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Intestinal protozoan and helminthic infections

Report of a
WHO Scientific Group

World Health Organization
Technical Report Series
666

World Health Organization, Geneva 1981

ISBN 92 4 120666 7

PRINTED IN SWITZERLAND

81/5002 – Schüler S.A. – 8000

CONTENTS

WORLD HEALTH ORGANIZATION

TECHNICAL REPORT SERIES

No. 666

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CORRIGENDA

Page 19, line 13:

Delete: about 10^{14} *Ascaris* eggs

Insert: about 9×10^{14} *Ascaris* eggs

Page 51, lines 10–12:

Delete: Used in a single dose of 500 mg or a daily dose of 200 mg for 5–7 days, they cure 70–85% of patients; single doses have been reported to be more effective.

Insert: Used in a single dose of 1.5–2.0 g or a daily dose of 0.3–1.0 g for 5–7 days, according to the drug chosen, they cure 70–85% of patients; single doses have been reported to be more effective.

Page 59, line 11:

Delete: about 10^{14} *Ascaris* eggs

Insert: about 9×10^{14} *Ascaris* eggs

WHO SCIENTIFIC GROUP ON INTESTINAL PROTOZOAN AND HELMINTHIC INFECTIONS

Geneva, 27 October – 1 November 1980

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INTESTINAL PROTOZOAN AND HELMINTHIC INFECTIONS

Report of a WHO Scientific Group

1. INTRODUCTION

A WHO Scientific Group on Intestinal Protozoan and Helminthic Infections met in Geneva from 27 October to 1 November 1980. The meeting was opened by Dr A. Davis, Director, Parasitic Diseases Programme, on behalf of the Director-General.

The public health importance of intestinal parasitic infections continues because of their high prevalence, their virtually global distribution, and their effects on both the nutritional and the immune status of populations, particularly those living in the tropical and subtropical areas. These latter effects are of obvious significance in undernourished populations because of the poor utilization of food, which is frequently in short supply, and deficiencies in energy intake, proteins, vitamins and trace elements. Immunosuppression may also lower the resistance of such populations to other infections and render active immunization procedures less effective. Intestinal parasitic infections mainly affect the physical and mental development of children, who are the most vulnerable, but polyparasitism is also common in that most disadvantaged sector, the rural poor of the tropics.

In the 1960s, the World Health Organization played a very active role in the international promotion of both research on intestinal parasitic infections and their control. In the 1970s, WHO's activities in the fields of intestinal infections were somewhat restricted because of manpower and budgetary constraints. The advances in knowledge of the last ten years have nevertheless made it practicable to consider a reactivation of the programme of research and control in human intestinal protozoan and helminthic infections.

The development of new and effective drugs against intestinal infections has now made population-based chemotherapy available as an important element in control measures. However, chemotherapy alone does not solve all the problems, and the role of other measures

such as sanitation, education and community participation should also be evaluated. The success or failure of control may depend a great deal on man's behavioural attitudes and customs; therefore the wholehearted participation of any communities involved in active programmes directed towards the improvement of their health and standard of living is of crucial importance.

Many of the basic public health activities necessary for a programme of parasitic disease control will be covered by other WHO programmes such as those concerned with diarrhoeal diseases, water supply and sanitation, essential drugs, and—given the widespread acceptance of the concept of "health for all by the year 2000"—primary health care. Optimum use of the available technical tools will lead to maximum benefits from all these programmes in different situations.

2. GENERAL CONSIDERATIONS

2.1 Definitions and terminology

This report concentrates mainly on the intestinal parasites of man that are of major public health importance, namely:

Entamoeba histolytica	*Necator americanus*
Giardia intestinalis	*Ascaris lumbricoides*
Ancylostoma duodenale	*Strongyloides stercoralis*

Other intestinal parasites of man are also described, according to their clinical and public health importance.

With regard to the human intestinal protozoa, the Group paid attention to *Entamoeba polecki, Dientamoeba fragilis, Isospora belli, Sarcocystis suihominis, S. hominis* and *Balantidium coli.* Other species of protozoa that inhabit the human intestinal tract (but are not regarded as pathogenic), such as *Entamoeba gingivalis, Iodamoeba buetschlii, Endolimax nana, Enteromonas hominis, Chilomastix mesnili* and *Pentatrichomonas (Trichomonas) hominis,* were not discussed.

Schistosoma mansoni and *S. japonicum* infections, which present important public health problems, have been dealt with separately in a recent report (*1*) and in various publications of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases.

Clonorchis sinensis, Opisthorchis felineus, O. viverrini, and *Fasciola hepatica* are parasites of the liver and are of public health importance only in certain areas of the world; they require further study by experts in the affected areas. Several other flukes parasitizing the human gut, such as *Fasciolopsis buski* and *Echinostoma, Heterophyes, Metagonimus* and *Gastrodiscoides* species, are included in this report.

With regard to the cestodes, *Taenia solium, T. saginata, Hymenolepis nana* and *Diphyllobothrium latum* (*Bothriocephalus latus*) were discussed by the Group, but not *Hymenolepis diminuta, Dipylidium caninum,* and *Raillietina, Inermicapsifer* or *Bertiella* species, which occur only sporadically.

From among the intestinal nematodes that parasitize man, *Trichuris trichiura (Trichocephalus trichiuris), Ancylostoma ceylanicum, Trichostrongylus* species, *Oesophagostomum* species, *Capillaria philippinensis, Angiostrongylus (Morerastrongylus) costaricensis, Enterobius vermicularis, Anisakis* and *Phocanema* species were also discussed.

The Group did not deal with several species of tissue parasites for which the gut is only an entry to the human organism (e.g., *Toxocara* species, *Toxascaris leonina, Paragonimus* species, *Echinococcus* species and *Angiostrongylus cantonensis*). *Trichinella spiralis,* whose adult reproductive stages are spent in the human gut, may be regarded as a human tissue parasite since most of the pathology of trichinellosis is related to the muscle phase of invasion. Many of these infections were described in a recent WHO report on the parasitic zoonoses (*2*).

The list of parasites (considered here) that are found in the human gut is not complete for two reasons. First, the taxonomic position and life-cycle of some human parasites, such as *Strongyloides* in Papua New Guinea and *Taenia* in the Philippines, remain unclear. Secondly, man is exposed not only to specific parasites but also to many non-specific parasites which may occur accidentally in humans, e.g., the strobilocercus of *Taenia taeniaeformis.*

The nomenclature of some intestinal parasites is still a matter of controversy. For example, *Giardia lamblia* is well established in the USA as a synonym for the taxonomically correct name *Giardia intestinalis* (Lambl, 1859), but the synonym used in eastern Europe is *Lamblia intestinalis* (*22*). *Taeniarhynchus saginatus* is a synonym used in the literature of the USSR for *Taenia saginata.* There may be confusion also regarding the names of certain infections, e.g., giardiasis, taeniasis, trichuriasis. However, to avoid misunderstanding, *T. solium* taeniasis and *T. saginata* taeniasis are used for differenti-

ating between *T. solium* and *T. saginata* infections; ancylostomiasis is used for *Ancylostoma duodenale* infection, *A. ceylanicum* ancylostomiasis for *A. ceylanicum* infection, and necatoriasis for *Necator americanus* infection.

While there seems to be a great measure of unanimity as to what constitutes a parasitological case, this is not always so in relation to the clinical or epidemiological situation. As there is not always a clear-cut difference between infection and disease, the same terms are used for both, e.g., amoebiasis is used for the asymptomatic (carrier) infection and for the disease. The Group therefore recommended that clinicians should use more detailed descriptive terminology in individual cases, e.g., amoebic dysentery, amoebic non-dysenteric colitis, amoebic liver abscess, and asymptomatic amoebiasis, as defined by the WHO Expert Committee on Amoebiasis some years ago (*3*). The laboratory diagnosis should be amplified to cover the stages of the parasite, e.g., *Entamoeba histolytica* cyst, or haematophagous trophozoite. Similarly, the Group strongly urged that more exact terms should be used in the description of pathological changes and specimens.

2.2 Pathogenicity, mortality and morbidity

2.2.1 *Host factors*

In infectious disease, the causative pathology results from an interplay of host and parasite factors. Host factors, which have been relatively neglected in studies on intestinal parasites, may be classified as follows:

—environmental factors,
—innate susceptibility,
—nutrition,
—coexistent disease,
—immune competence, and
—drug therapy.

2.2.1.1 *Environmental factors.* The clearest host factors affecting the pathogenicity of intestinal parasitic infections are those linked with the conditions predisposing to infection and reinfection. For example, many millions of people in the tropics live in inadequate housing without proper water supplies or sanitation; waterborne infections (such as amoebiasis, giardiasis) are common because of the absence

of safe drinking-water, and the primitive conditions of food storage and handling result in frequent faecal contamination of food.

It is difficult to change the defaecation habits of people who have never known a toilet, and small children cannot be constantly supervised in this regard by their parents, who are preoccupied with gaining a subsistence living. The habit of using the same defaecation site should be discouraged; for example, the banana patch used at the back of houses is, because of the damp shady conditions, an excellent site for hookworm and *Strongyloides* transmission. These types of living conditions predispose to heavy loads of helminths and are a factor of fundamental importance when considering diseases such as ascariasis or hookworm infection.

Even in communities with good water and sanitation facilities, certain conditions favour disease transmission. Reports from institutions for mentally retarded children have shown how frequently intestinal parasitic infections occur in subnormal children. Homosexuals may also have frequent intestinal parasitic infections. Cooking habits can promote some infections; and occupational factors also affect prevalence, e.g., when agricultural workers come into close contact with soil contaminated with faeces of human origin.

2.2.1.2 *Innate susceptibility.* Two groups of conditions operate here with respect to the host: one relates to the host's intrinsic susceptibility, and the other to acquired modifications in the host which are encountered by the invading parasite.

Good examples of the first group can be found in experimental findings in amoebiasis. The different species of laboratory animals show a wide spectrum of resistance to amoebiasis. This could be expressed in part thus: cats > dogs > apes. Inbred rat strains differ in their susceptibility to caecal invasion and young male animals are often more susceptible. More work is needed, however, on isogenic animal hosts and their susceptibility to intestinal parasites. Just how far findings such as these can be extrapolated to man is debatable. Severe amoebiasis is common in Bantu in South Africa. Hepatic amoebiasis is seven times more common in adult males. There is some evidence that genetic markers, such as specific blood groups or the pattern of HLA antigens, may be linked with the acquisition of *Giardia.*

Among the examples of the second group of factors are:

(1) Hormonal influences. The change in the sex prevalence of patients with amoebic liver abscess occurs only after puberty. Although

experimental evidence is conflicting, both the sex hormones and cholesterol have been shown to influence the course of experimental amoebiasis. Raised corticosteroid levels are implicated in the severe invasive amoebiasis encountered under stress conditions and in pregnancy. Certainly concomitant cortisone therapy worsens the prognosis in amoebiasis and strongyloidiasis.

(2) Organisms already present in the intestine of man may modify the way the disease becomes manifest. The role of bacterial associates in promoting amoebiasis in the weanling guinea-pig has been extensively investigated and is undoubtedly important in this model. Little is known about the effect on new luminal parasitic infections of other parasites already *in situ.* A crowding-out phenomenon in tapeworms has been described and in severe multiple infections, individual worms are often stunted.

(3) There is evidence that physiological changes within the intestinal lumen (with respect to pH, oxidation reduction potentials, and certain organic substances) could influence the lumen-dwelling parasites.

2.2.1.3 *Nutrition.* Protein-energy malnutrition depresses cellular immune responses and may have a complex effect on intestinal parasite infection. In rats, protein deficiency predisposes to caecal ulceration with *Entamoeba histolytica* and carbohydrate supplementation of a protein-deficient diet favours infection but reduces tissue invasion. In hamsters, an iron-rich diet increases the incidence and severity of amoebic liver abscesses.

An important factor in the development of severe hookworm anaemia is the level of iron stores in the host tissues. The unexplained demand by *Diphyllobothrium latum* for cyanocobalamin (vitamin B_{12}) must place a strain on the host's requirement for this vitamin. Protein-losing enteropathy in infections with hookworm, *Strongyloides,* and *Fasciolopsis buski* results in hypoalbuminaemia.

2.2.1.4 *Coexistent disease.* There are many examples of coexistent disease which influences the outcome of intestinal parasitic infection. They include a large number of general conditions (e.g., hyperpyrexia, myxoedema) as well as wasting diseases that prejudice the host's immune responses (e.g., tuberculosis, lymphoma). Other diseases favour the parasite by target organ involvement which facilitates parasite invasion. Ulcerative colitis (in amoebiasis) and megacolon (favouring larval repenetration in strongyloidiasis) are examples.

Mice infected with either *S. mansoni* or *Trichuris muris* are more susceptible to amoebic tissue invasion. Colonic carcinomas in patients infected with *E. histolytica* seem to be parasitized more frequently. The genesis of amoeboma is unexplained but the unusual intense fibrosis may be due to a host factor. The general point should be made that there is often a difference between the host's response on first exposure to the parasite and on subsequent exposures.

