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Amoebiasis and Giardiasis

The Global Impact of Two Common Intestinal Protozoan Infections

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Summary

Intestinal protozoan infections involving humans are by no means confined to tropical/subtropical countries; however, it is here that maximal prevalence, and consequently, morbidity, assumes a major practical importance. Coccidial infections (*Cryptosporidium* spp., *Isospora belli*, *Sarcocystis hominis* and *Cyclospora* spp.), *and Blastocystis hominis* and *Microsporidium spp.*, which were previously underrecognised, have come to the fore in the present era, largely in association with HIV infection. Nevertheless, these organisms can induce self-limiting infection in travellers and other individuals in countries in which standards of sanitation/public health are less than satisfactory. Overall, however, there can be no doubt that *Giardia lamblia* (also known as *G. intestinalis* and *G. duodenalis*) and *Entamoeba histolytica* are numerically the most important protozoan parasites to involve the gastrointestinal tract. Whereas trophozoites of the former organism are virtually confined to the small intestinal lumen, the extra-intestinal manifestations of *E. histolytica* infection are of greater importance than its colorectal pathogenic properties.

This review concentrates on the prevalence of the 2 infections in different populations; this reflects both incidence and outcome, including duration of illness. Epidemiology is also addressed. Routes of transmission of the 2 infections are covered, together with morbidity and, in the case of *E. histolytica* infection, mortality also.

When viewed within a broad scenario, data collected over the last 15 to 20 years suggest that prevalence and transmission patterns of *Entamoeba histolytica* infection have not changed significantly during that period.[1] However, increasing migration of the population into urban areas in developing countries, coupled with an increase in the size of urban slums and wars (frequent in these countries), are probably accelerating the spread of infection; consequently, resultant disability might well be greater in future years. Whereas *E. histolytica* infection causes a great deal of morbidity and mortality (see sections 1.4 and 1.5), this is less than that caused by the major 'killers' in developing countries, such as diarrhoeal disease, *Plasmodium falciparum* infection, tuberculosis (and other respiratory tract problems), schistosomiasis and childhood infections.

1. *Entamoeba histolytica* Infection

1.1 Prevalence of *E. histolytica* Worldwide

In a world context, *E. histolytica* is very widespread and produces an enormous burden of morbidity and mortality resulting from colitis and invasive hepatic disease (liver 'abscess').[1] Some 10% of the world's population harbours this protozoan parasite (in most as the encysted form), al-

though only a small proportion experiences disease. In certain countries, e.g. Mexico, this infection constitutes one of the leading 10 causes of death. In 1984, it is probable that 500 million individuals were infected with *E. histolytica* and, of these, 40 million experienced resultant disabling colitis or extra-intestinal disease. Furthermore, at least 40 000 deaths during that year were estimated to have resulted from this infection.[1] The accuracy of reported prevalence rates depends very largely on the quality of diagnostic procedures, which are principally parasitological and serological, although seroepidemiological surveys are very important. Recorded prevalence is also dependent on the enthusiasm of medical/scientific workers in a particular locality in reporting disease, and in most developing countries this is inadequate. Many surveys have used a nonrepresentative population sample;[1] even hospitalised patients supposedly without intestinal symptoms can, for example, be construed as a representative sample in a survey of *E. histolytica* infection. Furthermore, most surveys conducted outside hospital do not mention the sampling technique used to ensure a random sample, and patient compliance is not always stated.

Those experiencing diarrhoea (or another illness) may be more willing to give a faecal and/or blood sample, in the hope of benefiting from the study. Interestingly, an age-related antibody response to *E. histolytica* has been documented in India.[2] It is also essential to appreciate that with both of the infections covered in this review excretion of cysts tends to be intermittent. In addition, many surveys do not state the number of faecal specimens examined, or indeed the interval between their collection. Arroyave et al.[3] have stressed that circannual variability of infection with *E. histolytica* in Mexico should be carefully taken into account in intervention studies.

There are huge differences in the prevalence rates of *E. histolytica* infection at different geographical locations. Using available serological techniques, reported differences in prevalence vary from 0 in Surinam blood donors to 57% in parts of rural Tanzania;[1] faecal parasitological surveys have given a range of 0 in rural parts of the Dominican Republic to 49.4% in urban Peru. No method assessing the potential pathogenicity of *E. histolytica* (i.e. its zymodeme status) is currently in routine use.

1.1.1 Geographical Evidence for Local E. histolytica *Prevalence*

Probably the most reliable data available for prevalence rates comes from Mexico and, to a lesser extent, other parts of South America. In northeastern Brazil, 24 of 334 serum samples examined by an enzyme-linked immunoabsorbent assay (ELISA) gave positive results for *E. histolytica* antibody.[4] In a suburban community with low socioeconomic status in Venezuela, *E. histolytica* infection prevalence (as assessed by faecal microscopy) was 8.7% (29 of 342), with most cases being cyst carriers.[5] The Republic of South Africa is another country from which extensive data are available.[6] In a series of 5087 adult admissions involving invasive amoebiasis to a medical unit at Durban, intestinal and liver disease accounted for 60% and 40%, respectively, and the mortality rate for both forms of disease was 1.9%. In the same city, the overall mortality rate from intestinal disease was 27% (see also section 1.4).

In a study carried out on the Thai-Cambodian border, the highest incidence of amoebic dysentery was 63 per 1000 (6.3%) in children 12 to 23 months old.[7] On the West Bank of Jordan, during 1981-1986, of 22 900 faecal samples obtained from patients attending the Central Medical Laboratory at Nablus,[8] *E. histolytica* was present in 22.9%. As with other intestinal parasites, the peak incidence occurred during the summer and early Autumn, with a low incidence in winter and early spring. In a survey at Mahe, Seychelles, 21 of 313 cultures grew *E. histolytica,*[9] and when zymodeme analysis was employed, 8 organisms were pathogenic and 40 nonpathogenic (*E. dispar* as defined by Brumpt[10] see section 1.3.1).

