0	0	0	1	2	1	0	0	4
Total	91	58	34	29	40	54	21	22	15	364

The majority of the non-local people (from other nationalities) were aged between 25 and 40 years, but the Pakistani people were older (Table 3.2). The local people were nearly equal in numbers of males and females (the male to female ratio 1:1), but in the other nationalities the people were all male, apart from the Sri Lankans who were all females.

3.3.2.3.The education in the Wadihaloo village

46.2% of the population in Wadihaloo village were educated. Most of the educated people in the village were aged between 7 and 24 years, (Table 3.3). The percentage of educated males and females were equal. But the percentage of non educated males (67%) were nearly double that of the non educated females, due to influex of non educated male expatriates to the village.

3.3.2.4. Occupation of the people in the village

A large number (42%) of the people in the village were without work (including the children below 6 years, unmarried women, housewives and the old people).

Most of the people in the village (22.3%) were working on the farms or considered as a second group after the students (23.1%). The rest of the people in the village were working as policemen (6.6%), housemaids (1.7%), labourers (1.4%), salesmen (0.8%), teachers (0.8%), a tailor (0.3%), and a driver (0.3%).

Table 3.3. Population in Wadihaloo village in age groups related to education in three demographic survey rounds

Rounds	1st Round			2nd Round			3rd Round		
Education / Age groups in years	Educ.	Non.Educ.	Total	Educ.	Non.Educ.	Total	Educ.	Non.Educ.	Total
7-12	50	0	50	53	1	54	57	1	58
13-18	21	1	22	29	1	30	33	1	34
19-24	20	7	27	22	13	35	21	8	29
25-30	4	19	23	7	23	30	11	29	40
31-40	1	32	33	2	46	48	3	51	54
41-50	0	13	13	1	15	16	1	20	21
51-60	0	23	23	0	22	22	0	22	22
60+	0	11	11	0	15	15	0	15	15
Total	96 (47.5%)	106 (52.5%)	202 (100%)	114 (45.6%)	136 (54.4%)	250 (100%)	126 (46.2%)	147 (53.8%)	273 (100%)

(children aged between 0-6 years were not included).

Table 3.4. Population in Wadihaloo village in age groups related to occupation in 3rd demographic survey round

Occupation	Age Groupings (years) 0-6	7-12	13-18	19-24	25-30	31-40	41-50	51-60	60+	Total
Farm worker	0	0	0	4	16	33	10	8	10	81 (22.3%)
Housewife	0	0	2	5	11	7	6	11	1	43 (11.8%)
At home	89	1	8	5	0	2	1	0	4	110 (30.2%)
Student	2	57	23	2	0	0	0	0	0	84 (23.1%)
Policeman	0	0	1	10	6	4	2	1	0	24 (6.6%)
Watchman	0	0	0	0	1	0	0	2	0	3 (0.8%)
Salesman	0	0	0	0	0	2	1	0	0	3 (0.8%)
Teacher	0	0	0	0	0	2	1	0	0	3 (0.8%)
Labourer	0	0	0	0	2	3	0	0	0	5 (1.4%)
Tailor	0	0	0	0	1	0	0	0	0	1 (0.3%)
Housemaid	0	0	0	3	2	1	0	0	0	6 (1.6%)
Driver	0	0	0	0	1	0	0	0	0	1 (0.3%)
Total	91 (25%)	58 (15.9%)	34 (9.3%)	29 (8%)	40 (11%)	54 (14.8%)	21 (5.8%)	22 (6.1%)	15 (4.1%)	364 (100%)

At home (including the children below 6 years, unmarried women and the old people).

The farm workers in the village were usually over 19 years old, as were most of the policemen. At age above 6 years the children start going to the school in the village (Table 3.4).

The females in the village either worked at home or they went to school as students, and the rest of the work in the village was done by males.

The Pakistani and Bangladeshi people in the village were working as farm workers and labourers. The Indians were working as salesmen in the shops. The Sri Lankan women were working as housemaids and the Egyptians were working as teachers (Table 3.5).

Some of the housewives in Wadihaloo village were educated, but most of them were not. None of the farm workers, housemaids and the labourers in the village were educated.

61 (17%) of the population of Wadihaloo village were working outside the village, 42 (69%) of this group of people were males. 41 (49%) of the students were going to the school in next village. And 20 (83%) of the policemen in the village were working outside the village. 349 (96%) of the population in the Wadihaloo village were leaving the village from 2 to 240 times per year and returned back in the same day. 5 (1%) of them were leaving the village from 36 to 38 times per year and staying for 162 days outside the village. And 10 (3%) of the population were leaving the village up to 40 times per year and staying outside the village for 258 days per year (Table 3.6).

Table 3.5. The occupation of the population in Wadihaloo village related to nationality in the 3rd demographic survey round

Occupation	Nationality							
	U.A.E.	Pakistani	Bangladeshi	Indian	Sri Lankan	Egyptian	Total	
Farm worker	20	32	29	0	0	0	81	(22.3%)
Housewife	43	0	0	0	0	0	43	(11.8%)
At Home	110	0	0	0	0	0	110	(30.2%)
Student	84	0	0	0	0	0	84	(23.1%)
Policeman	24	0	0	0	0	0	24	(6.6%)
Watchman	3	0	0	0	0	0	3	(0.8%)
Salesman	0	0	0	3	0	0	3	(0.8%)
Teacher	0	0	0	0	0	3	3	(0.8%)
Labourer	0	1	4	0	0	0	5	(1.4%)
Tailor	0	0	1	0	0	0	1	(0.3%)
House Maid	0	0	0	0	6	0	6	(1.6%)
Driver	0	0	0	0	0	1	1	(0.3%)
Total	284 (78%)	33 (9.1%)	34 (9.4%)	3 (0.8%)	6 (1.6%)	4 (1.1%)	364 (100%)	

Table 3.6. No. of times the people leaving the village per year and for how many days per year they stayed outside the village, in three demographic survey rounds

No.times leaving the village per year	1st Round				2nd Round				3rd Round			
	No. of days staying outside the village per year											
	0	162	258	Total	0	162	258	Total	0	162	258	Total
2	3	0	0	3	3	0	0	3	3	0	0	3
4	102	0	0	102	114	0	0	114	122	0	0	122
6	51	0	0	51	65	0	0	65	70	0	0	70
12	16	0	0	16	14	0	0	14	14	0	0	14
21	1	0	0	1	0	0	0	0	0	0	0	0
24	53	0	0	53	77	0	0	77	87	0	0	87
36	0	0	0	0	0	2	0	2	0	3	0	3
38	0	1	0	1	0	2	0	2	0	2	0	2
40	0	0	10	10	0	0	10	10	0	0	10	10
52	3	0	0	3	3	0	0	3	3	0	0	3
156	1	0	0	1	1	0	0	1	1	0	0	1
204	41	0	0	41	40	0	0	40	39	0	0	39
240	10	0	0	10	10	0	0	10	10	0	0	10

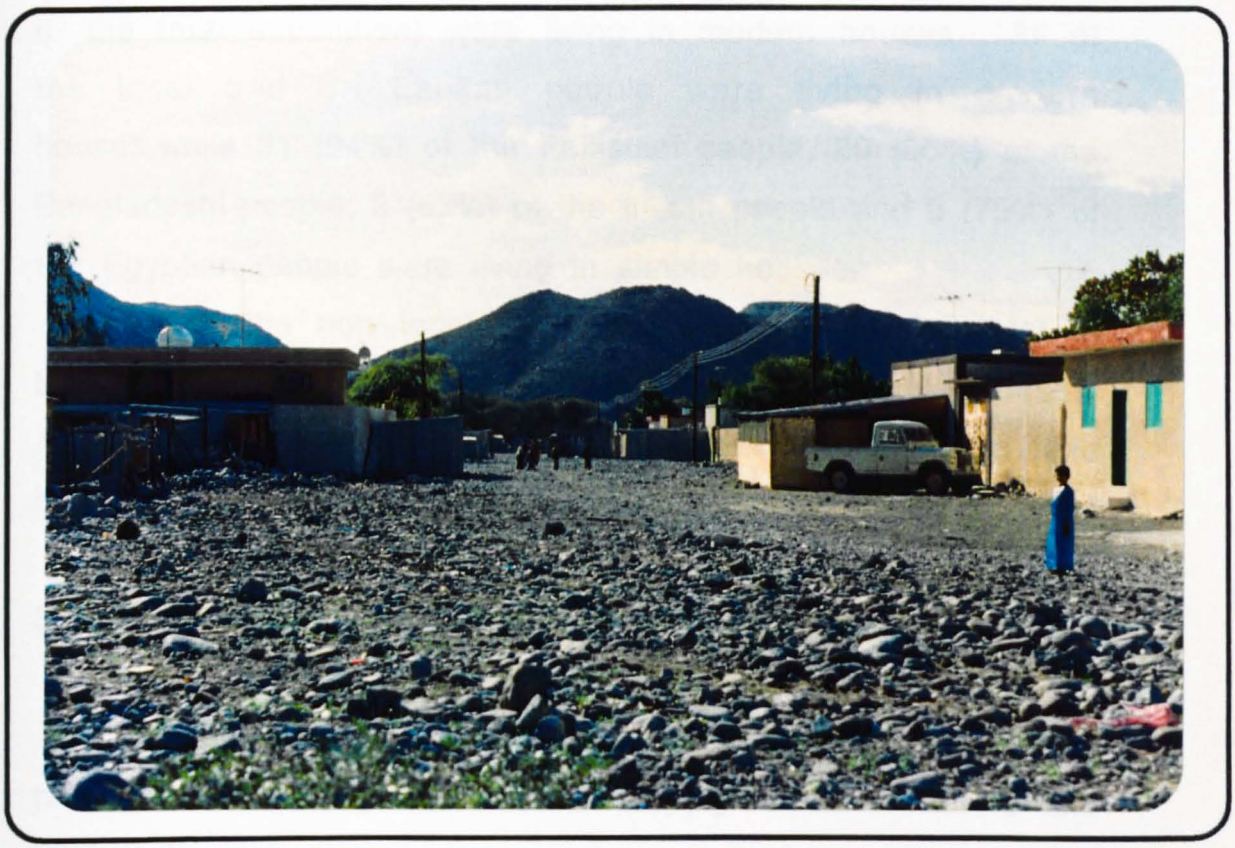


Figure 3.9 Showing the modern houses in Wadihaloo village

3.3.3. The housing in Wadihaloo village

3.3.3.1.Type of house

There were two types of houses in the village, modern houses which were built of blocks and concrete, and simple houses which were built of wood and aluminium sheets (Figure 3.9 and Figure 3.10).

The majority of the population in the village 317 (87% of the total population) were living in modern houses. All of the local and Sri Lankan people were living in modern houses,while 31 (94%) of the Pakistani people, 29 (85%) of the Bangladeshi people, 2 (67%) of the Indian people and 3 (75%) of the Egyptian people were living in simple houses.

All the non local farm workers (75% of the total of the farm workers) were living in the farms in simple houses. Also the salesmen and the teachers were living in simple houses.

3.3.3.2.Source of drinking water in Wadihaloo village

The people in the village were drinking water from two sources, firstly the central supply which reached the houses through closed pipes, and secondly from different wells on the farms. The modern houses were all supplied with drinking water from the central source. 320 (88%) of the population in the village were drinking water mainly from the central supply, and 44 (12%) of them were drinking water from different wells in the farms. Only 4 (8%) of the population living in the simple houses were drinking water from the central source, while 43 (92%) of them were drinking water from different wells.

31 (97%) of the Pakistani and 29 (85%) of the Bangladeshi people in the village were drinking water from the wells on the farms.

Source of drinking water, was related to occupation. 61 (75%) of the farm workers in the village were drinking water from the wells on the farms.

3.3.3.3.Type of latrine

The people in the village were either using modern latrines, which open into a septic tank, (Figure 3.11) or they have no latrine. The majority of the people in the village who lived in modern houses used the modern latrines, but 44 (94%) of the people who lived in simple houses had no latrine.

31 (94%) of the Pakistani people, 29 (85%) of the Bangladeshi people and 2 (67%) of the Indian people in the village did not use latrines.

The majority of farm workers 61 (75%), and 2 (67%) of the salesmen in the village did not use latrines.

3.3.4. The farms in the Wadihaloo village

The farms were scattered around the village (Figure 3.12). The number of the farms increased from 36 farms in the first survey to 47 farms in the third survey round. Mainly they grew tobacco with some vegetables and fruits, which were irrigated with water pumped from the well to large cement reservoirs, then the water was distributed through small channels to the plant (Figure 3.13). Usually the non local farm workers were living on the farm in small simple houses with no latrines or central water supply (Figure 3.10).

Figure 3.10 Showing a simple house in the farm in Wadihaloo village

Figure 3.11 Showing the type of latrine in the modern houses in Wadihaloo village

Figure 3.12 Showing the farms in Wadihaloo village

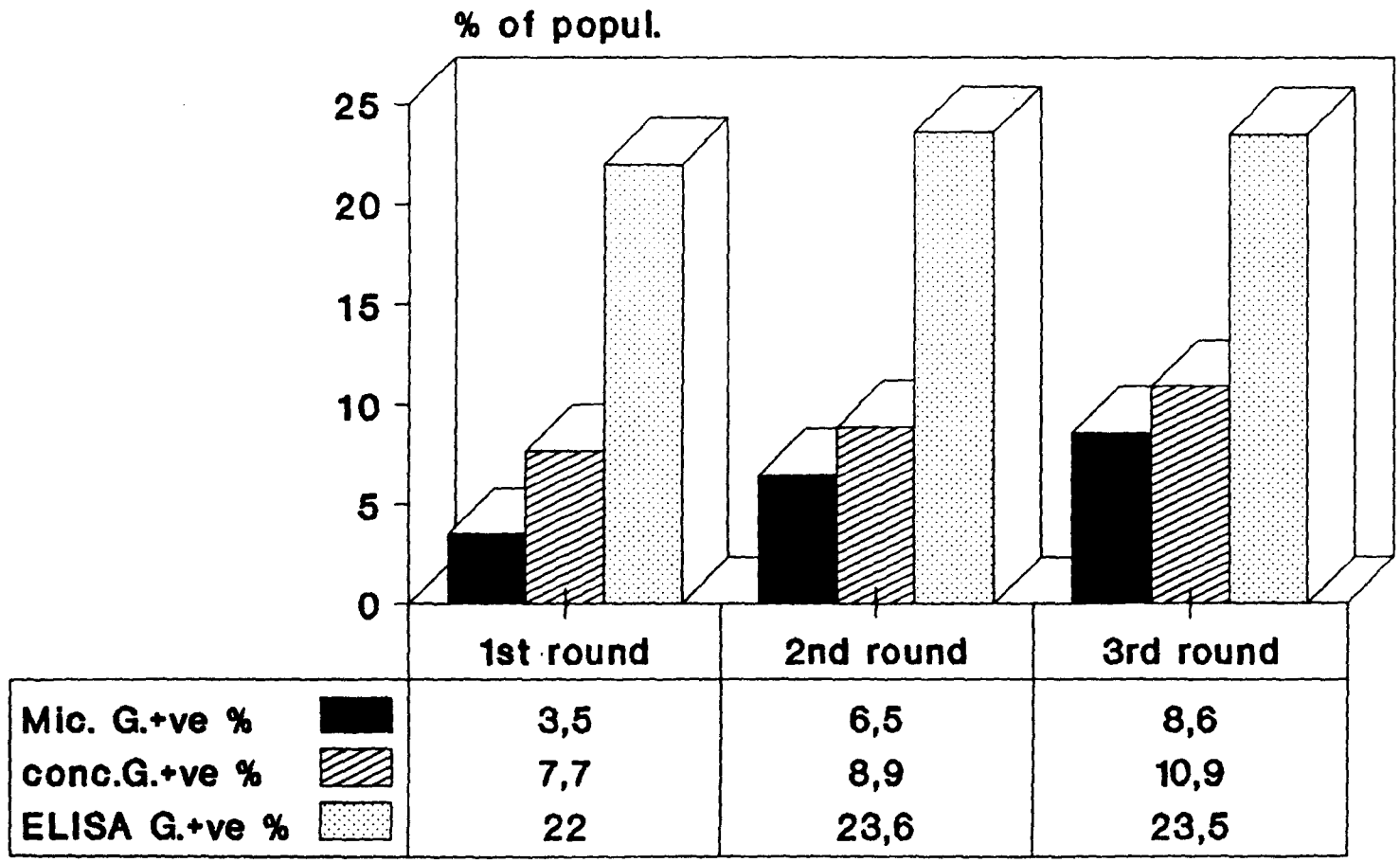
Figure 3.13 Showing cement water basin reservoir in the farm in Wadihaloo village

3.3.5. Examination of stool samples from Wadihaloo village (Figure 3.14)

A total of 982 stool samples were collected from Wadihaloo village in the three survey rounds. 286 stool samples were collected in the first survey round with 6 refusals to give stool samples. 339 stool samples were collected in the second survey round, when there were 2 refusals to give stool samples. 357 stool samples were collected in the third survey round when 7 refused to give stool samples. The stool samples were examined by three diagnostic methods to detect Giardia intestinalis (G.intestinalis).

3.3.5.1. Routine Microscopical examination

Stool samples were examined for Giardia detection by routine microscopy. Some of the positive cases were treated after each survey round, even so the percentage of positive cases was found to have increased in the following survey (Table 3.7.). The percentage of positive cases for other intestinal parasites was found to be nearly equal in the three survey rounds, despite treatment of some positive cases after each survey round.



	1st round	2nd round	3rd round
Mic. G.+ve %	3,5	6,5	8,6
conc.G.+ve %	7,7	8,9	10,9
ELISA G.+ve %	22	23,6	23,5

Fig.3.14 Percentage of Giardia cases detected with 3 def. diag. methods in the village in three survey rounds.

Table 3.7 **Comparing the results of examing stool samples with three different diagnostic methods (routine microscopy, concentration test and ELISA test to detect stool antigen) , in three survey rounds**

Methods of Stool Examination	First Round			Second Round			Third Round		
	G +ve	Other intes. parasites +ve	Total No. of stool samples	G +ve	Other intes. parasites +ve	Total No. of stool samples	G +ve	Other intes. parasites +ve	Total No. of stool samples
Routine Microscopy	10 (3.5%)	13 (4.5%)	286 (100%)	22 (6.5%)	14 (4.1%)	339 (100%)	30 (8.4%)	19 (5.3%)	357 (100%)
Concentration Test	22 (7.7%)	32 (11.2%)	286 (100%)	30 (8.9%)	37 (10.9%)	339 (100%)	39 (10.9%)	39 (10.9%)	357 (100%)
ELISA	63 (22%)	-	286 (100%)	80 (23.6%)	-	339 (100%)	84 (23.5%)	-	357 (100%)

3.3.5.2. Microscopical examination with concentration test

The concentration test was found to be more sensitive in detection of Giardia and other intestinal parasites than routine microscopy (Table 3.7). The same pattern was found with the concentration test for the percentage of Giardia positive cases and other intestinal parasites, as with routine microscopy.

3.3.5.3. ELISA test to detect Giardia antigen in stool samples

982 stool samples collected during the three survey rounds were examined with ELISA test.

The case was considered as positive if the O.D. value was above the mean of negatives samples +3 standard deviations. The results of the concentration test were compared with ELISA data to evaluate ELISA sensitivity and specificity. Nine false-negative samples were detected of the total collected stool samples. And eighteen stool samples were positive by ELISA but negative by concentration test and were not attributable to non specific binding; all eighteen samples contained other intestinal parasites, but these have been shown not to cross-react in the assay (Green et al. 1985). The sensitivity of the ELISA for the total examined stool samples was found to be 90% (Table 8.1).

In the first survey 63 (22%) of the stool samples were positive for Giardia. The sensitivity of the ELISA was found to be 95.5%.

In the second survey 80 (23.6%) of the stool samples were positive for Giardia. The sensitivity of the ELISA was found to be 86.7%.

In the third survey 84 (23.5%) of the stool samples were positive for Giardia. The sensitivity and the specificity of the ELISA was found to be 89.7%.

3.3.5.3.1. Number of people in Wadihaloo village related to sex and G.intestinalis detected with ELISA test

The prevalence of Giardia with faecal ELISA in the three survey rounds was found to be higher amongst the females than the males. (Chi-square = 2.27 with P >0.05).

In the first survey round 156 stool samples were collected from males, 29 (18.6%) of them were positive for Giardia. And 130 stool samples were collected from females, 34 (26.2%) of them were positive for Giardia.

Most of the people (except the children below 6 years) in the village were treated after the first survey round. The prevalence of Giardia amongst the males was found to increase in the second survey round. This may be due to not taking the medicine, arrival of new positive cases with the expatriates or an increase in the positive cases amongst the male children at age below 6 years. Amongst the females the prevalence of Giardia was decreased, but still higher than in males.

191 stool samples were collected from the males, 43 (22.5%) of them were positive for Giardia. And 148 stool samples were collected from the females, 37 (25%) of the samples were positive for Giardia.

In the third survey round the prevalence of Giardia

amongst the males and the females were found to be nearly the same as in the second survey round, this may be due to equal numbers of new positive cases and the treated cases. 204 stool samples were collected from the males, 45 (22%) of stool samples were positive for Giardia. 153 stool samples were collected from the females, 39 (25.5%) of the stool samples were positive for Giardia.

3.3.5.3.2. Number of people in Wadihaloo village related to education and G.intestinalis detected with ELISA test

The prevalence of Giardia was found to be higher amongst the educated people than in the non educated people, in the three survey rounds. (Chi-square = 2.62 with P >0.05).

This may be due to an increase of local transmission of the infection especially amongst the school children, and not proper treatment of the positive cases.

In the first survey round 99 stool samples were collected from educated people, 17 (17.2%) of the stool samples were positive for Giardia. And 102 stool samples were collected from non educated people, 16 (15.7%) of the stool samples were positive for Giardia.

In the second survey round 122 stool samples were collected from educated people, 32 (26.2%) of the stool samples were positive for Giardia. And 135 stool samples were collected from non educated people, 25 (18.5%) of the stool samples were positive for Giardia.

In the third survey round 126 stool samples were collected from educated people, 26 (20.6%) of the stool samples were positive for Giardia. And 142 stool samples

were collected from non educated people, 22 (15.5%) of the stool samples were positive for Giardia.

3.3.5.3.3. Number of people in the Wadihaloo village related to nationality and G.intestinalis detected with ELISA test

In each survey round, the prevalence of Giardia with faecal ELISA increased amongst the local people. (Chi-square for linear trend = 0.137 and P >0.05). This may be due to the increase of local transmission in the presence of non treated positive cases, especially amongst the children below 6 years. Also the prevalence increased amongst the non local people, especially amongst the Bangladeshi and Pakistani people, this may be due to local transmission and arrival of new positive cases.

In the first survey round 251 stool samples were collected from the U.A.E. nationality people, 57 (22.7%) of them were positive. 22 stool samples were collected from the Pakistani people, 3 (13.6%) of them were positive. 11 stool samples were collected from the Bangladeshi people, 3 (27.3%) of them were positive. 1 stool sample was collected from the Indian male which was negative, and 1 stool sample was collected from a Sri Lankan female and it was negative for Giardia.

In the second survey round 272 stool samples were collected from the U.A.E. people, 66 (24.3%) of them were positive. 30 stool samples were collected from the Pakistani people, 5 (16.7%) of them were positive. 27 stool samples were collected from the Bangladeshi people, 5 (18.5%) of them were positive. 2 stool samples were collected from the Indian

people, both of them were positive. 5 stool samples were collected from the Sri Lankan people, only one of them was positive. And 3 stool samples were collected from the Egyptian people, only one (33.3%) of them was positive for Giardia.

In the third survey round 281 stool samples were collected from the U.A.E. people, 71 (25.3%) of them were positive. 30 stool samples were collected from the Pakistani people, about 3 (10%) of them were positive. 25 stool samples were collected from the Bangladeshi people, 7 (28%) of them were positive. 3 stool samples were collected from the Indian people, only 1 (33.3%) of them was positive. 6 stool samples were collected from the Sri Lankan people, only 1 (16.7%) of them were positive. And 4 stool samples were collected from the Egyptian people, only one (25%) of them were positive for Giardia.

3.3.5.3.4. Number of people in the Wadihaloo village related to occupation and G.intestinalis detected with ELISA test

The highest prevalence of Giardia with faecal ELISA was found amongst the group without work, followed by the students, the farm workers, the housewives and the policemen. (Chi-square for linear trend = 2.596 P >0.05). In the third survey round, the prevalence of Giardia was found to decrease amongst the occupational groups, except amongst the group of people without work (Table 3.8).

Table 3.8 Prevalence of Giardia detected with ELISA test, amongst different occupational groups of people in Wadihaloo village, in the three survey rounds

Survey Rounds	Prevalence of Giardia among									
	Farm workers	Housewives	Without work	Students	Policemen	Watchmen	Housemaids	Salesmen	Labourers	Drivers
	(n)	(n)	(n)	(n)	(n)	(n)	(n)	(n)	(n)	(n)
	(52)	(39)	(104)	(62)	(24)	(3)	(1)	(1)	(0)	(0)
First	19.2%	18%	30.8%	21%	4.2%	0	0	0	0	0
	(72)	(43)	(104)	(79)	(24)	(3)	(5)	(2)	(4)	(1)
Second	15.3%	16.3%	27.2%	32.9%	12.5%	33.3%	20%	100%	0	100%
	(76)	(43)	(109)	(84)	(23)	(3)	(6)	(3)	(5)	(1)
Third	15.8%	11.6%	38.5%	21.4%	8.7%	0	16.7%	33.3%	40%	100%

(N = total number of examined stool samples)

In the first survey round 52 stool samples were collected from the farm workers in the village, 10 (19.2%) of them were positive, 39 stool samples were collected from the housewives in the village, 7 (18%) of them were positive. 104 stool samples were collected from the people without work, 32 (30.8%) of them were positive. 62 stool samples were collected from the students, 13 (21%) of them were positive. 24 stool samples were collected from the policemen, only 1 (4.2%) of them were positive. 3 stool samples were collected from the watchmen, none of them were positive. One stool sample was collected from the salesmen, but it was negative. And only 1 stool sample was collected from the housemaid but it was negative for Giardia.

In the second survey round, 72 stool samples were collected from the farm workers, 11 (15.3%) of them were positive. 43 stool samples were collected from the housewives, 7 (16.3%) of them were positive. 104 stool samples were collected from the people without work, 28 (27.2%) of them were positive. 79 stool samples were collected from the students, 26 (32.9%) of them were positive. 24 stool samples were collected from the policemen, 3 (12.5%) of them were positive. 3 stool samples were collected from the watchmen, only 1 (33.3%) stool sample was positive. Two stool samples were collected from the salesmen, both of them (100%) were positive. Two stool samples were collected from the teachers, both of them were negative. 4 stool samples were collected from the labourers, all of them were negative. One stool sample was collected from the tailor in the village, it was negative. 5 stool samples were collected from the housemaids, only one of them was positive. And only one stool

sample was collected from the driver, it was positive for Giardia.