2.2.1.5 *Immune competence.* Primary immunodeficiency diseases may play a role in influencing intestinal parasitic infections, but because of their rarity there is little information on the effects of their association with intestinal parasitic disease. It was suggested that lack of secretory IgA immunoglobulin may lead to increased bacterial colonization of the jejunum and consequently increased susceptibility to *Giardia* infection, but subsequent investigations did not confirm this finding.

Secondary immunodeficiency, resulting from drug therapy and influencing the pathogenicity of *Strongyloides,* is well documented. Indeed it poses a problem of clinical management in endemic areas in patients who are on immunosuppressive therapy; all such patients have to be screened for *Strongyloides* before commencing this therapy.

The nature of the host's immune response which influences the outcome of a parasitic infection has been studied extensively in schistosomiasis, and a fascinating picture has emerged. Both schistosomula and adult worms promote host immunity. Adult worms *in situ* influence the reception of later-invading schistosomula. The incorporation of host proteins into the adult worm's integument permits them to evade the host's immune response. Delayed hypersensitivity responses play an important role in egg granuloma formation. Factors such as these will influence the way the disease is expressed in man. In contrast to schistosomiasis, the information available on the mechanism of host immunity to other intestinal infections is meagre. While information on antibodies is well documented in invasive amoebiasis, such antibodies do not usually have a protective role and cell-mediated immunity has been little investigated. Repeated self-infections with *Necator americanus* have been induced, suggesting that, if protective immunity eventually does develop in natural conditions, it may be only partial.

2.2.1.6 *Drug therapy.* Specific drug therapy in intestinal parasitic infections is one of the success stories of modern chemotherapy, but

as individual treatment becomes more practical for more people, the possibility that this treatment may fail for other reasons must be considered. A study of the changes in the stability or bioavailability of drugs after storage in tropical conditions has been neglected. Defects of absorption may occur owing to abnormal gastric and intestinal motility or physicochemical factors in the small intestine. Some (or many) of these factors could influence the pathogenesis, morbidity or even mortality in intestinal parasitic infections.

2.2.2 *Parasite factors*

Pathogenic parasitic infections of the intestinal tract occur in degrees of severity ranging from inapparent to overt, and in some instances they may be life-threatening or fatal. However, even inapparent infections are important in maintaining endemicity. Among the human intestinal helminths, there are none that can be classified as nonpathogenic in the sense that they are invariably harmless. On the other hand, among the nine common intestinal protozoa of man, only three are regarded as both pathogenic and common (*Entamoeba histolytica, Giardia intestinalis, Balantidium coli*); in individual cases even these often fail to produce perceptible disease.

The parasite factors that influence pathogenicity, morbidity and mortality in protozoan and helminthic infections of the intestine can be considered under five main headings: (1) population density, (2) mode and pattern of entry, (3) virulence and adaptation to the human host, (4) responses to intercurrent and associated infections, and (5) responses to the modified host.

2.2.2.1 *Population density.* Intestinal parasites, like free-living organisms, require space for living. Although the intestine is large, the different species of parasite that inhabit it are adapted to living only in certain parts, and even within those parts there are areas that are ideal and others barely tolerable. This is especially apparent in experimental hookworm infections. Thus for each helminth or protozoan that lives in the intestine, there is a threshold of tolerance that establishes the size of the population that can be accommodated free of significant pathogenicity to a given host. A population exceeding that threshold will be pathogenic in proportion to its size.

The approximate size of this threshold population has been determined for *Ascaris, Necator* and *Trichuris,* and less precisely for *Ancylostoma,* but not for *Strongyloides, Enterobius,* the tapeworms, or the

trematodes. However, applicable to all is the general helminthological principle that worms in small numbers are relatively well tolerated, while heavy and massive infections cause signs and symptoms that are characteristic of each specific infection—namely, anaemia in hookworm disease and diarrhoea or dysentery in trichuriasis. In each instance, crowding affects the worm's normal feeding, mating and other activities, as well as the dispersion of toxic secretions and excretions; in the case of hookworms and whipworms, crowding results in excessive mechanical damage to the host's mucosa.

The pathogenicity of migrating larvae of *Ascaris* and hookworms in the tissues also is related to the size of the inoculum, at least in initial infections. A massive inoculation of *Ascaris* in pigs may produce few worms or none, while small inocula of 50 infective eggs consistently produce patent infections (*4*). However, massive inocula may cause severe pulmonary symptoms; for example, four male students were hospitalized with severe pneumonitis after eating food that had maliciously been seeded with large numbers of infective eggs of *A. suum* (*5*).

There is no principle of protozoology that corresponds with the crowding-effect principle of helminthology. Presumably when *G. intestinalis* causes diarrhoea and *E. histolytica* causes dysentery, the trophozoites are numerous and the colonized area is large, but in protozoan infections the terms "heavy" and "light" are inappropriate because the size of the colony may vary from day to day, and the number of organisms shed in the faeces frequently does not correspond to the extent or density of the trophozoite population. The size of the inoculum is a factor in establishing *E. histolytica* infection and causing ulceration in experiments on laboratory animals, but other factors appear to have a greater influence on pathogenicity in man.

2.2.2.2 *Mode and pattern of entry. Ancylostoma duodenale* infection can be acquired through the skin or by mouth. Larvae entering through the skin pass through the lungs, causing pulmonary symptoms. However, the best known pulmonary disease caused by *A. duodenale* larvae, the so-called Wakana disease, is caused by larvae entering by the oral route and the symptoms coincide with the period of development in the intestinal mucosa (*6*).

In Europe, where transmission of ascariasis is interrupted by the low temperatures of winter, and in Saudi Arabia, where it is limited to one brief rainy season, pulmonary disease (Löffler's syndrome) caused by the migrating larvae is common and may be severe even

when caused by a few larvae. In tropical areas, where favourable temperatures and rainfall permit continuous all-the-year-round transmission, ascaris pneumonitis is rare.

A pattern of seasonal transmission has been described in which larvae of *A. duodenale,* acquired late in the rainy season or early in the long dry season, are maintained in the intestine as larvae until a few weeks before the onset of the rainy season, when they mature and begin laying eggs (*7*). These observations may require reinterpretation as further studies are made on the fate or function of *Ancylostoma* larvae, sequestered in the muscles (*8, 9*).

2.2.2.3 *Virulence and adaptation to the human host.* With few exceptions, host specificity is relative rather than absolute. Among the parasites found in the human host, only a few occur naturally in that host alone. To some parasites, man is a final host; to others, an intermediate or paratenic (transfer) host.

As the paratenic host, human beings are most frequently infected with the larval stages of helminth parasites from their most closely associated animals—namely, the ascarids and hookworms of dogs and cats. In small mammals, such as rats and mice, which serve as hosts for these parasites in nature, the larvae and intermediate stages are relatively nonpathogenic; in man, they cause diseases that vary in severity according to the numbers present, and some species are more pathogenic than others, depending on special adaptations for migration, localization in certain organs, and longevity.

As the final host for many zoonotic intestinal parasites, human beings commonly become infected with the intestinal helminths of sheep and goats, and less commonly with those of pigs, dogs and cats. The diseases caused by such parasites generally are mild and transient. However, intestinal infections with zoonotic protozoa, which are well tolerated in their natural hosts (e.g., *Balantidium coli* of pigs), may be severe in man.

Geographically diverse strains of *E. histolytica* and *Giardia* are suspected of showing differences in pathogenicity, *E. histolytica* being more pathogenic in the tropics and *Giardia* unusually pathogenic in certain temperate regions. Strains of *E. histolytica* maintained by *in vitro* cultivation differ markedly in their pathogenicity in experimental animals (*10*). The loss of virulence in strains of *E. histolytica* under continuous cultivation can be restored. The *Strongyloides* infections acquired by European military personnel in south-eastern Asia have

more frequently produced migrating skin lesions (larva currens) than have infections from strains in other regions (*11*).

2.2.2.4 *Responses to intercurrent and associated infections.* In children with acute febrile illnesses, *Ascaris* is often seen migrating from the mouth and nose as well as from the anus. During such migrations the adult worms may migrate into the trachea, gallbladder, intrahepatic bile ducts, or pancreatic ducts. In regions where several kinds of intestinal parasite are endemic, polyparasitism is common and the most frequently observed combinations in some regions are triple infections with *Ascaris, Trichuris* and hookworms. Little is known about the interaction of these and other intestinal parasites, but it is reasonable to expect that a relationship does exist in certain combinations. There is evidence suggesting that the frequency of multiple infections is not random (*12*). Several observers have reported a high frequency of concomitant infections with *Trichuris trichiura* and *Entamoeba histolytica,* especially in young children (*13*).

2.2.2.5 *Responses to the modified host.* In the immunosuppressed, immunodefective, or debilitated host, certain parasites, unable to maintain normal host relations, overwhelm the host by internal autoinfection as in *Strongyloides. E. histolytica* infection may be affected by pregnancy and corticosteroid therapy.

Parenteral feeding of surgical patients may affect some kinds of intestinal parasite more than others. In parenterally fed rats, *Trichinella spiralis* can develop normally. The adult *Hymenolepis diminuta* can live and grow without exogenous food in the host intestine, but the cysticercoids of *H. diminuta* are unable to do so (*14*). The effects of parenteral feeding on worm infections in people are unknown.

In conclusion, the parasite factor of first importance in the pathogenicity of intestinal helminths is the parasite population density, i.e., the relative worm burden; among intestinal protozoa the chief disease factor probably is strain virulence. In hookworm, a secondary factor is the mode of entry (cutaneous or oral), and in ascariasis the seasonal exposure to infection is a contributing factor. For all intestinal parasites the effects of intercurrent and associated infections may be significant. Poor adaptation to the human host, especially among zoonotic species, and inability to adjust to a nonreacting or parenterally fed host may also influence pathogenicity.

2.3 Prevalence and intensity of infections

The global prevalence and intensity of human intestinal protozoan and helminthic infections show considerable variations in distribution and seasonal occurrence because of geographical and climatic factors and human activities (such as changing the environment and improving sanitation). These are well-known facts but other factors remain unclear, such as the reason why the prevalence and intensity of intestinal infections frequently show significant regional or local differences. Another basic problem is understanding the factors responsible for a higher intensity of infection, because the severity of the clinical pathology frequently depends on the number of parasites carried in the host. In epidemiological terms, the lack of direct multiplication in the host means that there are greatly overdispersed parasite populations, best described by a truncated form of the negative binomial distribution, in which there are some infected persons with much higher numbers of parasites, out of proportion to that which may be calculated on the basis of a probability of contacts (*15*).

A fairly stable level of endemicity of many protozoan and helminthic intestinal infections is a result of a dynamic process based on repeated reinfections. The frequency of reinfections depends mainly on two factors: the infection pressure and host susceptibility, in which immunological mechanisms play a most important role. The term "infection pressure" was used by Gemmell & Johnstone (*16*) in their studies on hydatidosis and cysticercosis and refers to the number of exposures per host in a certain area within a given time.

The variables influencing the infection pressure of parasitic intestinal infections may be divided into two groups: the environmental and the socioeconomic. There is obviously no clear-cut distinction between the two groups because the environment influences the socioeconomic status of people and human activities may change the environment. A useful simplification would be to accept that environmental factors are responsible for the development and spread of parasitic invasive stages, whereas socioeconomic factors are responsible for contamination of the environment with parasites by re-establishing contacts with the parasitic invasive stages.

2.3.1 *Environmental factors*

The environment has a crucial role in the transmission of many infections. An evolutionary trend to bypass the environmental hazard

by autoinfection is obvious in hymenolepiasis and strongyloidiasis; this has also been observed in human hookworm infections but transmammary infection or autoreinfection from filariform larvae (developed in submucosal "cysts" containing adult worms) probably plays only a minor role in the spread of hookworms.

The environmental factors that influence the infection pressure of intestinal parasitic infections are related to the distribution pattern, the latency period, the survival time and the eventual multiplication of the infective stages of the parasite. The degree of contamination of the environment with the products of intestinal parasites is enormous and depends largely on inadequate excreta disposal. Two facts illustrate the situation. The global external environment is contaminated daily by about 10^{14} *Ascaris* eggs. In 1975, in the rural sector of developing countries (excluding China), 1190 million people, i.e., 85% of the total population, were lacking adequate sanitation (*17*).

The contamination of the environment is uneven; in the case of ascariasis, it is concentrated around the houses, where small children are the most important disseminators of the infection; hookworm eggs are disseminated mostly by adolescents and adults, and the contamination is heaviest around the edges of cultivated fields. There are few comparable data on the selective environmental contamination in the case of other intestinal infections. The distribution of parasitic eggs and larvae on the ground is not only "horizontal" but also "vertical", i.e., "horizontal" spread of the invasive stages to plants above the ground surface occurs as does "vertical" penetration into the ground to survive unsuitable conditions on the soil surface, such as insolation or dryness. Invasive hookworm larvae may actively pass through a 30-cm layer of the soil.

Both the latency period and a long survival time may contribute to the further horizontal or vertical dispersion of the invasive stages in the environment. The latency period, which is necessary for maturation of the invasive stages, is short or nonexistent in the case of the protozoan *Entamoeba* and *Giardia* cysts; it is a question of days or weeks for hookworm, roundworm and whipworm eggs. It is much longer in the case of infections transmitted through intermediate hosts, such as capillariasis; in these infections, the invasive stages appear at a later stage but the parasite numbers are increased by a multiplication in the tissues of intermediate hosts. Sometimes, in unfavourable environmental conditions, the latency period is prolonged, e.g., a low temperature inhibits the development of *Ascaris* eggs.