Despite these findings, in some tropical countries the prevalence rate is low. In Jamaica, a recent report of 3 cases of such infection represented the

first documentation of amoebiasis there for more than 2 decades. Also, invasive hepatic disease can occur in individuals who have never travelled to an endemic area.[11] These authors have recorded a case contracted in Australia. In Europe and North America, the disease usually occurs in well defined groups, e.g. recent travellers[12] and immigrants, active homosexual men,[13,14] and those confined to institutions.[15]

1.1.2 Prevalence of E. histolytica *in Special Groups*

Analysis of faecal samples from 2700 individuals who travelled from a tropical to a temperate country [16] showed that 4.0% had evidence of *E. histolytica* infection, but in only 5 was a pathogenic zymodeme identified. The authors concluded that travellers to the tropics had a 0.3% (1:340) risk of acquiring invasive amoebiasis, and a 92.3% risk of an *E. histolytica* infection remaining asymptomatic. In a group of travellers to Phuket, Thailand, *E. histolytica* infection showed a significant relationship to consumption of drinks containing ice, ice-cream, and raw fruit in ice.[17] 525 Czechoslovaks who had worked in 50 tropical and subtropical countries in Asia, Africa, and Latin America were tested for evidence of *E. histolytica* infection.[18] A total of 74 (14.1%) were infected with one or more intestinal pathogen, and 3.8% of the organisms were identified as *E. histolytica.*

E. histolytica does not act as an 'opportunistic' infection in the presence of HIV infection. A recent study in Mexico has confirmed that patients with AIDS did not have an increased prevalence of infection.[19] In another study, 16.3% of homosexual men were infected.[16] Sorvillo et al.[20] have concluded that 'amoebiasis trends (as a result of the association with male homosexuality) may be a useful predictor of human (HIV) transmission and future rates of AIDS among gay men'.

218 residents in a nursery, a foster home, and a rehabilitation centre for handicapped children at Abha, Saudi Arabia, were examined for the presence of intestinal parasites.[21] Approximately 30% were shown to possess an asymptomatic infection with either *Entamoeba* spp. or *Giardia lamblia* (also known as *G. intestinalis* and *G. duodenalis*); *E. histolytica* was present in 18.4% of the residents at the rehabilitation centre. In a 190-patient institution for mentally retarded individuals in Japan, 20% had either cysts or trophozoites of *E. histolytica* in a faecal sample;[22] 38% of them were serologically positive.

1.2 Epidemiology of *E. histolytica* Infection

High prevalence rates of *E. histolytica* infection in developing countries are associated with poverty, poor sanitation, overcrowding, and a warm climate.[6] Available epidemiological data are limited for several reasons, including a) a tendency to include the most accessible people, i.e. those living nearest to the road; b) intermittency of cyst excretion; c) the fact that faecal analysis reflects prevalence at one point in time and not the actual prevalence of infection (see above), which might approach 100% in an endemic area; d) variability in the laboratory methods used;[23] and e) lack of suitable/consistent criteria for diagnosing the disease and defining its severity. Most data relating to prevalence have been obtained from faecal analysis and serological surveys. *E. histolytica*-specific serum IgG, IgA, IgM, and IgE antibodies might prove to be valuable indices in the detection of infection in an endemic area.[24]

Several host factors influence the prevalence/epidemiology of severe disease. Overall, the infection is more common in children than in adults,[6,25] and it is more often encountered in men, in pregnant women, during the summer months, and in association with undernutrition and altered iron status. A high prevalence of HLA-DR3 has been recorded in patients with invasive hepatic disease among Mexican children;[26] however, further studies are required. A study carried out in rural India[27] suggested that '... invasive *E. histolytica* infection (mostly asymptomatic) evokes good gut immunity in the host with clearing of the parasite from the colon and/or resistance to reinfection', and that a 'high prevalence of amoebic antibodies indicates good 'herd' immunity'. Moreover, pathogenic zymodemes are essential

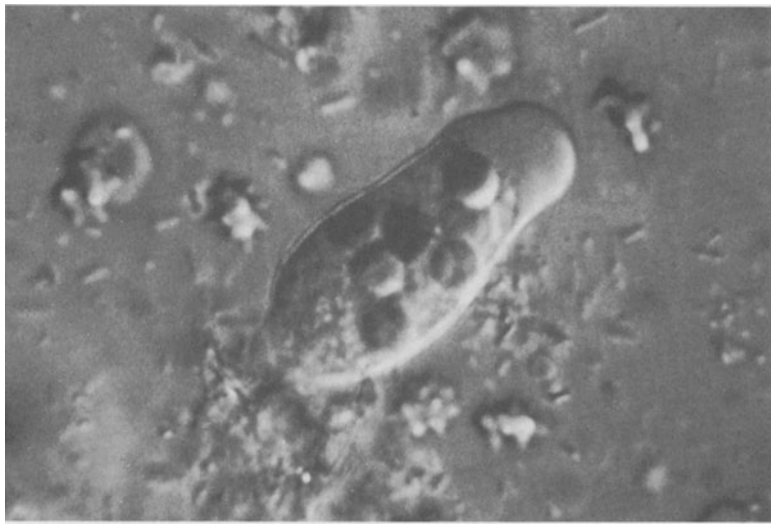

Fig. 1. *Entamoeba histolytica* trophozoite from amoebic dysentery faeces. The circular bodies in the cytoplasm are red cells. The parasite is actively motile and generally moves in one direction at a time. The background shows bacteria, red cells and degenerate white cells. Interference contrast × 1000. Enlarged by 9.6.(From Zaman[106]).

for invasive disease to occur and, indeed, demonstration of these is of paramount importance in any epidemiological study.[28-30] These reports were made in India and Mexico, respectively.

In endemic areas, some 12% of the population is infected with *E. histolytica*; however, only 10% present with acute disease,[6] and a similar percentage probably harbours noninvasive organisms (i.e. *E. histolytica* acting as a 'commensal'). Between these 'polar' forms of infection, many gradations of pathological change and clinical expression occur, although only sparse reliable data are available on this group, which warrants much greater study.

1.3 Transmission of *E. histolytica*

Various factors involved in the transmission of *E. histolytica* have been reviewed by Walsh.[31] With all pathogenic organisms, both parasite and host factors are involved. The mature quadrinucleate cyst is the stage in the life-cycle that is transmitted from one individual to another (less mature cysts have a lower survival) and cysts can remain viable in a damp/moist environment for several months. Desiccation following exposure to bright sunlight or a high ambient temperature significantly reduces cyst viability. Trophozoites, which are extremely sensitive to environmental influences, are unimportant with regard to transmission. They rapidly die on exposure to dry air and are also destroyed by gastric acid and other digestive enzymes. However, when trophozoites are directly introduced into the colon of another animal, dysentery results (fig. 1). Cyst excretion, either by convalescent or asymptomatic carriers, constitutes an important vehicle for transmission (fig. 2).

