

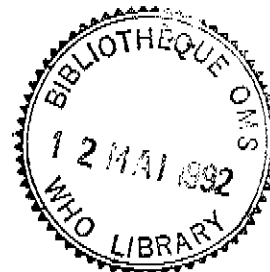


WHO/PAHO INFORMAL CONSULTATION
 ON INTESTINAL PROTOZOAL INFECTIONS

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1. INTRODUCTION

Intestinal protozoan infections are among the most common infections in humans worldwide, and are a significant cause of morbidity and mortality. Recent advances in the biology of these parasites will enable us to consolidate our knowledge of their importance and to approach control in a more effective way.

1.1 Intestinal protozoa of world importance

The amoeba Entamoeba histolytica, the flagellate Giardia intestinalis (- G. lamblia, G. duodenalis), and the coccidian Cryptosporidium parvum are found throughout the world and all cause diarrhoeal disease, although many infections may be asymptomatic¹. While the major intestinal protozoa are found both in developed and developing countries, the epidemiology of infections differs between them and so do the resources available for treatment and control. Deprived communities in the developed world may experience problems caused by intestinal protozoa in a similar manner and intensity to developing countries.

E. histolytica is one of the ten most common infections in the world today. In addition to diarrhoea it can cause dysentery and liver disease when it invades tissues, and the outcome can be fatal. It was estimated in 1984 that 500 million people were infected, of whom 40-50 million developed clinical amoebiasis each year, resulting in 40,000 to 100,000 deaths.

Giardia may cause diarrhoea, growth retardation and malnutrition in children and is an important cause of morbidity in adults. In the United States it is the most commonly reported intestinal parasite of man, having been identified from 1971-85 as the cause of 52% of waterborne outbreaks of diarrhoea in which an aetiological agent was found. Seven per cent of faecal specimens submitted to State diagnostic laboratories show Giardia. In 1989 in England and Wales, Giardia was the fifth most common identified cause of gastroenteritis.

Cryptosporidium can cause an acute, self-limiting watery diarrhoea, which in some cases can be as severe as cholera. The first case of human cryptosporidiosis was reported in 1976 in an immunocompetent child, and it was subsequently recognised as an important cause of diarrhoea, especially in children. Farm and domestic animals serve as animal reservoirs. It has been identified in 4.3% of Costa Rican children and in 10.8% of Venezuelan children suffering from diarrhoea. In 1989 in England and Wales, Cryptosporidium was the fourth most common of enteric pathogens

¹ Ideally it would be attractive to conform to conventional usage and use the terms "amoebiasis", "giardiasis" and "cryptosporidiosis" to indicate disease caused by infection with E. histolytica, G. intestinalis or C. parvum respectively. However, for amoebiasis this would be at variance with the approach taken in 1989 ("Amoebiasis" Technical Report Series; 421, Geneva, 1989) and in subsequent reports in the definition of amoebiasis. ("The condition of harbouring Entamoeba histolytica with or without clinical manifestations"). This terminology was not discussed during the present meeting, but it seems relevant to suggest that it be reconsidered on another occasion.

identified in gastrointestinal disease. The infection can be persistent and life-threatening in patients with the acquired immunodeficiency syndrome (AIDS).

Diarrhoeal illness due to another coccidian, Isospora belli is self-limiting and of little importance in the immunocompetent host, but may also, like cryptosporidiosis, persist in the immunocompromised. The prevalence of Isospora infection in HIV-infected individuals ranges from 0.2% in the USA and Italy to 15% in Haiti.

The ciliate Balantidium coli causes a rare zoonosis and is associated with domestic pigs, primarily in the tropics and subtropics. The pathogen may cause colitis, typhlitis or a dysentery, which may sometimes be mistaken for amoebic dysentery. Balantidiasis may have a fatal outcome.

A newly described microsporidian parasite of the intestine, Enterocytozoon bieneusi, has recently been implicated in the causation of diarrhoeal illness and wasting in AIDS. The organism is small in size and difficult to identify. The significance of microsporidia (phylum Microspora) in AIDS in developing countries is not yet known.

Direct costs of management of an estimated 58 million cases of childhood protozoan diarrhoea worldwide may be in the region of \$150 million per year.

1.2 Transmission

Waterborne transmission of G. intestinalis and C. parvum has been documented in some developed countries and there have been rare authenticated reports of waterborne transmission of E. histolytica and of B. coli. A waterborne route of transmission must be regarded as possible for all the protozoan parasites mentioned above. However, waterborne spread probably represents only a proportion of total faecal-oral transmission worldwide, the other routes of which are still uncertain and need to be determined.

1.2.1 Environmental classification

Feachem et al.¹ have discussed the determinants of transmission of excreta-related diseases and produced an environmental classification of water-borne pathogens. They conclude that for most intestinal protozoa direct faecal-oral transmission is probably more important than for geohelminths, since the ova of the latter need to mature outside the body before becoming infective. Although protozoan infective stages will persist in the environment, they are not as resistant as helminths. However, in contrast to bacteria, the infective stages of most intestinal protozoans resist inactivation by stomach acid, and so the infective dose is low (<100). Transmission of protozoa such as E. histolytica and G. intestinalis therefore takes place easily from person to person under poor conditions of personal and domestic hygiene, and infection can be

acquired from faecally contaminated water, food, etc. This conclusion would also appear to apply to E. bieneusi.

Epidemiologically, I. belli would be expected to have some similarity to geohelminths because a latent period of several days elapses after defaecation before the oocysts become infective. Improvements in faeces disposal may have a bigger impact on transmission here than improvements in personal and domestic hygiene.

C. parvum and E. coli have no latency period before the cysts become infective as also seen in E. histolytica and G. intestinalis, but in contrast to these organisms there are recognised animal reservoirs enabling the pathogens to multiply and persist outside the human host for an unlimited period. In these cases, human contact with both human and animal faeces should be avoided. Improvements in both personal hygiene and disposal of animal and human faeces are required for control of transmission.

Improvements in personal cleanliness, achievable by education and availability of clean water for washing, may have more marked effects on acquisition of infections like E. histolytica than improvements in disposal of faeces. But for control of transmission of I. belli, improvements in faeces disposal may be much more important.

1.2.2 Comment

Sanitary education, environmental approaches such as improved water supplies, and effective excreta disposal are likely to have long lasting benefits for prevention of all water borne intestinal disease. The provision of safe water supplies and the sanitary disposal of faecal waste have health and sociological implications far beyond the field of control of intestinal parasites. They are promoted within existing PHC structures. Community perception of need for parasite control is recognised to help promotion and monitoring of sanitation programmes.

1.3 Breast feeding

Lack of breast feeding is reported to be the single most important risk factor for diarrhoea in young children², and the encouragement of breast feeding may be one of the most cost-effective educational interventions possible in a PHC programme.

1.4 The importance of recent advances in molecular biology

Recent advances in molecular biology have provided techniques which, when applied to clinical and field studies in the next few years, could lead to a great expansion in our understanding of the epidemiology of intestinal protozoal infections which, in turn, will result in improved methods to control both infection and disease. For example, there is now compelling

evidence that what is currently recognised as E.histolytica actually comprises two morphologically indistinguishable species, only one of which is invasive. The use of new tools such as DNA probes and monoclonal antibodies allows us to distinguish between these organisms and is leading to a reassessment of the epidemiology of amoebiasis.

2. EPIDEMIOLOGY, CLINICAL AND THERAPEUTIC ASPECTS, AND RESEARCH NEEDS.

2.1 E.histolytica

2.1.1 Epidemiology and disease.

Entamoeba histolytica, multiplying in the large intestine of man, has long been thought to be a single entity with variable pathogenic capacity. Studies of lectin agglutination and erythrophagocytosis in the 1970's pointed to the possibility that virulent and non-virulent isolates showed biological differences^{3,4}. In 1978 the zymogram pattern of cultured E.histolytica was shown to differ depending on whether the culture came from a symptomatic or asymptomatic case⁵. In 1981 it was stated in a WHO Report that "isoenzyme electrophoretic patterns are proving useful in differentiating invasive from non invasive strains of E.histolytica"⁶. These and subsequent observations on phenotypic diversity have been supported and extended by studies using the techniques of molecular genetics^{7 8 9 10 11 12 13 14 15}. Compelling evidence now indicates that what is currently identified as E.histolytica actually comprises two morphologically identical species, differing genetically and in their capacity to cause disease. One species can be described as an invasive pathogen exhibiting varying degrees of virulence, the other as a non-invasive parasite which at the most may be capable of producing superficial erosions of the colonic mucosa. This species would thus be equivalent to E.dispar, a species proposed many years ago by Brumpt^{16 17}. The use of new tools such as DNA probes and monoclonal antibodies allows us to distinguish between pathogenic E.histolytica and these other organisms, and is leading to a reassessment of the epidemiology of amoebiasis.

The most recent estimate is that about 10% of the 500 million persons infected with E. histolytica develop clinical symptoms and 40,000 to 100,000 die each year¹⁸. If there are two species, of different pathogenic potential, a reevaluation of the accepted epidemiological model becomes necessary. The proportion of infections which produce disease may simply reflect the proportion of infections with "invasive" E.histolytica or (more likely) the relationship may be more complex. New approaches to both epidemiological research and control strategies will clearly be necessary.