The maximum survival time of invasive eggs in the external environment is quite long—up to 60 days for *Giardia* cysts in water, up to 122 days for hookworm larvae during the rainy season in India, and up to 6–9 years for *Ascaris* eggs. The main factors that affect survival time in a positive or negative way are the character of the soil, moisture, temperature, oxygen, pH, insolation, and the presence of organic matter and antagonistic organisms, e.g., fungi that attack *Ascaris* eggs. Besides these, there are many other factors that make one type of soil—sandy, for instance—more favourable for the development of hookworm larvae, and another—clay—for *Ascaris* eggs. In general, most of the parasites that have an environmental phase have specific requirements but they also have the possibility of adapting themselves to different conditions. The existence of a *Strongyloides* strain adapted to temperate zones is an example. Laboratory and field studies on these environmental variables are difficult because of the large number of factors and their complex interactions, and the lack of techniques to evaluate the invasiveness of the invasive stages, e.g., in *Giardia* and *Taenia.* Therefore, what is known about environmental factors is still very superficial and inadequate for the planning of realistic control measures.

Measurement of the contamination of the environment, together with behavioural studies, which may include cultural and religious activities, may provide a clearer understanding of the potential of parasitic transmission in a particular area.

2.3.2 *Socioeconomic factors*

What is known about the socioeconomic factors that are responsible for the transmission of intestinal parasitic infections is extremely scanty and superficial for reasons described by Dunn (*18*). There are many kinds of human behaviour that may influence the prevalence and intensity of intestinal infections. They can be classified into four major groups: deliberate and nondeliberate, each of these in turn either promoting health or contributing to ill-health. The variety and complexity of behavioural factors that influence a parasitic infection are best illustrated by the studies on man/water contact in schistosomiasis (*19*). There are two field methods of medical and behavioural research, each one having several limitations. The epidemiological type of survey deals with a limited number of variables or determinants in an unlimited number of social units, but it is horizontal or shallow;

the case study, on the other hand, can deal in depth with many variables but the number of social units examined is limited.

Most of the studies undertaken so far on intestinal parasitic infections have dealt with traditional social determinants such as age and sex; a few include density of population and occupational and ethnic factors. The social units examined were mostly families, rural versus urban parts of the population, and poor versus rich social classes. Models of complex studies of the ecological and sociological factors are the surveys on intestinal parasitism in Malaysian aborigines (*23*), and a rural study on the prevalence of intestinal parasites in Uttar Pradesh, India (*24*).

The shape of the age-prevalence curves may be related to the principal mode of transmission; young children contract ascariasis by playing on contaminated ground around the houses, older children contract fasciolopsiasis by crushing infected water chestnuts with their teeth, and young agricultural workers contract hookworm infection by working on contaminated plantations. When more communal factors, such as water, food and dust, are involved as carriers of the invasive stages, the age-related prevalences disappear or depend more on the immune status of the host than on environmental and behavioural factors.

Sex has less bearing on the mode of transmission and may be related to the role of females in society, which is different in various ethnic groups. Occupation is a more important indicator. The soil-transmitted helminthiases are primarily infections of agricultural societies. Soil-transmitted intestinal infections are usually less common in urban than in rural areas, or at least are less intensive. Infections such as amoebiasis, giardiasis, and hymenolepiasis, which are transmitted by direct contact or by contaminated water or food, are common in urban and rural populations, but they may be even commoner in densely populated suburban squatter areas. Soil-transmitted infections are often rare in nomad societies owing to the break in contact with the contaminated environment, which is caused by their frequent migrations. Occupation-related contact with faecal material causes intestinal infections to be more prevalent among sewage workers than in other workers; intestinal capillariasis and diphyllobothriasis are frequent among fishermen.

Clustering may be the result of secondary transmission from an index case or of a common risk factor. Clustering may occur in amoebiasis, giardiasis, hookworm infections and taeniasis. The prevalence of soil-transmitted helminthic infections is much higher in the

low socioeconomic classes; in Malaysia, 90.9% of children examined from 6 squatter kindergartens were infected in contrast to 10.0% of the children from upper- or middle-class kindergartens. The results of studies in Colombia suggest that the prevalence of ascariasis is significantly higher in the low-income section of the population but this is not true for trichuriasis. Human migration, whether organized or nomadic, is a factor influencing the prevalence of parasitic intestinal infections either by gaining or by losing contact with infected sites.

The effect of an improved general standard of living and sanitation on the prevalence of intestinal parasitic infections is best illustrated by comparison of the epidemiological situations after a period of decades. In Japan, following well-conducted campaigns of anthelmintic control, the prevalence of ascariasis fell from 51.8% in 1945 to 1.6% in 1970. In the Republic of Korea, between 1949 and 1971, the national prevalence of ascariasis dropped from 81% to 46%, trichuriasis from 87% to 47%, and hookworm from 39% to 7%. In Poland, the prevalence of ascariasis in schoolchildren was 8% in 1952 and 3% in 1972, and that of trichuriasis 60% and 34%, respectively; no changes were observed in the prevalence of giardiasis, which remained at a level of about 10%.

A rise in the socioeconomic level of a community, the greater availability of anthelmintic drugs, and the increasing habit of self-medication in many tropical countries may influence the prevalence as well as the intensity of intestinal parasitic infections.

2.4 Public health and socioeconomic importance

Intestinal parasitic infections represent large and serious medical and public health problems in the developing countries, particularly in tropical regions. Frequently physicians and public health authorities show little interest in them. The reasons for this tolerant attitude are probably the high rates of prevalence and the difficulties previously experienced in attempts at eradication or control.

Wherever there exists a high prevalence of soil-transmitted helminthic infections, the living conditions are characteristically poor. Thus the frequency of intestinal parasitism in human populations has been considered as a general indicator of the local level of development. On the other hand, the impact of parasitism on health conditions and particularly on children's growth and development, as well as on the working capacity of adults and on the social costs of medi-

cal assistance, creates a vicious circle that must be broken if social progress and welfare are to be improved.

Although previous estimates of global prevalences of intestinal helminthic infections indicated the presence of hundreds of millions of parasitized people, these figures should not be interpreted in an absolute sense, but they do indicate the major order of magnitude of the problem. Repeated surveys in recent years in numerous countries in Africa, Asia and South America have confirmed that the size of the problem remains the same. Efficient control in some areas and ecological changes and urbanization or other socioeconomic factors in other areas have introduced improved conditions in many countries and consequently eradicated or reduced the prevalence of these endemic infections. But the impact of progress has been minimal in the rural areas of developing countries, where the demographic explosion has considerably enlarged the population at risk. In Brazil, for instance, hookworm prevalence has been found to change from 77% in the earliest surveys to 42.5% after 30 years and to 28.8% after 45 years; yet the calculated absolute number of cases has remained at around 22.5–24 million (*20*). The same phenomenon has been observed in Venezuela, between 1926 and 1962.

2.4.1 *Impact of parasitic infections*

Intestinal parasitic infections can interfere with both social and economic aspects of regional or national living conditions through a number of different mechanisms, including the direct pathogenic action of some parasites which produce specific lesions and, consequently, disease or death.

The socioeconomic impact will, in such cases, be the consequence of:

(1) the incidence of new infections and the prevalence of disease in the general population, the clinical manifestations of parasitism usually being correlated with the parasite burden of infected people;

(2) the incidence and prevalence in particularly susceptible groups of the population, such as the low-income classes, the undernourished, or just the children and the pregnant women;

(3) the prevalence in economically important groups, such as farmers, fishermen, and cattle breeders.

2.4.1.1 *Nutritional implications of parasitism.* Nutritional aspects are of the utmost importance when the population at risk is suffering because of a low economic status or from malnutrition due to social, cultural or other causes. The mechanism of interference may vary and be complex, acting through one or more of the following factors: loss of appetite, malabsorption, decreased capacity for absorption of specific substances (iron, vitamins, etc.), anti-enzymic action of parasites, host/parasite competition for food materials (cyanocobalamin, for example), altered peristaltic activity (including diarrhoea), blood loss, and pathological changes in the portal circulation.

2.4.1.2 *Association of different pathogenic factors.* Different pathogenic factors can be observed in multiple parasitic infections or when intestinal parasites aggravate other pathological conditions. Classical examples are the association of malaria and hookworm infections in the production of severe anaemia, or the association of schistosomiasis and salmonellosis, which produces a chronic bacteraemia. Immunodepression is another important condition that can aggravate the pathogenicity of intestinal parasites. Strongyloidiasis and amoebiasis are both examples of infections that can assume a particular severity in patients during prolonged corticosteroid treatment, as well as in those with an incompetent immunological system or a malignancy.

Although the social impact of such highly prevalent debilitating and, at times, severe infections can be easily understood, quantification is very difficult because there are no measurements that assess adequately the ill-effects of intestinal infections, when traditional parameters (such as death, hospitalization, absenteeism, reduced productivity, and treatment costs) do not reflect all the aspects of the damage. The difficulty of collecting statistical data, not only on morbidity and mortality due to intestinal parasites but also on prevalence in most of the endemic areas, must further raise doubts about the feasibility of quantifying the socioeconomic consequences of intestinal infections.

The economic loss due to these infections is almost impossible to measure with accuracy: most of the infected population in the Third World are children; in adults, a significant proportion of those parasitized are unemployed or partially employed workers; the majority of the women have purely domestic activities. Self-medication, or treatment of children by parents without reference to medical services, which is an increasingly common practice in many tropical countries,

is rarely measurable and the costs incurred, which can be debited against a particular parasitic infection, are seldom taken into any accounting procedure.

The criteria for evaluating the importance of intestinal protozoan and helminthic infections are therefore essentially subjective and are based on available, though admittedly deficient, information on the prevalence and on past knowledge of their potential pathogenic effects.

2.4.2 *Estimated importance of some parasitic infections*

2.4.2.1 *Amoebiasis.* Although a cosmopolitan parasitic infection, amoebiasis has the peculiarity of presenting no correlation between prevalence and pathogenicity. In Canada, Europe, and the USA, cases are in general asymptomatic or benign, in spite of prevalences of 2–5%.

Asia and Africa are the continents where it assumes greater significance as a public health problem. Prevalences are particularly high, sometimes more than 30%, and pathogenicity is particularly high between latitudes 10 °N and 10 °S, although it may be severe in other latitudes (e.g., in Egypt and Morocco). In Asia, the most affected countries are Bangladesh, Burma, China, India, Iraq, the Republic of Korea, and Viet Nam. Amoebiasis is a serious problem in Mexico and some other Latin American countries.

Malnourishment may favour the severity of amoebiasis. Factors producing immunodeficiency may elicit or worsen the clinical manifestations of amoebic infections. Asymptomatic female carriers may develop severe amoebiasis during pregnancy or the puerperium.

Acute intestinal amoebiasis may assume considerable gravity, producing fulminating dysentery, with a death rate as high as 7%, as in an epidemic in Chicago, USA, in 1933. Most of the acute cases subside after one or more weeks and some assume a chronic form of amoebic colitis. Whether or not they began with acute or chronic manifestations or, even, were asymptomatic from the beginning, there is always a risk of extraintestinal involvement. Amoebic necrosis or abscess of the liver is very frequent in some areas, particularly in south-eastern Asia, India, northern Africa, and Mexico. Pulmonary and cerebral amoebic abscesses are less frequent but dangerous. Extraintestinal lesions are the principal cause of death from amoebiasis in Mexico, yet in other countries fulminating colonic infection appears to be the principal cause.

There is frequently grossly inadequate information about the real state of the problem. Technical difficulties in establishing a correct diagnosis and lack of sampling criteria are some of the problems encountered in obtaining sound and comparable data for an evaluation of the prevalence and public health importance of amoebiasis.

It is almost impossible to calculate the social costs of a disease such as amoebiasis: first, because of the polymorphic pathology that is presented, with a gradient of severity from asymptomatic to acute fulminating cases; secondly, because no quantification can be made. However, it is very suggestive that in the General Hospital of Mexico City before 1970 amoebiasis occupied the fourth place as a cause of death (*21*).

2.4.2.2 *Hookworm infection. Necator americanus* (prevalent in the Americas, equatorial Africa, south and south-eastern Asia, Polynesia and Australia) and *Ancylostoma duodenale* (common in northern Africa, northern and south-western Asia) are the two specific agents of human hookworm infections. Almost eradicated from Europe and the USA, these parasites are still infecting many millions of people throughout the world. For example, high prevalences have been registered in the following places (*25*): in the Dominican Republic there were 60% positive faecal examinations and in Puerto Rico 25%; in Mexico, 26% of the population was infected; a 30% prevalence rate was seen in surveys made in Costa Rica and Panama, and 70% in the llanos of Venezuela. In the last-named country, although the prevalence found in 1926 for the rural population was 69% and surveys carried out between 1950 and 1958 showed 58% positives (and recently only 40%), the absolute number of infected subjects has shown no substantial reduction. The situation in Brazil was similar (see section 2.4).