1.3.1 The Protozoan-Host Equilibrium

Viable cysts ingested in water and food or from faecally contaminated hands hatch in the intestinal lumen, producing an infection which may give rise to symptoms. The reservoir for infection lies in humans; however, evidence of transmission from a subhuman primate to man has been recorded.[33] Canine and feline amoebae have also been experimentally transmitted to many mammalian species.

Host susceptibility is complex, with immunological, age related, sexual, ethnic, nutritional, and

cultural factors all being involved. After tissue invasion, humoral antibodies and cell-mediated immunity result, but the degree of protection against further infection remains unclear.

On theoretical grounds, Brumpt suggested in 1925 that there are 2 strains of *E. histolytica: E. dysenteriae*, which produces invasive disease, and *E. dispar*, which is noninvasive.[10] Recent research using DNA technology has confirmed the hypothesis that pathogenic and nonpathogenic zymodemes are genetically distinct. It has also been suggested that transmission characteristics differ for pathogenic and nonpathogenic strains.[31] Nonvirulent strains might possess greater environmental resistance and be able to circulate under more hygienic conditions, and in less humidity and extremes of temperature. Moreover, the infecting dose may be lower and the persistence longer, and these strains may possess the ability to multiply in the intestinal lumen under more variable conditions, as well as being able to reproduce/mature more rapidly so that a larger proportion of cysts is produced.

An oral inoculum of 2000 to 4000 cysts consistently produces *E. histolytica* infection, but it is likely that a single cyst is sufficient under certain conditions. After ingestion, mature cysts appear in a faecal sample in approximately 5 days; however, the median incubation period from ingestion to the production of symptomatic disease is usually much longer (of the order of 2 to 6 weeks), and depends on the inoculum size. Invasive hepatic

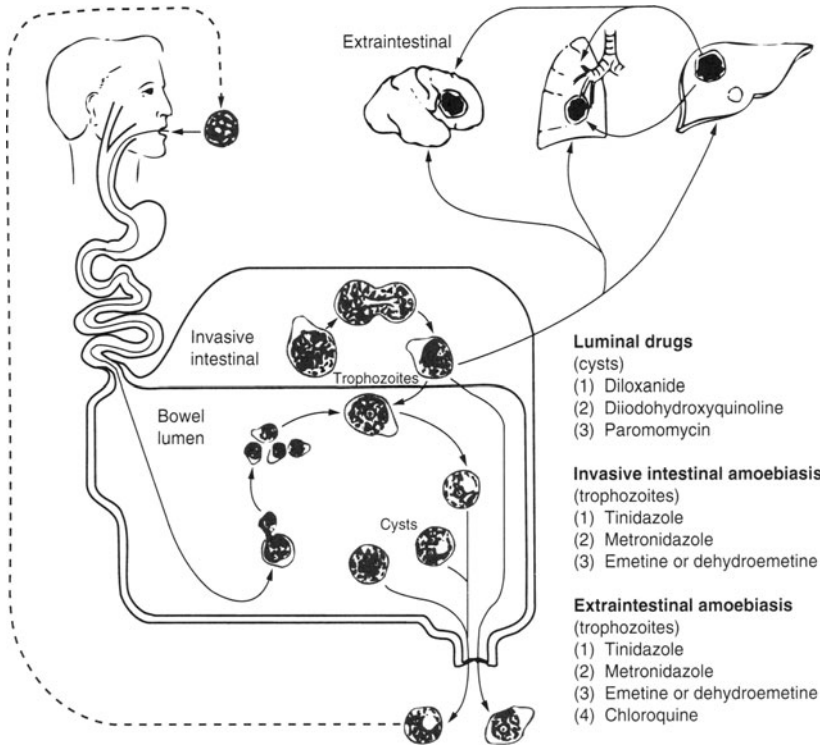

Fig. 2. Life-cycle of *Entamoeba histolytica* showing transmission, site of infection, and sites of action of chemotherapeutic agents.[32]

disease may not occur until many years later. Excretion of cysts is erratic, and can amount to 15 million daily.

1.3.2 Risk Factors for E. histolytica Carriage

A variety of factors are involved in *E. histolytica* infection, and many of these are interconnected. Individuals of low socioeconomic status are usually exposed to poor sanitation, inadequate water supplies, poor hygiene, crowded living conditions and, in addition, are poorly educated. Most of these factors operate in a developing country setting. Seasonability and environmental conditions, and sexual practices are also involved in transmission.

Personal hygiene and attention to hand washing are of paramount importance in the prevention of amoebiasis. Culturally appropriate health education must be instituted and children in particular must be taught to defecate in a latrine. Personal hygiene is crucially important in mental hospitals and other institutions. The value of installing a purified water supply with regard to transmission rates has received a great deal of attention. It is probable that only a minor proportion of cyst transmission takes place via contaminated water; infection more often follows ingestion of contaminated food.

1.3.3 Methods of E. histolytica Transmission

The importance of the food handler in conveying infection is well established.[31] In one study, the author concluded that '... the infected mother bears most of the responsibility for infection of young children with this parasite in Nigeria'. A closely related means of transmission is direct contamination of the oral cavity by faecally soiled hands and fingernails. This is especially important in children, and in mental hospitals.

In developing countries, sewage contamination of foodstuffs is important, and human night-soil is frequently used as a crop fertiliser. Also, freshening vegetables and skinned fruit with polluted water, should be actively discouraged. These foodstuffs should be carefully washed using filtered/purified running or boiled water. Treatment of water with iodine, chlorine, or silver solutions kills *E. histolytica* cysts only if high concentrations are used over a long period. Although information on the most appropriate methods for sewage treatment is limited, the viability of cysts is substantially reduced by exposure to bright sunlight and a warm temperature. In a hot dry climate, cyst survival may be less than 24 hours, and dry weather for 3 days kills cysts on the surface of crops that have been irrigated with polluted water or fertilised with night-soil.

Water also plays a significant role in the transmission of *E. histolytica* infection, and 2 factors are important: 1) the purity of the supply, which must be free from faecal contamination; and 2) adequate water per capita and easy accessibility. Personal hygiene is not directly affected by the quality of the water supply. Although *E. histolytica* cysts have only rarely been isolated from the domestic water supply, infections are common when the water is faecally contaminated. In an endemic area, person to person transmission is probably more important than water-borne infection.