In the third survey round 76 stool samples were collected from the farm workers, 12 (15.8%) of them were positive. 43 stool samples were collected from the housewives, 5 (11.6%) of them were positive. 109 stool samples were collected from the people without work, 42 (38.5%) of them were positive. 84 stool samples were collected from the students, 18 (21.4%) of them were positive. 23 stool samples were collected from the policemen, about 2 (8.7%) of them were positive. 3 stool samples were collected from the watchmen, all of them were negative. 3 stool samples were collected from the salesmen, only one (33.3%) of them was positive. 3 stool samples were collected from the teachers, all of them were negative. Five stool samples were collected from the labourers, two (40%) of them were positive. One stool sample was collected from the tailor, it was negative. 6 stool samples were collected from the housemaids, only one (16.7%) of them was positive. One stool was collected from the driver, it was positive for Giardia.

3.3.5.3.5. Number of the people in the Wadihaloo village related to age group and G.intestinalis detected with ELISA test

The highest prevalence of Giardia with faecal ELISA was found amongst the children at age group 0-6 years. (Chi-square for linear trend = 36.521 with significant $P < 0.05$). The prevalence was decreased until the age of 18 years (amongst the local people). At age above 19 years the prevalence rate became variable, this may be due to new arrival of positive

cases.

In the first survey round 88 stool samples were collected from age group 0-6 years, 31 (35.2%) of them were positive. 50 stool samples were collected from age group 7-12 years, 10 (20%) of them were positive. 22 stool samples were collected from the 13-18 years age group, 2 (9.1%) of them were positive. 27 stool samples were collected from 19-24 year age group, 5 (18.5%) of them were positive. 22 stool samples were collected from the age group of 25-30 years, 3 (13.6%) of them were positive. 31 stool samples were collected from age group of 31-40 years, 5 (16.1%) of them were positive. 13 stool samples were collected from age group of 41-50 years, 4 (30.8%) of them were positive. 23 stool samples were collected from the 51-60 year age group, 3 (13%) of them were positive. And 10 stool samples were collected from the age group of 60 and above years, all of them were negative for Giardia.

In the second survey round 90 stool samples were collected from the 0-6 year age group, 27 (30%) of them were positive. 54 stool samples were collected from the 7-12 years age group, 19 (35.2%) of them were positive. 30 stool samples were collected from the age group of 13-18 years, 6 (20%) of them were positive. 35 stool samples were collected from age group of 19-24 years, 8 (22.9%) of them were positive. 30 stool samples were collected from the 25-30 years age group, 7 (23.3%) of them were positive. 47 stool samples were collected from the 31-40 years age group, 8 (17%) of them were positive. 16 stool samples were collected from age group of 41-50 years, all of them were negative. 22 stool samples were collected from 51-60 years age group, 4 (18.2%) of them were positive. And 15 stool samples were

collected from age group above 60 years, only one (6.7%) of them was positive for Giardia.

In the third survey round, 91 stool samples were collected from the age group of 0-6 years, 37 (40.7%) of them were positive. 58 stool samples were collected from age group of 7-12 years, 13 (22.4%) of them were positive. 33 stool samples were collected from the age group of 13-18 years, 8 (24.2%) of them were positive. 29 stool samples were collected from age group of 19-24 years, 4 (13.8%) of them were positive. 39 stool samples were collected from the 25 to 30 year age group, 8 (20.5%) of them were positive. 50 stool samples were collected from age group of 31-40 years, 8 (16%) of them were positive. 21 stool samples were collected from age group of 41-50 years, 2 (9.2%) of them were positive. 22 stool samples were collected from the 51-60 years age group, 4 (18.2%) of them were positive. 16 stool samples were collected from age group of above 60 years, none of them was positive for Giardia.

3.3.5.3.6. Number of the people in the Wadihaloo village related to place of work and G.intestinalis detected with ELISA test in the three survey rounds

In general the prevalence of Giardia with faecal ELISA was higher amongst the people working inside the village, than that amongst the people working outside the village. (Chi-square = 2.43 and P >0.05).

In the first survey round, 224 stool samples were collected from the people in the village who were working inside the village, 57 (25.5%) of them were positive. 62 stool samples were collected from the people who were working outside the village, 6 (9.7%) of the stool samples were

positive for Giardia

In the second survey round, 277 stool samples were collected from the people who were working inside the village, 64 (23.1%) of them were positive. And 62 stool samples were collected from the people who were working outside the village, 16 (25.8%) of them were positive for Giardia.

In the third survey round, 297 stool samples were collected from the people who were working inside the village, 72 (24.2%) of them were positive while 225 (75.8%) of them were negative. 60 stool samples were collected from the people who were working outside the village, 12 (12%) of them were positive for Giardia.

3.3.5.3.7. Number of the people in the Wadihaloo village related to type of house and G.intestinalis detected with ELISA test in the three survey rounds

Amongst the people who were living in modern houses, the prevalence of Giardia with faecal ELISA was found to increase in each survey round, and higher than that amongst the people who were living in simple houses. (Chi-square = 2.55 and P >0.05).

In the first survey round, 252 stool samples were collected from the people who were living in modern houses, 57 (22.6%) of the stool samples were positive. 34 stool samples were collected from the people living in simple houses, 6 (17.6%) of them were positive for Giardia.

In the second survey round, 283 stool samples were collected from the people who were living in modern houses, 68 (24%) of them were positive. 56 stool samples were

collected from the people who were living in simple houses, 12 (21.4%) of them were positive for Giardia.

In the third survey round, 295 stool samples were collected from the people living in modern houses, 75 (25.4%) of them were positive. 62 stool samples were collected from the people living in simple houses, 9 (14.5%) of them were positive for Giardia.

3.3.5.3.8. Number of the people in the Wadihaloo village related to source of drinking water and G.intestinalis detected with ELISA test in the three survey rounds

The prevalence of Giardia with faecal ELISA amongst the people who were drinking water from the central supply was found to be higher than that amongst the people who were drinking water from the wells. (Chi-square = 3.21 and P >0.05).

In the first survey round, 253 stool samples were collected from the people drinking from the central supply, 57 (22.5%) of them were positive. 33 stool samples were collected from the people who were drinking from the wells, about 6 (18.2%) for Giardia.

In the second survey round, 287 stool samples were collected from the people who were drinking water from the central supply, 70 (24.8%) of them were positive. 52 stool samples were collected from the people drinking water from the wells, 10 (19.2%) of the stool samples were positive for Giardia.

In the third survey round, 300 stool samples were collected from the people drinking water from the central supply. 76 (25.3%) of them were positive. 57 stool samples

were collected from the people drinking water from the wells 8 (14%) of them were positive for Giardia.

3.3.5.3.9. Number of the people in the Wadihaloo village related to type of latrine and G.intestinalis detected with ELISA test in the three survey rounds

The prevalence of Giardia with faecal ELISA was found to increase in each survey round, amongst the people who were using latrines it was found higher amongst them than that amongst the people who were not using latrines. (Chiq-square = 2.92 with marginal significant P-value = 0.08).

In the first survey round, 252 stool samples were collected from the people using modern latrines, 57 (22.6%) of them were positive. 34 stool samples were collected from people who were not using latrines, 6 (17.6%) of them were positive for Giardia.

In the second survey round, 285 stool samples were collected from the people using modern latrines, 68 (23.9%) of them were positive. 54 stool samples were collected from the people who were not using latrines, 12 (22.2%) of them were positive for Giardia.

In the third survey round, 248 stool samples were collected from the people using modern latrines, 75 (25.2%) of them were positive. 59 stool samples collected from the people who were not using latrines, 9 (15.2%) of them were positive for Giardia.

3.3.5.3.10. Number of the people in the Wadihaloo village related to their movements to and from the village, and positive G.intestinalis detected with ELISA test in the three survey rounds

The prevalence ratio of Giardia with faecal ELISA amongst the group of people living in the village, and travelling to and from the village in one day was 24%. A small group (1.4% of the population) were staying for more than one day outside the village, the prevalence rate of Giardia with faecal ELISA among them was variable (Table 3.9).

In the first survey round, the numbers of people with Giardia infection, leaving the village and returning back the same day were found to be 26 (4 times/year), 16 (6 times/year), 3 (12 times/year), one (21 times/year), 10 (24 times/year), one (52 times/year) and 5 (204 times/year). One person leaving the village for 38 times/year and staying outside the village for 162 days was found to be positive for Giardia.

In the second survey round the numbers of people with Giardia infection, leaving the village and returning back the same day were found to be 24 (4 times/year), 19 (6 times/year), 5 (12 times/year), 15 (24 times/year), one (52 times/year), 14 (204 times/year) and one (240 times/year). One person leaving the village for 40 times/year and staying outside the village for 258 days was found to be positive with Giardia.

Table 3.9. **No of people in the Wadihaloo village positive for Giardia with faecal ELISA test related to number of times left village per year and number of days stayed outside the village per year in the three survey rounds**

No. of times leaving the village per year	1st Round			2nd Round			3rd Round			
	No. of days staying outside the village per year									
	0	162	Total	0	258	Total	0	162	258	Total
4	26 (25.5%)	0	26	24 (21.1%)	0	24	30 (24.6%)	0	0	30
6	16 (31.4%)	0	16	19 (29.2%)	0	19	21 (30.0%)	0	0	21
12	3 (18.8%)	0	3	5 (35.7%)	0	5	5 (35.7%)	0	0	5
21	1(100.0%)	0	1	0	0	0	0	0	0	0
24	10 (18.9%)	0	10	15 (19.5%)	0	15	16 (18.4%)	0	0	16
38	0	1(100%)	1	0	0	0	0	1 (50.0%)	0	1
40	0	0	0	0	1(100%)	1	0	0	1(100%)	1
52	1 (33.4%)	0	1	1 (33.4%)	0	1	0	0	0	0
156	0	0	0	0	0	0	0	0	0	0
204	5 (12.0%)	0	5	14 (35.0%)	0	14	9 (23.1%)	0	0	9
240	0	0	0	1 (10.0%)	0	1	1 (10.0%)	0	0	1
Total	62	1	63	79	1	80	82	1	1	84

(%) prevalence of Giardia in each group

In the third survey round, the numbers of people with Giardia infection, leaving the village and returning back in the same day were found to be 30 (4 times/year), 21 (6 times/year), 5 (12 times/year), 16 (24 times/year), 9 (204 times/year) and one (240 times/year). One person leaving the village 162 times, and another person for 40 times/year, and staying outside the village for 258 days were found positive for Giardia.

3.3.5.3.11. Number of the people in the Wadihaloo village related to other intestinal parasites found with microscopical concentration test and G.intestinalis detected with ELISA test in the three survey rounds

The prevalence of other intestinal parasites with concentration test was 11%, and nearly 25% of the stool samples with other intestinal parasites contain Giardia (Table 3.10).

In the first survey round, 254 stool samples were negative for other intestinal parasites, 52 of them were positive for G.intestinalis, 18 stool samples were positive for Entamoeba coli (E.coli), only four of them were positive for Giardia. And 9 stool samples were positive for Hymenolepis nana (H.nana), seven of them were positive for Giardia.

In the second survey round, 302 stool samples were negative for other intestinal parasites, 73 of them were positive for Giardia. 18 stool samples were positive for E.coli, 3 of them were positive for Giardia, 4 stool samples were

positive for H.nana, one of them was positive for Giardia.. Three stool samples were positive for Enterobius vermicularis (Enterobius), one of them was positive for Giardia. And 8 stool samples were positive for hookworm, two of them were positive for Giardia.

In the third survey round, 318 stool samples were negative for other intestinal parasites, 74 of them were positive for Giardia. 22 stool samples were positive for E.coli, two of them was positive for Giardia, 5 stool samples were positive for H.nana, two of them was positive for Giardia, two stool samples were positive for both Giardia and Entamoeba histolytica (E.h.). Two stool samples were positive for both Giardia and Enterobius, and 11 stool samples were positive for hookworm, 3 of them were positive for Giardia.

3.3.5.3.12. Number of the people in the Wadihaloo village with positive G.intestinlis detected with ELISA test related to number of times of loose motion and number of days with loose motion

21.4% of the total stool samples were associated with diarrhoea. The number of loose motions ranged between 3 to 7 times a day, and remained from 2 to 7 days. 27.1% of the diarrhoeal cases were associated with Giardia 25.1% of the positive Giardia cases were associated with diarrhoea and only 5.8% of the total examined stool samples were associated with Giardia and diarrhoea (Table 3.11).

In the first survey round, 63 stool samples were collected from the people in the village with loose motions, 16 (25.4%) of them were positive for Giardia.

Table 3.10 No of the people in the Wadihaloo village related to other intestinal parasites detected with microscopical concentration test, and Giardia detected with ELISA test in the three survey rounds

Other Parasites with conc. test.	1st Round			2nd Round Detection of Giardia with ELISA test			3rd Round		
	G -ve	G +ve	Total	G -ve	G +ve	Total	G -ve	G +ve	Total
Negative	202	52 (20.5%)	254	229	73 (24.2%)	302	244	74 (23.3%)	318
E.Coli	14	4 (22.2%)	18	15	3 (16.7%)	18	20	2 (9.1%)	32
H.nana	2	7 (77.8%)	9	3	1 (25.0%)	4	3	2 (40.0%)	5
E.h.	2	0	2	2	0	2	0	2 (100.0%)	2
Enterobius.	2	0	2	2	1 (33.4%)	3	0	2 (100.0%)	2
H.w.	1	0	1	6	2 (25.0%)	8	8	3 (27.3%)	11
Trichuris	0	0	0	2	0	2	1	0	1
Ascaris	1	0	1	2	0	2	0	0	0

E.coli - Entamoeba coli; E.h. - Entamoeba histolytica; H.nana - Hymenolepis nana; Entro - Enterobius vermicularis; H.w. - hookworm; Trichuris - Trichuris trichuria; Ascaris - Ascaris lumbricoides

Table 3.11 Number of the people in the Wadihaloo village with or without Giardia detected with faecal ELISA test related to number of times of loose motion and number of days with loose motion in the three survey rounds

No. of times of loose motions/day	ELISA Test	1st Round				2nd Round				3rd Round			
		No. of days with loose motions											
		0	2-4	5-7	Total	0	2-4	5-7	Total	0	2-4	5-7	Total
0	G-ve	176	0	0	176	204	0	0	204	222	0	0	222
	G+ve	47	0	0	47	67	0	0	67	56	0	0	56
3-4	G-ve	0	31	1	32	0	21	0	21	0	22	5	27
	G+ve	0	8	0	8	0	6	0	6	0	8	6	14
5-8	G-ve	0	12	3	15	0	33	1	34	0	22	2	24
	G+ve	0	7	1	8	0	6	1	7	0	11	3	14
Total		223	58	5	286	271	66	2	339	278	63	16	357

Chi square = 2.44 P >0.05

In the second survey round, 68 stool samples were collected from the people in the village with loose motion; 13 (19%) of them were positive for Giardia.

In the third survey round, 79 stool samples were collected from the people in the village with loose motions, 28 (35%) of them were positive for Giardia. .

3.4. Discussion

The Wadihaloo village is typical of many villages in U.A.E. especially those in the central plateau and east coast, they were similar in house structure, electric and water supply, presence of some farms with foreign farmers, and the habits of the people. The Wadihaloo village was chosen as a study area because it was typical, with most of the important facilities (the presence of the clinic, school, grocery, shop and the mosque), and semi-isolated (its location between the mountains and bad conditions of the road which connects the village with other villages and the city).

The population of the Wadihaloo village was mainly formed from local people (from U.A.E.) with small groups of people from other nationalities such as from Pakistan, Bangladesh, India, Sri Lanka and Egypt. The population in the village was increasing every year with an average 11.8%, mainly in males at age group between 25 to 40 years due to the influex of the male expatriates into the village at this age group. This made the population pyramid of the village, nearly the same shape as that of UAE (Figure 3.3). The local men in the village were working either as farmers (inside the village) or as policemen mainly outside the village. All the housewives

in the village were working at home, but they may help their husbands in the farms. The non local people were all working inside the village, mainly as farm workers (from Pakistan and Bangladesh), labourers (from Bangladesh), housemaids (from SriLanka), salesmen (from India), teachers (from Egypt).

The school in the village was opened in September 1987, before that the students at age above 6 years went to school in a nearby village, most of the students left the school usually at the age of 14 years for females and 16 years of age for the males. Nearly one third of the population in the village were educated. The children in the village were either playing at home or in the street.

In the past the people in the village lived in old simple houses (Figure 3.5). In recent years the UAE goverment built new low income modern houses for all local people in the village. These are supplied with electricty and central water, and contain modern latrines. The non local farm workers lived in simple houses in the farms with no latrine, and they drank water from the wells.

The prevalence of Giardia amongst the villagers increased from 3.5% (with routine microscopy), to 7.7% (with concentration test), and 22% (with ELISA test). The ELISA test was more rapid, easy and reliable than routine microscopy and concentration test in examining large numbers of stool samples.

Giardia is endemic to most developing countries, it was found in the traditional and transitional communities of the developing world (Oyerinde et al. 1977; Develoux et al. 1990). The pattern of the Giardia prevalence curve (with microscopic concentration test and faecal ELISA test) in the first survey round (Figure 3.15) was similar to previous

findings (Stibbs et al. 1988). The highest prevalence rate was amongst the children below 6 years (Feachem et al. 1983), then it fell until the age of 18 years. This indicates reduced transmission at this age or some degree of acquired resistance to infection. In older age groups the prevalence was irregular, this can be explained by; arrival of new positive cases among expatriates at age groups above 18 years (see Chapter 5) or reduction of immunity amongst the adults, leading to rise in new positive cases due to local transmission.

The prevalence of Giardia with faecal ELISA test in the village was variable from one survey round to another, (Figure 3.14), this can be explained by; increase of untreated positive cases (especially in the group of children below 6 years), these were left untreated to follow the effect of Giardia on their growth, (which will be discussed in Chapter 4), and they may have acted as a source of infection to other people in the village. Some asymptomatic positive cases especially among the adults refused treatment, they may act as a source of infection.

33.6% of the local people in the village were in the age group from 0-6 years. The prevalence of Giardia amongst this group of children (11.4% with microscopic concentration test) was found lesser than that in previous studies in Guatemala (21.5%) (Gupta and Urrutia, 1982), in Zimbabwe (19.4%) (Mason and Patterson, 1987) and in Bangkok (20%) (Janoff et al 1990), and in Bangladesh (51%) (Gilman et al. 1985). The higher prevalence in young children may be due to the high risk of exposure to Giardia; They were less immune to Giardia infection, so more susceptible to severe infection. Loose motions, may lead to spread of infection to other children; They were not going to school, so they had more

chance to play in the street, and become infected from dirt with hand-to-mouth method; They had more chance to go with their mothers to the farms, and become infected through food, water or with direct methods; And they liked to eat sweets (candy) which may be contaminated with dirt or from flies.

In Niamey (Niger) the prevalence of Giardia was similar between males and females (Develoux et al. 1990). While in Wadihaloo village the prevalence with faecal ELISA test was higher amongst the females, this may be due to; The females had more contact with the risk group of children (below 6 years); And most of the females left the school at age of 14 years and stayed at home, taking care of the children.

Mostly because one third of the local people were children (below 6 years) the Giardia prevalence with faecal ELISA test was higher than that amongst other nationalities. There were some Giardia cases amongst the expatriates either imported or indigenous, especially amongst the people from Bangladesh.

The Giardia prevalence rate with faecal ELISA test was higher amongst the educated people. Most of the people do not know what Giardia is and how it is transmitted, and do not consider simple hygienic processes such as hand washing before food. The prevalence among the students in the village was higher than that found among the students in Abha (Asir), Saudi Arabia (Omar et al. 1991).

All four food handling groups (farm workers, housewives, housemaids and salesmen) had a high prevalence rate with faecal ELISA test (Table 3.7), which may enhance the spread of infection in the village. During the study period some lectures in health education were given to the housewives in the village, this may reflect on the Giardia

prevalence amongst this group, which was reduced from 18% in the first, to 11.6% in the third survey round (Table 3.7).

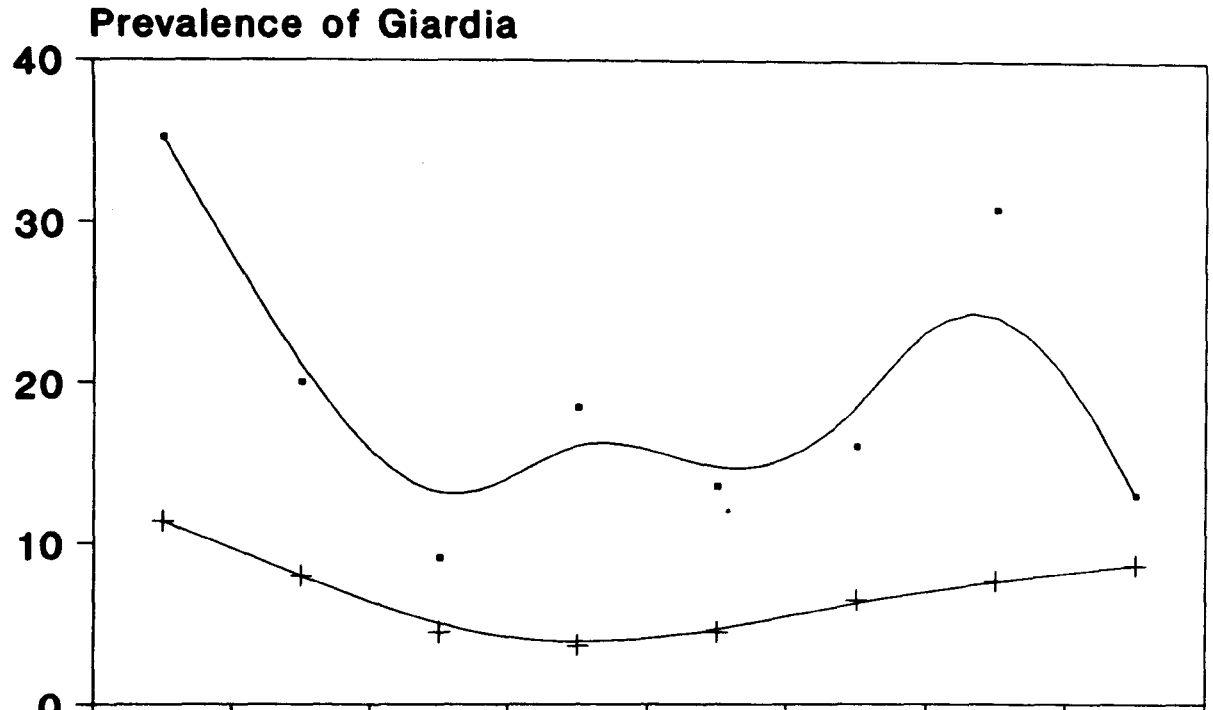
The possibility of getting infection with Giardia from outside the village is low, since most of the people working outside the village came back during the same day. The prevalence among them was low. A small group of people (1.4% of the population) were staying for more than one day outside the village, and prevalence among them was variable (Table 3.9).

The role of type of houses, source of drinking water and type of latrines in transmitting Giardia infection was not clear, since both groups of people mixed together in the same conditions.

Some people from the group who lived in modern houses, drank from the central water supply and used the modern latrines, would go from time to time to the farms, eat there, drink water from the well, and pass a stool on the farm (the source of infection will be discussed in Chapter 6).

Giardia is not considered the main cause of diarrhoea in the village, as some (27.1%) of the diarrhoeal cases were associated with Giardia and 25.1% of the positive Giardia cases were associated with diarrhoea. (the cause of diarrhoea will be discussed in Chapter 4).

The people in the village not only suffered from Giardia infection, but also from other intestinal parasite infections with a prevalence of 11% (with miscroscopic concentration test).



	0-6	7-12	13-18	19-24	25-30	31-40	41-50	51-60
ELISA test	35,2	20	9,1	18,5	13,6	16,1	30,8	13
Conc. test	11,4	8	4,5	3,7	4,5	6,5	7,7	8,7

Fig.3.15 Prevalence of Giardia detected with conc.test and ELISA antigene test related to age groups in the village.

CHAPTER 4

GIARDIA INTESTINALIS INFECTION OF PRESCHOOL CHILDREN IN THE VILLAGE AND ITS IMPACT ON PHYSICAL GROWTH

4.1. Introduction

Giardia intestinalis is the most common human enteric protozoan pathogen (Petersen, 1972). The organism is found worldwide in industrialised and technologically sophisticated societies (Keystone et al. 1978; Craun, 1979 and Phillips et al. 1981), and in the traditional and transitional communities of the developing world (Oyerinde et al. 1977; Melvin and Mata, 1971; Chhuttani et al. 1973 and Moore et al. 1966). Infection with Giardia may either be entirely asymptomatic or result in chronic diarrhoea with or without malabsorption. The reasons for variations in pathogenesis are not fully understood (Court and Anderson, 1959; Hoskins et al. 1967). Children infected with Giardia may have initial symptoms of chronic diarrhoea, malabsorption, poor weight gain or weight loss (Veghelyi, 1938; Boe and Rinvik, 1943; Cortner, 1959). The role of Giardia as a pathogenic agent for diarrhoea and malabsorption has been well accepted (Meyer and Jarroll, 1980). Most of the workers have reported occurrence of acute diarrhoeal symptoms in Giardia infected subjects (Wright et al. 1977a; Lopez et al. 1980). Infections may be asymptomatic and self limited, or they may last for years with symptoms such as severe diarrhoea, malabsorption and failure of children to thrive (Hartong et al.1979; Wolfe, 1978, Wright,

1980).

Various causes for the symptoms of Giardia have been suggested, such as the mechanical barrier formed by trophozoites to the transport of nutrients (Veghelyi, 1940; Tandon et al. 1977), injury to the intestinal mucosa with or without invasion by the parasite (Yardley et al. 1964; Brandborg et al.1967; Cowen and Campbell, 1973), and adherence of trophozoites to the mucosal surface causing destruction of the fuzzy coat which is essential for digestion and absorption of nutrients (Holmes, 1971).

Relatively little is known about the natural history of this infection in early life and its impact on subsequent physical growth and development, such information is best obtained by prospective, longitudinal observation of children in their own ecosystem (Mata et al. 1972; James, 1972; Rowland et al. 1977).

In many countries, malnutrition and infectious diseases commonly occur in the same child and together they play a major role in causing the high morbidity and mortality rates in children. The factors that depress the nutritional state of the susceptible child seem to be the very factors that magnify the severity of the infectious disease he acquires. Generally, dietary intake and the state of nutrition alter susceptibility to infectious disease (James, 1972). Andrews and Hewlett (1981) found that immune milk contains specific IgA and IgG antibody to Giardia, which may be the mediators of the demonstrated protection, also (Gillin et al. 1983a; Gillin et al. 1983b; Gillin et al. 1985) have shown and others have confirmed (Hernell et al. 1986) that non immune human milk (NHM) kills Giardia trophozoites in vitro in the presence of sodium cholate, but Zenian and Gillin, (1987) showed that

intestinal mucus protects Giardia from killing by human milk by inhibiting lipase activity and by decreasing the toxicity of products of lipolysis.