Hookworm infection is a public health problem of great magnitude in many African and Asian countries and produces morbidity by blood loss, with consequent iron deficiency anaemia and hypoproteinaemia. In addition, there is frequently poor iron intake in the diet and the resulting combination of events can lead to serious manpower and economic losses among rural farming populations.

2.4.2.3 *Ascariasis. Ascaris lumbricoides* infections are frequent in tropical and subtropical areas, where the prevalence is usually higher than that of hookworm infections. Prevalence rates of the order of

50–75% have been registered in many Asian and Latin American countries. Children in particular are affected.

While it seems probable, according to the evidence from animal studies, that heavy infections have an adverse effect on nutrition, studies in man have given variable results and there is a great need for further investigations; the latter should be designed, conducted and evaluated in accordance with accepted biostatistical principles to give more precise data and correlations.

Other forms of pathology result from adult *Ascaris* migrations and may require surgical intervention. For example, masses of worms may cause mechanical or spastic obstruction of the intestine and produce serious or fatal illness. Migration of a single worm into the pancreatic or the biliary ducts, the appendix, the bronchi, the Eustachean tube and other organs, although quite rare, can be responsible for severe cases of acute obstructive pancreatitis, suppurative cholangitis, liver abscesses, appendicitis, etc. Even in the case of low-grade infections, the risk of ectopic complications is always present, and requires preventive measures. In the USA, where the problem is of much less intensity than in highly endemic areas, it was estimated that the rate of intestinal obstruction in children of 1–5 years of age was 2 per 1000 cases of parasitism.

2.4.2.4 *Strongyloidiasis.* Strongyloidiasis, although not as common as the other intestinal nematode infections, has a patchy global distribution. Undoubtedly, the technical difficulties in diagnosing this infection have contributed to underestimates of prevalence. The infection is, however, becoming increasingly recognized as a potentially serious health problem, particularly in association with transplant surgery and the concomitant use of immunosuppressant drugs. Screening of patients for strongyloidiasis before the use of immunosuppressant therapy should be the normal practice rather than the exception. There are several areas in South America and the Caribbean where strongyloidiasis is recognized as a major public health problem, necessitating a much higher dose of tiabendazole in treatment than is usual.

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To quantify the impact of intestinal parasites on health is difficult and improved techniques are urgently required. Notwithstanding the sparse information on the frequencies of symptomatic and pathological states and on the severity of clinical cases, the importance of these

infections can be judged from the following facts:

(1) as young populations are particularly vulnerable, their physical and mental development can be affected by malabsorption, blood and protein loss, diarrhoea, or chronic dyspeptic syndromes, which are quite often generated by polyparasitism;

(2) the risk of complications, including a severe or fatal outcome, is a permanent threat to parasitized populations, particularly when a breakdown of natural or immunological resistance is provoked by other pathological situations, drugs, or simply by nutritional difficulties or other stressful social conditions; and

(3) intestinal parasites are prevalent in areas where other conditions contribute to maintain a precarious or even unbalanced nutritional situation.

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3. REVIEWS OF RECENT ADVANCES IN KNOWLEDGE

3.1 Major protozoan infections

3.1.1 *Amoebiasis*

Knowledge of the biology of *Entamoeba histolytica* has recently been well developed as a result of improvements in cultivation techniques, intensification of biochemical studies, and utilization of various techniques for studies on the virulence of different *E. histolytica* strains.

3.1.1.1 *Cultivation.* Notable advances in culturing *E. histolytica* have been made in the field of axenic cultivation. First reported in 1961, axenic culture remained little more than a laboratory curiosity for the next few years. The complexity of the medium, its diphasic nature,

and the low yields of amoebae obtained tended to discourage its use. In 1968, a monophasic liquid medium, free of gross particulate matter, was introduced. Automated counting and mass cultivation of the amoebae under axenic conditions became feasible.

Exploitation of axenically cultivated amoebae rapidly ensued. They were used as a source of highly sensitive and specific antigens for serodiagnosis, for immunological, biochemical, and pathobiological studies, and for *in vitro* screening of amoebicidal drugs. The latest medium to be devised, called TYI-S-33, has permitted the attainment of 50-fold increases in amoebic yields and the reduction of generation times to levels approximating to those obtained with conventional amoebae/bacteria cultures, long the benchmark for amoebic reproduction (*1*).

Development of a cloning procedure using a semi-solid nutrient agar provides a simple, rapid means of obtaining cloned colonies of *E. histolytica* with minimal selective genetic pressure. Moreover, the sensitivity of the method and the excellent proportionality obtained between the number of viable cells and the colony-forming efficiency make it especially useful in studies requiring quantitative assessment of amoebic survival, such as drug testing and the effects of temperature and oxygen on growth and survival of the amoebae.

Refinement of the currently available media can be expected to result in improved amoebic yields, but acceptable levels of growth have now been attained for most research purposes.

3.1.1.2 *Biochemistry and molecular biology. E. histolytica,* a microaerophilic organism, derives energy principally by the glycolytic conversion of glucose to pyruvate along a metabolic pathway differing from that of the classical Embden-Meyerhof. When oxygen is provided, it is consumed avidly by the amoebae. Recent investigations have clearly established that this parasite—though lacking mitochondria, a functional tricarboxylic acid cycle, and haem iron—has the capacity for an active respiratory metabolism which appears to be important physiologically. A chain of carriers, analogous to, but not identical with, the respiratory chain of mammalian cells and anaerobic bacteria, transfers electrons from respiratory substrates to molecular oxygen. The following components of the amoebic respiratory chain have been identified: pyridine nucleotides, flavins, nonhaem iron, and ubiquinone. Nonhaem iron in the form of iron-sulfur proteins has been shown to play a major role in the electron-transfer chain of the amoebae. The most recent study suggests that iron-sulfur proteins

(or perhaps other metallo-proteins) may serve as the terminal carriers replacing in amoebic cells the function of cytochromes (*2*).

In vitro consumption of oxygen by *E. histolytica* has been demonstrated only in the resting trophozoite stage. Actively growing cells, paradoxically, tolerate only small amounts of the gas. Investigations have shown that, *in vitro,* the parasite has a limited capability to detoxify the products of oxygen reduction, and that it is dependent for survival on the presence of reducing agents such as cysteine and ascorbic acid in the medium. *In vivo, E. histolytica* survives both in an environment of extremely low oxygen tension, as in the lumen of the lower bowel, and in the relatively oxygen-rich environment of tissues.

The metabolism, composition, and distribution of nucleic acids in *E. histolytica* have received considerable attention. Monoxenically and axenically cultivated parasites have been used for these investigations. Labelled precursor incorporation studies have demonstrated the existence of a *de novo* pyrimidine pathway, but not a purine pathway. Preformed purines and pyrimidines and their corresponding nucleosides were utilized by the amoebae for RNA and DNA synthesis. Entrance of adenine, adenosine, guanosine, uridine and cytidine into the amoebic cell was carrier-mediated. Four transport sites were identified: adenine-adenosine, adenine-guanosine, uridine-cytidine, and uridine-guanosine. Uracil, cytosine thymine, and thymidine appeared to enter the cell by passive diffusion. The ability of *E. histolytica* to transport several purines and pyrimidines into its nucleic acid precursor pools would enhance its capacity to compete for needed substrates. This would be particularly advantageous during tissue invasion where the amoebae would be in competition with undamaged host cells (*3*).

Autoradiographic studies of tritiated-thymidine incorporation showed DNA to be distributed randomly in small amounts throughout the nucleus of *E. histolytica* during the interphase. In other instances, extensive labelling of the substrate was detected around the endosome, suggesting that this was a site of DNA condensation, probably coincident with nuclear division. Autoradiography and pulse labelling of RNA precursors were employed to demonstrate that the peripheral nuclear chromatin was the site of RNA synthesis and possible storage. These results substantiate and add to earlier findings based on cytochemical studies.

Genome size and DNA base composition have been used to differentiate bacterial and protozoal species. In a study of several *Entamoeba* of the *histolytica* group, i.e., those which form 4-nucleated

cysts, significant differences in genome size were observed for "classical" *E. histolytica, E. histolytica*-like amoebae and *E. moshkovskii.* Additionally, significant differences in DNA base composition as determined by thermal denaturation were found among strains considered to be of the same species. Thus classical *E. histolytica, E. histolytica*-like amoebae, and *E. invadens* were found to be composed of more than one genospecies. In another study in which DNA base composition was derived from buoyant-density measurements, significant differences were also observed between strains of classical *E. histolytica* and between strains of *E. histolytica*-like amoebae. However, there were discrepancies between the two studies which may be due to differences in the techniques employed by the two groups for determination of DNA base composition (*4, 5*).

Studies to relate the surface properties of *E. histolytica* to virulence currently form an active field of research, in which there has been progress in two important areas: concerning cell surface chemistry and membrane dynamics, and understanding the mechanisms whereby the amoebae avoid immune responses by the host.

Trophozoites of *E. histolytica* were shown to be sensitive to agglutination by concanavallin A (Con A). Several prominent surface antigens were demonstrated to be Con-A-binding glycoproteins. Binding of Con A to living trophozoites induced capping, redistribution of Con-A receptor complexes, and endocytosis. Capping was inhibited by cytochalasin B and low pH, and accelerated by inducing phagocytosis. Cytochemical and freeze-fracture ultrastructural studies revealed the Con-A-binding antigens to be peripheral membrane components. It was also shown that the relationship between membrane structure and cell surface was extremely complex relative to other cells. Peripheral membrane components appeared to move independently of integral components, and surface determinants were redistributed independently of others.

Con-A reactions are similar in many respects to antigen/antibody reactions. In *E. histolytica* and *E. invadens,* Con-A-antigen complexes, after aggregating in the uroid area, were released from the cells by exocytosis. Capping and shedding of surface antigens was also observed after exposure of the parasites to sera from patients with amoebiasis, and sera from immunized rabbits. Furthermore, the amoebic surfaces could not be depleted of antigens after successive cycles of reaction with Con A or antibody followed by shedding (*6*). Capping and shedding have been proposed as a mechanism for avoiding the host immune system.

In studies attempting to relate surface properties of *E. histolytica* to virulence, five different factors were examined: (1) phagocytosis of erythrocytes, (2) production of cytopathic effect on cultured epithelial cells, (3) cloning efficiency in semi-solid agar, (4) agglutinability by Con A, and (5) adherence to apical epithelial cells *in vitro*. Only the first two were correlated with virulence. The failure to relate Con A agglutinability to virulence is at variance with the findings of earlier studies, in which a direct correlation was found between agglutinability and virulence.

3.1.1.3 *Virulence.* Several advances have been made in recent years in both *in vivo* and *in vitro* models for assaying the virulence of axenically cultured *E. histolytica.* The *in vivo* models include two simple and highly sensitive assays: intracerebral inoculation of the newborn mouse and intrahepatic inoculation of the newborn hamster. *In vitro* assay systems include an assay employing baby hamster kidney cells, the chromium release assay using Chang liver cells, and the MDCK cell line, which forms as a monolayer with morphological and electrophysiological features of an epithelium. These systems, which measure the damage to cultured mammalian cells by amoebic trophozoites, have permitted the study of factors influencing the virulence of different strains of *E. histolytica.*

The potential effect of amoebic viruses (see section 3.1.1.4) on the induction of virulence changes in *E. histolytica* have been investigated. Modest changes in virulence were observed in amoebic cultures. These changes were unpredictable and apparently were not correlated with the virulence of the amoebic strain serving as the virus donor for the susceptible recipient amoebic strain. Another study suggested a possible genetic control of the susceptibility of embryonated eggs to strains of *E. histolytica.*

Cell-free extracts of *E. histolytica* were shown to produce, *in vitro,* a cytopathogenic effect on mammalian cells in the absence of serum (the latter neutralized the toxic effect). This material was also shown to have an enterotoxic effect in ileal loops of the rabbit and to be a protein of a relative molecular mass of about 30 000. More recently, the amoebic "toxin" was shown to have a lectin-like agglutination effect on mammalian cells; this effect was neutralized or reversed by serum fetuin, or *N*-acetyl-galactosamine. The toxin was further shown to induce specific IgG antibodies in patients with invasive amoebiasis (*7*).

A lectin (isolated from several strains of *E. histolytica*) which agglutinates fixed erythrocytes is apparently different from previously described lectins. Preliminary studies suggest that this lectin may play a role in the pathogenesis of amoebic disease.

Isoenzyme electrophoretic patterns are proving useful in differentiating invasive from noninvasive strains of *E. histolytica.* Eighty-five cultural isolates of the amoebae were compared in regard to three isoenzymes: glucosephosphate isomerase (EC 5.3.1.9), phosphoglucomutase (EC 2.7.5.1), and malate dehydrogenase (decarboxylating) (NADP+) (L-malate: NADP exidoreductase) (EC 1.1.1.39). Four electrophoretic isoenzyme patterns were distinguished. One of them was associated exclusively with isolates derived from the 18 cases of clinical amoebiasis included in the study group (*8*). Further studies are planned to confirm these findings in various geographical regions and to search for additional enzyme variations in *E. histolytica.*

E. histolytica is one of a small group of organisms identified as having above average requirements for iron *in vitro.* Moreover it has been shown that axenically cultivated amoebae concentrate this metal far above the level present in the culture medium, and that iron-sulfur proteins play an important role in the organism's respiratory metabolism. The avidity of *E. histolytica* for erythrocytes undoubtedly reflects the importance of iron in the economy of the parasite.