E. histolytica cysts can be mechanically transmitted by insects, including cockroaches and flies, which contaminate food with their faeces and/or vomit. Although the importance of insect transmission is not well established, transmission by house flies is certainly possible under field conditions. *E. histolytica* cysts have been detected on the external surface of flies 4 minutes after exposure, in the intestinal tract for up to 240 minutes, in vomit for 64 minutes, and in faeces for 254 minutes. In the cockroach, cysts can survive both in the gut and faeces for 48 hours after feeding on infected human faeces. Overall, however, it seems likely that the role of insects in transmission is relatively small, and insect control should therefore not receive major attention in control strategies.[1]

1.3.4 Identification of Transmitters of E. histolytica

Although any effective control strategy should concentrate on major excreters of cysts, to date no satisfactory method for their identification has become available. Such individuals a) excrete a large number of mature virulent cysts; b) have poor hygiene, especially in food preparation and food handling for others; c) have poor excreta disposal

habits (children and institutionalised individuals are especially relevant); and d) although carriers, feel well and circulate freely among a susceptible population.[31] If major transmitters could be easily identified, they could be given a luminal amoebicide to prevent or minimise cyst excretion. Alternatively, a vaccine (when available) would induce local immunity and reduce cyst excretion. The proportion of carriers in an endemic equilibrium is a measure of transmissability within the population.

At present, mass chemotherapy of open populations is not feasible; available chemotherapeutic agents require several days to several weeks of administration to eliminate carriage in the majority of those treated. If a satisfactory short course luminal amoebicide were produced, mass chemotherapy might be possible.

E. histolytica infection differs from a viral disease, in which an accumulation of immune individuals limits the overall number of cases: a) infected individuals recover spontaneously, but only several months to several years after exposure; b) because infection does not impart full immunity, and after recovery (spontaneous, or after chemotherapy) individuals are immediately susceptible to a further infection; and c) spread of infection is by the person to person route, either directly or less frequently via water, food, or flies.

1.4 Morbidity Associated with *E. histolytica* Infection

A major problem in delineating morbidity (and mortality) involves difficulty in diagnosing disease and in defining mild, moderate, and severe cases.[1] Disease produced by *E. histolytica* infection is often indistinguishable from that caused by other intestinal pathogens. Unless clear definitions of 'dysentery', 'colitis', 'subacute colonic amoebiasis', and 'carrier state' are established, it is exceedingly difficult to compare different studies of morbidity (and mortality), incidence, treatment outcome, and serological response. There is, for example, good evidence that the cyst carrier rarely experiences significant clinical disease;[34,35] spontaneous eradication within 5 months is perhaps the usual sequel. Data obtained in Mexico in 1984 (population 77 million) indicated that 3.9 million people seroconvert for *E. histolytica* annually, and 5.5 to 7.0 million moderate to severe cases of clinical disease result.[1] A similar number will probably have had mild, transient diarrhoea (5 to 7 days) resulting in a visit to a health worker. In Mexican children, up to 15% of cases of acute diarrhoea necessitating hospitalisation are associated with *E. histolytica* infection. At Caracas, Venezuela, 11% of those suffering from colorectal disease were infected.[1]

Available evidence indicates that the prevalence of *E. histolytica* infection in most of Asia is lower than that in southern America, although satisfactory data are often sparse.[1] In sub-Saharan Africa, however, the proportion of the population suffering from invasive disease is probably comparable with that in Mexico. Therefore, morbidity is likely to be high, a conclusion that is supported by personal observation. The overall effect of *E. histolytica* infection on a population may be more significant than the mortality rate, as those more severely infected are often in their economically productive years of life.

There can be no doubt that because of the substantial morbidity (and mortality) associated with *E. histolytica* infection, this disease warrants far greater study with regard to preventive methods, diagnosis, and satisfactory chemotherapy.

1.5 Mortality Associated with *E. histolytica* Infection

One estimate of the mortality associated with *E. histolytica* infection is that amoebiasis causes 10 000 to 30 000 deaths annually in Mexico alone,[1] the majority in men during the third or fourth decades of life. Pregnant and postpartum women also have an excessive risk of severe disease and death. Furthermore, the death of a mother in a developing country often results in malnutrition and death in her young children.[1]

For every case of liver 'abscess', some 1 to 5 adults are admitted to hospital with colitis. Inva-

sive amoebiasis, involving liver and/or colon, frequently presents as an acute abdominal emergency.[36] The case fatality rate of liver 'abscess'/colitis sufficiently severe to necessitate hospitalisation may reach 13%.[1]

Some data for the mortality rate resulting from invasive disease in South Africa are provided in section 1.1.1. In Mexico, the mortality rate for patients with hepatic amoebiasis before 1970 varied between 9% and 12.8%.[6] After the introduction of the 5-nitroimidazoles, this dropped to 3.8% in the major centres. Elsewhere, the mortality rate remains high in fulminating colitis (72%), amoebic appendicitis (20%), and amoeboma (6.4%).

2. *Giardia lamblia* Infection

In common with *E. histolytica*, *G. lamblia* is very widely distributed in both tropical and temperate countries. It is, however, not an invasive protozoan; although it accounts for significant morbidity it is only very rarely (if ever) associated with mortality. All aspects of the infection have recently been reviewed.[37]

Giardia spp. have widespread distribution in the animal kingdom. The organisms have remarkable morphological similarities (this applies especially to the cysts) and controversy exists concerning the number and identity of *Giardia* spp. in different species.[38] It is therefore difficult to know which species contribute to human disease via water contamination. *Giardia* spp. isolated from beavers and calves are, for example, indistinguishable by light microscopy from the organism causing human disease. Early differentiation was based on the morphological appearance of the 'median body', an organelle shown by ultrastructural studies to be composed of microtubules. However, from more recent observations based on molecular biology, it is clear that the entire classification of the genus *Giardia* requires urgent revision.

2.1 Prevalence of *G. lamblia* Infection

As with *E. histolytica*, the reported prevalence rate is largely dependent upon methodology. In one study in India, *G. lamblia* was detected in the first faecal sample in 73% of patients, and in 85% when 3 consecutive samples were examined. In contrast, *G. lamblia* was detected in 44% of duodenal fluid aspirates obtained from patients with a positive faecal result.[39] Simultaneous estimation of infection rate, cure rate, and detectability of *G. lamblia* infection was carried out in Kenyan children by use of a new statistical model.[40] In a study in central Arkansas, the authors examined records from 3 clinical laboratories over a 7-year period and detected a circannual rhythm.[41] This was considered to be '... important for the prevention, diagnosis and treatment of this infectious order'. Such a cycle has also been described by workers in Mexico.[42] A further factor to be taken into account is the change in geographical patterns of infection. A study in the USA by Kappus et al.[43] demonstrated 'changes in rates of identification and in geographic patterns compared with state laboratory data collected a decade earlier'.