E. histolytica is cosmopolitan in its distribution, but the major impact on health is seen in developing countries. Transmission is probably by the faecal-oral route in the majority of cases and is facilitated in communities with a high population density and inadequate sanitation. There is no animal reservoir of E. histolytica. The cysts are killed by drying within ten minutes on the surface of the hands, but survive for periods of up to 45 minutes in faecal material, lodged under the fingernails. They are killed by freezing and remain viable in moist conditions or in water for about 100h at 25° C, but their survival is shorter at higher temperatures. A residual chlorine concentration of 3mg/L is necessary to kill cysts (exposure 30 min). This resistance allows the cysts to remain infective in water subjected to antibacterial chlorination. Cysts may be removed from water by slow sand filtration.

Several environmental studies have shown a relationship between prevalence of E. histolytica, and poor sanitation, housing, etc., and a smaller number of studies have shown no correlation¹⁹.

Clinical pattern

In many developing countries intestinal amoebic disease is frequently overdiagnosed, since clinicians may confuse it with other dysenteric or gastro-intestinal disorders. This erroneous approach is also seen among the patients themselves, who procure antiamoebic treatments without a prescription in countries where these drugs can be obtained over the counter.

Clinical intestinal amoebiasis may be more severe in children than in adults, with concomitant higher mortality, and also in pregnant women. A correct diagnosis is important to avoid unnecessary treatment and to exclude amoebiasis in suspected inflammatory bowel disease.

Clinical complications, such as fulminating necrotic colitis and intestinal perforations, are the main cause of death in cases of invasive intestinal amoebiasis. These complications are commonly seen in undernourished children. The development of a hepatic abscess is approximately four to five times more common in adult males as in females. This is in contrast to the similarity in incidence of intestinal amoebiasis in males and females.

Although invasive amoebiasis may be found in persons with anti-HIV antibodies or with AIDS, the incidence of disease has not been found to be higher than normal in such persons. A series of autopsies carried out in Mexico has shown no difference in post mortem characteristics of invasive amoebiasis in HIV1 +ve or HIV1 -ve persons.

2.1.2 Diagnosis

Laboratory tests²⁰ are essential for diagnosis, although colonoscopy may be helpful, and X-ray examination is valuable in cases of fulminating colitis, amoeboma, peritonitis or liver abscess. Microscopy is currently the most effective and widely available technique for diagnosing E.histolytica infections of the intestine. Detection of motile haematophagous trophozoites by immediate examination of material from rectosigmoidoscopy, rectal smears, or from blood or mucus in a stool specimen confirms a clinical diagnosis of amoebic disease. The stool in amoebic dysentery will often be acidic, with few leukocytes and may contain Charcot-Leyden crystals. Cysts can be detected in faeces by means of formol-ether, formol-ethyl acetate or formol-petrol (gasoline) concentration if the stool is formed or semi formed. Liquid stools should be examined directly for trophozoites. Staining of the cysts with Lugol's iodine or other stains is essential, but unstained preparations should also be examined for easier detection of chromidial bars. Microscopical measurement is essential, particularly to distinguish the cysts of E.hartmanni from those of E.histolytica²¹. Stools can be preserved for transport with merthiolate-iodine-formalin (MIF), polyvinyl alcohol (PVA) or sodium acetate formalin (SAF).

Faecal antigen detection systems of high sensitivity and specificity have been developed using enzyme-linked immunosorbent assay (ELISA) technology²². These have been made specific for invasive organisms using selected monoclonal antibodies²³. Serological tests²⁴ are valuable to detect extraintestinal disease, where antibody levels are raised on presentation in 95% or more of cases. Use of a recombinant fusion protein as an antigen in serological testing has recently been reported²⁵. This advance will avoid the need for preparation of diagnostic antigen from axenic cultures and make serological testing more readily available. In endemic areas, the distinction between background levels of antibody in currently uninfected persons and raised levels in persons suffering from intestinal disease is often not clear in up to 50% of cases²⁶. The use of selected antigens may solve this problem. (See reference²⁷ for diagnostic techniques applicable to Primary Health Care Services).

2.1.3 Treatment

Antiamoebic drugs can be divided into lumen and tissue amoebicides.

(a) Lumen amoebicides are not well absorbed from the gut, and act on the trophozoites in the intestinal lumen; they are not effective against parasites in the bowel wall or in tissues. They include dichloroacetamide derivatives such as diloxanide furoate, halogenated hydroxyquinolines such as diiodohydroxyquinoline and antibiotics such as paromomycin. The dichloroacetamide derivatives

are the agents of choice since, apart from causing flatulence they are free of serious side effects and there are no known contraindications (caution is advised during the first trimester of pregnancy).

If lumen amoebicides are used to treat clinical intestinal amoebiasis, rather than mere infection with *E.histolytica*, then they should be combined with a tissue amoebicide.

(b) Tissue amoebicides are readily absorbed from the small intestine and act primarily in the bowel wall, liver and other extraintestinal tissues; they do not reach high enough concentrations in the large intestinal lumen to eliminate the amoebae found there. Because the drugs, which include 5-nitroimidazole derivatives such as metronidazole, have low activity against parasites in the intestinal lumen, they must be used in conjunction with a lumen amoebicide, when they give clinical and parasitological cure in over 90% of intestinal cases. The nitroimidazole derivatives are generally administered orally, but parenteral metronidazole and ornidazole and suppository metronidazole are available. As well as the common but transient side effects of nausea and vomiting, 5-nitroimidazole drugs may induce a confusional state, flushing, headache, nausea, vomiting, drowsiness, or fall in blood pressure if alcohol is ingested during, or shortly after, treatment ("antabuse or disulfiram effect"). These drugs are mutagens in the *Salmonella* Ames test system and at high oral dosages for long periods metronidazole was tumorigenic in mice. The drug crosses the placenta and is excreted in the milk. These drugs are therefore best avoided during the first trimester of pregnancy and during lactation. Nevertheless metronidazole has been widely used throughout the world for the last twenty years and there is no documentation of carcinogenicity or teratogenicity in humans. An advantage of tinidazole and secnidazole is their longer half-life, permitting longer intervals between administration. The latter has higher activity in the lumen than the other 5-nitroimidazoles, producing up to 56% clearance of infection from asymptomatic cyst-passers.

Certain forms of intestinal and liver amoebic lesions may require surgical treatment²⁸.

2.1.4 Control and prevention

Individual approaches to amoebiasis control include therapy of clinical disease with drugs²⁸. Treatment of drinking water by boiling, and of salad vegetables with strong vinegar (5% acetic acid), iodine solutions (3 ppm) or hot water (>60° C) and personal and domestic cleanliness, may be individually effective in prevention. As mentioned above, sanitary education and improved water supplies may be necessary for prevention of transmission, as well as more effective excreta disposal. A desirable aim would be

the provision of sufficient water of a quality acceptable for washing hands and food.

According to a mathematical model developed by Knight³⁰, for a population where prevalence of infection is 50%, halving the hypothetical "transmission constant" by improvements in hygiene should ensure the virtual disappearance of infection over 5 or more years. Implementation of plans to improve hygiene requires a long term approach for funding, planning and evaluation. The use of a mass chemotherapy approach at the beginning of such a programme could be considered. It has been suggested that mass chemotherapy combined with sanitary improvements and education should enable a low prevalence to be maintained. However, doubt exists on the suitability of currently available drugs for such a programme. The relatively innocuous luminal amoebicides are thought to be unlikely to eliminate disease, while the 5-nitro-imidazoles are unlikely to be acceptable for mass administration because of adverse side effects.

Community-based treatment

It is known that prevalence of infection can be reduced using a single course of a lumen amoebicide³¹, but the reduction may not be long lasting, and the effects of such measures on morbidity have unfortunately not been studied. The widespread use of 5-nitro-imidazole drugs, with the problem of side effects, may have discouraged a repetition of this type of control initiative.

It would be valuable to set up amoebiasis control studies using tissue amoebicides whilst monitoring the incidence of clinical disease, especially in view of the new information on invasive and non invasive E. histolytica. This might enable the use of therapy directed to those carrying the invasive species of the organism.

2.1.5 Research needs

1. To reevaluate the epidemiology of amoebic infections in view of the new data on the invasive and non-invasive species, using serological and molecular probes.
2. To continue to develop new and simple diagnostic methods for field laboratories, and to evaluate them.
3. To investigate the feasibility of directed chemotherapy against invasive E. histolytica in field settings using "case" detection by antigen-capture ELISA or other techniques of similar sensitivities.
4. To develop more acceptable drugs capable of curing amoebic disease, ideally as a single dose, and applicable to large scale use.

5. To encourage the development of collections of field isolates of E. histolytica and their deposition in international type culture collections (e.g. American Type Culture Collection, Rockville Maryland, USA).
6. To reinvestigate the efficacy of simple disinfection procedures for uncooked food (vinegar, iodine, lime juice, etc.).

2.2 G.intestinalis

2.2.1 Epidemiology and disease.

G. intestinalis, multiplying in the small intestine, is one of the most common protozoan infections worldwide³². In some developing countries studies have shown that virtually all children have been infected by three years of age³³. In developed countries, infection appears to occur less frequently, but Giardia infections nonetheless pose a significant public health problem. In the United States for example, where the prevalence of infection appears to be increasing, Giardia is the leading known cause of diarrhoeal disease outbreaks associated with drinking water (with over 100 documented), is responsible for an estimated minimum of 4000 hospital admissions per year (usually taken to be 10% of the actual number of cases of illness).