Parasites, unlike free-living organisms, rely exclusively on the host for iron and compete vigorously with host cells for the metal. Several studies have shown that administration of large amounts of iron to experimental animals, beyond their capacity to sequester the metal, reduced the LD_{50} of bacteria and enhanced their multiplication in tissues. Similar studies with *E. histolytica* have demonstrated that iron overloading of hamsters enhanced infection. Injection of axenically cultivated amoebae into the liver of these animals resulted in higher incidences and greater severity of lesions, compared to untreated controls.

Acute infectious diseases of bacterial and fungal origin are characterized by a lowering of the host's serum iron levels and total iron-binding capacity, and saturation of transferrin with iron. These changes in iron status are believed to be part of the host's defence mechanism and serve to deprive the invaders of iron. Significant reductions in these mechanisms were found in 50 adult male patients, acutely ill from amoebic liver abscess (ALA) in a Mexico City hospital study. The findings indicate that this disease is accompanied by changes in

iron status similar to those found in patients with acute bacterial or fungal infections (*9*).

3.1.1.4 *Viruses of* Entamoeba histolytica. Three morphologically distinct virus-like bodies have been identified in cultured *E. histolytica:* an icosahedral particle, a filamentous particle, and a beaded structure. Evidence for their viral nature was based on: (1) ability to be continuously passaged in susceptible amoebic hosts, causing lysis, (2) failure to be retained by 0.22 μm filters, and (3) in the case of the icosahedral and filamentous particles, their resemblance to known viruses. The third particle was shown to have a novel structure of 14 beads, arranged linearly (*10*). Acridine-orange staining and metabolic-inhibitor studies were used to demonstrate the double-stranded DNA nature of these viruses. The filamentous and beaded viruses appeared to be nuclear viruses, and the icosahedral particle to be cytoplasmic.

The viruses were never observed in amoebae from clean, well-established axenic cultures. They were detected by incubation of cell lysates of amoebal strains (donors) with sensitive heterologous strains (recipients). Amoebae so infected lysed and, when examined by transmission electron microscopy, were seen to contain viral particles. One or more of these viruses were detected by means of cross-infection experiments in each of 10 strains studied. Attempts to transmit the icosahedral and filamentous viruses to animals or cultured mammalian cells have been, so far, fruitless.

Nine of the 10 amoebic strains studied for viruses were isolated from patients with invasive intestinal or extraintestinal disease. The one strain isolated from an asymptomatic cyst passer proved to be virulent when assayed in the rat. There appear to be no reports of viruses in noninvasive strains isolated from cyst passers, very likely because no attempts have been made to search for them.

3.1.1.5 *Immunological studies and serodiagnosis.* Lymphocyte transformation has been demonstrated in patients with clinical amoebiasis upon stimulation of those cells with various *E. histolytica* antigens. In a study of both intestinal and extraintestinal disease (amoebic liver abscess (ALA)) in an area of high endemicity in the Gambia, it was shown that on the whole the former group did not possess circulating populations of lymphocytes capable of reacting to amoebic antigen although the antiamoebic globulin (IgG) levels were raised (*45*). In contrast, the ALA group possessed circulating populations of trans-

formable lymphocytes and high levels of specific IgM and IgG. In other studies dealing only with ALA patients, humoral antibodies (precipitins) were also consistently present. It was suggested that primary invasion of amoebae into the bowel tissue was associated with a high degree of specific cellular immunodepression. When spread to the liver occurred, the immunological challenge was greater, the parasite antigen being present in tissues and the circulation, and a typical cellular response was always produced. On the other hand, in a study of cases of ALA using the migration-inhibition factor and skin testing to assess cell-mediated immunity (CMI), it was determined that the patients on admission to the hospital showed diminished CMI according to both tests. Ten days after treatment and discharge, all patients displayed active CMI response. Humoral antibodies (precipitins) were present throughout the period of investigation. A study of T lymphocyte transformation stimulated with "liposomal" antigen prepared from *E. histolytica* showed the presence of transformable cell populations in patients several months after presentation of symptoms.

Immunodepression produced by administration of cyclophosphamide or antimouse lymphocyte serum (ALS) was studied for the effects on intestinal amoebic lesions in mice. An unexpected result was the production of hepatic lesions in all the animals treated with ALS. Occurrence of such lesions in rats, mice, and guinea-pigs after intracaecal inoculation is not common. Since ALS depresses the T lymphocytes predominantly, it was suggested that these cells might play a role in protecting the liver from amoebic invasion.

Investigation of serum immunoglobulins in intestinal amoebic disease and amoebic liver abscess (ALA) revealed IgG levels to be significantly higher in the former. IgE concentrations, although comparatively higher in intestinal disease than in ALA, were much higher in both groups of patients compared to normal levels. No significant difference in the IgA and IgM levels between patients and controls was found. A seasonal study of immunoglobulins in a Gambia population revealed a positive correlation between the levels of IgG and IgM and the monthly prevalence of *E. histolytica* (measured by faecal examination). A similar positive correlation was found between parasite prevalence and specific IgE, although the total circulating IgE did not change (*45*).

Coproantibodies have been demonstrated by means of the indirect haemagglutination test in a high proportion (80%) of cases of intestinal amoebiasis. Testing for coproantibodies appeared to be more

useful than testing for humoral antibodies in the detection of asymptomatic cyst passers. Antiamoebic IgA and IgG were identified in the faeces of children with invasive intestinal disease.

E. histolytica has been reported to activate the alternative pathway of complement, the reaction products of which destroy the amoebae, but its significance in the pathogenesis of amoebiasis is unknown. No correlation between the virulence of an amoebic strain and activation of the alternative pathway has been found. Sera from patients with ALA contain substances that activate the pathway. Conceivably, complement activated by the normal or alternative pathways could lyse amoebae *in vivo*. It is also possible that activated complement, complement cleavage products, and amoebic enzymes combined could contribute to the lytic and reactive changes observed in tissues invaded by the parasite (*11*).

Although serious efforts to diagnose amoebic disease by detection of humoral antibodies date back more than half a century, it has been only within the last 15 years or so that serology has become a useful diagnostic tool. Among the factors that contributed to this development are: (1) the refinement of the older techniques, and development of new ones, (2) availability of axenically cultivated amoebae as a source of highly sensitive antigens, (3) recognition that *E. histolytica,* living as a noninvasive commensal in the lower bowel, does not stimulate humoral antibody production, and (4) that antibodies, once formed, may persist for years after amoebic invasion has ceased. Virtually every type of serological procedure has been adapted for diagnosis of amoebiasis. The major tests that are most commonly used include indirect haemagglutination, gel diffusion precipitation, immunofluorescence, counterimmunoelectrophoresis, immunoelectrophoresis, latex agglutination, and complement fixation. Each has its advantages and disadvantages. Some are more useful that others in clinical practice, and some more suitable for epidemiological studies. A recently introduced enzyme-linked immunosorbent assay (ELISA) shows promise. An excellent general review of the subject has been published (*12*).

Immunofluorescent techniques have been effectively employed in detecting *E. histolytica* in tissues. A procedure by which antigens of *E. histolytica* present in faeces are detected with enzyme-labelled antibody will, it is hoped, become an important diagnostic tool complementing microscopy.

Sensitive and highly reproducible serodiagnostic techniques, requiring a minimum of training to perform, are now available even to

the most modestly budgeted laboratories. Epidemiological data, essential for estimating the need for public health control measures and assessing their effectiveness, can now be obtained at relatively low cost. In one very large national survey conducted in Mexico, sera from over 19 000 individuals in 46 communities were collected and examined for antiamoebic antibodies using the counterimmunoelectrophoresis test (*46*).

3.1.1.6 *Development of a vaccine.* During the last decade, serious efforts to develop a safe and effective antiamoebic vaccine for human use have been made.

Recent investigations have been carried out both on small rodents and on nonhuman primates. Living amoebae cultivated monoxenically or axenically, crude aqueous extracts, and antigenic fractions of amoebae were employed to induce immunity in hamsters and guinea-pigs. The immunizing agents were administered in a variety of ways: subcutaneously and intraperitoneally, intradermally, intramuscularly, and intrahepatically. Challenge amoebic doses, with one exception, were introduced via intrahepatic inoculation. The exception was an intracaecal challenge. The following general conclusions can be drawn from these studies: (1) the introduction of living amoebae or crude aqueous amoebic extracts into hamsters and guinea-pigs affords protection against challenge with virulent strains of *E. histolytica,* and (2) amoebic fractions (soluble or particulate) provided a higher degree of protection than the crude extracts.

Studies using nonhuman primates are in their infancy. Specific humoral antibodies against *E. histolytica* were obtained in spider monkeys (*Ateles paniscus*) and green monkeys (*Cercopithecus sabens*) after inoculation with lysosomal antigen. Skin reactivity after intradermal inoculation of histolyticin indicated the development of cell-mediated immunity in these animals. Immunization with lysosomal antigen has provided protection against intrahepatic challenge with virulent *E. histolytica* (*13*).

3.1.1.7 *Clinical aspects.* Clinical studies and observations have recently been focused on the problems of invasive and noninvasive amoebiasis, fulminating and protracted forms of intestinal amoebiasis, intestinal and extraintestinal sequelae of infections, as well as on pathomorphological aspects of amoebiasis.

Asymptomatic form. The presence of saprophytic minuta forms of *E. histolytica* in the colon may continue at subclinical level for many

years but may at any time develop into a progressive disease—invasive amoebiasis with magna forms of *E. histolytica.* Prevalence rates in the endemic countries (obtained from routine stool examinations of the apparently healthy population) are generally below 10% (e.g., in Ivory Coast, Indonesia, Senegal, Thailand); but occasionally they are much higher—50% in Colombia and 72% in Costa Rica.

Other assessments have been based on serological surveys, but the evaluation of some of these tests remains a matter of controversy. Some authors maintain that intestinal amoebiasis, even when progressive, gives a positive reaction in only 30% of cases and then at low or non-significant levels. Investigations of a mixed sample of sick and healthy subjects gave a positive result (at titres of 1/128 or higher, using the indirect haemagglutination test) in 58% of cases in Bangkok and 76% of cases in Calcutta, India (*14*). Moreover, nonconformity between subjects with positive serological findings and those with positive coprological findings is not uncommon.

Acute intestinal amoebiasis. Whether it is a primary infection, an exacerbation of the progressive form of the disease, or a reinfection, the common acute amoebic colitis may occur as a diarrhoea and not as a dysentery. The stool is sometimes normal in appearance, but most often mucus and blood are present.

There are a number of topographic variants of amoebiasis: bipolar (caeco-sigmoid) forms, which are the most frequent; amoebic proctitis, in which the tenesmus is particularly painful; and amoebic typhlosis, which may (rarely) lead to a true amoebic appendicitis. There are two other forms with deceptive symptoms, but these are also rare: the pure febrile form mimicking a typhoid or tuberculous fever, and the pseudorenal form with lumbago. In young children especially, the disease is commonly accompanied by general signs: fever, vomiting, dehydration.

When an amoebic infection and a shigellosis coexist, as frequently occurs in the tropics, the association may remain asymptomatic; but in up to 5% of patients, the conditions aggravate each other to produce a severe mixed colitis. These forms, characterized by copious diarrhoea, high fever and pronounced dehydration, can generally be cured.

Fulminating forms of intestinal amoebiasis. Fulminating intestinal amoebiasis, which accounts for 5–10% of acute intestinal amoebiasis cases admitted to hospital, mainly affects women during pregnancy and the puerperium. The colon is riddled with ulcerations, about half of which perforate; the clinical picture is dominated by a toxic syndrome; the entire abdomen is painful, sometimes contracted

by a peritonitis, sometimes distended by a paralytic ileus; the condition progresses in one continuous process and within a few days or weeks results in death. However, parenteral administration of high doses of dehydroemetine or ornidazole, with or without colectomy, may lead to recovery. A related clinical form of segmented colic necrosis is important but has a less serious prognosis.

Protracted intestinal amoebiasis. It is rarely possible to forecast how an amoebic colitis will develop in the medium or long term; it may heal, never to return, or the subject may remain a healthy carrier indefinitely, or sooner or later long-lasting manifestations may appear. Such manifestations are all too frequently referred to as chronic amoebiasis and account for 10–20% of hospital cases. Apart from the severe forms, the various indispositions complained of by many former amoebiasis patients should be attributed to nonspecific functional colonopathies, whether spasmodic or not. These are quite common, even in temperate countries. The amoebae do not seem to be responsible at all and the repetition of amoebicidal treatment achieves nothing. When a cancer of the colon or rectum occurs, amoebiasis cannot be held responsible.

In some patients, however, bowel movements are disrupted, either permanently with alternating phases of diarrhoea and constipation, or by so-called relapsing episodes of diarrhoea with or without blood, mucus or constipation occurring at varying intervals. The development of a genuinely chronic dysenteric state is unusual. Numerous nonspecific and varied manifestations, such as dyspeptic disorders, biliary or pyloric dyskinesia, and sensitivity to cold, may occur either in isolation or associated with disorders of bowel movement.