Certain human populations are at especial risk for *G. lamblia* infection. Prevalence rates of ≥ 20% have been recorded in Bangladesh (21 to 33%), Guatemala (20%), Thailand (21%), Seychelles (43%), India (20%), Egypt (35%), and Zimbabwe (22%).[44] Other risk factors are age (5- to 10-year-old children are especially at risk), male sex, impaired nutritional status, gastric hypoacidity,[45] an immunodeficiency disorder (e.g. hypogammaglobulinaemia), lack of breast-feeding in infancy, dietary factors, travel to an endemic area,[46] pregnancy, high carbohydrate intake, living in an urban environment, exposure to cool and wet weather, high population density, and a poor socioeconomic environment.[44] Although *G. lamblia* infection is more common in male homosexuals, there is no satisfactory evidence that this protozoan parasite constitutes an 'opportunistic' agent in AIDS.

Giardiasis is by no means uncommon in Australia and New Zealand. In the former country, prevalence of *G. lamblia* was documented in an aboriginal community at Kimberley, western Australia,[47] where 32.1% of children and 12.5% of adults were infected. Incidentally, a high prevalence rate in cats and dogs was documented in this

community. In Canterbury, New Zealand, an overall attack rate of 4.0 per 10 000 population per year has been documented.[48] Major mechanisms of infection were probably the same as those previously identified overseas. At Denver, Colorado, a 16% prevalence in the toddler age group was recorded.[49] Risk factors for all children in the sample included travel to the Colorado mountains, large family size, and attending a daycare centre.

In a rural village in Kenya, a *G. lamblia*-infected group was delineated in children aged 19 to 24 months.[50] At Niger, a 28.5% prevalence has been documented.[51] In institutionalised children (1 to 61 months old) at a Thai orphanage, 20% were infected with *G. lamblia*.[52] Prevalence has also been documented for a residential housing estate in Malaysia.[53] At Cairo, Egypt, infants and children showed a progressively elevated antibody titre, which reached the adult level at 16 years.[54]

Numerous studies have documented high rates of infection in children, especially preschool groups[55-59] and those at daycare centres in many parts of the world.[60-64] High rates have also been documented at a nursing home,[65] and in lactating women in Egypt.[66]

2.2 Epidemiological Aspects of *G. lamblia* Infection

G. lamblia infection is probably the most widespread intestinal parasitic infection to involve humans worldwide,[67] and it is by no means confined to developing countries. The fact that giardiasis persists in a technologically advanced country in the face of measures to prevent transmission 'suggests that there are important features (involving) its biology and epidemiology which contribute to its transmission in human populations living in very different circumstances'.[67]

Since 1965, over 100 outbreaks of waterborne *G. lamblia* infection have occurred in the USA.[38] Up to 21 million individuals in that country may be at risk because their drinking water is derived from unfiltered supplies. One report indicates that over 80% of surface water samples from 66 sites in northern America contain *G. lamblia* cysts.

The infectious stage of *Giardia* spp. is the cyst, which measures 10μm × 6μm. After excretion in faeces, the cyst is immediately infectious to a new host, no period of maturation or latent period being necessary. Furthermore, the organism cannot multiply outside the host, and an intermediate one is not required for transmission. An infected individual excretes up to 4×10^8 cysts daily, and this can continue for several months[67] although excretion is intermittent. Cysts can survive for several weeks in cool water, but not after chlorination, drying, or heating. Filtration is recommended for the removal of cysts from water collected from surface sources such as lakes and rivers. Volunteers given as few as 10 cysts have been shown to be infected.[67]

The biology of *Giardia* spp. (fig. 3), the variety of possible modes of transmission, and the variability of human host responses are all involved in the complex epidemiology of this infection. Some of the factors responsible for its ability to remain endemic in most countries of the world are as follows: a) the infection period of *G. lamblia* is relatively long, typically lasting for months; b) a very small number of cysts (<10 cysts) can establish an infection; c) cysts can survive in the environment for several weeks, assuming that conditions are appropriate; and d) infections do not render protective immunity, at least not in all individuals.[67]

The latent period (i.e. that interval between the moment of infection and the beginning of infectiousness) is equivalent to the prepatent period (i.e. the time of infection and appearance of the infective stage in a faecal sample). The incubation period for this infection is difficult to determine because many cases are asymptomatic; the precise reasons for this are largely unclear. However, most studies indicate that the incubation period is usually 1 to 2 weeks after exposure to infection. It is important to draw a distinction between infection with *G. lamblia*, and being diseased as a result of this infection.[67] Adequacy of case definition is essential in epidemiological studies.[68]

Patterns of *G. lamblia* infection have been summarised by Hall.[67] In developed countries, whilst endemic in institutions, epidemics also oc-