4.2. Method and materials

A group of preschool children (below 6 years) from Wadihaloo village were chosen to be included in this study and the aim of this study was explained to the mothers of the children.

Questionnaires were prepared which included the child number, family code number, date of birth of the child, number and date of visits, sex, weight in kg, height in cm, type of feeding, result of physical examination. The mothers were asked to bring their children to the clinic every two weeks during the study period (From January 1989 to June 1990) in each visit the mother was asked if the child had any complaints, loose motions, had she taken him to other clinics, or had she given him any medicine since the last visit and what type of food she had given him, then the child was physically examined, the weight and height were recorded (if the child was unable to stand the weight was taken by weighing him with the nurse, and the height in the specifically scaled box (Figure 4.1). A stool sample was also collected and a new stool container will be given to the mother to collect stool samples from the child for the next visit. If the child was suffering from a disease treatment was given, except in the case of Giardiasis. If the child had loose motions and was being breast fed the mother was asked to continue breast feeding together with some apple, carrot, banana, rice water, tea and oral rehydration salt (ORS). The stool samples were

coded with the childrens number, the family number and visit number and were then taken to Kalba hospital to be examined by the laboratory technician with direct smear microscopy for Giardia and other intestinal parasites. If the child had loose motions then part of the stool sample was examined with culture to exclude any bacteriological cause of diarrhoea. The stool samples were stored at 4° overnight. On the following day the negative samples were re-examined using direct microscopy. Then part of the faecal samples were mixed with 10% formaldehyde, filtered through wire mesh (size 250μm), ether was added, shaken vigorously for a few minutes, left to stand for 30 minutes, the fatty plug was loosened from the wall of the tube with a stick, the supernatant fluid was poured into chloros, and the deposit stained with Lugol's iodine was examined by microscopy. The negative samples were re-examined by Khorfakkan Hospital laboratory technicians.

For ELISA examination part of the faecal samples were suspended in phosphate buffer saline (PBS pH7.2) at a concentration of 10 gm% mixed properly and large particulate matter removed by filtration through wire mesh, then left to stand for 30 minutes and the supernatant was collected and stored at -20°C. The ELISA procedure was that outlined in Chapter 2 then positive-negative cut off value for the ELISA was carried out, the arithmetic mean of the negative samples +3SD.

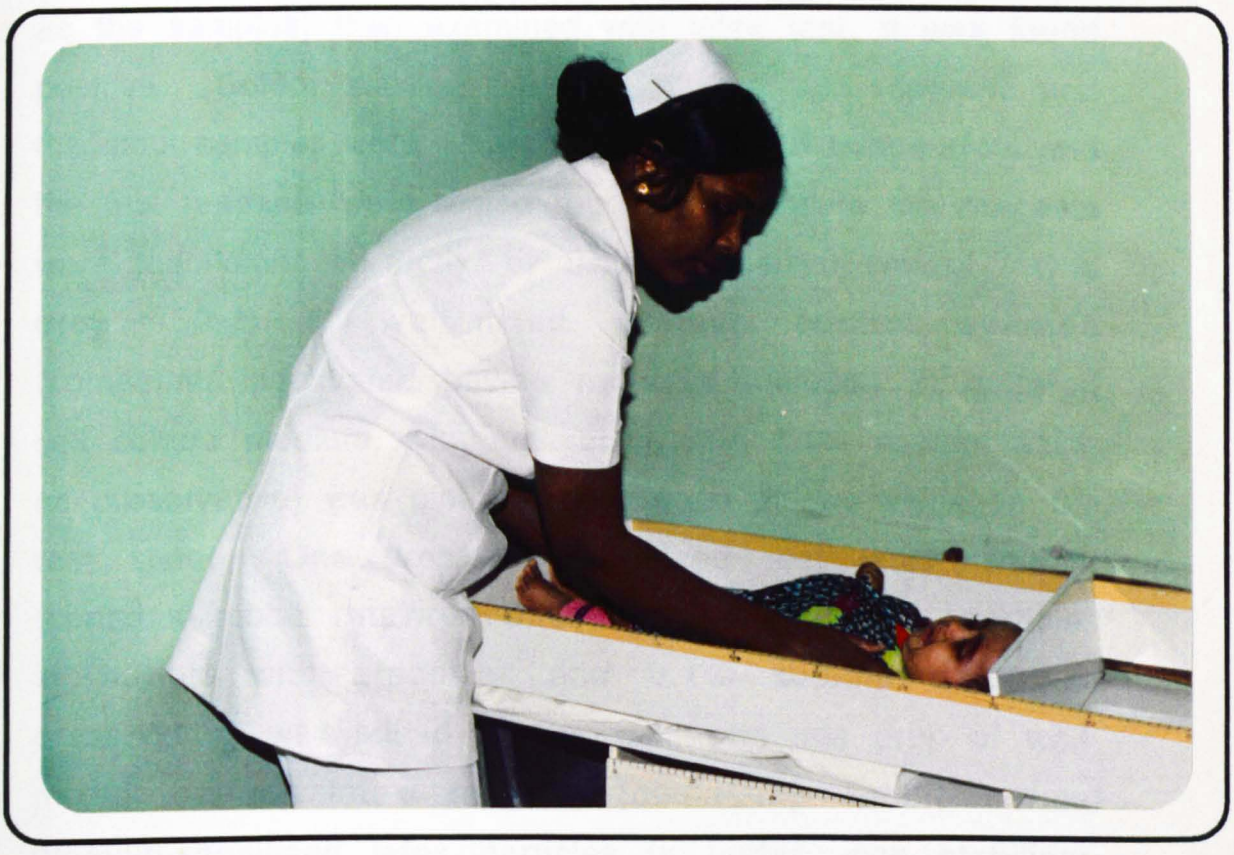


Figure 4.1 Measuring the height of small baby with specifically scaled box

For the cases with diarrhoea, part of the frozen faecal samples which were suspended in phosphate buffer were examined with latex test to detect rotavirus antigens in the stool. Stool with positive rotavirus detected with electron microscopy (supplied from microbiology department - Royal Liverpool Hospital) was mixed with PBS pH7.2, 10gm% and stored at -20°C for one week (to be under the same condition as the samples) then examined with latex test, it was found positive. Before starting the test, all the test reagents and the stool samples were allowed to reach room temperature and the test reagents were tested regularly to ensure the reagents were functioning correctly, by using a positive control. One drop (50µl) of well-mixed positive control solution (containing inactivated bovine rotavirus antigens in buffered cell culture medium, with antibiotics, and 0.1% sodium azide as preservative) was pipetted onto each of two wells on the test slide. One drop of well-mixed test latex reagent (contains rabbit rotavirus antibody-sensitised latex particles in buffer, with stabiliser and 0.1% sodium azide as preservative) was added to one well, and one drop of well-mixed control latex reagent (containing rabbit normal globulin-sensitised latex particles in buffer, with stabiliser, and 0.1% sodium azide as preservative) was added to the other. The contents of each well was mixed by using separate mixing sticks for each sample, covering the entire area of the well. The test slide was gently rocked and observed for agglutination for up to two minutes. A positive result was indicated by agglutination of the test latex reagent with no agglutination of the control latex reagent; the result was negative if no agglutination of either the test latex reagent or

the control latex reagent is observed within the two minutes test period, and agglutination of the control latex reagent was evidence of a non-specific reaction and means that the specimen was unsuitable for testing by this method. The stool samples were tested by repeating previous steps, and replacing the positive control by the stool samples.

The data was arranged on spreadsheets using Integrated 7 software (Version 1.5, 1987), and Lotus 1-2-3 software (release 2.01, 1987). Then the data was statistically analysed using Minitab software (release 7.2, 1989), and Epi Info software (Version 5, 1990).

Anthro software for calculating paediatric anthropometry (Version 5, 1990), using standard recommended by Center for Disease Control (CDC) and World Health Organisation (WHO), was used to measure the Z-scoare.

The Z-score for the reference population has a normal distribution with a mean of zero and standard deviation (SD) of one. For example, if a study population has a mean weight for height Z-score (WHZ) of zero, this would mean that it has the same median WH as the reference population. The Z-score cut off point recommended by WHO and CDC to classify low anthropometry levels is less than -2SD units from the reference median for the three indices.

Also the height and weight of the children were compared with standard height and weight (WHO, 1983).

4.3. Results

4.3.1. The group of children in the study

88 children were in the 0-6 years age group at the end of the first survey round, 68 children (33 male and 36 females)

were at suitable age for study (they were too young to enter school during study period), 9 children (3 males and 6 females) of this group refused inclusion in the study. 17 children (11 male and 6 females) were born during the study period, 9 (7 males and 2 females) included and 8 (4 males and 4 females) were refused inclusion into the study. The total number of children included in the study was 68 (37 males and 31 females).

The youngest child in the study was aged 0.4month and the oldest child was aged 56.3 months, 24 (35.3%) children (13 male and 11 female) were aged between 0-11 months, 11 (16.2%) children (7 male and 4 female) were aged between 12-23 months and 16 (23.5%) children (8 male and 8 female) were aged between 24-35 months. 10 (14.7%) children (5 males and 5 females) were aged between 36-47 months, and 7 (10.3%) children (4 males and 3 females) were aged between 48-59 months.

4.3.2. *Giardia* infection amongst the group of children

4.3.2.1. Prevalence of *Giardia* amongst the group of children

1816 stool samples were expected to be collected from the 68 children, 1719 (94.7%) of them were collected, and 97 (5.3%) of the samples were missed. The average number of stool samples obtained from each child was 27, with an average two week interval between each sample.

Maximum number 391 (22.8%) of the stool samples were collected from children aged between 12-23 months, and less number 148 (8.6%) of the stool samples were collected

from the children aged between 60-71 months (Table 4.1).

Faecal samples were positive for Giardia with (1) routine microscopic examination - 152 (8.84%). (2) formal-ether concentration - 253 (14.72%) and (3) faecal antigen capture ELISA - 331 (19.26%). The results of microscopy were compared with ELISA data to evaluate ELISA sensitivity and it was found 96.4% (Table 8.1).

Faecal samples examination showed that 29 (42.7%) of the children were infected with Giardia. The youngest child infected with Giardia was female aged 8 months.

The prevalence of Giardia was found to be higher amongst females (20.8%) than males (17.8%). The prevalence of Giardia was highest (46.6%) at age between 60-71 months, followed by (25.6%) at age between 24-35 months (Table 4.1).

The ELISA test detected Giardia infection earlier than routine microscopy or faecal concentration test (El Kadi et al. 1992). 22 children were positive by capture ELISA on average 2-4 stool samples earlier than microscopical and concentration test. One child was positive by capture ELISA in one stool sample after microscopical confirmation test, and another child was positive only by capture ELISA. Five children were positive by capture ELISA as well as concentration test at the same time (Figure 4.2 from El Kadi et al. 1992).

Table 4.1. Prevalence of Giardia with faecal ELISA amongst age groups of children related to sex

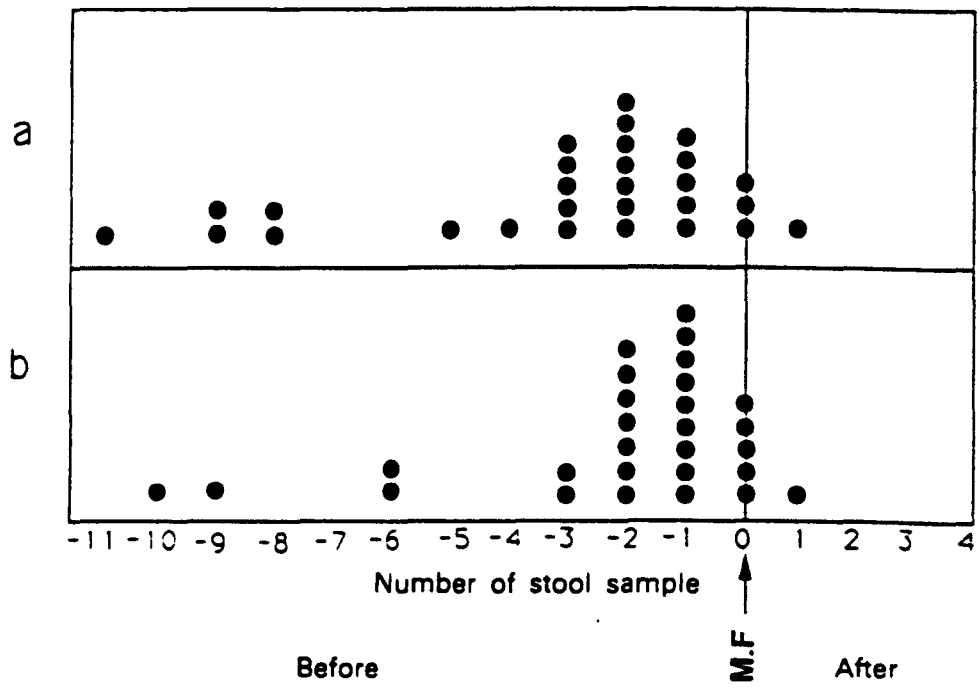
Age Groups in Months	Male children		Female children			
	Total No. Stool samples	+ve stool with Giardia (Prevalence)	Total No. Stool samples	+ve stool with Giardia (Prevalence)	Total Stool samples	Total +ve Stool with Giardia (Prevalence)
0-11	148	2 (1.4%)	91	6 (6.6%)	239	8 (3.4%)
12-23	188	41 (21.8%)	203	32 (15.8%)	391	73 (18.7%)
24-35	161	38 (23.6%)	151	42 (27.8%)	312	80 (25.6%)
36-47	161	37 (23%)	188	3 (1.6%)	349	40 (11.5%)
48-59	150	20 (13.3%)	130	41 (31.5%)	280	61 (21.8%)
60-71	63	17 (27%)	85	52 (61.1%)	148	69 (46.6%)
Total	871	155 (17.8%)	848	176 (20.8%)	1719	331 (19.3%)

4.3.2.2. Seasonal occurrence of Giardia infection

The study period was started in January 1989 (for 18 months) and ended June 1990, there were 59 children at the beginning of the study and at the end there were 68 children. When the study started one child was found to be infected with Giardia, and during the study period 29 children were infected with Giardia. Infection with Giardia occurred over the year usually mainly between August and April (with the highest incidence rate) the prevalence of Giardia infection was increased every month due to accumulation of positive cases. The lowest number of stool samples were collected in June 1989 and the highest number of stool samples were collected in June 1990 (Table 4.2).

4.3.2.3. Giardia infection related to the diarrhoea

Only 75 (4.4%) of the total collected stool samples were found diarrhoeic. Not all of them were related to Giardia infection, only 15 (20% of the total diarrhoeic stool samples) were found associated with Giardia infection. 10 stool samples (13.3% of the total diarrhetic stool samples) which were associated with Giardia infection were collected from patients with mild diarrhoea (3-4 times of loose motions per day), while 5 stool samples (6.7% of diarrhetic stool samples) were collected from patients with severe diarrhoea (5-8 times of loose motion per day) (Table 4.3).



Detection of *Giardia* antigen by ELISA in relation to microscopical detection

Fig.4.2 The time of detection of *Giardia* antigen by enzyme-linked immunosorbent assay, in stool samples taken at two-weekly intervals, in relation to the time of detection of the parasite by microscopical examination of stool samples (M.F). a, *Giardia* detected by routine microsopical examination; b, *Giardia* detected by formol-ether concentration. One child was positive by enzyme-linked immunosorbent assay only.

Table 4.2. Number of new infected children with Giardia and prevalence of Giardia with faecal ELISA in each month of the study period

	Number of children per month					Number of stool samples per month		
	New cases with Girdia +ve	Total with Giardia +ve	Total	Giardia Incidence (%)	Giardia Prevalence (%)	Stool Samples Giardia +ve	Total stool samples	Giardia Prevalence (%)
1989								
January	1	1	59	0	1.7	2	77	2.6
February	0	1	59	0	1.7	1	77	1.3
March	0	1	59	0	1.7	1	88	1.14
April	2	3	60	3.4	5	5	90	5.6
May	0	3	60	0	5	4	85	4.7
June	0	3	60	0	5	4	70	5.7
July	1	4	62	1.7	6.5	7	105	6.7
August	3	7	62	4.8	11.3	8	82	9.8
September	3	10	63	4.8	15.9	14	100	14.0
October	0	10	65	0	15.4	16	99	16.2
November	3	13	65	4.6	20	21	105	20.0
December	4	16	65	4.6	24.6	21	98	21.4
1990								
January	4	19	65	4.6	29.2	27	88	30.7
February	2	21	66	3.1	31.8	38	124	30.7
March	4	24	68	4.6	36.8	39	106	36.8
April	4	27	68	4.4	39.7	40	110	36.4
May	4	29	68	2.9	42.7	40	94	42.6
June	2	29	68	0	42.7	53	126	42.1

Diarrhoea was found more common in younger age, as the percentage of diarrhoeic stool samples was found to decrease from younger age (8.8% among children aged 0-11 months) to older age (2% among children aged 60-71 months), also 80% of the diarrhoea stool samples were collected from the children aged below 3 years, while 20% of them were collected from the children aged between 3-6 years. It was also found the Giardia infection caused more diarrhoea in younger ages (12.5%) than in older ages (2.9%), while the diarrhoea was caused more by Giardia infection in older ages (25%) than in younger ages (4.8%) Giardia was not the only cause of diarrhoea (among the children in the village), as 17.3% of the diarrhoeic stool samples were found to be associated with Shigella dysenteriae (Shigella shigae) and 18.7% of the diarrhoeic stool samples were associated with Rotavirus. (Table 4.4).

Table 4.3 **Number of stool samples related to number of times of loose motion per day and causative organisms.**

Causative Organism	No. loose motion per day 0	3 - 4	5 - 8	Total
Negative	1328	20	21	1369
Giardia	316	6	3	325
Shigella	0	7	2	9
Rotavirus	0	6	2	8
Giardia + Shigella	0	2	0	2
Giardia + Rotavirus	0	2	2	4
Shigella + Rotavirus	0	1	1	2
Total	1644	44	31	1719

Diarrhoea related to Giardia Chi-square = 0.03 P-value >0.05
Diarrhoea related to Shigella Chi-square = 287.13 P-value <0.05
Diarrhoea related to Rotavirus Chi-square = 309.4 P-value <0.05

Table 4.4 **Number of stool samples without or with loose motions, related to Giardia detected with ELISA test, and related to age group**

	Age groups in Months						
	0-11	12-23	24-35	36-47	48-59	60-71	Total
Without loose motion							
Giardia -ve	211	300	220	303	216	78	1328 (80.8%)
Giardia +ve	7(3.2%)	69 (18.7%)	75 (25.4%)	38(11.1%)	60 (21.7%)	67 (46.2%)	316 (19.2%)
Total	218 (13.3%) (91.2%)	369 (22.5%) (94.4%)	295 (17.9%) (94.6%)	341 (20.7%) (97.7%)	276 (16.8%) (98.6%)	145 (8.8%) (98%)	1644 (95.6%) (100%)
With loose motion							
Giardia -ve	20	18	12	6	3	1	60 (80%)
Giardia +ve	1(4.8%)	4 (18.2%)	5 (29.4%)	2(25%)	1(25%)	2 (66.7%)	15 (20%)
Total	21 (28%) (8.8%)	22 (29.3%) (5.6%)	17 (22.7%) (5.4%)	8 (10.7%) (2.3%)	4 (5.3%) (1.4%)	3 (4%) (2%)	75 (4.4%) (100%)
Total	239 (100%) (13.9%)	391 (100%) (22.7%)	312 (100%) (18.2%)	349 (100%) (20.3%)	280 (100%) (16.3%)	148 (100%) (8.6%)	1719 (100%)

4.3.2.4. *Giardia* infection related to other diseases

271 stool samples (15.8% of the total collected stool samples) were collected from children with other diseases, 14.8% of them were associated with *Giardia*.

Giardia was found not to be the cause of abdominal pain, as only 1.2% of *Giardia* cases were associated with abdominal pain (Table 4.5).

4.3.2.5. *Giardia* infection related to other intestinal parasites

55 stool samples (3.2% of the total stool samples) were found positive for other intestinal parasites. *Giardia* was not found to be associated with other intestinal parasites, as only 3.3% of the total stool samples with *Giardia* were found positive for other intestinal parasites mainly *Entamoeba coli* (Table 4.6).

4.3.2.6. *Giardia* infection related to bacteria and rotavirus infections

Only 75 diarrhetic stool samples (4.4% of the total stool samples) were examined with both culture test (to exclude any bacterial cause of diarrhoea) and latex test for rotavirus.

Table 4.5. Results of stool examination with ELISA to detect *Giardia* antigens, in relation to result of medical examination among the group of children

Medical Examination	Giardia negative	Giardia positive	Total
Normal	1157	291	1448 (84.2%)
Common Cold	88	16	104 (6%)
Toothache	6	3	9 (0.5%)
Upper respiratory tract infection	46	7	53 (3.1%)
Tonsilitis	32	2	34 (2%)
Otitis media	14	1	15 (0.9%)
Conjunctivitis	21	2	23 (1.4%)
Abdominal pain	9	4	13 (0.8%)
Stomatitis	6	3	9 (0.5%)
Dermatitis	5	2	7 (0.4%)
Otitis externa	4	0	4 (0.2%)
Total	1388 (80.7%)	331 (19.3%)	1719 (100%)

Table 4.6. Results of stool examination with ELISA to detect Giardia antigens, in relation to other intestinal parasite diagnosed by concentration test, among the group of children

Stool Examination for other intestinal parasites	Giardia negative	Giardia positive	Total
Negative	1344	320	1664
Entamoeba coli	36 (80.0%)	9 (20.0%)	45
Hymenolepis nana	4 (66.7%)	2 (33.3%)	6
Entamoeba histolytica	7	0	7
Enterobius vermicularis	2	0	2

Bacteria and rotavirus were found to play a role in causing diarrhoea among the children in the village, since 17.3% of the diarrhoeic stool samples (0.8% of the total stool samples) were found positive for Shigella dysenteriae, while 18.7% of the diarrhetic stool samples were positive for rotavirus. 15.4% of Shigella dysenteriae positive stool samples were associated with Giardia, while 28.6% of rotavirus positive stool samples were associated with Giardia (Table 4.7).

4.3.3. Type of feeding among the group of children

4.3.3.1. Type of feeding related to sex of child

Breastfeeding was more common with the male (72.3%) than female (27.7%) children. Also breastfeeding supplemented with bottle and normal food was more common amongst males (17.9%) than females (8.4%) (Table 4.8).

1816 visits were done for the group of children in this study, 916 (50.4%) of them were done for the males and 900 (49.6%) were done for the females.

83 (4.6%) of the total visits were related to children who were breast feeding only, 60 (72.3%) and 23 (27.7%) of them were related to males and females respectively.

10 (0.6%) of the total visits were related to children who were bottle feeding only, one (10%) and 9 (90%) of them were related to male and females respectively.

1031 (56.8%) of the total visits were related to children with normal food only, 506 (49.1%) and 525 (50.9%) of them were related to males and females respectively.

Table 4.7. Results of stool examination with ELISA to detect *Giardia* antigens in relation to the results of culture test and latex test for rotavirus, among the group of children

	ELISA test		
	Giardia negative	Giardia positive	Total
Culture test			
Not done	1328	316	1644 (95.6%)
No growth	49	13	62 (3.6%)
Shigella 11	2	13	(0.8%)
Total	1388 (80.7%)	331 (19.3%)	1719 (100%)
Latex Test for Rotavirus			
Not done	1328	316	1644 (95.6%)
Negative	50	11	61 (3.6%)
Positive	10	4	14 (0.8%)
Total	1388 (80.7%)	331 (19.3%)	1719 (100%)

Table 4.8. Number of visits among the group of children related to type of feeding and sex

Type of Feeding	Male	Female	Total
Breast only	60 (72.3%)	23 (27.7%)	83 (100%) (4.6%)
Bottle only	1 (10%)	9 (90%)	10 100%) (0.5%)
Food only	506 (49.1%)	525 (50.9%)	1031 (100%) (56.8%)
Breast and bottle	68 (73.1%)	25 (26.9%)	93 100%) (5.1%)
Breast and food	42 (64.6%)	23 (35.4%)	65 (100%) (3.6%)
Bottle and food	185 (40.9%)	267 (59.1%)	452 (100%) (24.9%)
Breast, bottle and food	54 (65.9%)	28 (34.1%)	82 (100%) (4.5%)
Total	916 (50.4%)	900 (49.6%)	1816 (100%)

93 (5.12%) of the total visits were related to children with breast and bottle feeding, 68 (73.1%) and 25 (26.9%) of them were related to the males and the females.

65 (3.6%) of the total visits were related to children with food and breastfeeding, 42 (64.6%) and 23 (35.4%) of them were related to the males and females respectively.

452 (24.9%) of the total visits were related to children with food and bottle feeding, 185 (40.9%) and 267 (59.1%) of them related to male and the female children respectively. 82 (4.5%) of the total visits were related to children with food, bottle and breast feeding, 54 (65.9%) and 28 (34.1%) of them were related to the male and the female children respectively.

4.3.3.2.Type of feeding related to age groups

Early weaning was observed amongst the children in the village, since the majority (81.7%) of the children in the first years of age were being breastfed, while in the second year only a few of them (28.3%) remained on breastfeeding.

In the first year of life, 32.3% of the children were on breastfeeding only, either they were below 4 months or their food was not supplemented.

The bad habit of busy mothers in the city (feeding the children with bottle instead of breast) has started to appear amongst the mothers in the village, since 38.7% of the children in the first and 18.7% of the children in the second year of life were fed on the bottle as well as on the breast. While 18.3% of the children in the first year and 68.8% of the children in the second year of life were fed on the bottle with no breastfeeding (Table 4.9).