When confronted by such clinical pictures, it is important to find out whether the colitis is "inhabited" or "deserted". Since stool, endoscopic and radiological examinations do not always give a definite answer, it may be necessary to resort to a challenge treatment. If, as in a small number of such cases, it is a true or pure chronic amoebic colitis, the condition will return to normal. If not, it could be either an interlinked amoebiasis, in which case there will be a partial but only temporary improvement, or (as in the great majority of cases) a "deserted" meta-amoebic colonopathy. In the latter two instances, an effort must be made to discover the factors causing the disorders to persist: these factors may be either individual (associated infections, unsuitable diet, underlying organic colonopathy, etc.) or community-linked (inadequate living standards, lack of water, etc.).

An amoeboma, which is located mainly in the caecum and only

occasionally in the sigmoid, is rare. For this condition, specific serological tests are positive and medical treatment is remarkably effective.

Extraintestinal forms of amoebiasis. Hepatic involvement is often the presenting condition, and frequently appears to be the primary condition, since questioning reveals a history of colitis in only 30–40% of cases. In a few patients, the liver lesions are associated with a progressive infection of the colon.

The classical form—acute, of medium intensity, with a painfully enlarged liver, fever, and biological, radiological and echotomographic signs—accounts for between 65% and 80% of cases. Chronic alcoholism or prolonged corticosteroid therapy are conducive factors. The controversy surrounding the presuppurative stage, which was claimed to precede the gathering of the abscess, now seems outdated. X-ray scanning followed, as required, by exploratory puncture, or alternatively echotomography, which confirms the fluid content of the lacunae, shows that these foci, which may be resorbed following specific treatment, are already necrotic gatherings. While the majority of abscesses heal without drainage, there are also complicated cases and cases not seen until a late stage; bursting of the abscess then leads to vomiting, thoracic lesions, peritonitis, subphrenic abscess, or pyopericarditis. In children, a picture of protein-energy malnutrition may lead to a wrong diagnosis.

Two other types of progressive disease can be singled out:

—the superacute form, or fulminating abscess of Rogers, usually with several foci, often associated with a colitis, likewise superacute; the prognosis is fatal and it is not uncommon for serological tests to prove negative in such cases;

—the chronic abscess, around which a thick capsule forms and which sometimes contains hepatic sequestra; the pus is putrid and turns yellowish. Hepatotomy is no longer sufficient, and excochleation or a partial hepatectomy is now essential. Some hepatomegalies are torpid and apyretic, and simulate a liver cancer.

Finally, there are deceptive forms which ought to be mentioned: pure febrile forms, pseudovesicular forms, icteric forms, or even undeveloped forms, which have in common the feature that they do not draw attention to the liver, even though they may account for 10–20% of cases in some hospital statistics.

Pleuropulmonary amoebiasis may be the presenting condition, and in endemic areas it should be borne in mind whenever there is a pleuropulmonary syndrome of the base of the right lung. It must be distinguished from the pleuroparenchymatous reaction of the base of

the right lung, which is normal in hepatic amoebiasis. In the vast majority of cases, pleuropulmonary amoebiasis is produced by perforation across the diaphragm from a manifest hepatic focus. Usually the pus is coughed up via the bronchial system. However, in some cases a pyothorax or pulmonary abscess develops which then requires surgical intervention. Much more rarely metastatic abscesses occur. The diaphragm is neither elevated nor immobile; the lesions are no longer linked to the copula; the sputum has the crushed raspberry appearance rather than that of anchovy sauce or chocolate. Contrary to generally accepted ideas, it is not unusual to find haematophagous amoebae in the sputum or in the pleural pus.

Pericardial involvement is generally a postmortem discovery. The bursting of a hepatic abscess into the pericardial cavity produces an acute cardiac tamponade, leading rapidly to death. However, a cure may on rare occasions be effected by pericardial puncture to draw off the fluid. Apart from these purulent lesions, which are rarely encountered, it should be borne in mind that some hepatic abscesses are accompanied by a neighbouring pericardial reaction, with no effusion but with an audible friction rub on auscultation.

Cutaneous amoebiasis deserves special mention because of the rapidly spreading and extensive ulcerations and its lower susceptibility to specific treatment. Fortunately, it is rare and observed solely in malnourished or debilitated subjects. It predominates in the perianal region or around an abdominal incision; it may spread to the penis in males and to the vulvo-vaginal region in females.

There are also rare cases of abscesses of the brain, unrelated to the primary meningoencephalitis caused by *Naegleria,* which carry a serious prognosis. Abscesses of the spleen, muscles, and bones occur infrequently.

In conclusion, it should be recognized that, provided it is identified early in a subject whose general condition is good and who has an acceptable standard of living, amoebiasis is nowadays a benign condition with no sequelae. On the other hand, in an unhealthy subject or when the socioeconomic context is unsatisfactory, it can show formidable tenacity and indeed severity. Deceptive, mixed clinical pictures seem to be more common in temperate countries, where amoebiasis (in the disease form) occurs sporadically.

3.1.1.8 *Pathomorphology of amoebiasis.* Recent retrospective studies of cases of amoebiasis have led to an increase in knowledge about the pathomorphological changes.

In some cases of intestinal amoebiasis, there are discrete, minute button ulcers, probably corresponding to the sites of entry into the mucosa. These buttons are seen as very small, oedematous, reddened papillae, at times with evidence of haemorrhage, and the advancing lesions resemble the neck of a micro-flask. In other cases, there is usually marked undermining of the mucous membrane, giving rise to overhanging and swollen edges to the ulcer. The ulcers tend to spread along the mucosal folds so that they are broader than they are long. In about a quarter of the cases, the mucous membrane between individual ulcers appears normal. In the remaining three-quarters with suspected superimposed secondary bacterial infection, there is congestion and sloughing, the base of the ulcers being covered with a necrotic dark-coloured material. In other cases, there are changes which may be regarded as chronic forms.

Macroscopically, chronic lesions take the form of shallow weeping ulcers, as extensive as a centimetre in diameter, with a hyperaemic raised margin. Sometimes the lesions show a diffuse granulating mucosa, covering over a number of invaded mucosal glands, but show no evidence of ulceration. More commonly, these chronic changes take the form of raised nodules with sharply delimited edges and minute depressed yellowish pores surrounded by a reddened ring and opening into small but enlarged bases filled with gelatinous material and necrotic tissue.

Histologically, in most proven cases, there is marked lytic necrosis and stromal oedema. The histological appearances of the lesions are remarkable for lack of infiltration of the neutrophilic leukocytes or of macrophages. The ulcers show invasion of the mucosa and underlying tissues by the amoebae, with necrotic degeneration and some lymphocytic infiltration. Polymorphs appear in significant numbers only in cases where the gross pathomorphological appearances are suggestive of possible superimposed bacterial infection. In many cases in which the initial tissue invasion occurs, the lesions are limited to the mucosal layer and only superficial ulceration develops. In the chronic cases, the edge of the ulcer consists of a matrix of fibrous tissue. The underlying cellular components are made up of a dense infiltrate of neutrophil leukocytes extending into the submucous or muscular coats and sometimes providing a relatively compact wall around the margins of the necrotic base.

This pathomorphological type of chronic bowel lesion may not be a distinct entity, but represents an advanced picture usually resulting from the superimposed invasion of enteric bacteria. It is conceivable

that the latter may provide a stimulus for host cell infiltration because, in uncomplicated cases, colonies may establish themselves in the mucosa with remarkably little reaction on the part of the tissues.

Hepatic amoebiasis is perhaps the commonest complication of intestinal amoebiasis. Gross pathomorphological studies have shown that the smallest abscesses are usually a few millimetres in diameter. They are solid, white and still resemble the normal liver tissue. In slightly larger abscesses, the contents become gelatinous and yellow, and still larger ones contain a reddish-brown fluid and shreds of necrotic tissue. In cases that come to autopsy soon after the onset of the disease, it appears that the abscess has no opportunity for development of a fibrous capsule, but in chronic cases there is usually a limiting wall. The majority of the single abscesses develop in the right lobe of the liver. This usually occurs either just below the diaphragm or at the lower aspect near the surface. Solitary abscesses can be as large as a grapefruit and may have trabeculated strands of more resistant stroma. Microscopically, there are three recognizable zones: an outer zone of relatively normal tissue which is in the state of being invaded by amoebae, a median zone in which the vital tissues have been destroyed and only the stroma remains, and a necrotic centre. The wall of the abscess is usually composed of a shaggy fibrous lining, surrounded by a fibrovascular area which is infiltrated by a few mononuclear leukocytes.

Other focal changes may occur in apparently unaffected parts of the liver. These changes consist microscopically of thrombi composed of a fibrinous filament and leukocytes. These are usually found in close contact with the hypertrophic Küppfer cells. In addition, there may be lytic necrosis and occlusion of some of the blood vessels with an extension into the lobule, and at times the central collecting vein may be involved in the erosive process. These areas are usually without any inflammatory reaction, which in more advanced cases of liver abscess is found as an extensive infiltration of large numbers of neutrophilic leukocytes.

In pleuropulmonary amoebiasis, the right cupola of the diaphragm is usually raised in cases of amoebic liver abscess and direct extension of the abscess to the pleural cavity or base of the right lung is not uncommon.

In pleuropulmonary amoebiasis, there is localized pneumonitis with the formation of an abscess containing a gelatinous mass and blood. Occasionally, a fistula opens into a large bronchiole or bronchus so that the reddish-brown contents of the pulmonary abscess,

and at times of the hepatic abscess, are coughed up and discharged. In cases in which the amoebae have reached the lung by the bloodstream, the diaphragm is not involved and the pulmonary abscess at first lies wholly within the parenchyma of the lung. Histologically, there is localized pneumonitis with abscess formation and marked vascular congestion with an inflammatory cellular infiltrate made up mainly of large mononuclear cells from the walls of the alveoli.

Amoebiasis of the brain is very uncommon compared with hepatic and pleuropulmonary infection. There have been 56 reports of cerebral amoebiasis in the literature, of which 29 were from Egypt. However, in one series of deaths from amoebiasis, 8.1% of 210 autopsied cases had brain involvement. Macroscopically, the affected area of the amoebic brain abscess appears oedematous and congested. The outer wall of the abscess is thin, its inner aspect fuzzy and the contents are of a chocolate-coloured pasty material. The brain abscess is characteristically sterile. Congestion and thrombosis of the blood capillaries occurs in the contiguous tissue. Macroscopically, there is lytic necrosis and abscess formation with degenerate tissue cells, erythrocytes and leukocytes in the abscess cavity. In the inner portion of the wall of the abscess are usually found many lymphoid cells, degenerate nerve cells, and amoebic trophozoites.

The skin is the fourth most common anatomical location outside the large bowel for the development of *E. histolytica.* Macroscopically, there is an oedematous elevation of the involved skin with induration of the margin of the developing ulcer. The characteristic features of this lesion include a rapidly spreading ulcerative process with irregular margins and an overhanging edge of gangrenous epidermis. The advancing zone on the periphery of the ulcer has a dusky-red hue which gradually merges with the normal skin. The ulcer itself contains blood-tinged material of fetid odour and has a dirty greyish necrotic base. Microscopically, in the necrotic tissue amoebae are found advancing into the peripheral zone.

3.1.2 *Giardiasis*

In recent years, *Giardia intestinalis* has again been recognized as an organism capable of causing human disease. Recent reviews have dealt with the epidemiology, public health importance, medical aspects, and clinical and laboratory diagnosis of the disease (*15–18*). Knowledge about the biology of *G. intestinalis* (including its taxonomy, hosts, cultivation, excystation, and immunology) has greatly increased

and some new ideas concerning the clinical and epidemiological aspects of giardiasis have emerged.

3.1.2.1 *Taxonomy.* In a recent review of the classification of *Giardia* species, it was concluded that there has been a tendency to name *Giardia* species after the host in which they are found. The consensus, however, would seem to indicate that in mammals, at least, there are probably two species: *G. muris,* found in the mouse, rat and hamster, identifiable by the presence of smooth, rounded, median bodies in the cyst; and *G. intestinalis* (synonyms: *G. lamblia* and *Lamblia intestinalis*), identified by the presence of median bodies shaped like the claws of a claw hammer (*48*). This type of structure is seen in *Giardia* species described from man, guinea-pig, ox, dog, cat, rabbit, and other mammals. The inclination to believe in a single species of *Giardia* in man and certain other mammals is borne out by recent studies on cross-transmission of *G. intestinalis.*

3.1.2.2 *Hosts.* Using *G. intestinalis* cysts of human origin, it has been possible to infect at least one animal in the following groups fed the organisms: rat *(Rattus norvegicus),* gerbil *(Gerbillus gerbillus),* guinea-pig (*Cavia porcellus*), dog (*Canis familiaris*), raccoon (*Procyon lotor),* bighorn × mouflon sheep *(Ovis canadensis* × *O. musimon),* and pronghorn antelope *(Antilocapra americana).* Negative results were obtained when human-derived cysts were fed to hamsters, domestic rabbits, laboratory mice, deer-mice, black bear, wapiti, mule-deer, domestic sheep, cattle, and white-tailed deer.