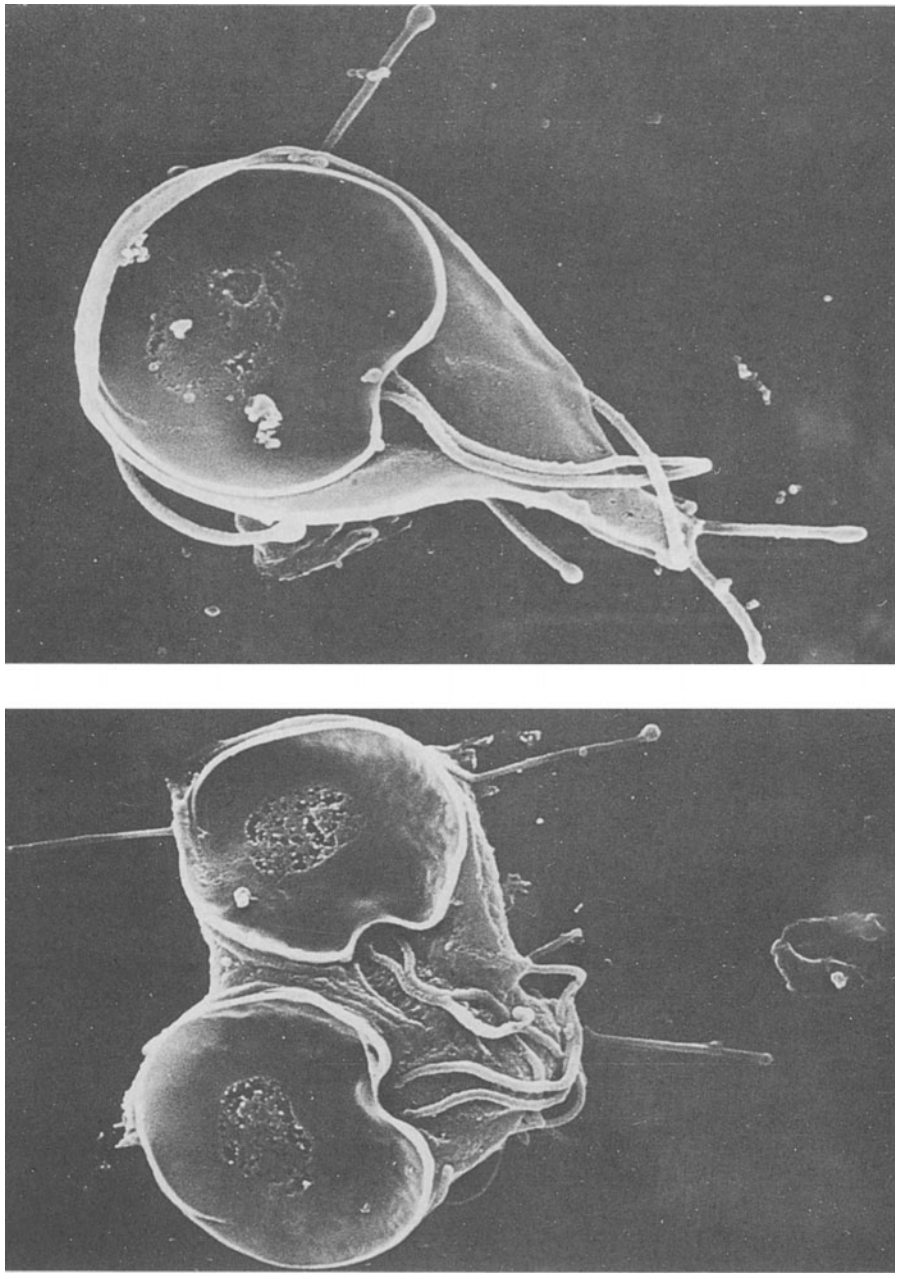

Fig. 3. (top) *Giardia* trophozoite from culture. The ventral surface is characterised by a large disc at the anterior end. Scanning electron micrograph × 6000. (below) *Giardia* trophozoite undergoing division × 6000. (From Zaman[106]).

cur, and these are usually associated with a contaminated water, or possibly food supply. In developing countries, infection is usually acquired in early childhood. Reinfection is possible immediately after eradication of infection. Seasonability of infection is another factor that must always be taken into account.[69]

Owing to intermittent excretion of cysts, *G. lamblia* infection is underdiagnosed, especially in developing countries, where faecal samples are usually used for diagnosis. A capture enzyme immunoassay (EIA) has been shown to provide an efficient means of processing large numbers of samples for prompt and accurate assessment of a *Giardia* spp. epidemic.[70] It also facilitates rapid tracing of epidemic sources. Levels of parasite-specific IgA seem to accurately reflect *G. lamblia* infection in Colorado and Thailand.[71]

2.3 Transmission of *G. lamblia*

Faecal transmission of *G. lamblia* from person to person is a major problem, although the scale remains unclear.[38,67] It seems probable that in a developing country setting, where hygiene is poor and sanitary conditions inadequate, this constitutes the major mode of transmission. While much evidence indicates that a contaminated water supply is the most common vehicle for a human *G. lamblia* infection,[72,73] other sources should also be considered. Food contamination is undoubtedly important in some outbreaks. Venereal spread is a further possibility. The importance of an animal reservoir remains unclear. With regard to domestic animals, the dog has received most attention, and although 'man's best friend' can certainly be infected,[74] the role of this reservoir in the context of human disease remains to be elucidated.

2.3.1 Contaminated Water Supply – A Vehicle for G. lamblia Infection

The importance of a contaminated water supply in the transmission of *G. lamblia* infection seems clear, although it is difficult to balance the frequency of this against person to person (faecal-oral and venereal) spread.[75] Economic, regulatory, recreational, environmental, and social aspects of waterborne infection should also be considered. Details of waterborne outbreaks have been well documented in the USA, Canada, Australia, New Zealand, Scotland, and Sweden.[75-78] Whereas most outbreaks have been traced to sewage contamination of the water supply, the importance of beaver and muskrat faeces remains a possibility in some. In addition to health aspects, other factors including economic costs, lost tourist revenues, lowered workforce productivity, and maintenance costs should also be considered.[75]

Giardia spp. cysts have been shown to remain viable in natural, tap, and distilled water for periods of up to several weeks.[75] Cold temperatures enhance survival, but other water-associated factors seem relatively unimportant. In unchlorinated tap water, the maximum survival time for *G. lamblia* has been shown to be 77 days at 8°C; the maximum survival time in natural water for *G. muris* is 56 to 84 days at 3.3°C.

The methodology for detecting *Giardia* spp. cysts has been reviewed.[75] At Dunedin, New Zealand, the incidence rate ratio for a population receiving unfiltered (microstrained) water relative to one using sand-filtered water was 3.3 (90%, CI = 1.1, 10.1), and in a parallel cross-control study of incident cases, the ratio for *Giardia* spp. infection and unfiltered (microstrained) water supply was 1.8 (90%, CI = 0.5, 6.9).[79] Not all studies, however, have shown an association between endemic *Giardia* spp. infection and a drinking water source.[80] A questionnaire used to assess risk factors in the greater Vancouver water district showed that the significant ones were a <6-year-old child in the household, and travel (both within British Colombia and internationally). Interestingly, shallow wells have been implicated in *G. lamblia* infection in New Hampshire, USA.[81]

The CDC [(US) Centers for Disease Control (and Prevention)] and US Environmental Protection Agency recently undertook a collaborative surveillance programme on the occurrence of waterborne diseases.[82] Over the previous 5 years, the number of outbreaks had not changed substan-

tially, although *Escherichia coli* 0157:H7 and *Cryptosporidium* spp. were being reported more frequently and from new settings.

Few data are available on the prevalence of *G. lamblia* cysts in raw and treated sewage. The removal efficiency of various treatment systems appears to be very high, and the viability of cysts may increase as they pass through the treatment system.