In developing countries, the risk factors for acquiring an infection have not been well-defined. Infections occur more frequently in children than adults; it is unclear whether this is primarily a function of greater childhood exposure to the organism or of the development of immunity in late childhood after repeated exposures. However, infant mice are more susceptible to infection with G.intestinalis than adults. Breast-fed infants are less likely to be infected, and a marked increase in prevalence is observed at the age of weaning. The low rates of infection in breast-fed infants may be due to protective effects of human milk or to a lower chance of ingestion of cysts.

Giardia may be transmitted by water, food, and by person-to-person, faecal-oral routes. However, in both developed and developing countries, little is known about the relative importance of different routes of infection.

In developed countries, risk factors for infection include being in the one to four year age group, attendance at child day care centres, drinking unfiltered surface water, travel to countries where Giardia is highly endemic, oral-anal sex practices, and the presence of certain medical conditions such as hypogammaglobulinemia³⁴.

Reservoirs, biotypes and zoonotic potential.

Giardia is possibly the most ancient eukaryote³⁵. Morphologically identical Giardia organisms are found in a variety of animals. In experimental studies, several animal species have been infected with isolates from humans and other animal species. Although beavers have been implicated in waterborne outbreaks of giardiasis, it is unclear whether humans can be infected by animal isolates of Giardia. The identification of animal reservoirs may have important implications for control of Giardia transmission³⁶. Recent studies have shown differences between Giardia isolates from man³⁷ and that Giardia undergoes a high degree of antigenic variation³⁸. Studies are in progress to determine whether differences in virulence and persistence in man are linked to genetic type.

Clinical pattern

The clinical course of infection with Giardia is variable, ranging from asymptomatic infections to severe diarrhoea, and appears to be related to both host and parasite factors. Signs and symptoms of giardiasis can be difficult to distinguish from those of other gastrointestinal diseases. Infection with Giardia does not always result in diarrhoea; in fact, abdominal pain, cramps and bloating may occur more frequently than diarrhoea³⁹.

Following chemotherapy, stool examinations may fail to identify Giardia for a short period of time, but the organism can reappear in the faeces some time later. It is unclear whether this represents reinfection or recurrence of the infection after treatment.

In developing countries, Giardia tends to be endemic with seasonal fluctuations in prevalence. In some developed countries, Giardia also appears to be epidemic as well as endemic. In areas where Giardia is endemic, infection is often not associated with diarrhoea. The reasons for this observation are unclear but probably include the effects of maternal factors (such as breast-feeding), immune modulation of infection, other host factors such as nutrition, diet, and concomitant infections, and biochemical differences in Giardia strains.

Giardia is recognised as a cause of rapid weight loss, fat malabsorption and loss of dietary energy, and has been linked with growth faltering in children in developed countries. However, studies in developing countries of the impact on growth and nutritional status of treating Giardia infections are still indecisive. The effect of Giardia infection on nutritional status and growth of children⁴⁰ is difficult to evaluate because of the presence of multiple pathogens that could also explain differences in growth^{41 42}.

2.2.2 Diagnosis

Although microscopical examination of stool is the standard and most effective method to establish the presence of Giardia infection, cyst excretion can be erratic, which can lead to the infection being missed. Jejunal aspiration is a sensitive procedure, but the string test (Enterotest) is a practical and much less invasive alternative⁴³. Microscopical examination can be time-consuming and requires considerable training and experience. It may be particularly ill-suited to field projects which require accurate discrimination between infected and uninfected persons.

ELISA tests have been developed for the detection of Giardia antigen in stools⁴⁴. These tests have comparable sensitivity and specificity to high quality microscopical examinations, but are easier to perform and are less time-consuming when large numbers of samples have to be tested. However, commercial assays are expensive and reagents are difficult to obtain in developing countries.

A variety of novel technologies have shown promise in recent evaluations. For example, methods have been developed to extract nucleic acids from cysts⁴⁵ and a PCR assay using appropriate primers can detect these and determine the biotype of Giardia present. This assay may be useful in addressing issues such as the presence of virulence factors, the determination of routes of transmission, and the identification of sources of outbreaks of giardiasis. Sensitive assays using fluorescent dyes and photon detection have also been described for the detection and identification of individual cysts.

The detection of the presence, species and viability of cysts in water supplies is a pressing need but this technology has not yet been fully evaluated.

2.2.3 Treatment

The 5-nitro-imidazoles are the drugs of choice. It has been reported that one of these, tinidazole, may be effective as a single dose⁴⁶, but this drug is not universally available. In preliminary studies, albendazole, a broad-spectrum anthelmintic known to have anti-giardial activity in vitro⁴⁷, appears to be effective when given in a single dose⁴⁸, but the comparative efficacy compared with other agents, and optimal dosing regimens, remain to be determined. The activity against a broad range of helminths may make albendazole particularly useful in developing countries. Additional research is needed to develop and evaluate effective single-dose therapy. There is a need to develop palatable paediatric formulations (e.g., flavoured syrups) for a range of antiparasitic drugs.

Persons with symptomatic Giardia infections should be treated. It is often incorrectly assumed that infected persons who do not have diarrhoea are "asymptomatic". Particularly for infected children under five years of age, a careful history may be required to assess the presence of nondiarrhoeal symptoms. Although conclusive evidence is lacking, clinical and laboratory studies indicate that Giardia infection may contribute to the poor nutritional status of malnourished children, even in the absence of frank diarrhoea or other obvious gastrointestinal symptoms. Therefore, therapy may be justified for all malnourished children who are infected with Giardia.

Treatment of children with asymptomatic Giardia infection is controversial. It is generally recommended that children with asymptomatic infection should not receive antimicrobial treatment. However, in certain situations, treatment of an asymptotically infected infant or toddler may be warranted on the basis of public health considerations, for example, in controlling outbreaks of giardiasis in day care centres when other preventive measures are ineffective, or to prevent infection in household members at increased risk of severe disease⁴⁹.

2.2.4 Control and prevention

Public health efforts to control Giardia infection have been hampered by a lack of knowledge about the biology, natural history, ecology, and transmission of the organism, risk factors for infection in various settings, and immunological and clinical responses to infection in the human host.

No single control measure is likely to be entirely effective in the prevention or control of infection. The basic strategy for control of transmission of Giardia is to prevent or reduce exposure to infective faeces. Methods to accomplish this may be sophisticated or simple, and should be adapted to local situations.

In some developed countries, infection and disease have been associated with outbreaks in relatively well-described risk groups. Even in these countries, however, the majority of Giardia infections are sporadic rather than outbreak-related, and the source of infection is unknown. Giardia infections associated with municipal water supplies can be prevented by adequate water filtration and treatment and by protecting and improving watershed areas. Measures such as washing hands, good personal hygiene, use of latrines, and containment of faeces are recommended. Under adequate hygienic conditions, adult-to-adult transmission appears to be uncommon. Models of transmission have not been well-defined. As mentioned above, environmental classification suggests that, in endemic areas, human-to-human transmission most likely plays an important role and control measures should be directed at person-to-person transmission. Sanitary education promoting personal hygiene,

supplies of safe water and effective disposal of faeces are all important. The importance of animal reservoirs is not yet clear and needs further investigation.

Epidemiological and clinical evidence for acquired immunity exists, and some epitopes associated with Giardia surface proteins may be linked with the development of immunity. A successful vaccine does not seem feasible in the near future. A better understanding of gut immunity in general, and of gut immunity against Giardia is needed.

Community-based treatment

The only likely approach at present is the use of albendazole in periodic anti helminth treatment of selected communities. Giardia prevalence and morbidity should be examined in addition to the helminths of interest.

2.2.5 Research needs

1. The impact of Giardia infection and disease on nutritional status and growth of children in developing countries needs further study and evaluation.
2. Research is needed to find consistent and reproducible means, which do not require growth of the organism in vitro, to distinguish between strains of Giardia.
3. There is a need to develop sensitive, reliable, non-invasive, inexpensive, and simple diagnostic tests for detection of infections in humans and viable cysts in the environment.
4. Host and parasite factors which control the development of disease should be investigated. The biological significance of antigenic variation, and its relationship to persistent Giardia infections is an area of great interest and importance.
5. The economic impact of morbidity associated with Giardia infections needs to be assessed.
6. Routes of transmission and their relative importance in establishing human infections should be determined.
7. The role of animal reservoirs as sources of human infection in developed and developing countries, particularly in terms of the strains of organisms involved, should be evaluated.

2.3 C.parvum

2.3.1 Epidemiology and disease

Cryptosporidium infection has been described in man and his livestock animals from more than forty countries of the world, including both developed and developing areas. It is recognised that C.parvum is the species involved, but there has been a recent report of C.bailevi (normally found in birds) in a diarrhoeic HIV +ve man⁵⁰.

Infection occurs in the small intestine in both immunocompromised and immunocompetent subjects. In the former, especially in AIDS patients, the infection is life-threatening; in the latter, infection in previously unexposed subjects may have marked morbidity⁵¹. Up to 10% of identified cases require hospital admission. In acute infections organisms may colonise the bile duct, the colon and the respiratory tract. The stools of persons at the onset of Cryptosporidium diarrhoea are often watery and later change to a slimy consistency, although these characteristics are not unique to cryptosporidiosis.

Developed countries

In developed countries, cryptosporidiosis is reported to develop in 2 to more than 10% of AIDS patients. They become at risk of persistent cryptosporidial diarrhoea when the CD₄ cell count falls below 100 cells/mm³.