Because of epidemics of waterborne giardiasis in towns implicating beavers, interest has focused on these animals. *Giardia* cysts of beaver origin fed to hamsters, guinea-pigs, mice, and rats gave negative results, but in 4 out of 4 dogs and 2 out of 3 human beings, infections were obtained. An infection also occurred in a volunteer fed *Giardia* cysts from a mule-deer, but not in dogs. These results of studies on cross-transmission (*47*) will undoubtedly stimulate further research better to define the *Giardia* species complex.

3.1.2.3 *Cultivation. G. intestinalis* was successfully cultured from man and from the rabbit *in vitro,* using *Candida guilliermondi* or *Saccharomyces cerevisiae* as concomitant organisms. The goal of axenic cultivation of Giardia was reached in 1970 with the maintenance of cultures of *Giardia* from the rabbit, chinchilla and cat for more than one year. True axenic cultivation was established after the *Giardia*

were grown in tubes with the yeast initially confined to a dialysis tubing, then later without the yeast at all. In 1976, an isolate of *Giardia intestinalis* (obtained from the duodenal aspirates of a female patient) was reported to be successfully cultivated *in vitro,* and maintained for over one year (*19*). A medium, designated HSP-1, was prepared from a basic broth (phytone peptone, glucose, L-cysteine hydrochloride, and Hanks' solution) to which was added Seitz-filtered serum, potassium penicillin G and streptomycin sulfate. *G. intestinalis* has also been cultivated in the TPS-1 medium of Diamond, which was used for over 12 years to grow *E. histolytica.* The presence of human serum in the HSP-1 medium precluded the use of organisms grown in it for serological tests. Pooled bovine serum has now been substituted. A recent variation in *in vitro* culture of *Giardia* has been the use of a solid agar medium.

3.1.2.4 *Excystation.* The increasing interest and success in the *in vitro,* axenic cultivation of *G. intestinalis* from human beings and isolates from animals have stimulated investigation of excystation of the organisms for subsequent growth *in vitro* as measures of viability. Viability of *Giardia* cysts has been determined largely by the eosin exclusion test. This has been used to determine the viability of organisms after exposure to high temperatures for thermal death-point measurements, for exposure to low temperatures, and to measure the effects of giardiacidal concentrations of iodine and chlorine in both individual and municipal water treatment experiments.

Using hydrochloric acid at pH 2.0, excystation can be induced in *Giardia* cysts obtained from man, monkeys, dogs, rats, and mice. It was shown that eosin exclusion gave a higher cyst viability than that through excystation and subsequent cultivation. Storage of cysts at 8 °C permitted cyst survival for up to 77 days, at which time the supply of cysts was exhausted. Cysts stored at 21 °C retained viability for variable periods, from 5 to as many as 24 days, with different lots of cysts, but cysts stored at 37 °C did not survive for more than 4 days. Freezing and thawing resulted in a low level (<1%) of cyst survival for 14 days. Cysts exposed to boiling water were immediately incapable of excystation (*20*).

3.1.2.5 *Immunology.* The immunogenic activity of *Giardia* does not seem to be high. The findings of specific serum antibodies in giardiasis are rather confusing with respect to their specificity.

Significant decreases of serum IgA and diminished levels of IgG and

IgM were detected in some patients with persisting giardiasis but were not confirmed in others. In some cases with dramatically low IgA (351–619 mg per litre), the infection was reported to be resistant to all active antigiardial drugs including tinidazole. There is often a significant elevation of native DNA-binding capacity of serum, detected by the radioimmunological technique, and an inhibited reaction to levamisole stimulation that has been observed using the phytohaemagglutinin skin test (*21*).

The role of the thymus in mice in immunity to *G. muris* is still not clear. In one series of experiments, athymic nude mice showed cyst excretion peak levels of 18 days with reduced but persistent levels well beyond day 46, while thymus-intact mice had peak levels of excretion at days 7–14 with undetectable levels after day 42. On the other hand, fatal *G. muris* infections in nude mice have been reported by others. Other studies indicated that the natural intestinal resistance is lost in mice during lactation.

In children, the immunodeficient condition in patients with giardiasis seems to correlate in some way with allergic disorders observed among them. There are numerous reports on the frequency of allergic symptoms in those infected by *Giardia* and on the higher proportion of giardiasis among allergic subjects. However, a more carefully controlled study has shown that the frequency of allergic symptoms among *Giardia*-infected and noninfected children was the same and in both cases it did not differ from the global rate.

In children infected with *Giardia,* there is an increased number of intraepithelial lymphocytes which may be T lymphocytes, attesting to the role of cell-mediated immunity in giardiasis.

Giardiasis apparently does not affect an individual's immunity to intestinal bacteria. In a carefully checked study, no difference was found in the clinical symptomatology of bacterial dysentery in children aged 2–14 years, infected or uninfected with *Giardia.*

Genetic studies in human giardiasis have shown a higher than expected frequency of HLA antigens A_1 and B_{12} and a higher than expected frequency of phenotypes A_1/A_2 and B_{12}/B_{27} (*22*).

3.1.2.6 *Epidemiology.* In recent years giardiasis has been diagnosed during epidemic gastrointestinal illnesses with increasing frequency. Suspected and confirmed epidemics of giardiasis have been recognized in both Europe and the USA. The occurrence of giardiasis has been especially troublesome in international travellers including those going overland.

In the USA, *G. intestinalis* is the parasite most commonly identified. Between 1977 and 1979, 4% of stool specimens submitted to state health department laboratories were positive for *Giardia.* Other surveys have demonstrated *Giardia* infection rates ranging from 1% to 20% depending on the geographical location and age of persons studied. However, person-to-person transmission is the principal means of spread of giardiasis in day-care centres, where it has become a significant public health problem.

Twenty-three waterborne outbreaks of giardiasis affecting 7009 persons have been reported in the USA since 1965. Most of the outbreaks occurred as the result of consuming untreated surface water or surface water treated with chlorine only, but they can also occur from filtered water supplies. Well water was not found to be contaminated. The watersheds for the surface water sources were well isolated, had no human habitation, and had extremely limited human activity. Three *Giardia*-positive beavers were found within foraging distance of water intakes in one instance.

Waterborne outbreaks of giardiasis seem to involve two distinct groups of people, either visitors and campers or the usual residents of the area. There appears to be a seasonal trend of outbreaks in visitors during the summer months. This implies that there is either increased contamination of these water supplies at that time or, if it is assumed that the supplies are always contaminated, a larger number of susceptible individuals use them during the period in question.

To protect against transmission of giardiasis, all surface water should receive pretreatment preferably with sedimentation and filtration in addition to chlorination. Clearly, the long-term solution to control of waterborne giardiasis will undoubtedly involve widespread improvements and use of water filtration.

Increased recognition of this infection because of reports of giardiasis in international travellers has no doubt been responsible for increased surveillance, investigation, and reporting by public health authorities.

The epidemiology of giardiasis in the tropics is little known. Studies carried out in India seem to indicate that this disease frequently causes intestinal symptoms in children, e.g., diarrhoea, and that it may interfere with bacterial enteric infections as well as nutrition.

3.1.2.7 *Clinical aspects.* The clinical significance of *G. intestinalis* has been many times revised and re-evaluated.

A small proportion of the adult population is predisposed to rein-

fection or persistent giardiasis. Among adult hospital patients, giardiasis was shown to be 1.5 times more frequent than in healthy controls. But in patients with gastrointestinal disorders or bacterial intestinal infections, the prevalence of giardiasis goes up to 2.5–11.3% in adults and to 16.2% in children.

In children, in addition to diarrhoea, nausea, abdominal pains, headache, malaise and enhanced irritability occur. In adults with giardiasis, dyspeptic disorders and sometimes malaise and a diminished capacity for work are found.

The most severe pathological condition observed in patients with giardiasis is malabsorption, which has been considered to be a result of the direct toxic effect of the parasite on the glycocalyx of the small intestine, the enhanced bacterial colonization, and the general protein, energy and folate deficiency that occurs in tropical and subtropical areas. Histologically there is shortening of the villi, crypt hypertrophy, and plasma cell and lymphoid infiltration of the lamina propria of the mucous membrane. By electron microscopy, dystrophy of the parietal cells in the gastric fundal mucosa and epimembranous damage of glycocalyx have been observed, as well as a normal mucosa in spite of *Giardia* colonization. Morphological changes have also been found in the gastric mucosa and in distal parts of the small intestine.

Impaired absorption of fat, D-xylose and cyanocobalamin was reported in about 50% of patients with giardiasis in one study but was not confirmed in others.

There is a suggestion that giardiasis may present in two clinical forms:

(1) primary infection, as a diarrhoeal disease with both clinical and laboratory signs of malabsorption, which is of short duration and usually self-limiting in a hygienic environment or readily treated; and

(2) prolonged or repeated *Giardia* infection, which occurs even in a highly hygienic environment in patients with a damaged resistance of the intestinal mucosa of a specific or nonspecific nature.

3.1.2.8 *Diagnosis.* When the number of cysts is high, *G. intestinalis* is easily found by direct examination of the faecal smear. Concentration techniques must be used in diagnosing infections with only a small number of cysts in the faeces. As the discharge of *G. intestinalis* cysts may be irregular, multiple coproscopic examinations should be carried out when giardiasis is suspected. However, in some cases it is only possible to make a diagnosis by the use of Beal's string test, by examining freshly obtained duodenal contents, or by jejunal bi-

opsy, but there must be good indications for undertaking such procedures.

Of the different serological tests for diagnosing giardiasis, the indirect fluorescent antibody test has been found to be the most valuable. *In vitro* allergic tests such as blast transformation and macrophage migration inhibition have been reported to be highly specific and sensitive.

3.1.2.9 *Treatment.* Modern antigiardial drugs are highly effective. Most of them, such as metronidazole, nimorazole, ornidazole, tinidazole, are nitroimidazole derivatives. Used in a single dose of 500 mg or a daily dose of 200 mg for 5–7 days, they cure 70–85% of patients; single doses have been reported to be more effective.

3.2 Other protozoan infections

3.2.1 *Balantidiasis*

Balantidium coli is the only ciliate parasitizing man; its incidence is low. Many mammals, including pigs, are naturally infected with *B. coli.* Contact with infected pigs may increase the human infection rate, although the biological characteristics of the *Balantidium* parasitizing man and pigs are not clear. Waterborne epidemics of human balantidiasis have been reported.

Many human infections are self-limiting and asymptomatic. In some individuals, especially malnourished children, *B. coli* causes deep penetrating ulcerations of the colon, resulting in a dysenteric syndrome. A fulminating dysentery, intestinal perforation, haemorrhage, and shock, though rare, are serious and sometimes fatal complications of human balantidiasis. Laboratory diagnosis can be readily made by faecal examination. The disease is usually easily treated with tetracyclines. Nitroimidazole and paromomycin are alternative drugs.

3.2.2 *Sarcocystosis*

Until 1972 the organism inducing human coccidiosis had been referred to as *Isospora hominis* or *Isospora belli.* Recent findings introduced a new, improved classification: *Isospora hominis* has been replaced by *Sarcocystis hominis* and *S. suihominis* (*23, 49*). The parasites have an obligatory heterogenous type of life-cycle; man is

the final host and cattle or pigs are strictly specific intermediate hosts. The final host passes in the faeces free sporocysts with sporozoites which are ingested by the intermediate host. The sporozoites initiate an asexual multiplication forming, in cardiac or skeletal muscles, sarcocysts containing thousands of merozoites (cystozoites and bradyzoites). The merozoites, after being eaten within the muscle tissue by the final host, complete the sexual stages in the subepithelium. In the final host there is no asexual multiplication preceding gametogony, the oocyst sporulates within the intestinal tissue and free sporocysts are usually shed in the faeces.

Until recently human intestinal sarcocystosis had been rarely diagnosed. By the application of suitable diagnostic techniques, the intestinal infection has been shown to be widespread in man in most parts of the world, with an incidence of between 6% and 10%.

No more than three dozen human muscular sarcocystosis cases have been reported. *Sarcocystis* cysts were found in the cardiac, laryngeal and skeletal muscles of patients.

The incidence of sarcocystosis in intermediate hosts may reach 100% in some parts of the world. *Sarcocystis* is most commonly found in the cardiac muscle and oesophagus of older cattle and pigs. The incidence is usually shown to be higher when a trypsin digestion technique is used rather than trichinoscopy. Experimental infection of domestic animals as intermediate hosts has made it possible to describe the fine structure of the cysts of different species of *Sarcocystis;* the cyst wall structure can be used for differentiation of species in the same host. Observations of this characteristic showed that infections with *S. hominis* or *S. suihominis* accounted for half the *Sarcocystis* infections found in animals.

3.2.2.1 *Epidemiology.* Man is the final host of *S. suihominis* and *S. hominis* and becomes infected by eating raw or undercooked pork or beef. The strict intermediate-host specificity prevents the chance of man-to-man transmission of the parasite by faecal contamination. On the other hand, the excretion of mature sporocysts greatly increases the chances of transmission to the intermediate host, these sporocysts being able to resist climatic conditions that are unfavourable to sporulation.