Table 4.9 **Number of visits among the group of children related to type of feeding and age groups in months**

Type of Feeding	Age groups in Months 0-11	12-23	24-35	36-47	48-59	60-71	Total
Breast only	81 (97.6%) (32.3%)	2 (2.4%) (0.5%)	0	0	0	0	83 (100%) (4.6%)
Bottle only	9 (90%) (3.6%)	1 (10%) (0.2%)	0	0	0	0	10 (100%) (0.5%)
Food only	0	9 (0.9%) (2.2%)	205 (19.9%) (61.9%)	371 (36%) (100%)	295 (28.6%) (100%)	151 (14.6%) (100%)	1031 (100%) (56.8%)
Breast & Bottle	75 (80.7%) (29.9%)	18 (19.3%) (4.3%)	0	0	0	0	93 (100%) (5.1%)
Breast & Food	27 (41.5%) (10.7%)	38 (58.5%) (9.1%)	0	0	0	0	65 (100%) (3.6%)
Bottle & Food	37 (8.2%) (14.7%)	289 (63.9%) (68.6%)	126 (27.9%) (38.1%)	0	0	0	452 (100%) (24.9%)
Breast, Bottle & Food	22 (26.8%) (8.8%)	60 (73.2%) (14.4%)	0	0	0	0	82 (100%) (4.5%)
Total	251 (13.8%)	417 (23%)	331 (18.2%)	371 (20.4%)	295 (16.3%)	151 (8.3%)	1816 (100%)

251 (13.8%) of the total visits involved the group of children at age group 0-11 months , 81 (32.3%), 9 (3.6%), 75 (29.9%), 27 (10.8%), 37 (14.7%) and 22 (8.8%) of them were children fed on breast only, bottle only, breast and bottle, breast and food, bottle and food and breast, bottle and food respectively

417 (23%) of the total visits involved the group of children at age group 12-23 months, 2 (0.5%), one (0.2%), 9 (3.2%), 18 (4.3%), 38 (9.1%), 289 (69.3%) and 60 (14.4%) of them were done by children fed on breast only, bottle only, food only, breast and bottle, breast and food, bottle and food, and breast, bottle and food respectively.

331 (18.2%) of the total visits involved the group of children at age group 24-35 months, 205 (61.9%) and 126 (38.1%) of them were done by children fed on food only and bottle and food respectively.

371 (20.4%), 295 (16.2%) and 151 (14.7%) of the total visits involved the group of children of children in age group (36-48), (48-60) and (60-71) months respectively, all of them were done by children fed on food only.

4.3.3.3.Type of feeding related to Giardia infection

Breast feeding may play a role in the protection of the children in the village from Giardia infection, since no child with breast feeding only was infected, while 7.5% of Giardia positive stool samples were collected from children with breast feeding supplemented with other food. Giardia infection was more common among children eating ordinary food (Table 4.10)..

Table 4.10 Number of the stool samples examined with ELISA to detect Giardia antigen, related to type of feeding among the group of the children

Type of feeding	Giardia negative	Giardia positive	Total
Breast only	79 (100%) (5.7%)	0	79 (100%) (4.6%)
Bottle only	10 (100%) (0.7%)	0	10 (100%) (0.6%)
Food only	766 (78.4%) (55.2%)	211 (21.6%) (63.8%)	977 (100%) (56.8%)
Breast and bottle	87 (100%) (6.3%)	0	87 (100%) (5.1%)
Breast and food	53 (88.3%) (3.8%)	7 (11.7%) (2.1%)	60 (100%) (3.5%)
Bottle and food	331 (77.7%) (23.8%)	95 (22.3%) (28.7%)	425 (100%) (24.7%)
Breast, bottle and food	62 (77.5%) (4.5%)	18 (22.5%) (5.4%)	80 (100%) (4.7%)
Total	1388 (80.7%)	331 (19.3%)	1719 (100%)

1388 (80.8%) of the stool samples were negative to Giardia, detected with ELISA test, 79 (5.7%), 10 (0.7%), 766 (55.2%), 87 (6.3%), 53 (3.8%), 331 (23.8%) and 62 (4.5%) of them were related to children with breast feeding only, bottle feeding only, food only, breast and bottle feeding and breast, bottle feeding and food respectively.

331 (19.3%) of the stool sample were positive to Giardia, detected with ELISA test, 211 (63.8%), 7(2.1%), 95 (28.7%) and 18 (5.4%) of them were related to children with breast feeding only, bottle feeding only, food only, breast and bottle feeding, breast feeding and food , bottle feeding and food and breast, bottle feeding and food respectively.

4.3.3.4. Type of feeding related to diarrhoea

Diarrhoea was found to be more frequent amongst the children on breast feeding, either on breast feeding alone (11.4%) or on breastfeeding supplemented with other food (22.5%). Most of the diarrhoeic stool samples in this group were collected from children with mild diarrhoea (Table 4.11).

75 (4.5%) of the total collected stool samples were related with loose motions.

9 (12%) of the stool samples related to loose motions were collected from children with breast feeding only, one (11.1%), 2 (22.2%), 4 (44.4%) and 2 (22.2%) of them were collected from children within 3 times, 4 times, 5 times and 6 times loose motion per day respectively.

Table 4.11 Number of stool samples related to number of loose motions per day and type of feeding

No. of loose motions per day	Type of Feeding							
	Breast only	Bottle only	Food only	Breast and Bottle	Breast and Food	Bottle and Food	Breast, Bottle and Food	Total
3 times	1 (11.1%)	0	3 (10.7%)	0	2 (40%)	3 (14.3)	1 (20%)	10 (13.3%)
4 times	2 (22.2%)	0	15 (53.6%)	4 (57.1%)	2 (40%)	9 (42.9%)	2 (40%)	34 (45.4%)
5 times	4 (44.4%)	0	7 (25%)	3 (42.9%)	1 (20%)	8 (38.1%)	2 (40%)	25 (33.3%)
6 times	2 (22.2%)	0	2 (7.1%)	0	0	1 (4.8%)	0	5 (6.7%)
8 times	0	0	1 (3.6%)	0	0	0	0	1 (1.3%)
Total	9 (12%) (11.4%)	0	28 (37.3%) (2.9%)	7 (9.3%) (8%)	5 (6.7%) (8.3%)	21 (28%) (4.9%)	5 (67%) (6.2%)	75 (4.4%)
Non-diarrhetic stool samples	70 (88.6%)	10 (100%)	949 (97.1%)	80 (92%)	55 (91.7%)	405 (45.1%)	75 (93.8%)	1644 (95.6%)
Total	79 (4.6%)	10 (0.6%)	977 (56.8%)	87 (5.1%)	60 (3.5%)	426 (24.7%)	80 (7.7%)	1719 (100%)

28 (37.3%) of the stool samples related to loose motions were collected from children on solid food only, 3 (10.7%), 15 (53.6%), 7 (25%), 2 (7.1%) and 1 (3.6%) of them were collected from children with 3 times, 4 times, 5 times, 6 times and 8 times loose motions per day respectively.

7 (9.3%) of the stool samples related to loose motions were collected from children with breast and bottle feeding, 4 (57.1%) and 3 (42.9%) of them were collected from children with 4 and 5 times of loose motion per day respectively.

5 (6.7%) of the stool samples related to loose motions were collected from children with breast feeding and solid food, 2 (40%), 2 (40%), and 1 (20%) of them were collected from chilren with 3 times, 4 times and 5 times of loose motions per day respectively.

21 (28%) of the stool samples related to loose motions were collected from children with bottle feeding and solid food, 3 (14.3%), 9 (42.9%), 8 (38.1%) and one (4.8%) of them were collected from children with 3 times, 4 times, 5 times and 6 times loose motions per day respectively. 5 (6.7%) of the stool samples related to motions were collected from children with breast, bottle and food feeding, one (20%), 2 (40%) and 2 (40%) of them were collected from children with 3 times, 4 times and 5 times of loose motions per day respectively.

4.3.3.5. Type of feeding related to other intestinal parasites.

55 stool samples (3.2% of the total collected stool samples) were found positive for other intestinal parasites.

The other intestinal parasites were rarely found amongst the children with breast feeding, while most of them were found amongst the children with ordinary food (Table 4.12).

Only one of the stool samples collected from the group of children with breast feeding was found positive for Entamoeba coli. Only one of the stool samples collected from the group of children with bottle feeding was found positive for Entamoeba coli.

34 (75.6%) of the Entamoeba coli, 6 (100%) of the Hymenolepis nana, 7 (100%) of the Entamoeba histolytica and 2 (100%) of the Enterobius vermicularis were found in the stool samples collected from children on solid food.

Only one of the stool samples collected from the group of children with breast and bottle feeding and bottle was found positive with Entamoeba coli.

8 of the stool samples collected from the group of children with bottle and solid food were found positive for Entamoeba coli.

4.3.3.6. Type of feeding related to intestinal bacterial and rotavirus infection

75 diarrhetic stool samples (4.4% of the total collected stool samples) were examined with culture test and latex test.

Only 13 stool samples (0.7% of the total collected stool samples) were found positive for Shigella dysenteriae and 14 stool samples were found positive for Rotavirus.

Table 4.12 Number of stool samples from the group of children, related to type of feeding and other intestinal parasite diagnosed with concentration test.

Stool Examination for other intestinal parasites	Type of Feeding							
	Breast only	Bottle only	Food only	Breast and Bottle	Breast and Food	Bottle and Food	Breast, Bottle and Food	Total
Entamoeba coli	1 (2.2%)	1 (2.2%)	34 (75.6%)	1 (2.2%)	0	8 (17.8%)	0	45 (100%)
Hymenolepis nana	0	0	6 (100%)	0	0	0	0	6 (100%)
Entamoeba histolytica	0	0	7 (100%)	0	0	0	0	7 (100%)
Enterobius Vermicularis	0	0	2 (100%)	0	0	0	0	2 (100%)
Negative	78 (4.7%)	9 (0.5%)	933 (56.1%)	86 (5.2%)	60 (3.6%)	418 (25.1%)	80 (4.8%)	1664 (100%)

Breast feeding did not play a role in protection against bacterial infection, since the prevalence of Shigella amongst the feeding groups were nearly equal. While rotavirus infection prevalence (even though it was low), was found more amongst the children with breast feeding (Table 4.13).

One (1.3%) and 4 (5.1%) of the total stool samples collected from the group of children with breast feeding only, were found positive for Shigella dysenteriae and rotavirus respectively.

8 (0.8%) and 4 (0.4%) of the total stool samples collected from the group of children with food only were found positive for Shigella dysenteriae and Rotavirus respectively.

2 (2.3%) and 2 (2.3%) of the total stool samples collected from the group of children with breast and bottle feeding were found positive for Shigella dysenteriae and Rotavirus respectively.

One (0.2%) and 3 (0.7%) of the total stool samples collected from the group of children with bottle feeding and solid food were found positive for Shigella dysenteriae and Rotavirus respectively.

One (1.3%) and one (1.3%) of the total stool samples collected from the group of children with breast, bottle feeding and solid food were found positive for Shigella dysenteriae and Rotavirus respectively.

Table 4.13. Number of stool samples collected from the group of children related to type of feeding, intestinal bacterial infection and intestinal rotavirus infection

	Type of Feeding							
	Breast only	Bottle only	Food only	Breast and Bottle	Breast and Food	Bottle and Food	Breast, Bottle and Food	Total
Culture Test								
Not done	70	10	949	80	55	405	75	1644 (95.6%)
No growth	8	0	20	5	5	20	4	62 (3.6%)
Shigella	1 (1.3%)	0	8 (0.8%)	2 (2.3%)	0	1 (0.2%)	1 (1.3%)	13 (0.8%)
Total	79 (4.6%)	10 (0.6%)	977 (56.8%)	87 (5.1%)	60 (3.5%)	426 (24.7%)	80 (4.7%)	1719 (100%)
Latex Test for Rotavirus								
Not done	70	10	949	80	55	405	75	1644 (95.6%)
Negative	5	0	24	5	5	18	4	61 (5.6%)
Positive	4 (5.1%)	0	4 (0.4%)	2 (2.3%)	0	3 (0.7%)	1 (1.3%)	14 (0.8%)
Total	79 (4.6%)	10 (0.6%)	977 (56.8%)	87 (5.1%)	60 (3.5%)	426 (24.7%)	80 (4.7%)	1719 (100%)

4.3.4. Change in the weight among the group of children

4.3.4.1. Change in the weight related to Giardia infection

When the centile distribution of the median of the weight for age of the group of children was calculated there was a slight increase in the median of the weight in the group of children with Giardia negative than with Giardia positive. When the process of calculation was repeated for the females of the group of children only, it was found that there was a slight increase in the median of the weight amongst the group with Giardia negative. But when the process of the calculation was reported for the males of the group of children only, it was found there was a slight increase in the median of the weight among the group with Giardia positive, (Table 4.14).

The mean and standard deviation were calculated of the weight for age in the group of the children at all ages with or without Giardia detected with ELISA test, it was found there was a slight decrease in the mean value of the weight of the children with Giardia infection.

Also when the mean and standard deviation were calculated of the weight for age for the female children only. It was found there was a slight decrease in the mean value of the weight among the children with Giardia infection. But it was found there was a slight increase in the mean value of the weight among the males children with Giardia infection, (Table 4.15).

Table 4.14 **Centile distribution of the median of weight for age, height for age and weight for height of group of children related to sex and ELISA results of stool examination**

Sex	Stool for Giardia	Weight for Age	Height for Age	Weight for Height
Both	Giardia Negative	19.98	15.32	39.09
	Giardia Positive	14.83	7.69	38.73
Males	Giardia Negative	20.02	15.25	42.30
	Giardia Positive	22.58	9.98	41.4
Females	Giardia Negative	19.96	15.38	37.68
	Giardia Positive	14.15	6.87	15.85

When the mean and standard deviation were calculated of the weight for age among the group of children with Giardia infection, three months before and after the infection with Giardia detected with ELISA test, it was found there was a slight decrease in the mean value of the weight of these group of children after the infection (Table 4.16).

29 (42.7%) of the children were found infected with Giardia (with faecal ELISA) during the study period, one of them was found infected with Giardia from the first visit. The percentage gain weight of the infected children three months before and after Giardia infection was compared with an equal number of controls of similar ages who were not infected with Giardia (Figure 4.3). Weight velocity for each child was calculated as follows:

$$\frac{b - a}{a} \times 100 = \%$$

a = the weight in the beginning of the 3 month period

b = the weight at the end of the 3 month period

The means of weight velocity in all age groups among the children with Giardia (detected with faecal ELISA) three months after infection, were lower than that of three months before infection. And also were lower than that of non-infected children in the same age group and in the same period, except in the age group 6-71 months. This can be explained by the infection being less severe in the older age groups, which may be due to repeat infections and developed immunity.

Table 4.15 **The Z-score for the mean and standard deviation of weight for age, height for age and weight for height, of the group of children related to sex and ELISA results of stool examination**

Sex	Stool for Giardia		Weight for Age	Height for Age	Weight for Height
Both	G.Negative	Mean	-0.842	-1.039	-0.176
		STD deviation	0.885	1.053	0.992
	G.Positive	Mean	-0.944	-1.094	-0.323
		STD deviation	0.861	1.060	0.910
Males	G.Negative	Mean	-0.801	-1.120	-0.065
		STD deviation	1.022	1.156	1.063
	G.Positive	Mean	-0.693	-1.117	0.009
		STD deviation	0.813	1.139	0.587
Females	G.Negative	Mean	-.0886	-0.953	-0.294
		STD deviation	0.709	0.922	0.895
	G.Positive	Mean	-1.159	-1.073	-0.609
		STD deviation	0.846	0.990	1.035

Table 4.16 The Z-score for the mean and standard deviation of weight for age, height for age and weight for height of the children with positive Giardia infection with faecal ELISA, three months before and after infection

Giardia infection		Weight for Age	Height for Age	Weight for Height
Before	Mean	0.021	0.029	-0.020
	STD deviation	0.501	0.929	0.420
After	Mean	-0.021	-0.248	-0.132
	STD deviation	0.551	0.681	0.519

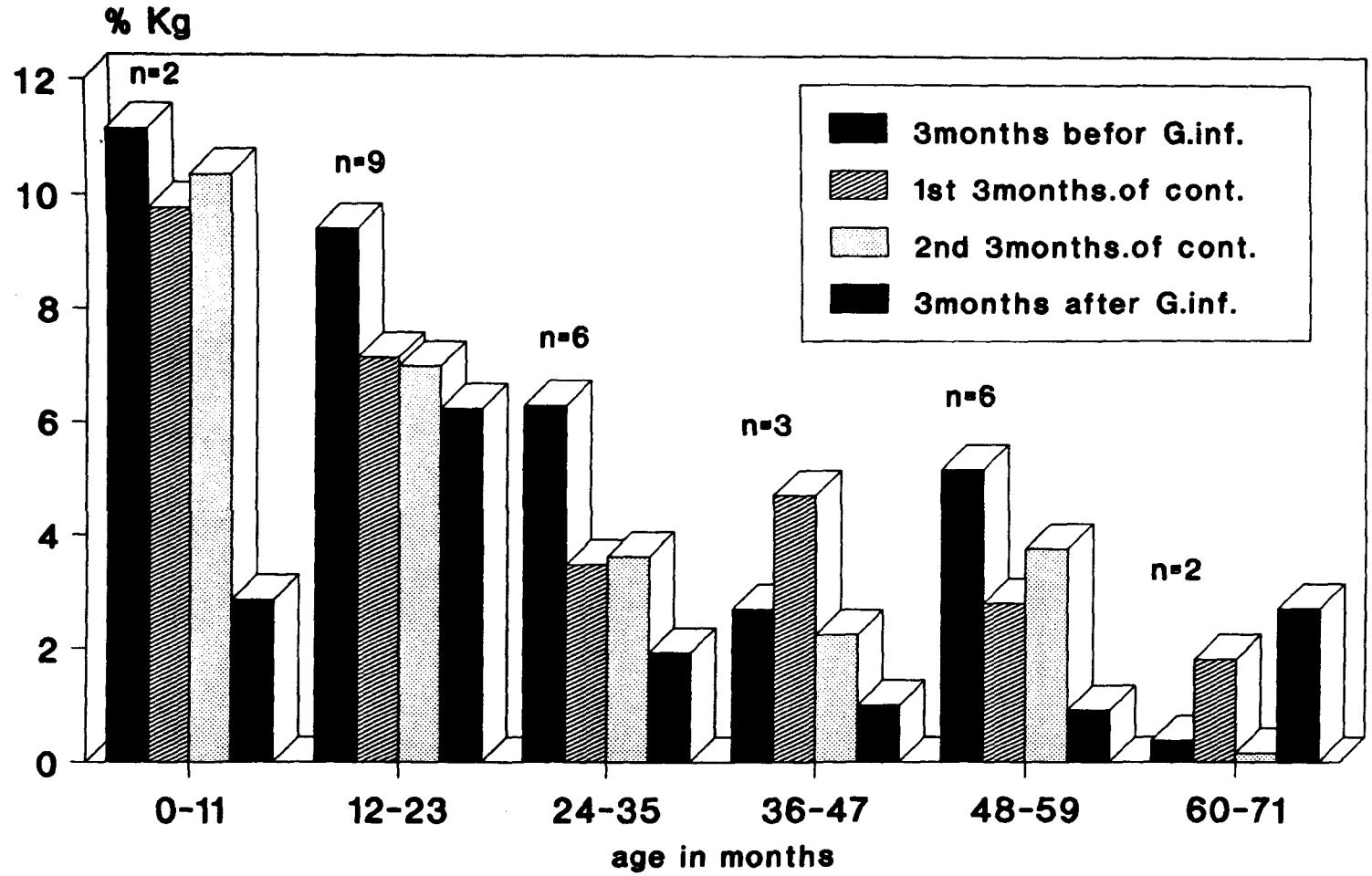


Fig.4.3 Comparison between the means of percentage gain in children's weight three months before and after Giardia infection (detected with ELISA),and noninfected controls.
(n=number of infected children in each age group.)
*one child was infected with Giardia from the first visit.

When the means of weight were calculated for the children without and with Giardia infection (with faecal ELISA), and compared with the means of the standard weight (WHO, 1983), (Figure 4.4).

It was found that the effect of the Giardia appears mainly at the third year of the childs life. This could be explained by rapid growth of the child in the first two years of life.

4.3.5. Increase in the height among the group of children

4.3.5.1. Increase in the height related to Giardia infection

When the centile distribution of the median of the height for age were calculated in the group of the children it was found to be decreased among the children with Giardia infection. Also it was found decreased with infection when it was calculated for both males and females separately (Table 4.14). The mean and standard deviation were calculated of the height for age among the group of children at all ages, with or without Giardia infection. It was found there was a slight decrease in the mean value of the height among the children with Giardia infection. Also the mean calculated for the female children only. But it was found there was a slight increase in the mean value of the height among the male children with Giardia infection, (Table 4.15).

The mean and standard deviation were calculated of the height for age, among the group of children with Giardia infection, three months before and after the infection, was

detected with ELISA test. Table 4.16 found a slight decrase in the mean value of the height group of children after the infection.

29 (42.7%) of the children were found to be infected with Giardia (with faecal ELISA) during the study period, one of them was found to be infected with Giardia from the first visit. The percentage gain in height of the infected children three months before and after Giardia infection was compared with an equal number of controls of similar ages who were not infected with Giardia, (Figure 4.5). Height velocity for child was calculated as follows:

$$\frac{b - a}{a} \times 100 = \%$$

a = the height in the beginning of the three month period

b = the height in the end of the three month period.

The means of height velocity in all age groups among the children with Giardia (detected with faecal ELISA) three months after infection, were lower than than of three months before infection. And also were lower than that of non-infected children in the same age, and in the same period. Except for the children in age group 60-71 months. This could be explained by the infection being less severe in oldere age groups which may be due to repeat infection and development of immunity.

When the means of the height were calculated for the children without and with Giardia infection (with faecal ELISA), and compared with the means of the standard height (WHO, 1983), (Figure 4.6). There were no significant changes in the means of the height of the children without or with Giardia infections.

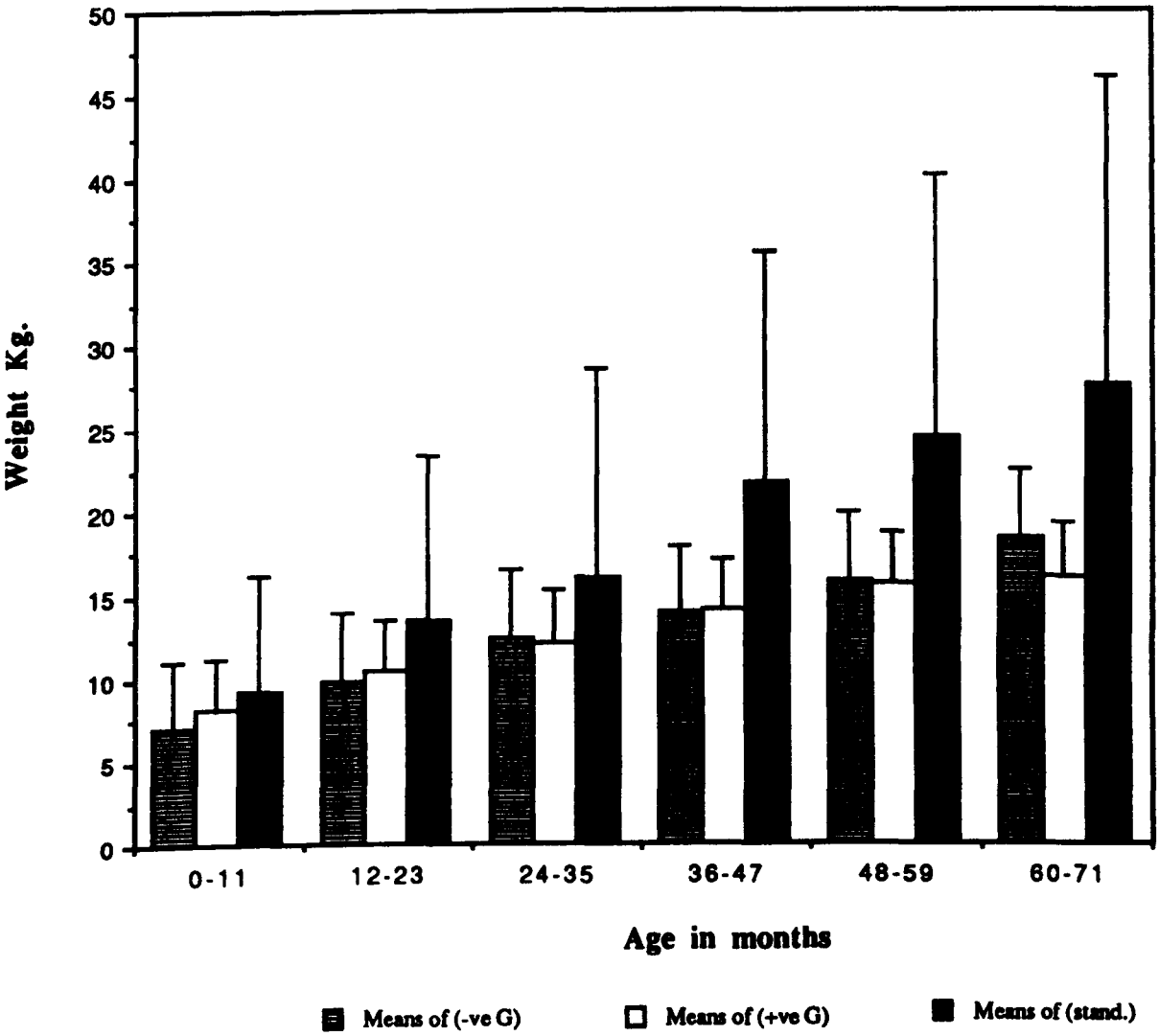


Fig.4.4 Comparison between the means of the weight for the children without or with Giardia (detected with ELISA),and standard weight (WHO 1983),related to age groups.