Recent advances in the understanding of the transmission dynamics of this and other parasitic infections of the intestinal tract have been reviewed.[83]

2.3.2 Food Contamination: A Source of G. lamblia Infection

Strict hygiene is clearly an important factor in preventing *G. lamblia* infection. In a recent outbreak involving a family party of 25 individuals,[84] 9 who had eaten fruit salad became ill compared with one who had not. The preparer of the salad had a diapered child and kept a pet rabbit at home; both were infected with *G. lamblia*. A further outbreak highlighted a similar problem in a commercial setting.[85] A total of 18 laboratory confirmed and 9 suspected cases were recorded in insurance company employees, and the infection was traced (using case controls) to a food handler infected with *G. lamblia*. In another outbreak, 27 (75%) of 36 training centre staff who had eaten at a restaurant became ill compared with 1 (3%) of 31 who had not done so. Although no specific food item could be incriminated, circumstantial evidence strongly suggested that ice contamination by a food handler might have been the source of infection. Another group of workers has addressed the appropriateness of providing mass chemotherapy to food handlers.[86]

2.3.3 Is a Zoonotic Reservoir Important in Human Giardiasis?

Possible zoonotic sources of infection can be divided into natural (e.g. beaver, muskrat, vole, and other wild animals) and domestic ones (e.g. dog, cat, gerbil, neonatal mouse, and rat). *Giardia* spp. colonise and proliferate within the gastrointestinal mucosa of a wide variety of different animal species.[38,44] Binary fission (and encystment) of the parasite is stimulated by bile. It should be assumed, on the basis of available evidence, that any *Giardia* spp. detected in water (from whatever species they are derived) are potentially pathogenic to humans.

Cross-species transmission of *Giardia* spp. isolates from man has been demonstrated in experimental hosts, e.g. the Mongolian gerbil, the neonatal mouse, and the neonatal rat.[38] This does not, however, constitute evidence for zoonotic transmission of the infection.

Potential natural hosts and domestic animals[75] occupy ecological environments in wetlands enabling them, via their aquatic habits, to directly contaminate (via faecal pollution) watersheds used for human drinking water. In beavers, a prevalence of infection of 7 to 16% has been recorded in parts of the USA and, in muskrats, a 95% rate has been documented.[38] Both of these species can be infected with human *G. lamblia* isolates, although the usual animal strains appear phenotypically distinct. A study carried out in the northern states of Canada and in Minnesota showed a regional variation in the prevalence of *Giardia* spp. in beavers, but not in muskrats.[87] Other animals known to harbour *G. lamblia* are wading birds, such as blue herons, green herons, black-crowned herons, and egrets. Erlandsen[38] and her coworkers have recently demonstrated cross-species transmission between avian and mammalian *Giardia* spp. The possibility that at least some of these species may act as sentinel animals, being infected by human cysts discharged into their environment, should also be considered.

Despite this evidence, the possibility of the beaver,[75] or indeed of any other animal, being significantly involved in water-borne human infection remains circumstantial.

Dogs and cats have also been claimed to be important in human disease transmission.[38] In one study involving a Western Australian aborigine community, *G. lamblia* was detected in 16.5% of 182 dogs investigated, but in none of 33 cats.[39] However, the importance of *G. lamblia* infection in dogs in the context of human disease remains in

doubt.[44,88-90] Other domestic animals, including sheep, cattle, goats, pigs, and birds have also been implicated.[44] In one study, the prevalence of infection was 17.7% in sheep and 10.4% in cattle, and it was significantly higher in lambs (35.6%) and calves (27.7%).[91] The evidence suggested that these domestic animals might form a reservoir for human infection and vice versa.

Erlandsen[38] has outlined future directions that could be taken in an attempt to unravel the 'zoonotic dilemma'. Precise data are required on the species of *Giardia* recovered from a given host, the actual number of *G. lamblia* species in humans, and the organisms involved in water-borne outbreaks. Delineation of genetic diversity, possible taxonomy, and determination of the numbers of species involved will lie in several different molecular approaches, e.g. isoenzymic analysis, southern blots, and random amplified polymorphic DNA markers. One group has documented significant differences in molecular structure between 3 species: *G. lamblia*, *G. muris*, and *G. ardeae*.[38] These are based on nucleotide sequencing of the rRNA genes, their size in kilobases, and their guanosine-cytosine content. Other work, using the polymerase chain reaction (PCR), suggests that *G. psittaci* and *G. microtus* are distinct from the other 3. Epidemiologically, such techniques are of value in comparing *Giardia* cysts in faeces with those in environmental samples. Using fluorescent *in situ* hybridisation, it has been possible to detect a) human cysts in a model system containing *G. lamblia, G. ardeae*, and *G. muris*; b) multiple species of *Giardia* in the same sample by the use of 3-colour fluorescence detection of rDNA and immunological probes; and c) cysts in human faecal and environmental samples from sewage in lagoons.

One group of investigators has concluded, in the light of available evidence, that '... although mammals and man do not seem to possess their own unique species of *Giardia*, in reality the major methods of transmission ... probably remain basically host-specific'.[92]

2.3.4 Intervention Programmes and G. lamblia

The paucity of precise information on the biology and epidemiology of *Giardia* spp. has hindered the development of effective control measures.[44] However, the following factors should be considered in any viable programme: a) identification of reservoirs; b) diagnosis and treatment of infected cases; c) prevention of environmental faecal contamination; d) provision of safe excreta disposal and a safe water supply; e) health education aimed at improved personal and health hygiene; and f) improved host resistance, with attention to nutrition and possible immunisation.

As discussed above, *G. lamblia* infection is a major practical problem in daycare centres, although the efficacy of control strategies has not been systematically evaluated. When a concurrent *Cryptosporidium* spp. infection is present, symptoms may be worsened. Bartlett et al.[93] have carried out a prospective, randomised, controlled trial in an attempt to throw light on this problem. A total of 31 daycare centres were involved, incorporating 4180 child months of observation. In group A, exclusion and treatment of symptomatic and asymptomatically infected children were implemented; in group B, exclusion and treatment of symptomatic infections only were incorporated, and in group C, exclusion and treatment of symptomatic infections and treatment of asymptomatic infection were implemented. *G. lamblia* prevalence was monitored before intervention, and at 1, 2, 4, and 6 months later. Prevalence was 8%, 12%, and 7% for groups A, B and C, respectively, at 1 month, and 7%, 8%, and 8%, respectively, at 6 months. Therefore, a more strict and costly intervention programme did not result in a significantly better level of control.