In community-based studies, detection rates have varied from less than one per cent to more than thirty per cent of stools examined and are generally higher in young children and in less developed areas. Selection criteria for examination, the population studied and the methods used have varied widely and true denominator data have not always been reported. It is therefore difficult to compare findings. It is not known how the detection rates relate to true incidence or prevalence but they are undoubtedly underestimated.

In developed countries it is probable that the ratio of incidence to prevalence is closer to unity than in underdeveloped areas. The peak age for incidence is in the one to five years age group, but symptomatic infection also occurs in adults, especially as a result of waterborne outbreaks. Such point-source outbreaks are usually followed by propagated (secondary) spread among contacts of cases, especially among children. Outbreaks resulting from person-to-person transmission are common in families and in day care centres and are difficult to control.

Cryptosporidium is a recognised cause of c. 1% of cases of travellers' diarrhoea in developed countries.

Developing countries

In developing countries most people acquire infection early in life, particularly during the weaning period. Asymptomatic infection is more common and reflects poor hygiene and recurrent re-infection resulting from pollution with both human and animal faeces. It has recently been shown in a cohort study in Bangladesh that Cryptosporidium infection is associated with persistent diarrhoea episodes⁵².

Secondary (herd) immunity may be important in preventing waterborne outbreaks and limiting the water route of transmission to sporadic cases including travellers.

The relative contribution to morbidity is unclear, but in several surveys Cryptosporidium was among the five most commonly identified causes of gastroenteritis in some countries. In the one to five age group it has been reported in some surveys to be more common than Salmonella and Shigella infections.

Many reports have noted seasonal variability in human infection, with peaks generally associated with periods of maximal rainfall.

The relative contribution of human and zoonotic reservoirs to prevalence is currently uncertain. Host-related antigenic differences in the organism have been observed⁵³.

2.3.2 Diagnosis

Diagnosis in faeces

Patients with diarrhoea usually excrete large numbers of oocysts during the acute stage of illness but numbers may fluctuate in chronic disease (especially in patients with AIDS). Oocysts are difficult to see on direct wet mounts and do not stain with iodine like Giardia and Entamoeba. For these reasons stool concentration techniques, special stains or immunodiagnostic techniques are usually employed to confirm the diagnosis.

Faecal specimens for laboratory examination may be submitted fresh, in 10% formalin, or in sodium acetate acetic acid formalin. Polyvinyl alcohol (PVA) preservative should not be used for specimens in which Cryptosporidium is suspected because such specimens cannot be concentrated or stained by the current techniques that optimize oocyst detection. Sputum specimens may be submitted in 10% formalin or placed on a microscope slide and allowed to dry.

Sheather's sugar flotation, formalin-ethyl acetate, and a modification of the formalin-ethyl acetate method are the most commonly employed concentration methods. Concentration technique

may not be necessary, however, in acutely ill persons. Sheather's sugar flotation offers enhanced sensitivity, but the sugar interferes with stains commonly employed for diagnosis and is incompatible with some immunodiagnostic reagents unless the oocysts are thoroughly washed - an extra procedure that may result in decreased sensitivity through the loss of oocysts during multiple washes.

Formalin-ethyl acetate or formalin-ether concentration procedures, commonly used for other protozoa, are highly inefficient for Cryptosporidium diagnosis. More than 99% of the oocysts present in a specimen may be lost during the procedure. A modification of the formalin-ether technique improves oocyst recovery.

Popular stains for oocysts include modified Ziehl-Neelsen and modified Kinyoun acid-fast stains. Phenol-auramine/Carbol-fuchsin stain has greater sensitivity than the acid-fast stains and is particularly useful for examining stools of patients who may be excreting low numbers of oocysts⁵⁴. The major disadvantage of the auramine stain (and of the antibody stains mentioned below) is that they require the use of a fluorescence microscope.

Detection and identification of oocysts also can be achieved using specific polyclonal or monoclonal antibodies conjugated to fluorescein. Several tests of this type are now commercially available. Antigen assays that are not dependent on fluorescence should be a high research priority⁵⁵.

DNA probes to distinguish between species and isolates of Cryptosporidium are not yet available.

Quality Control

Microscopical identification of oocysts depends on size, shape and staining characteristics. Competence in these techniques usually requires additional specialised training for laboratory technicians. Quality control in the laboratory requires systematic review of a sample of specimens reported as positive and negative, and careful attention to the preparation of reagents and calibration of equipment.

Environmental Samples:

Fluorescein labelled monoclonal and polyclonal antibodies are superior to all other available diagnostic techniques for the detection of oocysts in water or other environmental samples. Because the number of oocysts in such samples is small compared with the numbers of oocysts found in stools of infected patients, large volumes of material must be collected and processed for examination. Water samples in the range of 1000 litres are generally recommended. Present processing and examination techniques are highly labour

intensive; six to eight hours of laboratory time is currently required to process and examine a single specimen. A major limitation of the available techniques is that the viability of the oocysts detected cannot be determined.

The more rapid techniques under investigation include:

- (1) concentration using antibody attached to magnetic particles;
- (2) detection using enhanced chemiluminescence and
- (3) polymerase chain reaction (PCR).

Serodiagnosis

Serodiagnosis, at the present time is primarily a laboratory research and epidemiological tool. It is not currently recommended for individual patient diagnosis.

Antibodies specific to Cryptosporidium have been identified by indirect immunofluorescent antibody (IFA) procedures in patients who recovered from confirmed infections, and for the presumptive diagnosis of cryptosporidiosis in water borne and hospital outbreaks⁵⁶.

Four immunoglobulin classes: IgM, IgG, IgA and IgE involved in Cryptosporidium, have been detected in IFA⁵⁷.

Specific anti-Cryptosporidium IgG and/or IgM have also been detected by ELISA in 95% of patients with cryptosporidiosis at the time of medical presentation and in 100% within two weeks of presentation⁵⁸.

Serological surveys indicate seropositivity rates (IgG) of more than fifty per cent in persons in developing countries and 20%-30% of persons in developed countries, with no evidence of current illness, suggesting that past exposure and asymptomatic infection is common.⁵⁹

Recently a sandwich ELISA was developed for monitoring circulating Cryptosporidium antigens in immunocompromised patients⁶⁰.

2.3.3. Treatment

At present, there is no proven, reproducibly effective treatment for cryptosporidiosis⁶¹. In healthy, immunocompetent individuals, the disease is usually self-limiting and medical management to prevent excessive fluid and mineral loss caused by the watery diarrhoea is the principal treatment required. However, in malnourished children, the elderly, persons with AIDS, cancer patients undergoing

chemotherapy, or transplant patients, Cryptosporidium infection can be a serious and life threatening condition. Affected individuals may develop respiratory cryptosporidial infections, acalculous cholecystitis, or pancreatitis.

Supportive therapy for rehydration is usually required for Cryptosporidium diarrhoea in the form of oral rehydration solutions (ORS) or parenterally administered fluids. In severe cases, especially with vomiting, total parenteral nutrition is necessary. Antidiarrhoeal compounds may be of some value in partially controlling symptoms⁶².

Direct chemotherapy of cryptosporidiosis has failed with over ninety reported drugs, including trimethoprim-sulfamethoxazole, metronidazole and erythromycin. Several new regimens have been investigated in recent years with no clear efficacy. Compounds which have failed critical clinical trial in cryptosporidiosis include: oral spiramycin, intravenous spiramycin and diclazuril. Recent case reports of symptomatic and microbiological cure have appeared using paromomycin⁶³ or (in calves) hyperimmune bovine colostrum. Controlled clinical trials are underway or are under development for: letrazuril, paromomycin, lyophilized hyperimmune bovine colostrum, and the hydroxynaphthoquinone BW 566C80.

Recent developments in cultivation and in mouse models of cryptosporidiosis will help the development of active drugs^{64 65}.

2.3.4 Control and prevention

The direct, obligate parasitic nature of Cryptosporidium life-cycle, favours control by breaking the faecal-oral route(s) of transmission by assuring safe water supplies, sanitary education for personal and household hygiene and also safe disposal of excreta and sewage from humans and animals.

Waterborne, fomites and person-to-person routes of transmission have been documented. Food borne transmission also probably occurs. The infective oocysts of Cryptosporidium can survive for prolonged periods, possibly several months in the environment. However they are unable to survive desiccation or prolonged freezing. Effective water treatment processes play a critical role in the control of water-borne spread.

The occurrence of Cryptosporidium oocysts in water is underestimated using current analytical techniques and in the presence of algae, suspended solid, turbidity, etc.

Few commercial disinfectants including chlorine, are effective in inactivating the oocysts. Free chlorine levels up to 16,000 mg/l (unusable) would be required to disinfect water under conditions common to water treatment. Ozone is much more effective than other disinfectants investigated to date. To disinfect surfaces, 10% formalin, 30% hydrogen peroxide, and 5% ammonia have been used successfully.

Below are the general guidelines to follow in investigation and control of an outbreak of cryptosporidiosis thought to be associated with supplies of drinking water.

1. Prompt epidemiological investigation should be conducted to determine reservoirs, modes of transmission, and priority actions to interrupt the cycle of transmission.
2. "Boil water advice" (elevate to 100°C) should be issued and household water for human consumption and food preparation should be stored in containers which prevent recontamination. The thermal death point of the organism is 60°C for four minutes.
3. Operation and maintenance of water treatment plants should be carefully controlled. Monitoring and surveillance of water quality should be intensified.
4. Cattle, sheep and other livestock should be excluded from the vicinity of water sources for human use especially upstream of abstraction/collection points.