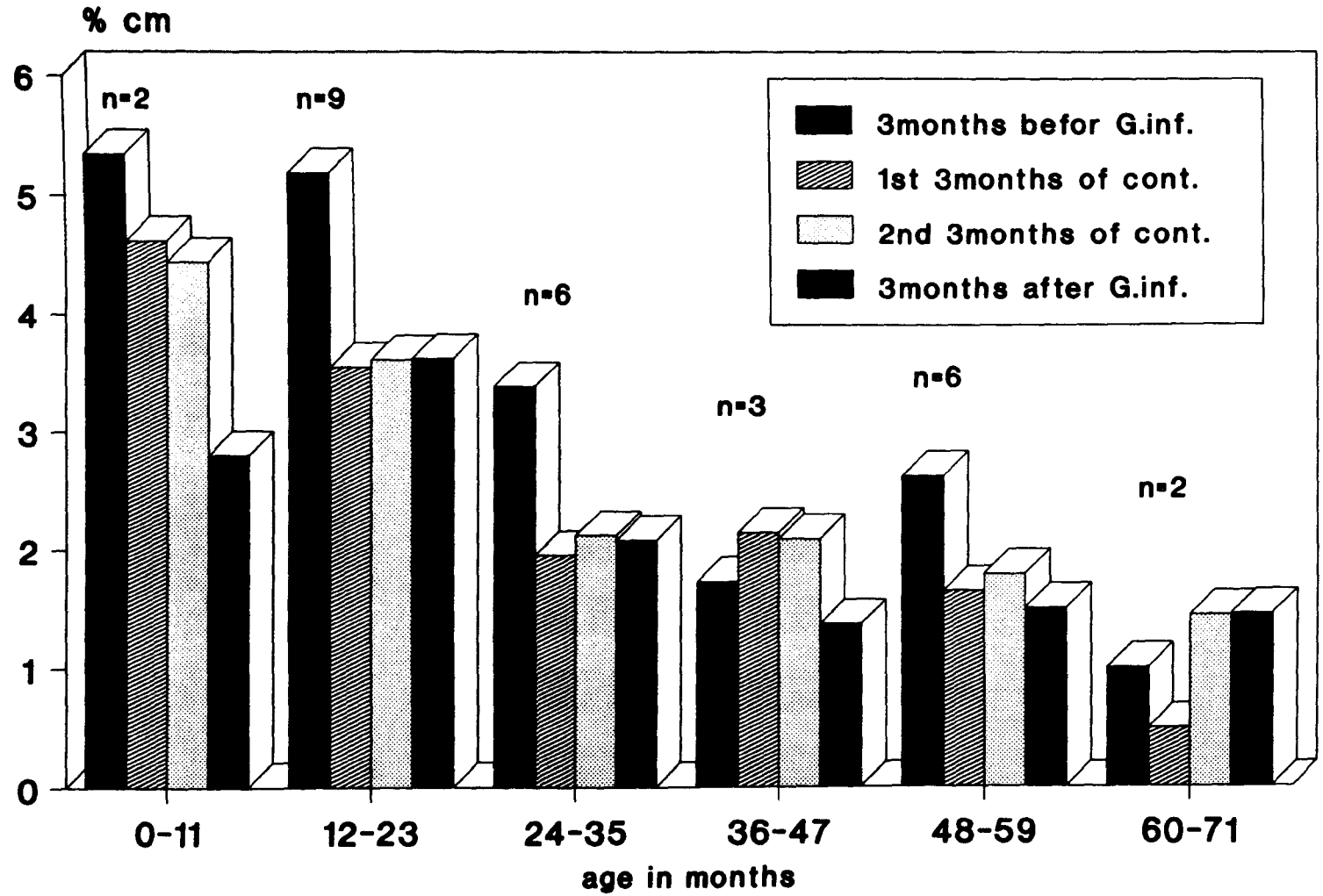


Fig.4.5 Comparison between the means of percentage gain in children's height three months before and after Giardia infection (detected with ELISA),and noninfected controls.
(n=number of infected children in each age group.)
*one child was infected with Giardia from the first visit.

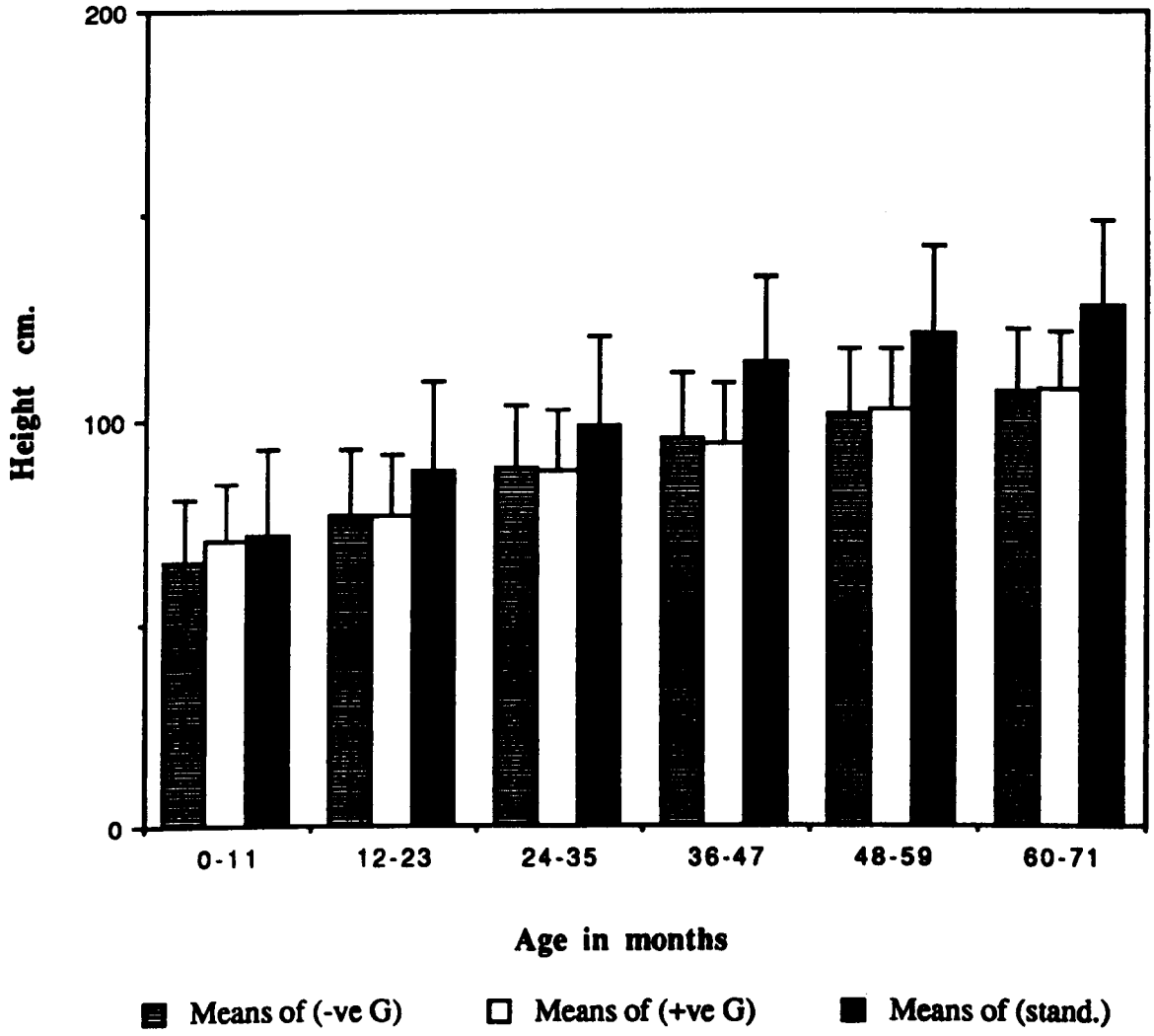


Fig.4.6 Comparison between the means of the height for the children without or with Giardia (detected with ELISA),and standard height (WHO 1983),related to age groups.

4.4. Discussion

4.4.1. Giardia infection associated with diarrhoea and related to infections

The prospective, longitudinal field study of the traditional village Santa Maria Cauque in the highlands of Guatemala (Mata, 1978) has for the first time provided data permitting complete description of the natural history of Giardia infection during early life (Farthing et al, 1986b). Because the diagnosis of Giardia infection rested entirely on the detection of Giardia forms in the stool, the apparent prevalence and incidence rates reported almost certainly underestimate infection with Giardia (Kamath and Murugasu 1974, Thornton et al, 1983).

68 children (37 males and 31 females) at pre school age were included in this longitudinal field study, the ELISA test to detect the Giardia antigens in the stool, with good sensitivity and specificity (Green et al, 1985) was used to avoid any underestimate of infection with Giardia. 17 children were born in the village during the study period (January 1989 to June 1990), and the mothers of 9 newborn children agreed to their children being included in the study. It was found that the youngest child in the study was aged 12 days, and this may be due to the mothers in the village not being used to leaving their houses within one month after delivery. The oldest child in the study group was aged 56.3 months, this is because the age of entering school in the village was 6 years and the study period was 18 months.

1719 stool samples were collected from 68 children by the end of the study period. The percentage for Giardia increased from 8.8%, 14.7% to 19.3% with routine microscopy,

formol-ether concentration and ELISA test respectively. The ELISA test was shown to be more useful in early diagnosis of Giardia in the stool of the group of children (El Kadi et al. 1992).

By the end of the period of the study 29 (42.7%) of the children were infected with Giardia, 16 (55%) of those infected were females. Giardia infection was uncommon at age below 8 months among the group of children. This may be because the child required close supervision by his mother, so he has no chance to play in the dirt, or may be due to breast feeding at this age, although there was one child with Giardia infection since first visit in the study.

Prevalence rates from cross-sectional surveys by necessity overlook this feature of the organisms impact on a community (Oyerinde et al, 1977; Moore, 1966; Black, 1980; Harter, 1982; Sealy and Schuman,1983). In this study the prevalence rate increased from 3.4% in the first year to 25.6% in the third year of life, this may be due to the child being released from his mothers control after the first year of life and getting a chance to acquire infection from playing outside the house (Figure 4.7). The prevalence rate decreased after the third year, indicating that clearance of the organism was more rapid as the child grew older, presumably due to the development of acquired immunity to the parasite (Farthing, et al 1986b). The high rise in the prevalence rate in the first 6 years of life, can be explained by (a) increase in the number of times exposed to the parasite, (b) early and highly diagnostic property of the ELISA test (Goldin et al 1990; Green et al 1985; Ungar et al, 1984). There are few community based surveys in infant and young children with which to compare these findings, a previous study in Central America reported

prevalence rates for Giardia in children with diarrhoea as 11.5-36.8% (Moore et al, 1966), prevalence of Giardia was lower in under five year old children in Nigeria (2.1-13.3%), (Oyerinde et al 1977), but higher in Washington State, (USA), where rates reached 7.1% in 1-3 year old children (Harter et al 1982) and in Guatemala where rates reached 20.2% in 1-3 year old children (Farthing et al 1986b).

The significance of the modest predominance of Giardia infection in females is not clear, Oyerinde et al (1977) found male predominance in Nigeria (Male/female ratio 1.2) when children and adults were analysed together, but in children of 1-5 years the ratio was reversed at 0.9, and Farthing et al (1986b) found male predominance in Guatemala (male/female ratio 1.3).

Seasonality has been described for some childhood infections (Mata et al 1983; Kapikian et al 1976). In this study we found little evidence that this was important in Giardia infection. Infection with Giardia usually occurred throughout the year mainly between August and April, in the cooler and wetter months. These climatic conditions are known to favour cyst survival (Jarroll et al, 1980).

Figure **4.7** Showing the children playing in the street in Wadihaloo village

It is well established that Giardia infection can occur in humans without the production of clinically evident diarrhoeal disease, the present study confirms this observation. Only 4.5% of the total positive stool samples for Giardia were diarrhoetic. But the pattern of diarrhoea associated with Giardia infection appeared to change during the first 6 years of life. In the first year only one (12.5%) of 8 observed Giardia infection (which occurred in the last third of the first year of life) was associated with diarrhoea. Breast feeding presumably played an important part in controlling the severity of these infections (Andrews and Hewlett, 1981; Gillin et al 1983a). In the second year of life, 4 (5.5%) of 73 observed Giardia infections were associated with diarrhoea. In the third year of life, 5 (6.3%) of 50 observed Giardia infections were associated with diarrhoea. After the third year of life the number of observed Giardia infections associated with diarrhoea decreased. Two (5%) of 40, one (1.6%) of 61 and 2 (2.9%) of 69 observed Giardia infection were associated with diarrhoea in the fourth, fifth and sixth year of life respectively. This finding supports our contention that after the third year of life the host is beginning to acquire protection against the parasite. We should state however, that a proportion of the diarrhoea associated with Giardia infections was not due to single infections, and other pathogens particularly Shigella species (Mata, 1978) and rotavirus (Mata et al 1983) were found simultaneously. We cannot ultimately be certain which agent was responsible for the production of diarrhoea on those occasions. Nor were exclude the possibility that Giardia excretion increased and thus became easier to diagnose microscopically during a

diarrhoeal illness due to another unrelated pathogen.

Single infections with Giardia often result in profound diarrhoeal disease (Farthing, 1984) and it seems improbable that all of the diarrhoea associated with Giardia infection in this study could be attributed to other agents.

4.4.2. Type of feeding related to Giardia infection

In this study it was found that most of the children were breastfed, either on the breast alone in early months of life or breast with bottle and food in later months of the first year of life, few of them were starting their life with bottle feeding only.

In the second year of life most of the children stopped breast feeding and continued on bottle and solid food feeding, because of the weaning at the end of the second year of life, no more children remained on breast feeding in the third year, only a few of them were bottlefed, and the majority of them were fed on ordinary food.

Andrews et al, (1981) demonstrated specific IgA and/or IgG antibody to Giardia in milk from immune, but not non immune mothers, consumption of immune milk was necessary at the time of innoculation and must continue for several (3-5) days thereafter, furthermore, once infection is initiated, immune milk has no effect, and consumption of immune milk does not result in induction of sustained immunity against Giardia infection.

In this study we found no one of the children on breast feeding only was infected with Giardia, but a few were infected with Giardia when they begin to supplement their food by ingestion of other material, which lead to decrease in the

consumption of immune milk, or the milk itself may not be immune (Stevens and Frank, 1978).

Our findings are compatible with data from other studies, where attacks of acute diarrhoea were comparatively infrequent in children during the first 6 months of age but most common in children from 6 - 24 months, in whom the incidence of infant diarrhoea increased to a maximum coinciding with the termination of breast feeding (Gordon et al 1963; Gordon et al 1964, Mata et al 1967). This well-recognised phenomenon, referred to as "weanling diarrhoea" is probably caused by a number of factors.

Bbreast feeding or administration of immune milk is above to reduce the incidence of infection with rotavirus (Banatvala et al, 1978), or the vaccine strain of poliovirus (Gonzaga et al 1963, Warren et al, 1964, Katz and Plotkin 1968). This protection against polio-virus has been a source of problems during attempts to immunise infants with oral poliovirus vaccine in developing countries (Plotkin, et al 1966). Duckett et al (1972) have demonstrated that protection against the intestinal phase of Trichinella spiralis was associated with suckling milk from previously infected mothers and that the protection was lost at the time of weaning. In this study it was found that the breast milk may protect against other agents of enteric infections, and the protection mechanisms may be similar to that against Giardia infection.

4.4.3. Change in weight and height related to Giardia infection

A number of isolated case reports and hospital derived

series of patients with giardiasis certainly indicate that the parasite can impair growth (Veghely; 1938; Boe and Rinvik, 1943; Cortner, 1959; Burke, 1975; Kay et al 1977). These reports generally relate to children whose symptoms were of sufficient severity to warrant a visit to a physician.

The impact of this parasite on child growth at a community level has been investigated. Firstly by Gupta (1980) attempting to demonstrate this by eradicating the parasite pharmacologically following which modest post treatment improvement in growth was observed. The design of his studies has inherent problems, namely that the anti-Giardia therapy used, metronidazole, is by no means monospecific. And secondly his subjects did not have single infections. Secondly Farthing et al (1986) attempted to demonstrate this by analysing longitudinal data which was collected more than 20 years ago for a cohort of Guatemalan children. They were faced with two problems in their study, namely that the diagnosis of Giardia was only done miscroscopically, this gave under estimates of infection, and secondly the other concurrent infections were not treated, this gave over estimation of the effects of Giardia infection on physical growth.

The advantages of our study were that a cohort of children were followed prospectively for 18 months, Giardia was properly diagnosed using ELISA test to detect Giardia antigens in the stool, and chemotherapy was used to treat any other concurrent infections. Despite the complexities of the study, we were able to demonstrate changes in the rate of gain of weight and the height before and after Giardia infection. We found slight reductions in the rate of growth of weight and height after Giardia infection by calculating the mean of the

weight and height with and without Giardia infection during the whole period of the study and during three months before and after Giardia infections, by calculating the rate of growth in the weight and height three months before and after Giardia infection and by comparing the weight values for infected and non infected children with standard values.

It was found the impact on their growth of diarrhoea associated with Giardia infection in some children was directed largely despite the presence of some concurrent infections in these children. We have been able to demonstrate what appears to be a separate Giardia associated reduction in physical growth in Giardia infected children.

We cannot exclude the possibility that Giardia infection is merely acting as a marker of another concurrent infection (known or unknown) which is actually the illness responsible for the growth retardation. In addition we should stress that we have only described associations between the presence of Giardia and growth disturbances and cannot therefore imply causation. Despite the difficulties and possible pitfalls in these analyses of this study we consider that this pathogen probably contributed to the morbidity of these children and was at least one factor in their failure to grow normally.

CHAPTER 5

NEWLY ARRIVED EXPATRIATES AS A SOURCE FOR IMPORTED GIARDIA IN UAE

5.1. Introduction

The recent socio-economic development which has taken place in the UAE as well as other Arab Gulf countries has been reflected on the demographic structure of the community. Demographic changes were mainly characterised and influenced by an influx of expatriates who came from many countries and cover various social strata (MOH, 1989(a)).

Most of the expatriates were poor and non educated, and are working as foodhandlers (such as cooks), housemaids, farmers, grocers, shepherds and fishermen. These jobs do not require education or proper experience and therefore the wages are less.

Some of the expatriates brought with them some infectious diseases from their countries, (Al-Lahham et al. 1990). For this reason the UAE government decided to examine every expatriate for infectious diseases before allowing him a visa (for 2 years) to work in the country. Every time the expatriate applied to renew his/her visa for a further two years the same examinations were carried out.

In this study we examine if newly arrived expatriates play a role in importing Giardia to UAE, and shared in its distribution or not.

5.2. Materials and Methods

A group of 1159 of newly arrived expatriates (mostly within 7 days) to UAE in the labour clinic in Kalba Hospital were selected to be included in the study.

Data was collected from everyone regarding age, nationality, date of arrival in the UAE, occupation and if he/she has any disease or any other complaint. Routinely the expatriates were physically checked, i.e. sent for x-ray, serum was collected for AIDS and stool was collected for Giardia and other intestinal parasites.

The collected stool sample was coded with the code number of the expatriate. Part of each stool sample was examined by laboratory technicians with direct smear microscopy for Giardia and other intestinal parasites, and the stool samples were stored at 4°C overnight. On the next day, part of each negative stool sample was re-examined with direct microscopy. Part of each stool sample was mixed properly with 10% formol saline, and the mixture was filtered through wire mesh (size 250µm). Ether was added to the mixture, shaken vigorously for a few minutes, left to stand for 30 minutes. The fatty plug was loosened from the wall of the tube with a stick, the supernatant fluid was poured into chloros, and the deposit which was Lugol's iodine-stained was examined by microscopy. The negative concentrated stool samples were re-examined by Khorfakkan Hospital laboratory technicians.

For ELISA examination, part of each stool sample was suspended in phosphate buffer saline (PBS pH7.2), at a concentration of 10gm% mixed properly. Large particulate matter was removed by filtration through wire mesh, then left to stand for 30 minutes. The supernatant was collected and

stored at -20°C. The ELISA procedure was that outlined in chapter 2. The positive-negative cut-off value was calculated as the arithmetic mean of negative samples plus 3SD.

The data was arranged in spreadhsheets using Integrated 7 software (Version 1.5, 1987), and Lotus 1-2-3 software (release 2.01, 1987). Then the data was analysed using Minitab software (release 7.2, 1989), and Epi Info software (Version 5, 1990).

5.3. The Results

5.3.1. Data Analysis

1,159 expatriates (within 7 days of arrival to UAE) were included in the study. 891 (76.9%) of them were males and 268 (23.1%) were females. Their ages ranged between 18 to 49 years. The largest number of them 304 (26.2%) were aged 22-25 years and a smaller number of them 8 (0.7%) were aged 46-49 years. The majority of the males 849 (95.1%) were aged 18-41 years, and the majority of the females 238 (88.8%) were aged 18-33 years).

The expatriates came from different countries. The largest number 354 (30.5%) of them came from Bangladesh. The largest number of the males 354 (39.6%) were from Bangladesh, and the largest number of the females 200 (74.6%) were from Sri Lanka (Table 5.1).

The possibility of the expatriates getting infection in UAE is increased every day since their arrival. The expatriates in this study group were found attending the labour clinic within different days (2-7 days) since their arrival in the UAE. The majority of both the males 711 (79.8%) and the females 206 (76.8%) attended the labour clinic within 5 days of their arrival.

Table 5.1. Number of expatriates newly arrived to U.A.E. related to nationality and sex, screened at Kalba Labour clinic

Nationality	Male		Female		Total	
Pakistan	184	(20.6%)	0		184	(15.9%)
Bangladesh	354	(39.7%)	0		354	(30.5%)
India	315	(35.4%)	20	(7.5%)	335	(28.9%)
Sri Lanka	8	(0.9%)	200	(74.6%)	208	(18%)
Egypt	14	(1.6%)	1	(0.4%)	15	(1.3%)
Philippines	2	(0.2%)	47	(17.5%)	49	(4.2%)
Syria	9	(1%)	0		9	(0.8%)
Iran	5	(0.6%)	0		5	(0.4%)
Total	891	(100%) (76.9%)	268	(100%) (32.1%)	1159	(100%)

The expatriates came to the U.A.E. to do different work. The majority of the expatriates in this study 732 (63.2%) were dealing with food, 469 (64.1%) of them were males (Table 5.2).

It was found that the majority 633 (86.5%) of the food dealers were aged between 18-33 years. The majority 196 (88.7%) of the cooks were aged between 18-29 years. The majority 232 (81.4%) of the farm workers were aged between 18-37 years. The majority 232 (88.9%) of the housemaids were aged between 18-33 years. The majority 42 (80.7%) of the fishermen were aged between 18-37 years, and the majority 42 (75.8%) of the shepherds were aged between 18-29 years (Table 5.3).

The majority of the Pakistani expatriates were working as farm workers and shepherds. The majority of the Bangladeshi expatriates were working as cooks, farmers and shepherds. The majority of the Indian expatriates were working as cooks, fishermen and food sellers, and the majority of the Sri Lankan and Philippino expatriates were working as housemaids.

5.3.2. Stool samples analysis

5.3.2.1.Stool samples analysis for Giardia

1,159 stool samples were collected from the expatriates and examined with three methods. The samples were examined by direct microscopy. 66 (5.7%) of the samples were positive for Giardia, 52 (78.8%) of the positive samples were collected from the males.

Table 5.2. Number of expatriates newly arrived to U.A.E. related to occupation and sex, screened at Kalba Labour clinic

Occupation	Male	Female	Total
Cook	219 (24.6%)	2 (0.7%)	221 (19.1%)
Farm worker	102 (11.4%)	0	102 (8.8%)
Housemaid	0	261 (97.4%)	261 (22.5%)
Fisherman	52 (3.8%)	0	52 (4.5%)
Grocer	34 (3.8%)	0	34 (2.9%)
Shepherd	62 (7%)	0	62 (5.4%)
Others	422 (47.4%)	5 (1.9%)	427 (36.8%)
Total	891 (100%) (76.9%)	268 (100%) (23.1%)	1159 (100%)

Table 5.3. Number of expatriates newly arrived to UAE related to occupation and age groups, screend at Kalba Labour clinic

Occupation	Age Groups (in years)								Total	
	18-21	22-25	26-29	30-33	34-37	38-41	42-45	+46		
Cook	71	95	30	13	4	8	0	0	221	(19.1%)
Farm worker	11	20	24	21	7	8	7	3	102	(8.8%)
Housemaid	51	62	61	58	19	10	0	0	261	(22.5%)
Fisherman	7	8	11	8	8	7	3	0	52	(4.5%)
Grocer	2	7	11	9	2	1	1	1	34	(2.9%)
Shepherd	15	22	10	5	5	2	3	0	62	(5.4%)
Others	48	90	104	85	51	24	21	4	427	(36.8%)
Total	205	304	252	199	96	60	35	8	1159	
	(17.7%)	(26.2%)	(21.7%)	(17.2%)	(8.3%)	(5.2%)	(3%)	(0.7%)	(100%)	

The stool samples were then examined with ELISA test to detect the Giardia antigens in the stool. 105 (9.1%) of the collected samples contained Giardia antigens, 87 (82.9%) of them were collected from the male expatriates. The results of concentration test of stool samples were compared with ELISA data to evaluate ELISA sensitivity (Table 8.1). The ELISA gave 100% sensitivty. The ELISA test detected 20 (19%) of the cases more than the concentration test (Table 5.4).

The prevalence of Giardia was highest (11.5%) among the younger ages, it decreased (2.9%) among older ages, and it was not found at age above 46 years (Chi-square for linear trend = 7.936, with significant P <0.05). This may be due to acquired immunity to Giardia infection in older ages (Table 5.5). 205 stool samples were collected from the expatriates aged between 18-21 years. 23 (11.2%) of the stool samples were positive for Giardia. 304 stool samples were collected from the expatriates aged between 22-25 years. 35 (11.5%) of them were positive. 252 stool samples were collected from group aged between 26-29 years. 24 (9.5%) of them were positive. 199 stool samples were collected from the expatriates at group aged between 30-33 years. 12 (6%) of them were positive. 96 stool samples were collected from the expatriates aged between 34-37 years, 6 (6.3%) of them were positive for Giardia. 60 stool samples were collected from the expatriates aged between 38-41 years. 4(6.7%) of them were positive. 35 stool samples were collected from the expatriates aged between 42-45 years, 1 (2.9%) of them were positive. And 8 stool samples were collected from expatriates aged 46+ years, but none of these stool samples were positive for Giardia.

The highest prevalence of Giardia was found among the Egyptian and Bangladeshi expatriates. The lowest prevalence of Giardia was found among the Philippino expatriates. No Giardia cases were found amongst the Iranian expatriates. The prevalence of Giardia was found low among the Pakistani expatriates, may be because most of them had come when they were old (Table 5.6).

Nearly all the occupational groups have the same rate of Giardia prevalence, except the housemaids who had the lowest prevalence rate of Giardia infection. This is due to the Philippino expatriates having the lowest prevalence rate of Giardia (as shown above). (Table 5.7).

The majority of the cooks and the farmers who were Giardia positive were from Bangladesh. The majority of the housemaids with Giardia were from Sri Lanka. The majority of the grocers who were Giardia positive were from India. And the majority of the shepherds with Giardia positive were from Pakistan.

19 (79.2%) and 5 (20.8%) of the positive stool samples with ELISA test from the cooks were from Bangladesh and Indian expatriates respectively.

7 (70%), 2 (20%), and 1 (10%) of the positive stool samples with ELISA of the farm workers were from the Bangladeshi, Pakistani and Egyptian expatriates respectively. 15 (83.3%), 2 (11.1%) and 1 (5.6%) of the positive stool samples with ELISA of the housemaids were from the Sri Lankan, Philippino and Indian expatriates respectively.

3 (75%) and 1 (25%) of the positive stool samples with ELISA of the grocers were from the Indian and Bangladeshi expatriates respectively.