In a study involving preschool children carried out in Lesotho, a developing African country, the authors concluded that the 'amount of water used for personal and domestic hygiene may be more important than the quality of drinking water'.[94]

In Mexico, the possible protective effect of breast-feeding against *Giardia* spp. infection has been assessed.[95] A total of 197 infants in a poor

area of Mexico City were followed up from birth to age 18 months, and symptoms and feeding status were recorded weekly. Lack of breast-feeding was a significant risk factor for the first *G. lamblia* infection at all ages. However, while an absence of breast-feeding was associated with a symptomatic *G. lamblia* infection, breast-feeding did not protect against chronic *G. lamblia* carriage. These authors showed that the presence of animals in the household and the use of water/nonmilk liquid for infant feeding were significant ($p = 0.005$ and $p = 0.035$, respectively) risk factors for *G. lamblia* infection.

The effectiveness of water treatment with chemicals, and filters for control of *Giardia* spp. cysts in areas where treated water is not available has been investigated.[96] Four filters and 7 forms of chemical treatment were evaluated for both clear and turbid water at 10°C, and contact disinfection devices were also investigated for cyst inactivation. The authors concluded that none of the devices provided significant cyst inactivation, although heating water to ≥ 70°C for 10 minutes was an acceptable alternative form of treatment. A *Giardia* spp. outbreak from a chlorinated, unfiltered surface water supply at British Columbia (population 25 000) over a 3-month period resulted in 363 confirmed cases of infection.[97] A reservoir containing *Giardia* spp.-infected beavers was incriminated as the source.

Microscopical detection methods have been adapted, using various filtration systems, for detection of *G. lamblia* cysts in water supplies; when 1 to 25% of a population is infected, levels of cysts in raw sewage have been estimated at 9.6×10^3 to 2.4×10^5 per litre. Orlon filters have been shown to be superior to cellulose acetate and polypropylene yarn-wound filters, and epoxy-fibreglass filter-tubes. Specific monoclonal antibody has been utilised to detect *G. lamblia*-antigen on the filter.

If the increase in waterborne *G. lamblia* outbreaks in the US is to be brought under control, public health officials will 'need to work with the water industry to ensure a risk of less than 1/10,000 for source waters with 0.7 to 70 cysts per 100 liters through treatment achieving reduction of 10 (–3) to 10 (–5), respectively, of Giardia cysts'.[98] In another study in Canada, *Giardia* spp. cysts were detected in 17% of 83 filtered water effluents.[99] Evaluation of the data indicated that '24% of the utilities examined would not meet a 1/10,000 annual risk of *Giardia* infection', and for cold water concentrations (0.5°C), 46% of the plants would not achieve the 1/10 000 risk level. Flanagan[100] has summarised the present position in the UK: 'the importance of potable water supplies as a source of infection ... is not clear, nor is the role of zoonotic spread. The apparent susceptibility to infection in certain population groups requires further exploration as does the role of the asymptomatically infected in transmission'.

High *G. lamblia* infection rates have been documented in malnourished children in many developing countries.[44,101] Clearly, therefore, an improvement in nutritional status is of paramount importance and this should be coupled with health education, which is especially important in developing countries where high levels of illiteracy, ignorance, and cultural taboos exist. Some evidence has been presented indicating that *G. lamblia* infection associated with malnutrition does not respond satisfactorily to chemotherapy.[101]

With regard to a potential vaccine, little progress has been made so far.[44] The effect of systemic oral immunisation with a 56 kDa protein of *Giardia* spp. has been tested in experimental mice, and although initially encouraging results have been obtained, further studies are required.

2.4 Morbidity Associated with *G. lamblia* Infection

A range of morbidity results from a *G. lamblia* infection – from travellers' diarrhoea (a self-limiting clinical syndrome) to severe malabsorption accompanied by weight loss and, in extreme cases, malnutrition.[102] However, the vast majority of cases remain asymptomatic.[100,103]

A surveillance programme carried out by means of a questionnaire at Bristol, England, revealed 22 patients with *G. lamblia* infection who had travelled abroad in the month preceding the onset of

symptoms;[104] most others were preschool children or individuals engaged in recreational water activities.

In individuals living in underprivileged conditions, *G. lamblia* constitutes a significant pathogen. Gracey[39] has reviewed the clinical significance of infection in Australian aboriginal children. In this group, *G. lamblia* infection was shown to be 2 to 3 times higher than in nonaboriginal children. Almost 30% of aboriginal children studied in the late 1960s had evidence of infection in faecal samples, and in a later study at south-east Queensland, 70% were infected. In a survey of 1600 faecal samples carried out in Western Australia, prevalence rates of 49% at 4 years of age and 36.7% at 9 to 10 years of age were documented. However, in a hospital-based study of aboriginal children in Western Australia, the isolation rate in those with diarrhoea was similar to that in those without.[39] This study also demonstrated that many other small-intestinal pathogens are of far greater significance than *G. lamblia*.

Hall[67] has summarised the possible nutritional consequences of a *G. lamblia* infection. Data obtained from case studies (e.g. 'failure to thrive'), cross-sectional studies, and prospective studies were assembled. The significant nutritional consequences of such an infection are anorexia and malabsorption, the pathogenesis of which remains unclear, despite much research. However, as Hall[67] has pointed out, most studies concerning the nutritional impact of *G. lamblia* infection contain serious flaws or limitations. Much of the evidence depends on clinical improvement after chemotherapy with a nitroimidazole compound. However, this agent also eliminates many concurrent organisms, including some bacteria. In addition, it must be remembered that improvement in a child's appetite, digestion, or absorption can only lead to improved growth if sufficient protein and energy are available.

Although failure to thrive, associated with infectious diarrhoea, is a common problem in aboriginal children at Kimberley, Western Australia, few reports satisfactorily record the prevalence of *G. lamblia*. Overall, Gracey[39] concluded that '... there is still disagreement about whether diarrhoeal disease is a major, community-wide cause of malnutrition in under-fives in developing countries'. In a further study, *G. lamblia*-infected children tended to achieve greater weight and height for age than those who were not infected.[103] These authors concluded that 'Healthy daycare children with asymptomatic (*G. lamblia*) infection show no disadvantage and perhaps even an advantage in nutritional status and freedom from other illnesses'. However, Cheek et al.[102] have studied aboriginal children at Yaluta, South Australia, and concluded that *G. lamblia* infection was prevalent, and that because it 'had been shown elsewhere that *G. lamblia* is capable of inducing malabsorption with resulting nutritional deficiencies', it probably accounted for at least part of the impairment of several nutritional indices.

2.5 Mortality Resulting from *G. lamblia* Infection

Mortality associated with *G. lamblia* appears to be exceedingly rare. Such an occurrence would presumably be related to malnutrition consequent upon a heavy small-intestinal infection with resultant malabsorption. One death attributed to acute jejunal ulceration has been documented.[105]

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