2.3.5 Research needs

1. The further development of both in vitro culture models and laboratory animal models for:
 - (a) testing new chemotherapeutic agents
 - (b) basic studies of the organism's biology and pathophysiology to identify target sites for therapy and the development of effective specific anticryptosporidial drugs.
2. Studies of cryptosporidial metabolic and synthetic systems to identify pathogenetic mechanisms.
3. Improvement of inexpensive, rapid methods of diagnosis that have minimal requirements for specialized equipment and training. Higher priority is needed for country surveys to determine public health significance relative to other diseases.

4. Improved surveillance using internationally standardized protocols (populations to be sampled and diagnostic tests used) to clarify understanding of the incidence and prevalence of Cryptosporidium infection, and distribution of disease, within individual countries. This will permit comparison of data with different regions of the same country and between countries.
5. Investigate more effective water treatment including,
 - (a) alternative means for disinfecting water containing oocysts including the use of mixed oxidants, forms of iodine, ozone, etc.
 - (b) Pre-oxidation of water with ozone as a preliminary treatment which will aid disinfection and enhance floc formation and make filtration more efficient.
 - (c) The use of filter cloths in the Schmutzdecke of slow sand filters to enhance removal of oocysts.
 - (d) The development of more effective methods to treat water in households and the design of safer storage containers.
6. An analysis of the genetic and antigenic diversity within Cryptosporidium and the development of molecular epidemiological methods and sero-epidemiology to clarify routes of transmission, the importance of zoonotic or human sources of infection, and the clinical significance of parasite heterogeneity.
7. Improve methods to monitor the presence of Cryptosporidium in water supplies (e.g. immunologically-based concentration methods) and to establish the viability of oocysts (e.g. organelle staining) and determine the risk of infection according to numbers of viable oocysts present and volume of water consumption.
8. Investigate the role of breast feeding in protection from infection.
9. Evaluate immunological therapies such as hyperimmune bovine or egg immunoglobulin.
10. Assess mechanisms of acquired immunity and the immunogenicity of parasite antigens.
11. Investigate the relationship between clinical disease, malnutrition and effects on growth and development.

12. Determine the economic cost of clinical cryptosporidiosis and of epidemics of cryptosporidiosis.
13. Evaluate the impact of integrated control strategies, particularly education, on disease transmission.

2.4 Isospora belli

2.4.1 Epidemiology and disease

Diarrhoea and illness due to Isospora belli⁶⁶ infection of the small intestine occurs in immunocompromised patients and ranges from self-limited enteritis to a severe illness resembling cryptosporidiosis. The prevalence of isosporiasis in HIV-infected individuals ranges from 0.2% in the USA and Italy to 15% in Haiti. No animal reservoir of infection has been identified to date. Infection follows the ingestion of the sporulated resistant cyst from faeces.

2.4.2 Diagnosis

Coprological examination shows the presence of the large, bottle-shaped oocysts, which are stainable by the same techniques as Cryptosporidium, and are detectable in preserved material. In cases where diagnosis is difficult, flotation techniques as used for Cryptosporidium may be used. The string test has been found valuable in some instances⁶⁷.

2.4.3 Treatment

The disease responds to chemotherapy with trimethoprim-sulfamethoxazole or pyrimethamine-sulfadiazine. Relapses occur in the immunocompromised and secondary prophylaxis is recommended. Intolerance to long-term therapy occurs fairly frequently in those suffering from AIDS, pointing to the need for safer, more specific drugs.

2.4.4 Control and prevention

The assumed route of infection is faecal-oral, so the control methods include avoidance of faecal contamination of foodstuffs and water. Personal hygiene may be less important than the safe disposal of faeces (see comments above on environmental classification). The life cycle is however not well understood⁶⁸ and further investigation is needed.

2.4.5 Research needs

This should include development of in vitro culture techniques and better definition of organism distribution and life cycle. New, more effective drugs are needed.

2.5 B. coli

2.5.1 Epidemiology and disease

Balantidiasis⁶⁹ is a zoonotic disease of the large intestine found world wide, but mainly in tropical and subtropical climates. It is primarily endemic, rarely epidemic. The pathogen, the ciliate Balantidium coli, is infrequently found in man. The primary reservoir of infection is the pig, but infections occur in other animals and non-human primates. The infection in humans and other primates may result in colitis, dysentery or typhlitis⁷⁰. The involvement of the liver and lymph nodes has been reported. The dysentery may resemble amoebic dysentery and is possibly underdiagnosed. Clinical disease has been associated with malnutrition.

This infection can be transmitted by drinking water contaminated with human or pig faeces or by direct contact with faeces of pigs or other reservoir animals. Where pigs are sheltered within the human habitation⁷¹, or have access to water supplies the risk of transmission is greatly increased⁷².

2.5.2 Diagnosis

The large ciliated and active trophozoites are detected in faeces, and the cysts in preserved or concentrated specimens.

2.5.3 Treatment

Tetracycline 500 mg 4 x daily for ten days is effective. In pregnancy and in children where tetracycline is contraindicated, paromomycin is an alternative.

2.5.4 Control and prevention

As discussed in the section on environmental classification, personal hygiene, availability of safe water and the safe disposal of human and animal faeces are important in the control of transmission.

Community:

1. Protect water supplies from pig faeces.
2. Isolate human faeces for a significant time to ensure inactivation of the protozoa before disposal to land.
3. Special training of health care workers in high risk areas in the identification and treatment of this disease.

Individual:

1. Avoid contact with pig faeces.
2. Treat water by boiling, slow sand filters, or diatomaceous earth filters and prevent recontamination.
3. Improve personal hygiene.
4. Cook foods thoroughly.

2.5.5 Research needs

1. Time/concentration curves for various disinfectants at different temperatures should be developed for the inactivation of B. coli cysts.
2. Investigate the prevalence of this infection in high risk areas and more accurately determine modes of transmission.
3. Investigate more accurate methods of diagnosis.

2.6 Microsporidiosis

2.6.1 Epidemiology and disease

In addition to the coccidians Cryptosporidium and Isospora, a newly described genus in the phylum Microspora⁷³, Enterocytozoon has been implicated in diarrhoeal illness and wasting in AIDS. The organism is small (1-2 um in size), located deep within the cytoplasm of cells of the small intestine, and is difficult to identify using conventional histopathological or faecal stains⁷⁴. It is likely that other microsporidia in addition to Enterocytozoon may be associated with diarrhoea in AIDS⁷⁵.

2.6.2 Diagnosis

Ultrastructurally examined small bowel biopsies of more than one hundred AIDS patients with diarrhoea or wasting of unknown origin have confirmed the presence of Enterocytozoon bienewsi in association with mucosal injury. Improvements in diagnosis of the organism in stool specimens include Giemsa-staining of processed faecal homogenates⁷⁶ and calcofluor staining. Histological detection of spores in human tissues can be aided by Brown-Brenn or Brown-Hopps tissue Gram stains, Giemsa, methylene blue-azure II with basic fuchsin, or Ziehl-Neelsen acid fast staining. Enterocytozoon cannot yet be cultured in vitro.

2.6.3 Treatment

Therapeutic responses have been reported with metronidazole at 250-500 mg b.i.d. for twelve to twenty-four days⁷⁷ and with albendazole at 400 mg b.i.d. for four to six weeks⁷⁸. Failures of metronidazole have also been reported.

2.6.4 Control and prevention

Mechanisms of transmission are not yet understood, and the only control measures available so far are of morbidity with available drugs. However, the presence of the spores in faeces suggests that these are a major source of infection. The organism, according to current limited knowledge, apparently falls into the same environmental classification category as E.histolytica so that personal hygiene, availability of safe washing water and also safe disposal of faeces are important.

2.6.5 Research Needs

Research is needed to:

1. Identify the prevalence and incidence of microsporidia in human disease;
2. develop in vitro culture and improved diagnostic techniques for human pathogens;
3. identify effective chemotherapeutic agents, and
4. develop an understanding of the epidemiology of microsporidiosis with emphasis on reservoir hosts and transmission.

3. GENERAL CONSIDERATIONS

3.1 Economic impact of intestinal protozoan infection

Apart from unquantifiable personal and family suffering, intestinal protozoal diseases have direct and indirect economic impacts. There is a direct cost for each case, of diagnosis, hospitalization, medical and nursing care, drugs, and costs of the death, should it occur.

The indirect costs include loss of the income of the breadwinner, or the services of the mother, for the period of the illness. If death should occur then these costs will be amplified, with the loss of working capacity permanent and an inbuilt cost to the state.

There is a shortage of data on the economic impact of infectious disease in general, even in developed countries where figures for prevalence and incidence are usually accessible.

The following are some estimates that are currently available:

- (1) In the United States Giardia is the leading known cause of diarrhoeal disease outbreaks associated with drinking water, is responsible for an estimated minimum of 4000 hospital admissions per year (usually taken to be 10% of the actual number of cases of illness) and annual direct costs of hospital treatment are estimated to amount to over 5 million dollars.
- (2) In 1984 it was estimated that 2% of all adults admitted to general hospitals in Mexico had an amoebic liver abscess, while the direct costs of amoebiasis were estimated to use 1.6% of the budget of the Mexican Ministry of Health.
- (3) The costs of treatment of a child with diarrhoea were estimated in Cebu in the Philippines in 1989 as \$2.50. Half the expenditure on childhood diarrhoea was incurred for hospital in-patients. It is estimated that 19,303,200 diarrhoea episodes took place in under fives in 1989⁷⁹, making the estimated cost of diarrhoea, assuming treatment was applied to all cases, of \$48 million annually. Only a proportion of these diarrhoeas would be caused by protozoa, but it is not unreasonable to assume that approximately 5% of this figure, \$2.5 million, could be ascribed in the Philippines to protozoan childhood diarrhoea, with Cryptosporidium a probable major factor. Globally, such a calculation leads from a childhood diarrhoea total of 1,172,614,500, to a total cost of \$2,931,536,250 and a protozoan component of 58,630,725 cases and around \$150 million per annum. This crude estimate can of course only act as a pointer to the true figure.