Table 5.4. Comparing the results of stool samples of expatriates examined with three different diagnositc methods, routine microscopy, concentration test and ELISA test to detect Giardia in relation to sex

Sex	Stool Examination Test: Routine Microscopy G.lamblia -ve	+ve	Total	Concentration Test G.lamblia -ve	+ve	Total	ELISA Test G.lamblia -ve	+ve	Total
Male	839 (94.2%)	52 (5.8%)	891 (100%) (76.9%)	823 (92.4%)	68 (7.6%)	891 (100%) (76.9%)	804 (90.2%)	87 (9.8%)	891 (100%) (76.9%)
Female	254 (94.8%)	14 (5.2%)	268 (100%) (23.1%)	251 (93.7%)	17 (6.3%)	268 (100%) (23.1%)	250 (93.3%)	18 (6.7%)	268 (100%) (23.1%)
Total	1093 (94.3%)	66 (5.7%)	1159 (100%)	1074 (92.7%)	85 (7.3%)	1159 (100%)	1054 (90.9%)	105 (9.1%)	1159 (100%

Table 5.5 Number of stool samples collected from newly arrived expatriates to UAE in Kalba Labour Clinic, examined withELISA test to detect Giardia antigens in relation to age groups

Stool examination with ELISA	Age Groups (in years)								Total	
	18-21	22-25	26-29	30-33	34-37	38-41	42-45	+46		
Negative	182	269	228	187	90	56	34	8	1054	
Positive	23	35	24	12	6	4	1	0	105	(9.1%)
	(11.2%)	(11.5%)	(9.5%)	(6%)	(6.3%)	(6.7%)	(2.9%)			
Total	205	304	252	199	96	60	35	8	1159	
	(17.7%)	(26.2%)	(21.7%)	(8.3%)	(8.3%)	(5.2%)	(3%)	(0.7%)	(100%)	

Chi-Square for linear trend = 7.936 P <0.05

Table 5.6. Number of stool samples collected from newly arrived expatriates to UAE in Kalba Labour Clinic, examined with ELISA test to detect Giardia antigens, in relation to nationality

Examination stool with ELISA	Pakistan	Bangladesh	India	Sri Lanka	Egypt	Philippines	Syria	Iran	Total	
Negative	172	309	308	192	13	47	8	5	1054	(90.9%)
Positive	12	45	27	16	2	2	1	0	105	(9.1%)
	(6.5%)	(12.7%)	(8.1%)	(7.7%)	(13.3%)	(4.1%)	(11.1%)			
Total	184	354	335	208	15	49	9	5	1159	
	(15.9%)	(30.5%)	(28.9%)	(18%)	(1.3%)	(4.2%)	(0.8%)	(0.4%)	(100%)	

Chi-Square for linear trend = 1.186 P >0.05

Table 5.7. Number of stool samples collected from newly arrived expatriates to UAE in Kalba Labour Clinic, examined with ELISA test to detect Giardia antigens, in relation to occupations

Stool examination with ELISA	Occupation							Total	
	Cook	Farm worker	Housemaid	Fisherman	Grocer	Shepherd	Others		
Negative	197	92	243	52	30	55	385	1054	(90.9%)
Positive	24 (10.9%)	10 (9.8%)	18 (6.9%)	0	4 (11.8%)	7 (11.3%)	42 (9.8%)	105	(9.1%)
Total	221 (19.1%)	102 (8.8%)	261 (22.5%)	52 (4.5%)	34 (2.9%)	62 (5.4%)	427 (36.8%)	1159 (100%)	

For all expatriates:

Chi-Square for linear trend = 0.061
P >0.05

For the food dealers only:

Chi-Square for linear trend = 0.557
P >0.05

3 (42.9%), 2 (28.6%) and 2 (28.6%) of the positive stool samples with ELISA of the shepherds were from the Pakistani, Bangladeshi and Indian expatriates respectively.

5.3.2.2. Stool samples analysis for other intestinal parasites

The concentration test is more effective in detection of other intestinal parasites than direct routine microscopy. The other intestinal parasite was found to be more common amongst males (73.6%) than females (26.4%). Ascaris lumbricoides (Ascaris) was found more common amongst the expatriates (238 (20.5%)), followed by Entamoeba coli (E.coli) was 15.6 (13.5%), hookworm (Hw) was 148 (12.8%), Trichuris trichuria (Trichuris) was 144 (12.4%), Entamoeba histolytica (E.h.) was 57 (4.9%), Hymenolepsis nana (H.n.) was 23 (2%), and Enterobius vermicularis (Enterobius) was 8 (0.7%) (Table 5.8).

Sri Lankan expatriates were found with the highest (61.5%) prevalence rate of other intestinal parasites. This was followed by the Bangladesh expatriates with a prevalence rate of 58.8%, then the Philippino expatriates with a prevalence rate of 55.1%, then the Pakistani expatriates with a prevalence rate of 48.4%, followed by the Indian expatriates with a prevalence rate of 47.2% and the Syrian expatriates with a prevalence rate of 44.4%. Both the Iranian and Egyptian expatriates with (40%) had equal prevalence rate of other intestinal parasites. Ascaris was found to be the commonest intestinal parasite amongst the expatriates of different nationalities (Table 5.9).

Amongst the food dealers, the farm workers were

found to be more infected (65.7%) with other intestinal parasites, followed by the cook group 64.7%, then the housemaids with prevalence rate of 61.7%. Then the fishermen with the prevalence rate of 53.8%. Followed by the shepherd with 50% of the prevalence rate. And the grocer with 44.1% of the prevalence rate of other intestinal parasites.

Also Ascaris was found to be the commonest intestinal parasite amongst the different food dealer groups (Table 5.10).

The majority of the stool samples 564 (48.7% of the total collected stool samples) were found infected with other intestinal parasites. 490 samples (42.3% of the total collected stool samples) were found negative. Small number of stool samples 58 (5% of the total stool samples) were found infected with Giardia and other intestinal parasites, while only 47 samples (4% of the total stool samples) were found positive with Giardia only (Table 5.11).

564 (53.5%) of the expatriates stool samples which were negative with ELISA test for Giardia antigen, were positive for other intestinal parasites with the concentration test, 218 (38.7%), 51 (9%), 8 (1.4%), 21 (3.7%), 141 (25%), 141 (25%) and 129 (22.9%) of them were positive for Ascaris lumbricoides, Entamoeba histolytica, Enterobius vermicularis, Hymenolepis nana, Hookworm and Trichuris trichiuria respectively. 121 (21.5%) and 443 (78.5%) of them were positive with mixed and single intestinal parasites respectively. 58 (55.2%) of the expatriates stool samples which were positive with ELISA test for Giardia antigen, were positive for other intestinale parasites with the concentration test, 20 (34.5%), 6 (10.3%), 2 (3.5%), 5 (8.6%) and 15 (25.9%) of them were positive for Ascaris lumbricoides, Entamoeba

histolytica, Hymenolepis nana, Hookworm and Trichuris trichiuria respectively, 10 (17.2%) and 48 (82.8%) of them were positive with mixed and single intestinale parasites respectively.

5.4. Discussion

5.4.1. Data analysis

A large number of the expatriates arrive in the UAE daily, 1159 of newly arrived (within 7 days to avoid any possiblity of getting infection in UAE) expatriates were included in this study, their ages ranged between 18 to 49 years, but the majority were aged between 20 and 40 years (at young active age) and the majority (76.9%) were males, this gave the population pyramid the special shape (Figure 3.3.). The expatriates came from different countries, but the majority of them came from Bangladesh, India, Sri Lanka and Pakistan, because of cheap labour in these countries.

The expatriates were working in different jobs, but the majority of them were working as food dealers (as cooks, housemaids, farm workers, fishermen, grocers and shepherd) maybe because these jobs do not need to be educated or experienced personnel. The Phillipino and Sri Lankan females work as housemaids, even the Phillipino are more educated, but the number of the Sri Lankans were higher, and this may be due to the cheap cost and less salaries.

Table 5.8. Number of positive stool samples for other intestinal parasites examined with routine microscopy and concentration test, collected from newly arrived expatriates to UAE in Kalba Labour Clinic, related to sex.

Intestinal parasite	Routine Microscopy			Concentration Test		
	Male	Female	Total	Male	Female	Total
E.coli (Entamoeba coli)	89	27	16	116	37	153
H.nana (Hymenolepis nana)	9	5	14	14	9	23
E.hist. (Entamoeba histolytica)	34	7	41	45	12	57
Enterobius V (Enterobius vermicularis)	1	0	1	5	3	8
Hookworm	96	27	123	126	32	158
Trichuris Trichiuria	75	38	113	94	50	144
Ascaris	154	45	199	183	55	238
Negative	515	150	665	433	104	537

Table 5.9. Number of positive stool samples for other intestinal parasites examined with concentration test, collected from newly arrived expatriates to UAE in Kalba Labour Clinic, related to nationality

Intestinal parasite	Nationality								
	Pakistan	Bangladesh	India	Sri Lanka	Egypt	Philippines	Syria	Iran	Total
E.coli (Entamoeba coli)	28	40	47	30	1	5	1	1	153
H.nana (Hymenolepis nana)	2	9	2	6	1	3	0	0	23
E.hist. (Entamoeba histolytica)	9	19	18	8	0	3	0	0	57
Enterobius V (Enterobius vermicularis)	1	4	0	3	0	0	0	0	8
Hookworm	25	44	46	26	1	4	1	1	148
Trichuris Trichiuria	11	48	36	35	1	11	2	0	144
Ascaris l.	28	110	37	44	2	9	2	0	232
Negative	95	146	177	80	9	22	5	3	537

Chi-Square for linear trend = 0.167 P >0.05

Table 5.10. Number of positive stool samples for intestinal parasites examined with concentration test, collected from newly arrived expatriates to UAE in Kalba Labour Clinic, related to occupation

Intestinal parasite	Occupation							
	Cook	Farm work	House-maid	Fisher-man	Grocer	Shepherd	Others	Total
E.coli (Entamoeba coli)	25	19	34	7	5	9	54	153
H.nana (Hymenolepis nana)	10	1	9	0	0	0	3	23
E.hist. (Entamoeba histolytica)	15	3	12	5	3	3	14	55
Enterobius V (Enterobius vermicularis)	2	0	3	1	0	0	2	8
Hookworm	36	22	32	7	5	6	40	148
Trichuris Trichiuria	26	9	50	6	2	6	45	144
Ascaris l.	68	26	55	10	4	15	60	238
Negative	78	35	100	24	19	31	250	537

For all expatriates: Chi-Square for linear trend = 48.002 P <0.05

For food dealers: Chi-Square for linear trend = 8.318 P <0.05

Table 5.11. The relation between the results of examined stool samples of newly arrived expatriates to UAE, with ELISA test to detect Giardia antigens and concentration test for other intestinal parasites.

Examination of stool samples with concentration test for other intestinal parasites	Examination of stool samples with ELISA for Giardia		
	Negative	Positive	Total
E.coli (Entamoeba coli)	134	19	153
H.nana (Hymenolepis nana)	21	2	23
E.hist. (Entamoeba histolytica)	51	6	57
Enterobius V (Enterobius vermicularis)	8	0	8
Hookworm	141	7	148
Trichuris Trichiuria	129	15	144
Ascaris l.	218	20	238
Negative	490	47	537

5.4.2. Stool Sample Analysis

5.4.2.1. Stool samples analysis for Giardia intestinalis

The number of positive cases with Giardia were increased from 66 (5.7%), 85 (7.3%) to 105 (9.1%) with routine microscopy, concentration test and ELISA test respectively. With the three diagnostic methods the percentage of positive cases for males and females was nearly equal.

The prevalence of Giardia were decreased with increasing age among the expatriates. This may be due to acquired immunity by repeated infection in older age.

Nearly all the expatriates from all countries had Giardia except those from Iran who did not. And prevalence was similar except the expatriates from the Phillipines who had a lower percentage.

Giardia was found in all food dealer groups of the expatriates, except in the fishermen group who were negative. Most of the cooks with Giardia were from Bangladesh and India, most of the farmers with Giardia were from Bangladesh, Pakistan and Egypt. Most of the housemaids with positive Giardia were from Sri Lanka, Phillipines and India, most of the grocers with positive Giardia were from India and Bangladesh, most of the shepherd expatriates with Giardia were from Pakistan, Bangladesh and India.

5.4.2.2. Stool samples analysis for other intestinal parasites

Giardia was not the only parasite imported by expatriates also other intestinal parasites, Ascaris

lumbricoides (20.5%), Entamoeba histolytica (4.9%), Entrobius vermicularis (0.7%), Hymenolepis nana (2%), Hookworm (12.8%) and Trichuris trichuria (12.4%), from Pakistan, (48.4%), Bangaldesh (58.8%), India (47.2%), Sri Lanka (61.5%), Egypt (40%), Phillipines (55.1%), Syria (44.4%) and Iran (40%), were found positive for other intestinal parasites. They were distributed among different occupational groups cooks (64.7%), the farm workers (65.7%), the housemaids (61.7%), the fishermen (53.8%), the grocers (44.1%), the shepherds (44.1%) and other jobs (41.5%) were found positve for other intestinal parasites.

53.5% of the expatriates stool samples which were negative with ELISA test for Giardia antigen, were found positive for other intestinal parasites with concentration test, and 55.2% of the expatriates stool samples which were positive with ELISA test for Giardia antigens, were found positive for other intestinal parasites.

5.4.3. Conclusion

Every day a large number of people (locals and expatriates) arrived to UAE from endemic areas (with Giardia). In this study we only examined a small number of these (1159), and the rest escaped the examination as the UAE government gives 30 days for the newly arrived expatriates to apply for a permanent visa after their arrival, and in this study only the newly arrived expatriates (those within 7 days) were included so a large number of the newly arrived expatriates were not included. When the children were included on their mothers passport, only the mothers stool was examined and not the children's stool. The UAE government gives a two year

visa for every expatriate working in the country, most of the expatriates renew their visa shortly before they go back home for 2 or 3 months leave, and when they come back, their visa is valid, so they are not checked for another 21 or 22 months. Kalba labour clinic is not the only clinic in UAE to check the newly arrived expatriates, there are several government and private clinics in the country who do this job, in some busy clinics the numbers of daily attendances was more than 500 expatriates (MOH, 1989b) and not only the expatriates may playing a role in importing Giardia to UAE, also the locals are sharing in this, especially the non educated people when they come back from an infected country and they do not have their stool checked.

In 1989 the Ministry of Health in UAE found 780 (3.5%) of 22132 stool samples which were collected from the expatriates were positive for Giardia (MOH, 1989b). This was less than our findings (9.1%) in this study, as examining only one stool sample from each expatriate by routine microscopy is insensitive and more than one stool sample may be needed (Kamath and Murugasu, 1974; Danciger and Lopez 1975; Wright et al 1977). Other methods of diagosis may be useful, such as formol-ether concentration method (Allen and Ridly 1970) or ELISA test as shown in this study the percentage of Giardia positivity was increased from 5.7% with routine microscopy to 7.3% and 9.1% with concentration test and ELISA test respectively.

From the results of this study we think that the expatriate is playing a role in importing Giardia to UAE, as 105 (9.1%) of 1159 stool samples were positive for Giardia. This is not a small number, especially as we know that each patient can excrete 900million Giardia cysts per day (Feachem et al

1983), and only 100 Giardia cysts is enough to transmit infection (Rendtorff, 1954; Rendtorff 1979; Rendtorff and Holt, 1954). This is especially important as 63.2% of the total expatriates worked as food handlers, and especially as the infection can be transmitted easily by the faecal-hand-mouth route.

CHAPTER 6

ANIMALS, WATER AND THE FARMS AS SOURCES OF INFECTION WITH G.INTESTINALIS IN WADIHALOO VILLAGE

6.1. Introduction

6.1.1. The animals

The reservoir of Giardia intestinalis is man, but there is some evidence that man may acquire infection from other animals. Many mammalian species harbour (their own) Giardia species whose relationship to Giardia intestinalis has not yet been properly elucidated. Giardia from man has been transmitted to some animals (Davies and Hibler 1979; Meyer and Radulsecu 1980). Giardia cysts from beavers and deer have caused infection in human volunteers (Davies and Hibler 1979). Woo and Paterson (1986) concluded that dogs are not the reservoir for infection in humans on the basis of negative cross infection experiments. But the fact that dogs were not susceptible to infection with human Giardia isolates does not prove that humans are thus refractory to infection with canine isolates. In attempts to excyst and culture Giardia isolates from humans and dogs under identical conditions Meloni and Thompson (1987) found that 44% of human isolates, but no canine isolates were established in axenic culture.

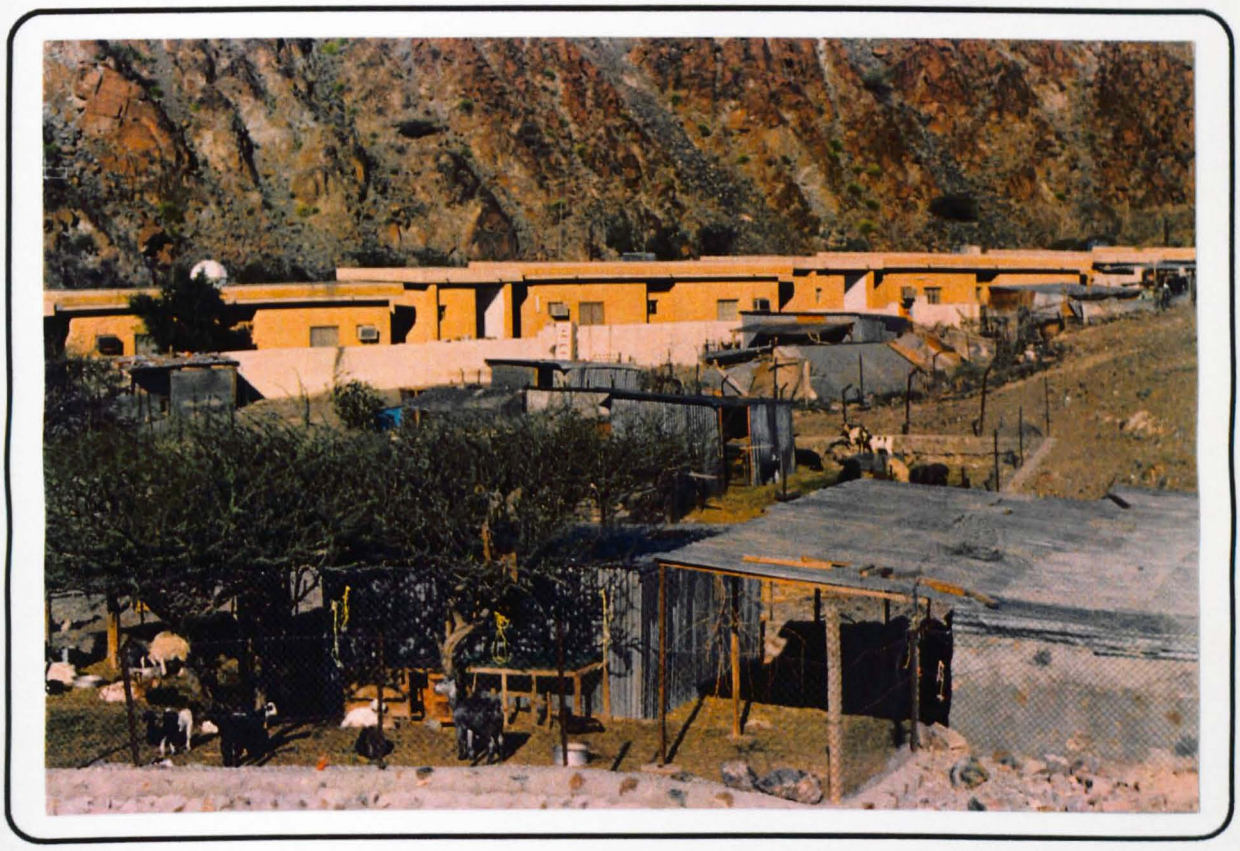


Figure 6.1 Showing the animals in the stable behind the houses in Wadihaloo village

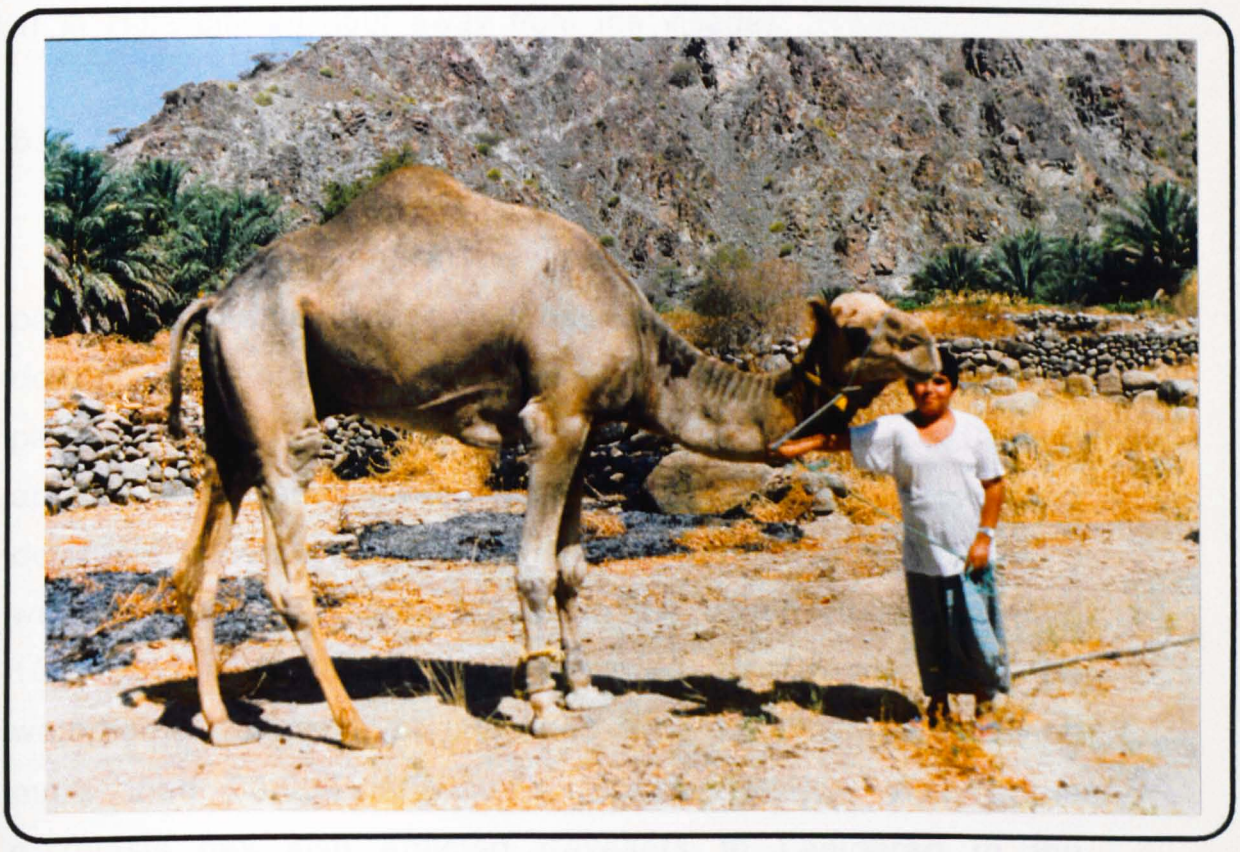


Figure 6.2 Showing the camel in the farm in Wadihaloo village

In Wadihaloo village there were sheep, goats and cows which were kept in stables behind the houses (Figure 6.1), with the chickens. There were a few donkeys and camels in the village (Figure 6.2). There were a very few dogs (mainly in the farms) and cats (mainly in the streets). The dogs and the cats are not allowed to enter the houses. (There was no veterinary clinic in the village, the nearest one was either in Kalba city or in Hata city an hour away from the village).

6.1.2. The Water

Giardia could be spread via drinking water, particularly surface water that has been untreated. The best documented cases have been in developed countries, particularly the USA and USSR. In an outbreak in a ski resort at Aspen, Colorado in 1965 (Moore et al 1969) fluorescent and detergent tracers placed in the sewers were detected in 2 of 3 water sources. Other outbreaks in the united States between 1965 and 1981 have been reviewed by Craun, when 53 waterborne outbreaks of infections were documented involving more than 20,000 individuals (Craun 1984). Waterborne giardiasis has also afflicted travellers to Leningrad in the USSR (Jokipii and Jokipii 1974). A waterborne outbreak has been reported in the United Kingdom following repairs to a water mains supply (Jephcott et al 1986). Recently Giardiasis was reported amongst the users of a water slide pool (Greensmith et al. 1988).

The drinking water in the village was supplied from the wells, by two systems. The first one (central) to the houses in the village in which the water was pumped from the

well (150-200feet deep in the ground) to the central water tank, through closed pipes then distributed to the small water tanks on the roof of the houses through closed pipes, then inside to the houses. There were two groups of houses and each of them have separate central water supplies. The second system of drinking water was supplied from scattered wells in the farms. The water was pumped from the well to the open cement reservoir. The drinking water was collected either direct from the pipe or from the open reservoir (Figure 3.13).

6.1.3. The Farms

The farms may help in transmission of Giardia infection to man through either contaminated food (lettuce) (Osterholm et al 1981) or contaminated water. It may help to increase the number of positive Giardia cases in the village either by attraction of new imported positive Giardia cases or by spread of the infection.

There were a number a farms scattered around the village, farmed by foreigners. Fruits, tobacco and vegetables were grown on these farms.

6.2. Materials and Methods

6.2.1. The animals

Fresh stool samples were collected from different types of animals in the village. The stool samples were coded with family number and animal type (one stool sample from each type of animal from each stable). The stool samples were taken to Kalba hospital to be examined by laboratory technicians with direct microscopy for Giardia and other

intestinal parasites. The stool samples were stored at 4°C overnight. On the next day the negative samples were re-examined with direct microscopy. Part of the faecal samples were mixed properly with 10% formol saline, filtered through wire mesh (size 250µm), ether was added, shaken vigorously for a few minutes, left to stand for 30 minutes. The fatty plug was loosened from the wall of the tube with a stick, the supernatant fluid was poured into chloros, and the deposit which was stained with Lugol's iodine was examined by microscopy. The concentrated stool samples were re-examined in the diagnostic laboratory and by veterinarians in LSTM.

For ELISA examination part of the faecal samples were suspended in phosphate buffer saline (PBS pH7.2) at a concentration of 10g% mixed properly and large particulate matter removed by filtration through wire mesh, then left to stand for 30minutes and the supernatant was collected and stored at -20°C, the ELISA procedure as outlined in Chapter 2.

6.2.2.1.The Water

In The Liverpool School of Tropical Medicine, we used a modification of the method described by Chang and Kabler (1956), to find Giardia cysts in drinking water. This test was checked by mixing 2×10^5 Giardia cysts with two litres of distilled water, a serial dilution was done from one litre until we ended with 1:16 dilution. One litre of the mixture was filtered through a 3.0µm pore size membrane in a glass 47mm filter holder (Milipore, Wolford, UK) with the aid of a vacuum pressure pump (Figure 6.3.). The filter was removed with forceps and washed in a 20ml tube with 10ml of distilled

water. The washed fluid was then centrifuged at 2000 rpm for 5 minutes after which the sediment was examined microscopically for Giardia cysts and counted by using a counting chamber. To examine the possibility of ELISA test to detect Giardia in water. Giardia cysts were mixed with distilled water or 50% distilled water/PBS (pH7.2). In different dilution, started with 10^4 cysts/ml (2000 cysts/well) and ended by 50 cysts/ml (10 cysts/well). The samples were examined with ELISA test. (The ELISA procedure as outlined in Chapter 2).

6.2.2.2.In the Field

Four litres of water samples were collected from 21 different sources of drinking water in the village especially from the two wells (the main source of water to the houses), (Figure 6.4). The samples were taken from some houses and farms where a child with positive stool for Giardia was living, from the school, police station and from the clinic.