3.2 Collaborative role of governments and international agencies

The view is often held by governments that the public will benefit more from economic and social developments than from public health interventions. A major constraint to the control of communicable and tropical diseases in developing countries is the relative lack of government support for public health activities. A national health system will ideally consist of two components, basic medical services (BMS) delivering curative therapeutic services to the individual and the public health service (PHS), which functions in epidemiological surveillance and local disease control. These functions complement the basic medical services. The absence of a public health service, or the running down of an existing one, reduces the ability of the country to control disease because population orientated tasks such as disease control, surveillance and immunization are neglected. Epidemiological surveillance is fundamental to a PHS, but the BMS are unwilling to carry out such tasks and may be too overloaded to do so. The PHS in some countries either does not exist as an independent entity or needs to be strengthened and allowed sufficient autonomy to carry out its functions. In addition, communication between the BMS and the PHS should be intensified since the two institutions are interdependent⁸⁰. International agencies should

endeavour to direct aid to strengthening the PHS in developing countries while supporting primary care activities.

Below we have listed several examples of cooperation and joint action by governments and international agencies against communicable and tropical diseases and malnutrition.

The Interministry (Ministries of Health) meeting of the South Cone Countries of South America on Chagas' Disease in which they established the priority of Chagas' Disease as a Public Health Problem (October, 1991).

Groups collaborating in parasite control initiatives at WHO include: Nutrition, Pharmaceuticals, Health Laboratory Technology, Control of Diarrhoeal Diseases, Community Water Supply and Sanitation and Veterinary Public Health. Regional activity has involved the development of national programmes, training support and resource mobilization in all WHO regions.

There are several recent WHO documents and meeting reports on parasitic disease control⁸¹.

The Interamerican Development Bank has a collaborative exploration with PAHO in implementing sanitation and clean water programmes.

UNICEF ICDC has implemented programmes for building national capacity⁸² in African countries.

Control programmes for intestinal parasites are supported by the governments of Venezuela, Malaysia, The Philippines and the Republic of Korea.

Linking control or research with other programmes, gives advantages in cost and sustainability. Cooperation of protozoal disease workers with the helminth control and the schistosome control programmes might be possible, but problems with evaluation of the impacts of the different approaches might emerge.

A subcommittee on nutrition of the United Nations Administrative Committee on Coordination, reporting in 1989 came to the conclusion that "treatment of intestinal parasites may often be a desirable accompaniment to food supplementation programmes."⁸³

3.3 Education

The United Nations Declaration of Rights of Children gives every child the right to health and to education. To facilitate the eventual global control of infectious and non-infectious diseases, a strategy of health education should be encouraged where health education should be an essential component of the education of every child. Basic education must include the promotion of health interventions. To this end every trained teacher should be additionally trained in health education. Hygienic and

sanitary education, should be integrated across the school curriculum and be linked to science, mathematics, civic studies and the arts. This will help people to take control of the determinants of their own health. To encourage community participation, education messages should be developed by and with the community to which they are addressed.

A recent UNESCO initiative in the school health area recognises the multiple linkages between health and education. For example, adequate sanitation and water supplies should be ideally provided in schools, to promote hygienic practices in children.

Attention is now being focused on promotion of quality of life for those children saved from childhood diseases during the Child Survival and Development Revolution programme (UNICEF/WHO EPI). The UNESCO international project to improve primary school performance, nutrition and health is of great importance here. "The struggle to save children's lives must go hand in hand with an effort to change the lives thus saved." (Director, UNESCO).

The importance of education in basic principles of hygiene of the general population and especially mothers and food handlers cannot be overemphasized. This is especially important because, in spite of major improvements in public works, indiscriminate defecation by children around houses and by agricultural workers, can still provide a source of infection, and there is scope for reduction of parasite transmission by behaviour modification in all but the worst environments. (See also reference ⁸⁴).

The Japanese organization for international cooperation in family planning (JOICFP) has demonstrated in cooperative programmes that parasite control can be used in promoting health education in general.

3.4 Research strengthening

Research strengthening should support scientists and physicians in developing countries who are carrying out medical and operational research on intestinal protozoal diseases of importance in those countries. The support should enhance their capacity to work independently or in collaboration with other research groups. A positive effort should be made to encourage research groups in developing countries to study diseases caused by intestinal protozoa. The aim should be to strengthen capacity in developing countries by transferring technology, training people, and supporting laboratories to develop programmes of practical research into indigenous problems.

A report has recently been produced by a panel of experts⁸⁵ which emphasises the importance of essential national health research in developing countries, international partnerships, financial support for research from international sources and an international mechanism to monitor progress and to promote financial and technical support for research.

4. RECOMMENDATIONS

General

- (a) In control programmes, there are three major areas where available resources need to be directed: childhood infections, infections in pregnancy and AIDS-related infections.
- (b) There is a need to strengthen local skills in evaluation and programme management at a senior level, in addition to and complementary to the local level of field implementation. As well as diagnostic laboratory work a managerial structure is needed to collate, analyse and interpret. Education and Health arms of government should be involved.
- (c) Sewage treatment should be encouraged for all sewered communities and latrine/septic tank programmes for those without sewers.
- (d) Guidelines already developed for the safe use of human excreta as agricultural fertilizer should be updated and additional studies should be undertaken.
- (e) Efforts should be made to encourage continued collaboration in parasite control activities between WHO and the initiatives of other UN agencies such as UNICEF and UNESCO. There may be scope for integration of intestinal protozoal disease control with initiatives against intestinal helminths.
- (f) Collaborations on zoonoses should be explored between human and veterinary health programmes.
- (g) A bank of isolates and strains of important intestinal protozoa should be established.

Chemotherapy

- (a) There is an urgent need for development and evaluation of single-dose treatments.
- (b) Palatable formulations of drugs need to be developed.
- (c) Industry's role in development of new drugs should be investigated and a list of interested firms maintained, together with an evaluation of new approaches.
- (e) Chemotherapy approaches should be situated within the context of health education programmes which promote improvements in sanitation.
- (f) Combination treatment for Giardia and helminths as part of the control approach to helminth infections should be further investigated.

Epidemiology and disease research requirements.

- (a) New molecular biological tools need to be developed and tested for evaluation of the extent and impact of infections with intestinal protozoa. Parasite-related and host-related components of disease need to be analysed.
- (b) Attempts should be made to clarify the contribution of intestinal protozoa to malnutrition, with special efforts to evaluate the contribution of other factors.
- (c) Surveillance and reporting of infections and clinical disease should be encouraged. Relationships between prevalence and morbidity need to be determined.
- (d) Mathematical models of transmission of important intestinal protozoa need to be developed to predict the effectiveness of interventions to control infection.
- (e) Where it is considered that control programmes can be developed, operational research should be carried out to feed back into modifications of existing programmes and the development of future programmes. For example, the impact of control on morbidity, and the mental and physical development of children should be assessed. It should prove possible in many cases to integrate such research with the control programme.

Education

- (a) Lack of breast feeding is the single most important risk factor for diarrhoea in young children. Since it appears probable that breast-feeding with delayed weaning prevents morbidity from cryptosporidiosis, giardiasis, and probably other intestinal protozoa, this practice should be encouraged.
- (b) Elementary hygienic practices should be encouraged and constantly reinforced in schools, health care units and the home through periodic campaigns using the mass media. The existing educational mechanisms developed for the National Diarrhoeal Disease Control Programmes should be used, and additional information (eg. related to the safety of food) should be addressed to the adult population.
- (c) Community based programmes for health educators should be implemented with special emphasis on hand washing. Health education should be included in school programmes.
- (d) Special training courses on important intestinal protozoa should be conducted in developing country settings for primary health care workers, workers in diagnostic laboratories, water treatment plant operators and workers in day care centres.

- (e) WHO published documentation needs updating and revision. New documents on chemotherapy and the assessment of health education are required. Ideally they should be incorporated into a field manual. Spanish translations should be produced.

Laboratory improvement.

- (a) There is a need to introduce initiatives for laboratory improvement programmes, accompanied by appropriate legislation and government regulations, with clear statements of laboratory performance in relation to staffing, technologies, quality control and financial support. This approach should be coordinated on national and international basis, with the development of standard techniques and methods of evaluation. Strategies for the training of health workers should not be neglected, at basic and higher levels.

REFERENCES

1. FEACHEM, R.G. et al (eds). Environmental classification of excreta-related infections. Sanitation and disease, pp. 23-41 (1983)
2. WHO. Programme for Control of Diarrhoeal Diseases. Interim programme report, p. 27 (1990)
3. MARTINEZ-PALOMO, A. et al. Selective Agglutination of Pathogenic Strains of Entamoeba Histolytica Induced by Con A. Nature New Biology 245, pp. 186-187 (1973)
4. TRISSL, D. et al. Surface Properties of Entamoeba : Increased Rates of Human Erythrocyte Phagocytosis in Pathogenic Strains. J.Exp.Med. 148, pp. 1137-1145 (1978)
5. SARGEAUNT, P.G. et al. The differentiation of invasive and non-invasive Entamoeba histolytica by isoenzyme electrophoresis. Trans.R.Soc.Trop.Med.Hyg. 72, pp. 519-521 (1978)
6. WHO Technical Report Series 666
7. STRACHAN, W.D. et al. Immunological differentiation of pathogenic and non-pathogenic isolates of Entamoeba histolytica. Lancet i, pp. 561-562 (1988)
8. GARFINKEL, L.I. et al. DNA probes specific for Entamoeba histolytica possessing pathogenic and non-pathogenic zymodemes. Infect.Immun. 57, pp. 926-931 (1989)
9. TANNICH, E. et al. Genomic DNA differences between pathogenic and non-pathogenic Entamoeba histolytica. Proc.Natl.Acad.Sci.USA 86, pp. 5118-5112 (1989)
10. TANNICH, E. et al. Differentiation of pathogenic from non pathogenic Entamoeba histolytica by restriction fragment analysis of a single gene amplified in vitro. J.Clin.Microbiol. 29, pp. 250-255 (1991a)
11. TACHIBANA, H. et al. Identification of a pathogenic-isolate specific 30,000 M_r antigen of Entamoeba histolytica by using a monoclonal antibody. Infect.Immun. 58, pp. 955-960 (1990)
12. EDMAN, U. et al. Characterization of an immunodominant variable surface antigen from pathogenic and non-pathogenic Entamoeba histolytica. J.Exp.Med. 172, pp. 879-888 (1990)
13. TANNICH, E. et al. Homologous cysteine proteinases of pathogenic and non-pathogenic Entamoeba histolytica: differences in structure and expression. J.Biol.Chem. 266, pp. 4798-4803 (1991b)

14. TANNICH, E. et al. Pathogenic and non-pathogenic *Entamoeba histolytica*: identification and molecular cloning of an iron-containing superoxide dismutase. Molec. Biochem. Parasitol. 49, pp. 61-62 (1991c)
15. CLARK, C.G. and DIAMOND, L.S. Ribosomal RNA genes of "pathogenic" and "non-pathogenic" *Entamoeba histolytica* are distinct. Molec. Biochem. Parasitol. 49, pp. 297-302 (1991a)
16. BRUMPT, E. etude sommaire de l'"*Entamoeba dispar*" n.sp., amibe a kystes quadrinuclees, parasite de l'homme. Bull. Acad. Med. (Paris) 94, pp. 942-952 (1925)
17. CLARK, C.G. and DIAMOND, L.S. The Laredo strain and other "*Entamoeba histolytica*-like" amoebae are *Entamoeba moshkovskii*. Mol. Biochem. Parasitol. 46, pp. 11-18 (1991b) (Amoebae generally referred to as "Laredo strains" or "*E. histolytica*-like", which will grow at 25°C. are now recognised to belong to the species *E. moshkovskii*.)
18. WALSH, J.A. Prevalence of *Entamoeba histolytica* infection in Amoebiasis: Human infection by *Entamoeba histolytica*. (ed. J.I. Ravdin) (1988)
19. FEACHEM, R.G. et al. *Entamoeba histolytica* and amebiasis. Sanitation and Disease - Health aspects of excreta and wastewater management. World Bank Studies in Water Supply and Sanitation 3, John Wiley & sons. Chichester. pp. 337-347 (1983)
20. WHO. Basic Laboratory Methods in Medical Parasitology, pp. 114 (1991)
21. GONZALEZ-RUIZ, A. and RUIZ-PALACIOS, G. *Entamoeba hartmanni*: missing or misidentified? J. Inf. Dis. 164, pp. 612-613 (1991)
22. UNGAR, B.L.P. et al. The use of a monoclonal antibody in an enzyme immunoassay for the detection of *Entamoeba histolytica* in faecal specimens. Amer. J. Trop. Med. Hyg. 34, pp. 465-472 (1985)
23. GONZALEZ-RUIZ, A. - in preparation.
24. MADDISON, S.E. Serodiagnosis of Parasitic Diseases. Clin. Microbiol. Rev. 4, pp. 457-469 (1991)
25. STANLEY, S.L. et al. Serodiagnosis of Invasive amebiasis using recombinant *Entamoeba histolytica* protein. Journal of the American Medical Association 266, pp. 1984-1986 (1991)
26. SHETTY, N. et al. Observations on the interpretation of amoebic serology in endemic areas. J. Trop. Med. Hyg. 91, pp. 222-227 (1988)
27. ANON. Diagnostic Techniques for Intestinal parasitic Infections (IPI) Applicable to Primary Health care (PHC) Services. PDP/85.2 (1985)

28. GUARNER, V. Treatment of amebiasis. MARTINEZ-PALOMO, A. Amebiasis. Human Parasitic Diseases, Vol. 2, pp. 189-212 (1986)
29. WHO. Drugs used in parasitic disease. (WHO model prescribing information.) (1990)
30. KNIGHT, R. Surveys for amoebiasis: Interpretation of data and their implications. Ann.Trop.Med.Parasitol. 69, pp. 35-48 (1975)
31. BOTERO, D. Estudio epidemiológico, terapéutico y quimioproláctico de amibiasis intestinal en el municipio de Apartadó. Antioquia Médica, 21, pp. 217- 227 (1971).

ZULUAGA, H., BOTERO, D., VELEZ, H. et al. Estudios terapéuticos y quimioprolácticos con la droga antiamebiana Win-13146. Antioquia Médica, 21, 559-574 (1971).
32. FEACHEM, R.G. et al. Giardia and giardiasis. ibid, pp. 349-356 (1983)
33. FARTHING, M.J.G. et al. "Giardiasis: impact on child growth" in Diarrhoea and malnutrition in childhood. (eds. J.A. Walker-Smith, A.S. McNeish) Butterworths London, p. 68-78, (1986)
34. AMENT, M.E. and RUBEN, C.E. Relation of giardiasis to abnormal intestinal structure and function in gastrointestinal immunodeficiency syndrome. Gastroenterology 62, pp. 216-226 (1972)
35. SOGIN, M.L. et al. Phylogenetic meaning of the kingdom concept: an unusual ribosomal RNA from Giardia lamblia. Science 243, pp. 75-77 (1989)
36. FAUBERT, G., BEMRICK, W.J. and ERLANDSEN, S.L. Is giardiasis a zoonosis? and Giardiasis:- is it really a zoonosis? Parasitology Today 4, pp. 66-71 (1988)
37. NASH, T.E. et al. Variant specific epitopes of Giardia lamblia. Mol.Biochem.Parasitol. 42, pp. 125-132 (1990)

NASH, T.E. et al. Restriction endonuclease analysis of DNA from 15 Giardia isolates obtained from humans and animals. J.Infect.Dis. 152, pp. 64-73 (1985)
38. NASH, T.E. Antigenic variation in Giardia lamblia. Exp.Parasit. 68, pp. 238-241 (1989)
39. HOPKINS, R.S. and JURANEK, D.D. Acute giardiasis: An improved clinical case definition for epidemiologic studies. Amer.J.Epidemiol. 133, pp. 402-407 (1991)
40. FARTHING, M.J. et al. Natural history of Giardia infection in infants and children in rural Guatemala and its impact on physical growth. Amer.J.Clin.Nutr. 43, pp. 395-405 (1986)

41. MATA, L. "Infections, malnutrition and growth", Journal of Gastroenterol.Hepatol Suppl., 1, pp. 32-43, (1990)
42. TOMKINS, A. and WATSON, F. Malnutrition and infection: a review. United Nations administrative committee on coordination/Subcommittee on Nutrition. State of the Art Series. Nutrition Policy Discussion Paper No. 5, (1989)
43. ROSENTHAL, P. and LIEBMAN, W.M. Comparative study of stool examinations, duodenal aspiration and pediatric enterotest for giardiasis in children. Journal of Pediatrics, pp. 278-279 (1980)
44. GOLDIN, A. et al. Efficient diagnosis of giardiasis among nurse and primary school children in Santiago, Chile, by capture ELISA for the detection of fecal Giardia antigens. Amer.J.Trop.Med.Hyg. 42, pp. 538-545 (1990)
45. ABBASZDEGAN, M. et al. Detection of Giardia cysts with a cDNA probe and application to water samples. Appl.Env.Microbiol. 57, pp. 927-931 (1991)
46. SPEELMAN, P. Single dose tinidazole for the treatment of giardiasis. Antimicrobial Agents and Chemotherapy, 27, pp. 227-229 (1985)
47. EDLIND, T.D. et al. Activity of the anthelmintic benzimidazoles against Giardia lamblia in vitro. J.Inf.Dis. 162, pp. 1408-1411 (1990)
48. HALL, A. and ANWAR, K.S. Albendazole and infections with Trichuris trichiura and Giardia intestinalis. S.E.Asian J.Trop.Med.Publ.Hlth. 22, pp. 84-87 (1991)
49. ADDISS, D.G., JURANEK, D.D., and SPENCER, H. Treatment of children with asymptomatic and non diarrhoeal Giardia infection. Pediatr.Infect.Dis.J. 10, pp. 843-846 (1991)

PICKERING, L.K. and MORROW, A.L. Commentary. ibid. pp. 846-848 (1991)
50. DITRICH, O. et al. The first finding of Cryptosporidium baileyi in man. Parasitol.Res. 77, pp. 44-47 (1991)
51. CASEMORE, D.P. Epidemiological aspects of human cryptosporidiosis. Epidemiology and Infection. 104, pp. 1-28 (1990)