The collected water samples were taken to the laboratory and concentrated with the same method which we used before. Half of the sediment was preserved in 10% formalin for microscopic examination. A drop of the sediment was transferred to a slide, stained with Lugol's iodine, and examined microscopically. The concentrated water samples were re-examined microscopically in the diagnostic laboratory (LSTM).

Figure 6.3 Showing the instruments used to concentrate water samples

Figure 6.4 Collecting the water sample from the main well in the village

For ELISA examination the other half of the sediment of the water samples were suspended in phosphate buffered saline (PBS pH 7.2) at a concentration of 10%, the supernatant was collected and stored at -20°C, the ELISA procedure outlined in Chapter 2.

6.2.3. The Farms

The data was collected about the farms in the village, stool samples were collected from the farm workers, and water samples were collected from some wells in the farms. The stool and water samples were examined for Giardia with the methods as described before.

6.3. The Results

6.3.1. The animals

Most of the stool samples were infected with different parasites when they were examined microscopically, one goat stool sample contained Giardia cysts (Table 6.1.). But all the stool samples were negative when they were examined with ELISA test to detect Giardia antigen.

6.3.2. The Water

By testing the membrane filter method in the laboratory (Chang and Kabler 1956). The percentage of recovery of cysts which were detected with the test were found to increase from 20% (in one dilution) to 32% (in 16 dilution) (Table 6.2).

Table 6.1. The results of animals stool sample with direct microscopical, concentration test and ELISA test

Sample No.	Animal Type	Microscopical examination	Concentration Test	ELISA test for G.lamblia
1	sheep	E.coli	E.coli	-ve
2	sheep	-ve	E.coli	-ve
3	sheep	Trichuris, HW	Trichuris, HW E.nana	-ve
4.	Goat	Trichuris	Giardia, Trichuris	-ve
5.	Goat	-ve	E.nana	-ve
6.	Goat	HW	E.nana, HW	-ve
7.	Goat	Trichuris, E.coli	Trichuris, E.coli, E.nana	-ve
8.	Cow	-ve	-ve	-ve
9.	Cow	-ve	E.nana	-ve
10.	Cow	HW, E.coli	HW, E.coli	-ve
11.	Cow	Trichuris, E.coli	Trichuris, E.coli	-ve
12.	Camel	Trichuris, HW	Trichuris, HW	-ve

E.coli - Entamoeba coli
Trichuris - Trichuris Trichiura
HW - Hookworm
E.nana - Endolimax nana
Giardia - Giardia intestinalis

Table 6.2. The Number and percentage of *Giardia* cysts detected by using membrane filter method (Chang and Kabler 1956) after several dilutions of the mixture

Dilution of the mixture	No. of Giardia cysts in 1 litre of Distilled Water	No. of Giardia cysts detected by the test	%
1:1	100000	20000	20
1:2	50000	10000	20
1:4	25000	6000	24
1:8	12500	4000	32
1:16	6250	2000	32

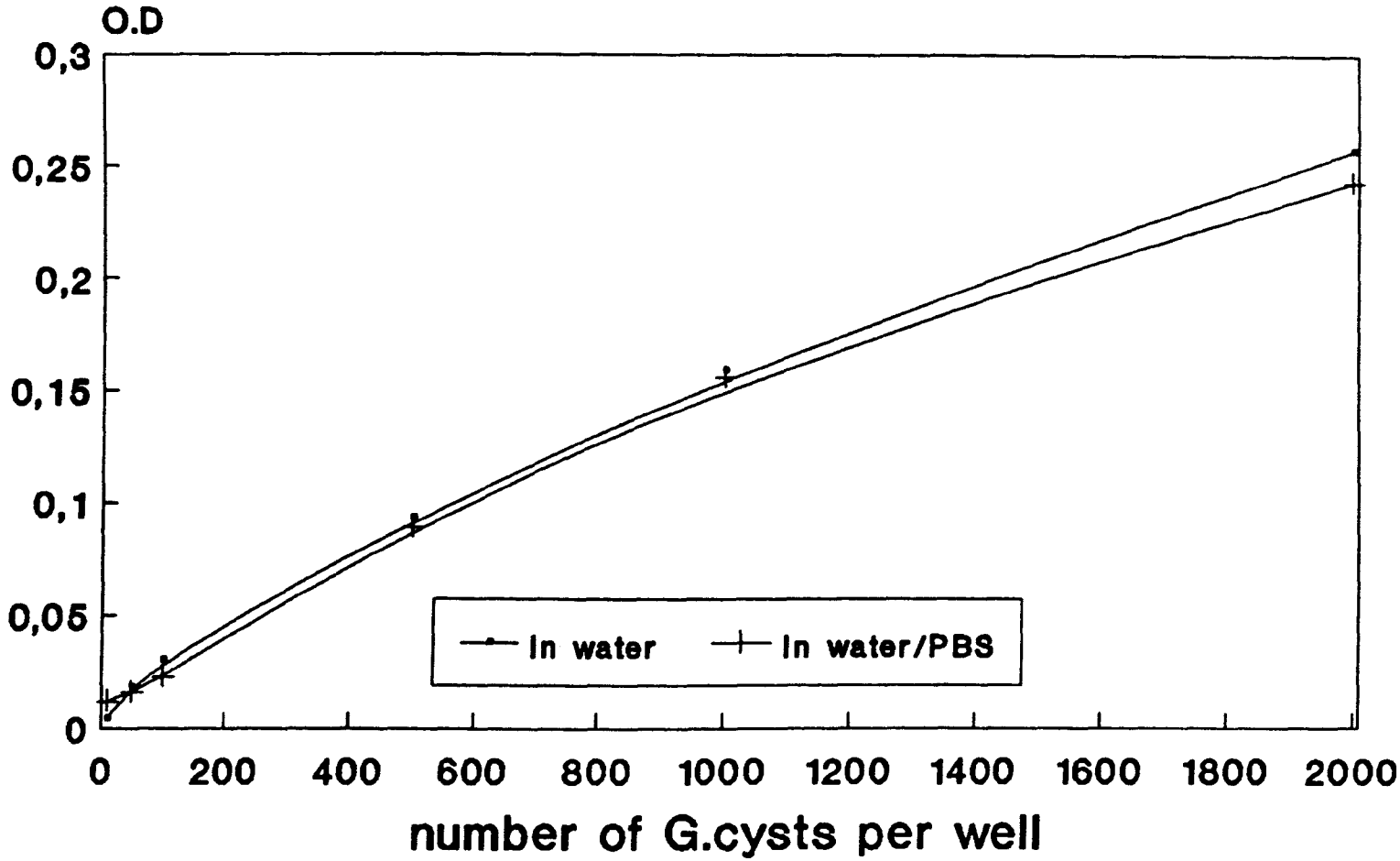


Figure 6.5 Comparing the O.D values of the number of Giardia cysts/well in water or in water/PBS.

By examining the serial dilution of Giardia cysts in distilled water or distilled water with PBS (pH7.2). 2500 cysts/ml (500 cysts/well) was the lowest number of Giardia cysts in water detected by ELISA test (O.D. = 0.091). Little difference in O.D. values was noticed, if the Giardia cysts were mixed with distilled water or with distilled water and PBS (pH7.2) (Figure 6.5).

After concentration of collected water samples (from different sources in the village) with the same method, the sediment was examined with direct microscopical concentration test and ELISA test to detect Giardia antigens; all the samples were negative.

6.3.3. The Farms

The number of the farms increased from 36 farms in August 1988 to 47 farms in October 1990. The number of the farm workers increased from 56 (19.1% of the population of the village), 36 (64.3%) of them were foreigners in August 1988 to 81 (22.2% of the population of the village), 61 (72.3%) of them were foreigners in October 1990. In August 1988 52 stool samples were collected from the farm workers, 33 of them were foreigners. 10 (19.2%) of the stool samples were positive for Giardia with ELISA, 6 (60%) of them were from the non local farm workers. In October 1990, 76 stool samples were collected from the farm workers, 49 of them were from the non local farm workers, 12 (15.8%) of the samples were positive for Giardia with ELISA, 8 (66.6%) of them were from the foreigners farm workers. 33% (of the total children aged 0-6 years) and 81.1% (of the infected children in the same age)

with Giardia infection were found to be related to the families who had farms, while only 5.5% of them were related to infected farm workers (Table 6.3). The collected water samples which had been collected from different wells of different farms (4 litres from each) (Figure 6.6) were examined microscopically and with ELISA test. All of the water samples were found negative with both tests.

34.1% (of the total children aged 0-6 years) and 83.8% (of total infected) with Giardia infection were found to be related to other infected members of the same family with Giardia, and 11% (of the total children aged 0-6 years) and 27% (of total infected) with Giardia were found related to infected mothers (with significant $P < 0.05$).

6.4. Discussion

6.4.1. The Farms

An increase in the number of farms in the village could increase the possibility of infection with Giardia via contaminated food (Osterholm et al 1981) or via contaminated drinking water.

Increase in the number of the farms will increase the number of foreign farm workers with positive stools for Giardia (even though they were found with a low prevalence of Giardia). They may pass the infection to the others via contaminated food by passing stools in the fields, or via contaminated drinking water with their dirty hands.

Increase in the number of the farms, will increase the use of infected animal waste as fertiliser, which contaminates the food and drinking water.

Table 6.3. **Number of the children (0-6 years) infected with Giardia with faecal ELISA related to other factors in the three survey rounds**

	Number of children (0-6 years)					
	1st Round (n = 90) (%)		2nd Round (n =- 91) (%)		3rd Round (n = 91) (%)	
	G+ve	G-ve	G+ve	G-ve	G+ve	G-ve
The mother with G+ve	9 (10)	7 (7.8)	10 (11)	6 (6.6)	10 (11)	3 (3.3)
The mother with G=ve	21 (23.3)	53 (58.9)	17 (18.7)	58 (63.7)	27 (29.7)	51 (56)
Other member in the family with G+ve	27 (30)	36 (40)	22 (24.2)	28 (30.8)	31 (34.1)	28 (30.8)
Other member in the family with G-ve	3 (3.3)	24 (26.7)	4 (4.4)	37 (40.7)	6 (6.6)	26 (28.6)
The family has a farm	18 (20)	39 (43.3)	22 (24.2)	54 (59.3)	30 (33)	43 (47.3)
The family has no farm	12 (12.3)	21 (23.3)	5 (5.5)	10 (11)	7 (7.7)	11 (12.1)
The farmworker with G+ve	7 (12.3)	7 (12.3)	7 (9.2)	18 (23.7)	4 (5.5)	13 (17.8)
The farmworker with G-ve	11 (19.3)	32 (56.1)	15 (19.7)	36 (47.4)	26 (35.6)	30 (41.1)

infected children related to the mothers, Chi square = 21.29 P <0.05
infected children related to other members, Chi square = 31.56 P <0.05
infected children related to the farms, Chi square = 0.13 P >0.05
infected children related to the farm workers, Chi square = 0.12 P >0.05

Figure 6.6 Collection of water sample from *different sources* in the village

An increase in the number of farms, will increase the chance of any infected person with Giardia visiting the farm (especially the children) passing a stool on the plants (because there is no latrine on the farm), then he will contaminate the plant

Food samples from the farms were not examined for Giardia.

6.4.2. Animals

There is some evidence that animals were positive for Giardia and man can get infection with Giardia from some animals (Davies and Hibler 1979; Buret et al. 1990; Karabiber, 1991). There were some animals living in the village e.g. sheep, goats, cows, camels and very few dogs, cats and donkeys, and they were not only kept in the stable but also allowed to walk freely in the village (Figure 6.7). The villagers grow up with cows, goats and sheep for their milk and meat, there was even milk and frozen meat in the grocers. If there was the possibility of infection with Giardia from animals to man in the village it would be in one of the following ways: The villages women were usually milking the cows in the stable where the milk could be contaminated with Giardia cysts, if the milk was not boiled properly, the infection could be transmitted to the members of the family and especially the children. If anyone entered the stable to feed the animals or to clean the stable, he may carry the Giardia cysts on his hands, especially below the finger nails, and he will infect himself if he put his hands into his mouth,

or he will infect the others if he touches the food or drinking water if he has not cleaned his hands properly. The free walking animals in the village may pass stools in the street, especially where the children play. The farmworkers in the village were usually using the animals waste as fertilizer for the plants, it could contaminate the plants. The flies may help in transmission of infection with Giardia as the flies may carry the cysts of Giardia mechanically with its wings or with its legs, from the infected stool to contaminate the food or drinking water.

As the results of the examination of animal stool samples were negative, the animals in the village could be excluded from sharing in the transmission of Giardia in the village.

6.4.3. The Water

All the houses in the village except that in the farms were supplied with closed system central supply in which the water is pumped from a drilled well (150-200 feet deep) to the closed system. The water inside the well could be contaminated with Giardia cysts from the sewage as the septic tanks are open from the side, and even the main water well is below the level of the septic tanks of the houses, but the possibility of Giardia cysts in the sewage reaching the water in the well is very low, due to the fact that the well was drilled type, very deep (150-200 feet) and the type of the soil (clay rocky type). The water in the cement reservoir could be contaminated with Giardia cysts, either from animals waste, mans waste or contaminated hands. The drinking water in the houses could be contaminated with Giardia cysts

between the tap water and the mouth from contaminated hands or contaminated flies.

The water samples which were collected from different sources and concentrated with membrane filter method were negative. It is not known whether this failure resulted from processing an insufficient volume of water, or because the water was truly free of cysts at the time of sampling. This failure was not due to inadequate methodology, because we tested the method, and it was used successfully by Sullivan et al (1988).

2500 Giardia cysts/ml water were the minimum number that can be detected with ELISA test in the laboratory. The negativity of the water samples with ELISA test was either due to:- Negative samples (no Giardia cysts in the water samples). OR The number of Giardia cysts in the water samples too low to be detected with ELISA test (each water sample (4 litres) should contain 10^6 Giardia cyst to become positive with ELISA test).

More recently a new method was discovered to detect Giardia cyst in water. And it could be more useful in future. This method can detect 1-5 cysts per ml by using cDNA probe (Abbaszadegan et al. 1991).

According to the results of the examination of the drinking water samples in the village, which were negative, the water in the village could be excluded from being the source of Giardia transmission in the village.

Figure 6.7 Showing the animals walking free outside the stable in the village

6.4.4. Conclusion

Because there were some Giardia cases in the village, especially amongst the people working inside the village, this meant there were some indigenous Giardia transmission with anyone of the methods of transmission.

Transmission of Giardia in the village via contaminated food was not clear.

The drinking water from the wells did not play a role in Giardia transmission in the village as no waterborne outbreaks of infection was found in the village, and no Giardia cysts were found in the collected drinking water samples.

Direct method of transmission (contaminated hand to mouth) mostly played a big role in transmission of Giardia in the village, because through our observation of Giardia cases in the village during the study period we found that the cases clustered amongst relatives especially amongst children.

CHAPTER 7

THE ROLE OF TREATMENT IN THE CONTROL OF GIARDIA INFECTION

7.1. Introduction

Giardia intestinalis was considered a non pathogenic inhabitant of the upper gastrointestinal tract until the 1950s. Since then its pathogenicity has been established, and Giardia has been recognised as an important cause of acute and chronic diarrhoea and intestinal malabsorption, and it was found worldwide (Davidson, 1984). The priorities given to controlling this parasite in the community include: (1) Environmental; water supply and sanitation control, adequate water filtration and proper sewage disposal; these expensive measures can be justified only as part of a comprehensive effort to control all waterborne enteric disease (Stevens 1985); (2) Research: Development of an effective vaccine for protection of high risk groups should have priority in research (3) Individual control: This should be done by two steps, first extensive public health education programmes, this will take a long time and depends on the level of peoples education, and secondly proper diagnosis and treatment of cases.

Several drugs were found to have an effect on Giardia in vitro (Boreham et al, 1984; 1987; 1988; Farthing and Inge, 1986; Inge and Farthing 1987; Meloni et al 1990) and in vivo with different side effects (Bassily et al, 1970; 1987; Al Waili 1988). Buelna (1989) found furazolidone (Furoxone) and metronidazole (Flagyl) were equally safe and effective in treating children with giardiasis. We treated the infected

children in our study group after the study period, to find the effectiveness of the drug in clearing the Giardia infection from the children controlled by ELISA test (Green et al. 1985) and to find out the role of treatment in parasitic control.

7.2. The Methods and Materials

The infected children with Giardia from the study group were identified and it was decided to treat them at the end of the study period. The mothers of the infected children were informed and stool containers were distributed to them. Two stool samples were collected from each child, the first stool sample was collected in the same day before the treatment and second one was collected 12 days after the beginning of the treatment.

Each child was treated with 25mg per kg body weight of metronidazole (Flagyl) syrup on three divided doses per day for seven days.

The collected stool samples from each child were coded with family number, child number and before or after treatment, then the stool samples were taken to Kalba hospital to be examined by the laboratory technicians with direct microscopy for Giardia and other intestinal parasites, then stool samples were stored at 4°C overnight. On the next day they were re-examined with direct microscopy then part of the stool samples were mixed properly with 10% formol saline, filtered through wire mesh (size 250μm), ether was added, shaken vigorously for a few minutes, left to stand for 30 minutes, the fatty plug was removed with a stick, the supernatant fluid was poured into chloros, and the deposit was exmained by microscopy after staining with Lugol's iodine. Some of the concentrated stool samples were re-examined by

laboratory technicians in Khorfakkan hospital, with direct microscopy for Giardia and other intestinal parasites.

For ELISA examination part of the stool samples were suspended in phosphate buffer saline (PBS pH7.2) at a concentration of 10gm% mixed properly and large particulate matter removed by filtration through wiremesh, then left to stand for 30 minutes and the supernatant was collected and stored at -20°C, the ELISA procedure as outlined in Chapter 2, then the positive-negative cut off value for the ELISA was carried out, the arithmetic mean of the negative samples +3SD.

7.3. Results

29 children from the study group in Wadihaloo village were found infected with Giardia at the end of the study (as shown in Chapter 5).

Stool samples from this group were collected immediately before the treatment with metronidazole (Flagyl) after examination for Giardia, it was found 19 (65.5%), 23 (79.3%) and 26 (89.7%) of them were positive with routine microscopy, concentration test and ELISA test respectively. And by examining the second group of the collected stool samples (12 days after the treatment) it was found none of the stool samples was positive for Giardia with the three diagnostic methods (Table 7.1). By comparing the results of microscopy with the results of the ELISA test it was found that sensitivity of the test was 91.3% (Table 8.1). The parasites were cleared from the children after treatment with metronidazole as indicated with low OD values of the stool sample with ELISA test (Figure 7.1).

Table 7.1. Comparing the result of stool examination with three different diagnostic methods (routine microscopy, concentration test and ELISA test) to detect Giardia before and after treatment

Method of Diagnosis for Giardia infection	Before Treatment			After Treatment		
	Giardia Negative	Giardia Positive	Total	Giardia Negative	Giardia Positive	Total
Routine Microscopy	10 (34.5%)	19 (65.5%)	29 (100%)	29 (100%)	0	29 (100%)
Concentration test	6 (20.7%)	23 (79.3%)	29 (100%)	29 (100%)	0	29 (100%)
ELISA test	3 (10.3%)	26 (89.7%)	29 (100%)	29 (100%)	0	29 (100%)

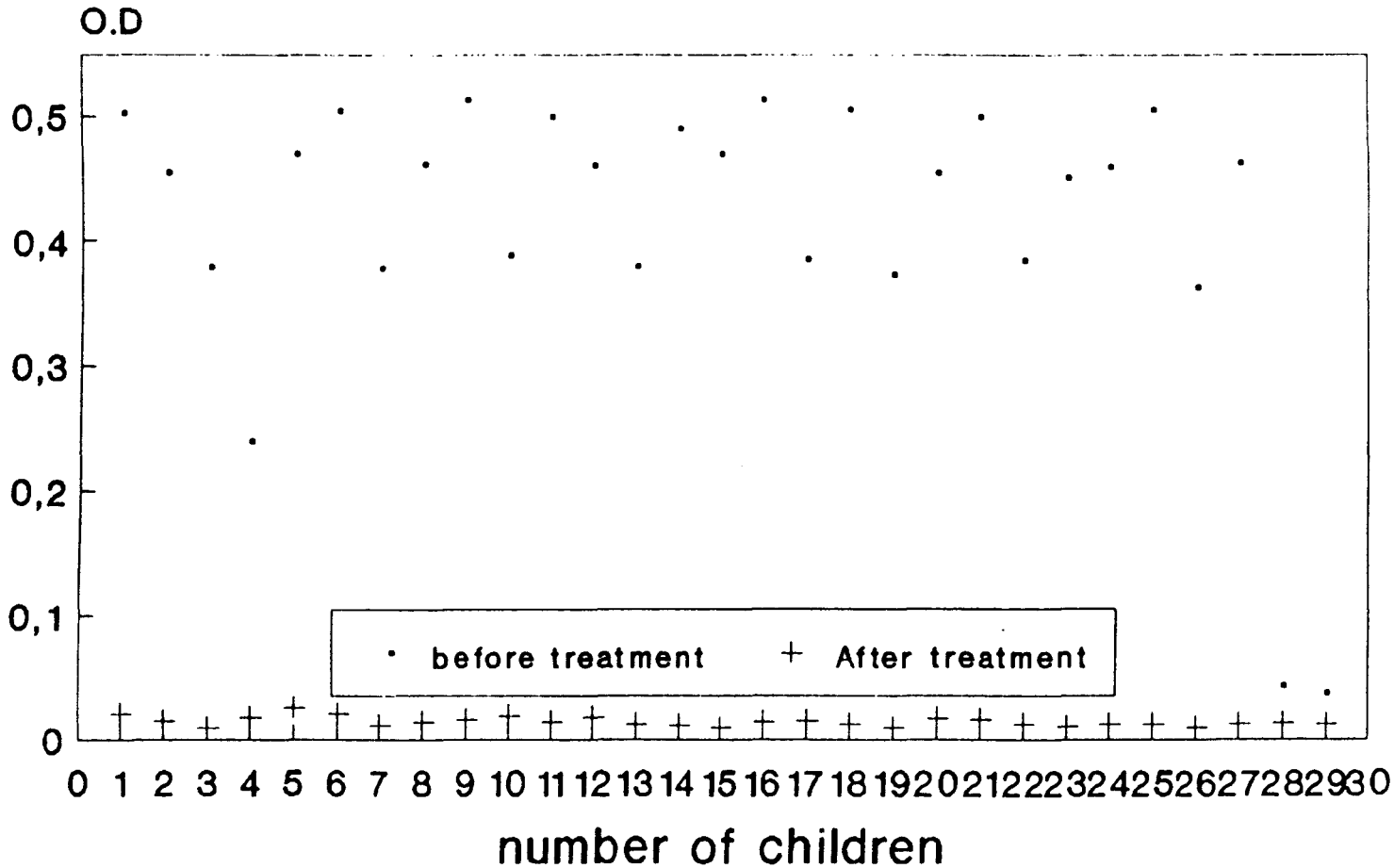


Figure 7.1 Comparing the O.D values of the stool samples,before and after treatment of Giardia infection.

7.4. Discussion

To control any parasite is to break its life cycle, either by eliminating the reservoir or by stopping transmission of the parasite between its life stages. Giardia is known to transfer by the faecal oral route, either by faecal contaminated water and food or by the easier direct faecal hand oral route. The latter becomes difficult to control, especially among children at preschool age, with uneducated parents, and especially if we know that the health education programme will take a long time, and the people will not respond completely to instruction. So the alternative way to control the parasite, is to treat the positive cases to reduce the source of infection and to cure the patient from the parasite infection. The effect of drugs on Giardia differ from one drug to another, either by affecting growth or both growth and adherence (Jokipii and Jokippi, 1980; Gillin and Diamond, 1981; Smith et al 1982; Boreham et al 1984). With the ELISA test we are able to demonstrate the clearance of the parasite from the patient using metronidazole.

Several drugs are available for effective treatment of positive cases, but therapeutic failures are seen occasionally (Levi et al, 1977; Kavons; 1979; Mendelson, 1980; Carft et al 1981; Upcroft et al 1990). No agent was recommended for mass chemotherapy, due to their side effects and expense. The treatment of positive cases can help in the parasitic control by reducing the source of infection.

CHAPTER 8

SUMMARY OF RESULTS

8.1. ELISA test to detect Giardia antigens in the stool

It was found that the ELISA test gave good results, when we used a Costar ELISA plate No.3590 (Centrified Surface Chemistry). The plates were coated with 200µl of affinity purified pooled Giardia antisera at a concentration of 15µg/ml (3µg/well) in carbonate coating buffer pH 9.6 in each well with incubation at 4°C overnight. 200µl of a mixture of equal volumes of FCS and a 10gm% suspension of stool sample in PBS was added to each well and incubated for one hour at 37°C. 200µl of affinity-purified rabbit-anti-Giardia-peroxidase conjugate at dilution 1:800 in 50% FCS in PBS-Tween was added to each well with incubation for 90 minutes at room temperature. 200µl of substrate solution (25ml citrate phosphate buffer pH5.0 + 8.5mg OPD + 2.5µl H_2O_2) was added to each well with incubation for 60 minutes in the dark at room temperature, 50µl of 2.5M HCl was added to each well to stop the reaction. The optical density was read with an ELISA plate reader at 492nm.

3918 stool samples (which were collected from different groups of people during the study period (Table 8.1) were examined with three different diagnostic methods to compare their results in detection of Giardia. We found that 7.6%, 11.5% and 17.5% of total stool samples were positive for Giardia with direct microscopy, formol-ether concentration test and ELISA test to detect Giardia antigens respectively.

The results of microscopy were compared with ELISA data and showed an ELISA sensitivity of 95.6% (Table 8.1).

Table 8.1. **Comparing the results of examining stool samples from different groups of people with three different diagnostic tests and the sensitivity of the ELISA test in each group**

Stool samples collected collected from:	Stool samples examined with Direct Microscopy			Concentration test			ELISA test				
	-ve	+ve	Total	-ve	+ve	Total	-ve	+ve	Total	False -ve	Sens-itivity
People in the village in first survey round	276	10	286	264	22	286	223	63	286	1	95.5%
People in the village in second survey round	317	22	339	309	30	339	259	80	339	4	86.7%
People in the village in third survey round	322	30	357	318	39	357	273	84	357	4	89.7%
the children in the village	1567	152	1719	1466	253	1719	1388	331	1719	9	96.4%
the children during the treatment time	39	17	58	35	23	58	32	26	58	2	91.3%
the expatriates newly arrived to UAE	1093	66	1159	1074	85	1159	1054	105	1159	0	100%
Total	3619	297	3918	3466	452	3918	3229	689	3918	20	95.6%

ELISA test was found detecting Giardia antigens on average 2-4 stool samples earlier than microscopical and concentration test (El Kadi et al. 1992).