CURRENT, W.L. and GARCIA, L.S. Cryptosporidiosis. Clinical Microbiological Reviews. 4, pp. 325-358 (1991)
52. WHO. Programme for control of diarrhoeal diseases: Interim programme report p. 25 (1990)
53. NICHOLS, G., SAMUEL, D., and MCLAUCHLIN, J. Characterisation of oocyst surface antigens of Cryptosporidium species. In Cryptosporidiosis. Proceedings of the first international workshop (ed. K.W. Angus and D.A. Blewett) Edinburgh Animal Disease Research Association. pp. 121 (1989)

53. NICHOLS, G., SAMUEL, D., and MCLAUCHLIN, J. Characterisation of oocyst surface antigens of *Cryptosporidium* species. In *Cryptosporidiosis. Proceedings of the first international workshop* (ed. K.W. Angus and D.A. Blewett) Edinburgh Animal Disease Research Association, pp. 121 (1989)
- MCDONALD, V., DEER, R.M.A., NINA, J.M.S., WRIGHT, S., CHIODINI, P.L. and MCADAM, K.P.W.J. Characteristics and specificity of hybridoma antibodies against oocyst antigens of *Cryptosporidium parvum* from man. Parasite Immunol. **13**, PP. 251 (1991)
54. CASEMORE, D.P. Laboratory methods for diagnosing cryptosporidiosis. Journal of Clinical Pathology, **44**, pp. 445-451 (1991)
55. UNGAR, B.L.P. Enzyme linked immunosorbent assay for detection of *Cryptosporidium* antigens in faecal specimens. Journal of Clinical Microbiology, **28**, pp. 2491-2495 (1990)
56. D'ANTONIO, R.G. et al. A waterborne outbreak of cryptosporidiosis in normal hosts. Ann.Intern.Med. **103**, pp. 886-888 (1985)
57. CASEMORE, D.P. The antibody response to *Cryptosporidium*, the development of a serological test and its use in a study of immunologically normal persons. Journal of Infection, **14**, pp. 125-134 (1987)
58. UNGAR, B.L.P. et al. Enzyme immunoassay detection of immunoglobulin M and G antibodies to *cryptosporidium* in immunocompetent and immunocompromised persons. J.Infect.Dis. **153**, pp. 570-578 (1986)
59. UNGAR, B.L.P. et al. Seroepidemiology of *Cryptosporidium* infection in two Latin American populations. J.Infect.Dis. **157**, pp. 551-556 (1988)
- UNGAR, B.L.P. et al. Serologic evidence of *Cryptosporidium* infection in US volunteers before and after Peace Corps service in Africa. Arch.Int.Med. **149**, pp. 894-897 (1989)
60. GOMEZ MORALES, M.A. et al. Detection of *Cryptosporidium* circulating antigen/s in human and calf sera. J.Protozool. **38**, pp. 182-183 (1991)
61. LAUGHON, B.E. et al. Summary of the workshop on future directions in discovery and development of therapeutic agents for opportunistic infections associated with AIDS. J.Infect.Dis. **164**, pp. 244-251 (1991)
62. COOK, D.J. et al. Somatostatin treatment for cryptosporidial diarrhoea in a patient with AIDS. Ann.Int.Med. **108**, pp. 708-709 (1988)
63. CLEZY, K. et al. Paromomycin for the treatment of cryptosporidial diarrhoea in AIDS patients. AIDS **5**, pp. 1146-1147 (1991)
- GATHE, J. et al. The effectiveness of paromomycin in the treatment of gastrointestinal cryptosporidiosis (GIC). AIDS Conference, Florence, Abstract MB2270, pp. 249 (June 1991)

65. MEAD, J.R. et al. Chronic *Cryptosporidium parvum* infections in congenitally immune deficient SCID and nude mice. J.Inf.Dis. 163, pp. 1297-1304 (1991)
66. LEVINE, N.D. and BAKER, J.R. The *Isospora-Toxoplasma-Sarcocystis* confusion. Parasitology Today 3, pp. 101-105 (1987)
- FRENKEL, J.K. Beyond the oocyst: over the molehills and mountains of coccidialand. Parasitology Today 3, pp. 250-251 (1987)
67. WHITESIDE, M.E. et al. Enteric coccidiosis among patients with the acquired immunodeficiency syndrome. Amer.J.Trop.Med.Hyg. 33, pp. 1065-1072 (1984)
68. MARKUS, M.B. Origin of extra-intestinal stages of *Isospora belli* in the acquired immune deficiency syndrome (AIDS). Medical Hypotheses, 35, pp. 278 (1991)
- RESTREPO, G., MACHER, A.M. and RADANY, E.H. Disseminated extraintestinal isosporiasis in a patient with acquired immunodeficiency syndrome. Am.J.Clin.Pathol. 87, pp. 536-542 (1987)
- MELHORN, H. and MARKUS, M.B. Electron microscopy of stages of *Isospora felis* of the cat in the intestinal lymph node of the mouse. Z.Parasitenk. 51, pp. 15-24 (1976)
69. FEACHEM, R.G. et al. (eds). *Balantidium* and balantidiasis. ibid. pp. 333-336 (1983)
70. LEE, R. et al. Typhlitis due to *Balantidium coli* in captive lowland gorillas. Rev.Inf.Dis. 12, pp. 1052-1059 (1990)
71. RADFORD, A.J. Balantidiasis in Papua New Guinea. Med.J.Austral., 60, pp. 238-241 (1973)
72. WALZER, P.D. et al. Balantidiasis outbreak in Truk. Amer.J.Trop.Med.Hyg., 22, pp. 33-41 (1973)
73. CANNING, E. and LOM, J. The microsporidia of vertebrates. Academic Press, London, (1986)
- CAVALIER-SMITH, T. Eukaryotes with no mitochondria. Nature 326, pp. 332-333 (1988)
- CANNING, E.U. and HOLLISTER, W.S. Microsporidia of mammals - Widespread pathogens or opportunistic curiosities? Parasitology Today 3, pp. 267-273 (1987)
74. ORENSTEIN, J.M. et al. Intestinal microsporidiosis as a cause of diarrhoea in human immunodeficiency virus infected patients. A report of 20 cases. Human Pathol., 21, pp. 475-481 (1990)

- CANNING, E.U. and HOLLISTER, W.S. Microsporidia of mammals - Widespread pathogens or opportunistic curiosities? Parasitology Today 3, pp. 267-273 (1987)
74. ORENSTEIN, J.M. et al. Intestinal microsporidiosis as a cause of diarrhoea in human immunodeficiency virus infected patients. A report of 20 cases. Human Pathol., 21, pp. 475-481 (1990)
75. WICHER, V. et al. Enteric infection with an obligate intracellular parasite, *Encephalitozoon cuniculi* in an experimental model. Infect. Immun., 59, pp. 2225-2231 (1991)
76. VAN GOOL, T. et al. Diagnosis of *Enterocytozoon bieneusi* microsporidiosis in AIDS patients by recovery of spores from faeces. Lancet, 336, pp. 696-697 (1990)
- ORENSTEIN, J.M. et al. Identification of spores of *Enterocytozoon bieneusi* in stool and duodenal fluid from AIDS patients. Lancet, 336, pp. 1127-1128 (1990)
77. EEF TINCK SCHATTENKERK, JKM et al. Clinical significance of small intestinal microsporidiosis in HIV1 infected individuals. Lancet, 337, pp. 895-898 (1991)
78. BLANSHARD, C. et al. Treatment of intestinal microsporidiosis with albendazole. VII Intl.Conf.AIDS Florence, Vol 2., pp. 248 (WB 2265) (1991)
79. WHO. Programme for control of diarrhoeal diseases. Interim programme report, (1990)
80. LIESE, B. The organization of schistosomiasis control. Parasitology Today 2, pp. 339-345 (1986)
81. WHO. Prevention and control of intestinal parasitic infections. Report of a WHO expert committee. WHO Technical Report Series. No. 749 (1987)
- WHO. PDP/85.1 1985: General strategies for prevention and control of Intestinal Parasitic infections (IPI) within Primary Health Care (PHC)
- WHO. PDP/85.2 1985: Diagnostic techniques for intestinal parasitic infections (IPI) applicable to primary health care (PHC) services.
- WHO. PDP/85.3 1985: Planning, Implementation, Monitoring and evaluation of the control of intestinal parasitic infections (IPI) programmes.
- WHO. PDP/85.4 1985: Surveillance and survey methodology for intestinal parasitic infections (IPI).
- WHO. Informal consultation on intestinal helminth infections.
WHO/CDS/IPI/90.1 (Geneva, 9-12 July 1990)

82. LEMMA, A. and VALKONEN, E. Towards national capacity building in Africa. Unicef International Child Development Centre, Florence, Italy (1989)
83. TOMKINS, A. and WATSON, F. Malnutrition and infection: a review. United Nations administrative committee on coordination/Subcommittee on Nutrition. State of the Art Series. Nutrition Policy Discussion Paper No. 5., (1989)
84. HALLORAN, M.E. et al. Infectious Disease and the UNESCO Basic Education Initiative. Parasitology Today 5, pp. 358-359 (1989)
85. Commission on Health Research for Development. Health Research: Essential Link to Equity in Development. Oxford University Press, U.S.A. (1990)

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