8.2. Epidemiology of Giardia in the village

The total number of population in Wadihaloo village were found to increase in the following survey rounds, as 292, 341 and 364 people in the first, second and the third survey rounds respectively.

The majority of the population in the village (78%) were local (from UAE), and the rest were expatriates from Pakistan, Bangladesh, India, Sri Lanka and Egypt.

46.2% of the population in the village were educated and they were mainly aged between 7 and 24 years.

22.2% of the population in the village respectively were working as farm workers and the rest of them were students, housewives, policemen, watchmen, salesmen, teachers, housemaids, labourers, tailor and driver. 21.2% of them were working outside the village.

There were two types of houses in the village, modern houses which were built of blocks and concrete, with electricity and central water supply and contain a modern latrine. And simple houses which were built of wood and aluminium sheets (mainly in the farms), with no latrines, no electricity and no central water supply. 87.3% of the population in the village (in the first and the third survey rounds respectively) lived in modern houses, and 83.2% to 87.7% of the population were drinking water mainly from the central supply, which reached the houses through closed pipes. The rest of the population were drinking water from different

wells in the farms.

The prevalence of Giardia among the people in the village increased from 22% in the first round to 23.5% in the third survey round. The prevalence of Giardia was found to be less common amongst the males (22%) than amongst the females (25.5%) in the third survey round.

17.2% and 20.6% were the prevalence of Giardia amongst the educated people in the village in the first and the third survey rounds respectively, while 15.7% and 15.5% were the prevalance among the non educated people in the first and the third survey rounds respectively.

The prevalence of Giardia was found to be more common amongst the local people (25.3%) than amongst the expatriates (19.5%) in the village.

The highest prevalence rate of Giardia was found amongst the people without work (including children below 6 years, unmarried women and old people). Also Giardia was found amongst all occupational groups. Giardia infection was found amongst all ages in the village, except in age above 60 years. The highest prevalence rate was found amongst the children aged between 0-6 years.

Indigenous Giardia was more common in the village as the prevalence of Giardia was higher amongst the people staying inside the village.

Both groups of people in the village (who lived in modern houses or in simple houses) were found to be infected with Giardia. The prevalence of Giardia was higher amongst the people who lived in modern houses.

No significant relationship was found between the source of drinking water and prevalence of Giardia. The prevalence rate of Giardia was high amongst the people drinking water from the central supply.

Giardia infection was found amongst the people in the village who either used or did not use the latrine. The prevalence of Giardia was higher amongst the people who used the latrine.

Giardia infection was not the main cause of diarrhoea in the village, as only 25% of the diarrhoea cases were associated with Giardia.

Not only the Giardia infection was found in the village, other intestinal parasites were also found with a prevalence rate of 11.2%. Some of the stool samples were found to contain Giardia with other intestinal parasites.

8.3. Expatriates as a source for imported Giardia

1159 newly arrived expatriates to UAE were examined in the labour clinic in Kalba hospital during the study period. 76.9% of them were males, the expatriates came from different countries. The largest number (26.2% of total) came from Bangladesh.

64.1% of the expatriates were working as food handlers. The largest number (35.6% of the food handlers) were housemaids.

The prevalence of Giardia among the expatriates was found to be 9.1%. The prevalence was high (10.8%) at younger ages (below 30 years) and it was low (5.8%) at older ages (above 30 years).

Giardia infection was found amongst the expatriates from all nationalities except the Iranian. The Bangladeshi expatriates were found to have the highest rate of Giardia prevalence.

8.6% of the food handlers were found to be infected

with Giardia.

The expatriates not only brought Giardia with them, they brought other intestinal parasites also as 53.7% of them were found to be infected with other intestinal parasites.

8.4 Giardia infection of preschool children in the village

68 children from the village at preschool age (below 6 years) were included in the study. The youngest child in the study was aged 0.4 months and the oldest child was aged 56.3 months.

1719 stool samples were collected from the 68 children. 331 (19.26% of the total stool samples) were found positive for the Giardia.

29 (42.7% of the total children) were found infected with Giardia, the youngest child infected with Giardia was female aged 8 months.

The group of children in this study were found to be infected with Giardia at all ages (from 1-6 years). The highest prevalence rate (46.6%) was found amongst the children aged between 60-71 months.

Giardia infection usually occurred throughout the year, but mainly between August and April.

15 stool samples (4.5% of the positive stool samples with Giardia) were found diarrhetic. 55 stool samples (3.2% of the total stool samples) were found positive for other intestinal parasites, 11 of the stool samples were found positive for Giardia also.

13 cases (0.8% of the total stool samples) were found positive for Shigella dysenteriae, two cases of them were found positive with Giardia as well.

14 cases (0.8% of the total stool samples) were found positive for rotavirus, four of them were found positive with Giardia as well.

203 visits (80.9% of the total visits done by the children at the first year of life were in breast fed children, 81 (39.9%), 75 (36.9%), 25 (12.3%) and 22 (10.8%) of them related to breastfeed only, breast and bottle fed, breast and food, and breast, bottle and food respectively. 118 visits (28.3% of the total visits done by the children in the second year of life) were in breast fed children, 2 (1.7%), 18 (15.3%), 38 (32.2%) and 60 (50.8%) of them related to breast fed only, breast and bottle fed, breast and food, and breast, bottle and food respectively.

Only 25 cases (7.5% of the total positive cases with Giardia were found in breast fed children, 7 (28%), and 18 (72%) of them related to breast and food fed and breast, bottle and food fed respectively.

26 cases (34.7% of the total cases with diarrhoea) were found related to breast feeding, 9 (34.7%), 7 (26.9%), 5 (19.2%) and 5 (19.2%) of cases were related to breast feeding only, breast and bottle fed, breast and food fed, and breast, bottle and food fed respectively.

3 cases of colitis (23.1% of the total colitis cases) were found related to breast fed, one (33.3%) and 2 (66.7%) of them were related to breast and food fed, and breast, bottle and food fed respectively.

2 cases (3.6% of the total cases positive for other intestinal parasites) were found related to breast feeding, one (50%) case was related to breast fed only and the other one was related to breast and bottle fed.

4 cases (30.8% of the total cases with Shigella dysenteriae) found related to breast feeding.

7 cases (50% of the total cases with rotavirus) were related to breast feeding.

A decrease was found in the Median and Mean of the weight and the height of the children with Giardia infection.

A significant change was found in the weight and the height among the children three months before and after Giardia infection.

CHAPTER 9

CONCLUSION, DISCUSSION AND FUTURE PROSPECTS

Giardia intestinalis is a common human intestinal protozoan parasite found in industrialised and less developed countries (Harter et al, 1982; Boreham et al, 1981). Giardia intestinalis was found in Wadihaloo village (UAE), with a prevalence rate with faecal ELISA of 22%. UAE was found to be suffering mainly from the indigenous transmission of Giardia, and to a lesser extent from imported Giardia which was brought in through the expatriates (with a prevalence rate of 9.1%).

Giardiasis may cause problems for both the patient and the physician, as a large proportion of infected individuals are asymptomatic (Lopez et al, 1980; Jokipii, 1971), and some of them have severe diarrhoea, intestinal malabsorption and weight loss (Dupont and Sullivan, 1986). The children (below 6 years) in the village were found to be infected with Giardia (with a prevalence rate with faecal ELISA of 32.2%), and this fact also appeared to have some effect on their physical growth. To give a clearer picture as to the actual effect this fact had on their growth it would have been necessary to assess a larger number of children in the study, who had all been the same age (i.e. from birth) and for the study period to have been from 3-5 years duration.

Andrews et al (1981) found that immune milk may protect from Giardia infection and our results concurred with this view as in the village most of the children up to 2 years old were breastfed and the prevalence of Giardia in this age

group was found to be less than other age groups.

The main problem with Giardia infection remains its easy transmission and difficulty in diagnosis. In the face of rising health care costs, medical parasitology laboratories need to reassess their approach to the diagnosis of enteric parasites (Isaac-Renton, 1991). The clinicians and the epidemiologists are also looking for reliable, rapid, easy and cost-effective diagnostic procedures for all enteric parasites of medical importance.

In our study we found that the ELISA test to detect Giardia antigens in the stool was more reliable and faster than direct microscopy and formol-ether concentration test (in the case of examining a larger number of stool samples). The ELISA test (using 45 samples in each plate) takes on average 4 hours, while examining 45 samples with microscopy takes approximately 6 hours (an average of 8 minutes for each sample). However, microscopy methods are much easier (when using a small number of samples), safer (no carcinogenic materials like OPD are used) and more cost effective as each stool sample costs on average £5.00 (1991 price) (PROSPECT/Giardia diagnostic ELISA test (Alexon, Inc, Montana View, Calif, USA). All of the afore mentioned diagnostic methods need to be carried out by skilled technicians.

The work done so far in our study suggests that there is a large amount of non particulate Giardia diagnostic antigen secreted in faeces of infected patients. Rapid visually readable slide based coagglutination methods have been used for detection of rotavirus (Hughes et al, 1984), and adaptation of the Giardia two-site ELISA to dipstick form, as has been done for many other enzyme immunoassays (Shekarchi et al, 1982; Norman et al, 1985), is still worthy of consideration.

Such methods would allow the economic processing of small numbers of, or individual samples by completely unskilled personnel in remote areas.

We found the best way to solve the problems of Giardia diagnosis in the clinical (for medical purposes), or in the field (for epidemiological study) were:

1. In the clinics: if any case of Giardia is suspected, the first stool sample will be examined using direct microscopy. If it is negative, after three days the second stool will be collected and part of it will be examined using direct microscopy, if it is negative, another part of the second sample will be examined using formol-ether concentration test, if this is negative, another part of the second sample will be examined using the ELISA test (in a group).

2. In the field: each stool sample of all the samples will be divided into three parts. The first part of all the stool samples will be examined using direct microscopy, the second part of the negative stool samples will be examined using formol-ether concentration test and the third part of the remaining negative stool samples will be examined using ELISA test.

Strategies for the control of giardiasis must centre on the effective elimination of faecal contamination and adequate recognition and treatment of carriers (Jones, 1991).

To control any imported positive Giardia cases to the country, it is necessary to collect a stool sample from every

expatriate, newly arrived or returning from his annual leave, and their attendant family, and even from the locals or the visitors and their attendant families (if they have come from known endemic areas), as soon as they arrive (within 3 days) to avoid any possibility of the infection spreading.

Stool samples from the food dealers will be re-collected every 6 months, and all of the stool samples will be examined in the same way as used in the clinic (see above), and all of the positive cases should be treated immediately.

Wadihaloo village is a small village, similar to several villages in the UAE, they all have similar housing structures, population profiles and habits. The number of local people increases every year, but their percentage of the total population decreases every year because the village becomes dependent on the expatriates.

We think most of the Giardia transmission in the village was caused by the direct method (with contaminated hand to the mouth), this is due to bad hygiene habits of people. The reasoning behind our thinking is the fact that no Giardia cysts were found in the water or in the stool samples of the animals.

We think Giardia infection could be controlled in the village if:

1. The people (especially the mothers) in the village were given health education about Giardia infection and the ways of protection from it.

2. In any case of abdominal pain or diarrhoea a stool sample should be examined for Giardia and if found positive, all family members should have stool

samples checked for the presence of Giardia cysts.

3. One stool sample of every person in the village should be taken once every year which should be examined for Giardia.

4. Stool samples of the children at pre-school age (below 6 years) should be examined every three months, and if any child is found to be positive stool samples from all family members should be checked.

5. All the stool samples should be examined using the method mentioned above for use in the field. All positive cases should be properly treated.

6. The mothers should be encouraged to continue breastfeeding their children up to the end of the second year of life.

7. The government should build a clean place with modern latrines for the children to play in rather than them having to play in the street.

8. The people should be encouraged to boil the drinking water for three minutes or use filters (pore size 3μm).

9. The main source of drinking water should be treated, and random water samples should be examined for Giardia cysts.

10. The animals waste should be dried for 7 days before being used as a fertilizer for the plants. The animals should be kept away from the houses and random stool samples from them should be checked for Giardia cysts.

APPENDIX

1. Giardia axenic culture medium: TY1-S-33 added bile (Keister, 1983)

Basic medium:

K_2HPO_4	2.0g
KH_2HPO_4	1.2g
Trypticase (bio Merieux - 54641)	40g
Yeast extract (Difcol Bacto, 0127-02)	20g
Glucose	20g
NaCl	4g
Cysteine HCl monohydrate (Sigma C7880)	4g
L-ascorbic acid	400mg
Ferric ammonium citrate	45.6mg
Dehydrated bovine bile (Sigma B83181)	2g

Corrected to pH 7.0 with 1M NaOH, volume made up to 1800ml with distilled water. Filtered through Whatman No.1, then filter sterilised (0.45µm), dispensed into 100ml sterile bottles and stored at -20°C.

This comprised basic medium with 10% v/v heat inactivated foetal calf serum (Sera-lab 001084), and gentamicin 100µg/ml, made up just prior to use.

2. Phosphate buffer saline (PBS) pH 7.2
(10x concentrate)

NaCl	400g
KH_2PO_4	10g
$Na_2HPO_4\ 12H_2O$	145g
KCL	10g

distilled water to 5 litres
The 10X stock solution was stored at room temperature

3. MRC Ringer (NaCl 0.9gm, KCl 0.02gm, $CaCl_2$ 0.02gm; H_2O 100ml)

4. Lysis buffer

Tris	0.242g
NaCl	0.82g

Nonidet P40 (Sigma N6507) *0.5ml*
Dist. H_2O added to 100ml, pH adjusted to 8.0
with 1M HCL

Protease inhibitors were added to the following final concentrations

PMSF (Sigma P7626)	1mM
TLCK (Sigma T7254)	10µg/ml
Iodacetamide (BDH)	1mM

5. Coupling buffer
0.1M $NaHCO_3$, pH 8.3 containing 0.5M NaCL

6. Sodium acetate buffer (0.1M, pH 4)

Solution A:	acetic acid 0.1M NaCL 0.5M	100ml
Solution B:	sodium acetate 0.1M NaCL 0.5M	100ml

to 83ml of solution A, was added solution B to adjust buffer pH to 4.0.

7. Carbonate coating buffer: pH9.6

Na_2CO_3	0.795g
$NaHCO_3$	1.465g

distilled H_2O to 500ml

8. ELISA substrate buffer: phosphate citrate buffer pH5

Citric acid	10.505g in 500ml distilled H_2O
Na_2HPO_4	7.095g in 500ml distilled H_2O

to make up substrate solution just prior to use 10mg table tof orthophenylene diamine (OPD, Sigma) was dissolved in 29.4ml of phosphate citrate buffer and then 3µl of 30% H_2O_2 (BDH) was added.

9. Questionnaire for Demographic Survey in Wadihaloo Village (UAE)

Round Number:

Family Code Number Personal Code Number

Date of Birth Day Month Year

Sex: 1 Male 2 Female

Nationality: 1 UAE 2 Pakistan 3 Bangladesh

4 India 5 Sri Lanka 6 Egypt

Relationship 1 Head of the House 2 His wife 3 His son
4 His Daughter 5 His father 6 His mother
7 His brother or sister 8 His employer 9 Others

Education Number of years

Occupation 1 Farmworker 2 Housewife 3 Without work
4 Student 5 Policeman 6 Housemaid
7 Watchman 8 Salesman 9 Teacher
10 Labourer 11 Tailor 12 Driver

Place of Work 1 Inside the Village 2 Outside the village

Place of living 1 In the same house 2 In the farm

Type of House 1 Modern house 2 Simple house

Drinking water 1 From central supply 2 From the well

Type of latrine 1 Modern latrine 2 No latrine

Number of times leaving the village times/year

Number of days staing outside the village days/year

Maximum bumber of times loose motion times days

Number of the farms belong to the family

10. Questionnaire for study the effect of Giardia on children physicial growth

Child Code Number Family Code Number

Date of Birth day month year

Sex 1 Male 2 Female

Number of Visits:

Date of Visit day month year

Maximum number of times of loose motion times days

Does the child take any medicine since last visit 1 No 2 Yes (name of medicine)

Feeding 1 Breast 2 Bottle 3 Food
4 Breast + Bottle 5 Breast + food 6 Bottle + Food
7 Breast + Bottle + Food

Medical examination

1 Normal	2 Pneumonia	3 Common Cold
4 Toothache	5 Bronchitis	6 Tonsilitis
7 Otitis media	8 Conjunctivitis	9 Colic pain
10 Stomatitis	11 Dermatitis	12 Otitis externa
13 Bronchial asthma		

11. Questionnaire to study the newly arrived expatriates in importing Giardia

Date Day Month Year

Sex 1 Male 2 Female

Date of Birth Day Month Year

Number of days since arrived in UAE: Days

Nationality

1 Pakistan 2 Bangladesh 3 India
4 Sri Lanka 5 Egypt 6 Philippines
7 Syria 8 Iran

Occupation

1 Cook 2 Farm workers 3 Housemaid
4 Fisherman 5 Grocer 6 Shepherd
7 Others

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Short Report

Early diagnosis of giardiasis by faecal antigens detection using capture ELISA in a cohort of children in the United Arab Emirates

I. A. Elkadi, D. H. Smith and M. Hommel
Department of Tropical Medicine and Infectious Disease, Liverpool School of Tropical Medicine, Liverpool, L3 5QA, UK

Giardia lamblia is a common human enteric protozoan pathogen causing a spectrum of disease which varies from asymptomatic to severe diarrhoea, intestinal malabsorption and weight loss (DUPONT & SULLIVAN, 1986). Infants and young children appear to be particularly susceptible to infection, which is believed to contribute to growth impairment in the developing world (FARTHING *et al.*, 1986), infections are also frequent in travellers, 'back-packers', and homosexuals. The organism is found worldwide in industrialized and technologically sophisticated societies, as well as in the traditional and transitional communities of the developing world (OYERINDE *et al.*, 1977).

Diagnosis of giardiasis relies largely on the microscopical detection of stages of the parasite (trophozoites and cysts) in faeces, but this is labour- and time-intensive and depends on the skill of an experienced observer (KAMATH & MURUGASU, 1974). It was found that, even with patients with known giardiasis, up to 15% of individuals may be microscopically negative after examination of 3 consecutive faecal specimens. False negative are due to the poor sensitivity of microscopical methods and also to the fact that cyst excretion rates are highly variable in patients from one day to another (DANEIGER & LOPEZ, 1975). The alternative methods of small bowel biopsy or duodenal aspiration are invasive and impractical, especially in children.

A reliable enzyme-linked immunosorbent assay (ELISA) for the detection of faecal antigens of *Giardia* would be expected to improve the detection of cases. A capture ELISA using affinity purified antisera to *Giardia* antigen has been described, with sensitivity and specificity of 98% and 100% respectively, in cases of clinical giardiasis in the United Kingdom (GREEN *et al.*, 1985). When the test was evaluated in a field study in Chile, the sensitivity and specificity of detecting faecal *Giardia* antigens were 99% and 96% respectively (GOLDIN *et al.*, 1990). Longitudinal studies can provide an idea of the value of the antigen capture assay as a method for the detection of giardiasis, at a time when parasites are not excreted. Here we report the application of the method for early detection of giardiasis in the longitudinal follow-up of a cohort of 68 children below 6 years of age, performed in the village of Wadihaloo in the United Arab Emirates.

Wadihaloo is an isolated mountain village, 35 km from the nearest town, Kalba. Stool samples were collected from each child every 2–3 weeks for a period of 18 months, and the following tests were performed on each sample: (i) fresh stool microscopical examination (2–4 h after collection); (ii) formol-ether concentration with staining by Lugol's iodine, performed on samples stored overnight at 4°C and (iii) an ELISA on faecal suspensions in phosphate-buffered saline (PBS, pH 7·2). Reagents for the ELISA were prepared as described by GREEN *et al.* (1985).

A total of 1719 stool samples was collected from the 68 children; the average number from each child was 27, with an average interval of 2 weeks between each sample.

The proportions of faecal samples positive for *G. lamblia*, by routine microscopical examination, formol-ether concentration, and faecal antigen capture ELISA were 8·84%, 14·72% and 19·26% respectively (Table). The positive-negative cut-off value for the ELISA was the arithmetic mean of the negative samples +3 standard deviations.

Table. Results of different stool examination methods applied to the diagnosis of giardiasis

Results	Stool examination for *Giardia* Routine microscopy	Concentration method	ELISA
Positive	152 (8·84%)	253 (14·72%)	331 (19·26%)
Negative	1567 (91·16%)	1466 (85·28%)	1388 (80·74%)
Total	1719 (100%)	1719 (100%)	1719 (100%)

The examinations showed that 29 of the children were infected with *G. lamblia* (Figure); 22 children were positive by capture ELISA, on average 2–4 stool samples earlier than by direct microscopy and or concentration. One child was positive by capture ELISA in one stool sample after microscopical confirmation by concentration, and another child was positive by capture ELISA only. Five children were positive by capture ELISA as well as by concentration at the same time.

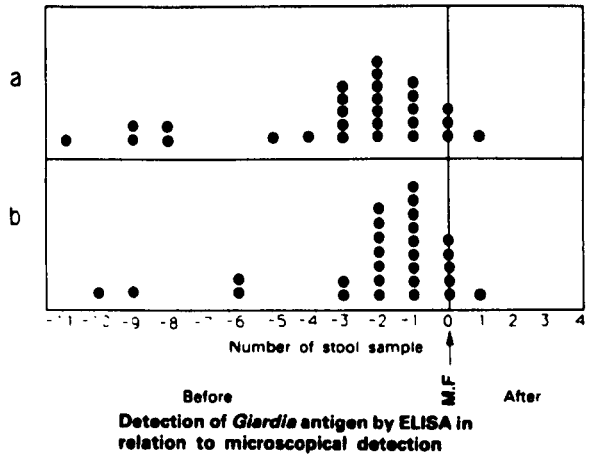

Figure. The time of detection of *Giardia* antigen by enzyme-linked immunosorbent assay, in stool samples taken at two-weekly intervals, in relation to the time of detection of the parasite by microscopical examination of stool samples M.F. a, *Giardia* detected by routine microscopical examination; b, *Giardia* detected by formol-ether concentration. One child was positive by enzyme-linked immunosorbent assay only.

We believe that the antigen capture ELISA is an effective means of diagnosis of giardiasis, applicable both for clinical case finding and epidemiological studies.

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Short Report

Induction of metronidazole and furazolidone resistance in *Giardia*

Steven M. Townson, Helen Laqua*, Peter Upcroft, Peter F. L. Boreham and **Jacqueline A. Upcroft**
Queensland Institute of Medical Research, The Bancroft Centre, 300 Herston Road, Herston, Brisbane, Queensland 4029, Australia

The nitroimidazoles are accepted as the drugs of choice for treating giardiasis in most countries (DAVIDSON, 1984). Furazolidone, a nitrofuran, which is especially useful in young children, is a recommended drug but is no longer available in Australia. Treatment failures with both classes of drugs are not uncommon and drug resistance has been indicated as one cause. Cross resistance between the 5-nitroimidazoles is a further complication to successful treatment (BOREHAM *et al.*, 1991).

Metronidazole is the most commonly used 5-nitroimidazole with potent antiprotozoal activity as well as activity against many obligate anaerobic organisms. However, resistance to metronidazole has been demonstrated in trichomonads and in *Bacteroides fragilis*, both in natural populations and induced in the laboratory under drug pressure (MEINGASSNER *et al.*, 1978; BRITZ & WILKINSON, 1979). Resistance to furazolidone has also been demonstrated in strains of *Salmonella enteritidis* isolated from poultry following prophylactic administration of nitrofurantoin (RAMPLING *et al.*, 1990).

Previously we reported a laboratory-induced metronidazole-resistant line of *Giardia intestinalis* which grows in low, sub-lethal concentrations of the drug (BOREHAM *et al.*, 1988). We report here on the laboratory induction of both metronidazole and furazolidone resistant lines of *Giardia* which grow in concentrations of drug lethal to the parent stock.

Drug-resistant lines were derived from 4 strains, BAC2, OAS1, WB1B and BRIS 87 HEPU 713, which differed in karyotype and in geographical and host origin (CAPON *et al.*, 1989) (Table 1). *Giardia* trophozoites were grown and harvested as previously described (BOREHAM *et al.*, 1984). Resistance to both metronidazole and furazolidone was induced in all 4 strains by the following methods:

(i) *Intermittent drug exposure.* Trophozoites were subjected to drug for 48 h *in vitro* and allowed to recover before further drug exposure. The initial concentration of drug was the respective ID_{50} value for each strain, i.e. the concentration of drug required to inhibit the uptake of [^{3}H]thymidine by 50% (BOREHAM *et al.*, 1984) (Table 1). At successive treatments the level of drug was increased to allow survival of 10–20% of the trophozoites. After 13 weeks, the exposure time was reduced to 24 h for each treatment and this regimen was continued for a further 17 weeks. Lines derived from intermittent metronidazole and furazolidone exposure are designated M1 and F1 respectively (Table 2).

(ii) *Constant drug exposure.* The 8 drug-resistant lines (4 metronidazole- and 4 furazolidone-resistant lines derived after intermittent drug exposure was completed) were

Table 1. Characteristics of *Giardia* strains used in this study

Strain	Host	Geographical origin	ID_{50} (μM)[a] Metronidazole	Furazolidone
BAC2	Cat	Perth, Western Australia	0·93	0·90
OAS1	Sheep	Calgary, Canada	0·75	0·90
WB1B	Human	Afghanistan	1·15	0·50
BRIS/87/HEPU/713	Human	Brisbane, Queensland Australia	0·85	1·40

[a]The concentrations of metronidazole and furazolidone which inhibit the uptake of [^{3}H]thymidine by 50% (BOREHAM *et al.*, 1984).

Table 2. The final concentration of metronidazole and furazolidone used in the induction of drug-resistant *Giardia* lines in the laboratory

Drug-resistant lines[a]	Final drug concentration (μM)
BAC2-M1	397
BAC2-M2	11·7
OAS1-M1	397
OAS1-M2	11·7
WB1B-M1	397
WB1B-M2	11·7
WB1B-M3	115
BRIS/87/HEPU/713-M1	397
BRIS/87/HEPU/713-M2	11·7
BRIS/87/HEPU/713-M3	85
BAC2-F1	45
BAC2-F2	8
BAC2-F3	34
OAS1-F1	40
OAS1-F2	8
OAS1-F3	56
WB1B-F1	40
BRIS/87/HEPU/713-F1	38
BRIS/87/HEPU/713-F2	8

[a]M1, M2 and M3 designate metronidazole-resistant *Giardia* lines induced by intermittent drug treatment, constant drug treatment and ultraviolet light mutagenesis, respectively. Similarly F1, F2 and F3 designate furazolidone-resistant lines.

*Present address: Richard Kirchner Str. 33, 3590 Bad Wildungen, Germany.
Address for correspondence: Dr J. A. Upcroft, Queensland Institute of Medical Research, The Bancroft Centre, P.O. Royal Brisbane Hospital, Brisbane, Queensland 4029, Australia.