The morphological identity of human and dog sporocysts (shed in the faeces) led to the suggestion that the dog might be a probable source of human infection. However, the lack of any correlation between the incidence of infection in man and the dog does not support this suggestion.

Experimental studies conducted recently have shown that several nonhuman primates could serve as final hosts for *S. hominis* or *S. suihominis.*

The ingestion of sporocysts infective to other primates or the sporocysts of porcine *Sarcocystis* may accidentally lead to muscular sarcocystosis.

3.2.2.2 *Clinical signs.* The intestinal *Sarcocystis*-induced coccidiosis in man appears to be practically asymptomatic. Nevertheless, in experimental infection of volunteers, some authors found symptoms such as nausea, abdominal pain and diarrhoea 3–6 h after the ingestion of raw or undercooked *Sarcocystis*-infected beef (*50*). During the peak shedding time of sporocysts, i.e., 14–18 days after such meat consumption, there were also symptoms such as diarrhoea and abdominal pain. Clinical symptoms become, however, more pronounced after ingestion of raw pork infected with *S. suihominis*; increasing nausea, bloating, lack of appetite, marked abdominal pain, unproductive retching and diarrhoea were observed 6–8 h after the infected meat had been eaten. The symptoms continued for up to 48 h. Some volunteers suffered from acute clinical symptoms, at first diarrhoea and vomiting, coldness and sweating, lasting up to between 12 h and 24 h. Clinicochemical investigations suggested acute infection combined with dehydration. There is no clear evidence that the clinical symptoms observed in cases of *S. hominis* (and particularly in *S. suihominis*) infections depend on the action of specific toxins (sarcocystin) which have been described. The clinical symptoms were observed, as a rule, after the consumption of heavily infected beef or pork.

Human muscular sarcocystosis is usually asymptomatic, and an inflammatory reaction associated with the presence of muscle sarcocysts has not been observed. However, some symptoms have been reported including intermittent swelling and weakness of muscles, muscular pain, focal myositis, eosinophilia, malaise and bronchospasm.

3.2.2.3 *Parasitological diagnosis.* Intestinal sarcocystosis in man can be diagnosed by the detection of mature sporocysts in fresh faeces, beginning on the ninth day following ingestion of raw meat. The cyst sizes can be used for species differentiation. As a rule, the number of cysts shed with human faeces is scanty, therefore concentration techniques are recommended—zinc sulfate flotation being the most efficient one.

There is still controversy as to the value of serological tests in the diagnosis of human intestinal sarcocystosis. Some authors observed that human serum samples examined with the indirect fluorescent antibody test, using bovine *Sarcocystis* antigen from muscle cysts, reacted with positive titres in the first weeks of infection. Nevertheless, the general opinion is that, at present, serological tests have little or no diagnostic value for human sarcocystosis; the presence of antibodies could indicate muscular infection, intestinal infection or both, or just be false positive reactions. Serological tests for muscular sarcocystosis in man are not likely to be helpful either.

3.2.2.4 *Therapy.* There is no specific therapy for human intestinal and muscular sarcocystosis. The opinion was expressed that treatment is rarely necessary as the intestinal infection is self-limiting, and the muscle infection is usually subclinical.

3.2.2.5 *Prevention and control.* Sarcocystosis can be eliminated by breaking the transmission of parasites as follows:
—by not consuming raw or undercooked beef or pork;
—by deep-freezing meat—the parasites are diminished in number by this procedure both in beef and in pork;
—by educating farmers in the mode of transmission of *Sarcocystis* to cattle and pigs; and
—by preventing contamination of pastures with human faecal material.

3.2.3 Isospora belli *infection*

I. belli of man does not need an intermediate host for transmission; it completes the schizogony stages in the epithelial cells of the human intestine and is shed as unsporulated oocysts. Transmission of oocysts follows the classical coccidian mode of intraspecific infection, i.e., man-to-man by the ingestion of mature faecal oocysts. Although relatively short periods of patency of *I. belli* are usual, there are reports of shedding of oocysts for longer periods. The existence of a reservoir in the visceral organs cannot be excluded, from which reinvasion of the intestinal epithelium may regularly occur, and a possible development of extraintestinal stages of *I. belli.*

The infection is probably more common in countries with tropical and subtropical climates. In Europe, cases of *I. belli* are noted sporadically and mostly in persons returning from the tropics.

The main clinical symptoms are diarrhoea, abdominal pain and loss of weight. Some cases of infection, however, are symptomless and self-limiting.

Infection with *I. belli* can be diagnosed by detection of unsporulated cysts in the faeces of man.

3.2.4 Dientamoeba fragilis *infection*

The evidence concerning the clinical and public health importance of *D. fragilis* is controversial. However, the mode of transmission, as well as the taxonomy of this parasite offer interesting problems to parasitologists. On the basis of recent data, *D. fragilis* can be considered as an amoeba-like flagellate (trichomonad) without flagella and somewhat structurally modified.

3.2.4.1 *Distribution and prevalence.* The distribution of *D. fragilis* is worldwide. Epidemiological investigations carried out in the last decade suggest that the incidence of *D. fragilis* may be higher than is usually found by routine coproscopic examination. By means of suitable examination procedures, the incidence of infection was found to be almost 40% in some investigations. Some authors even considered *D. fragilis* to be the most frequent intestinal parasite in children.

It is worth noticing that mixed infections of *D. fragilis* and *E. histolytica* are quite frequent.

3.2.4.2 *Epidemiology.* As cysts of *D. fragilis* are not likely to exist, the mode of transmission of the parasite is a matter of conjecture; *Enterobius vermicularis* might be a vector of *D. fragilis* and their eggs, and so act as a transmitting agent.

A very close correlation was found between the incidence of *E. vermicularis* and *D. fragilis* infections in man. Further confirmation of the vector theory has been found in the successful experimental transmission of *D. fragilis* by ingesting pinworm eggs from *D. fragilis* carriers, and also by determining the isoelectric-point identity of plasma and nuclear albumin of culture-forms of *D. fragilis* and cells found inside pinworm eggs (*51*).

3.2.4.3 *Clinical signs. D. fragilis* is often regarded as nonpathogenic. In the last decade, however, some evidence has been reported of a correlation with abdominal symptoms (mainly intermittent diarrhoea, meteorism and spasmodic abdominal pain) in patients with *D. fragilis*

and the disappearance of the symptoms after specific treatment. Some authors suggested that *D. fragilis* may be pathogenic only under conditions where there are persistent functional disturbances. It has also been observed that children with diarrhoea of unknown origin were infected with *D. fragilis* more frequently than those without diarrhoea. In some cases, the parasite was found in human bile duct material obtained either by surgery or by duodenal drainage.

3.2.4.4 *Parasitological diagnosis.* The diagnosis of *D. fragilis* infection can be made by demonstration of trophozoites in direct preparations of the stool. Using conventional techniques, the number of cases diagnosed is low, whereas improved techniques (proper staining of the fresh stool and culture methods) enhance the chances of detecting the parasite. For the exact differentiation of the parasite, it is necessary to fix the specimen for permanent staining. Some authors believe that examination of a purged stool is more productive than that of spontaneous stools.

3.2.4.5 *Therapy.* Treatment for *D. fragilis* is recommended only in cases with clinical symptoms; the most effective treatment is that used for amoebiasis.

3.2.5 Entamoeba polecki *infection*

E. polecki is described as almost exclusively a parasite of the large intestine of pigs and monkeys but has occasionally been found in man.

3.2.5.1 *Distribution and prevalence.* The actual number of the known human infections with *E. polecki* is no more than 300. Although most of these cases have been found in Papua New Guinea, the infection appears to be worldwide in distribution. Improved methods of examination would provide a much higher incidence than that reported so far. Some cases of amoebic infection diagnosed as being due to *E. histolytica* would most likely turn out, in the course of re-examination of specimens, to be due to *E. polecki.*

3.2.5.2 *Epidemiology.* Pigs in Papua New Guinea, particularly in the highland areas, where they live in the same compound as human beings, are the natural host of *E. polecki* and play an essential part in the transmission of the infection to man. Traditionally, young pigs may also be suckled by women. These facts account for the common as-

sociation of *E. polecki* with the human population in that country. Another most likely source of infection is the transmission of the parasite from monkeys. Some findings also suggest a person-to-person spread of infection.

3.2.5.3 *Clinical signs.* The parasite is apparently nonpathogenic; only a few cases have been reported in which the patient developed symptoms. The patients suffered mainly from occasional diarrhoea and abdominal pain. *E. polecki* is not invasive beyond the intestine.

3.2.5.4 *Parasitological diagnosis.* Permanent stained preparations are essential for the diagnosis of *E. polecki* because the cysts closely resemble those of *E. histolytica.* The uninuclear character and an abundance of chromatoid material in the *E. polecki* cysts enable the differentiation to be made from *E. histolytica.* Culture methods do not improve the diagnosis.

3.2.5.5 *Therapy.* Infection with *E. polecki* is rather refractory to therapy with amoebicidal drugs. Diloxanide furoate, in combination with metronidazole, has been successfully used in the treatment of *E. polecki* infection.

3.2.5.6 *Prevention.* The infection is associated with poor hygiene, as well as poor social conditions and habits. Improved housing (separate accommodation for human beings and pigs in Papua New Guinea) and preventing human food from being contaminated with animal faeces could solve the problem of *E. polecki* transmission.

3.3 Major nematode infections

3.3.1 *Ascariasis*

Ascaris lumbricoides is primarily a parasite specific for man. There are two conceptually separate populations and reservoirs of *A. lumbricoides*: one consists of adult *Ascaris* worms parasitizing man and the other of *Ascaris* eggs in the environment. The size of the former can be estimated as at least 7800 million of adult *A. lumbricoides* worms, which is obtained by multiplying the expected number of human hosts infected—1300 million (*24*)—by the expected mean number of parasites in a single host—6 (*25*). Among the various ecological factors, the physical ones are the most important in regulating the

population of *Ascaris* eggs outside man. Transmission to man depends mainly on socioeconomic factors and ascariasis has a tendency to patchy distribution in areas of low endemicity.

Ascaris infection is determined by a sequence of events in which the parasite tries to complete its life-cycle while the host safeguards the integrity of its own body. The response of the host depends on the stage of invasion and on its intensity. The host response to *Ascaris* at the larval stage is strong, and not infrequently hypersensitivity occurs. It is mainly in the larval migratory stage that the host regulates the intensity of invasion. Human hosts are frequently tolerant of intestinal infection with adult *Ascaris.* Man can react vigorously to an *Ascaris* allergen if he has not been desensitized in the course of infection. Complications due to migration of adult *Ascaris* worms or to intestinal obstructions are both unusual and rare consequences in man, but they are the most important causes of mortality in human ascariasis.

3.3.1.1 *Specificity of* Ascaris *for man. A. lumbricoides* is very closely related to *A. suum,* the pig roundworm. These two organisms have only minor morphological distinctions and physiological differences, but they evidently differ in the invasiveness to their natural or accidental hosts. *A. suum* develops better in the pig; in man it develops in the larval, tissue migratory stages but rarely reaches the intestinal adult stage (*26*).

The frequency of *A. suum* infection in man is not well known. In some parts of the world (e.g., Sri Lanka), there is no ascariasis in pigs whereas human ascariasis is common; in some countries (such as Papua New Guinea), where there is close contact between man and pigs, the relative frequency of the two infections is not known.

A. lumbricoides is practically the only cause of human ascariasis. However, in many regions of the world, man is frequently exposed to the invasive eggs of *A. suum* and *Toxocara* species; just how much the exposure to nonspecific but closely related species of *Ascaris* influences the pathology, immunology and epidemiology of ascariasis in man remains unknown.

Although *A. lumbricoides* infections have been induced in man experimentally, a great deal of our knowledge on human ascariasis is derived from experimental infections in animals by using *A. suum.* Pigs provide a good experimental model for studying both invasiveness and intestinal pathology in ascariasis (*27*). *A. suum* infections in small laboratory rodents are commonly used for immunological studies. Although the experimental models are excellent in many respects,

there are obvious limitations in the interpretation of results in relation to *A. lumbricoides* infection in man, because the unnatural hosts usually respond more strongly to the invasion of unusual parasites. Since the clinical and epidemiological observations also have their limitations, many aspects of the man/parasite relationship in human ascariasis are still not understood.

3.3.1.2 Ascaris *eggs in the environment.* The reproductive potential of each *Ascaris* female worm is extremely high—about 240 000 eggs per day, which counterbalances the heavy losses in viability and infectivity of these eggs in the environment. The global external environment is thus contaminated daily by about 10^{14} *Ascaris* eggs, many of which develop to the invasive stage.

Of the various ecological factors (landscape, weather, type of soil) regulating the population of *Ascaris* eggs outside the human host, the most important are the physical ones: temperature, moisture, oxygen pressure and ultraviolet radiation from sunlight. Depending on their action, *A. lumbricoides* eggs can survive for more than six years in a temperate climate but for only a few hours in some tropical conditions. These factors, when unfavourable, are responsible for seasonal breaks in transmission, e.g., in Saudi Arabia (because of the arid and warm climate most of the year) and in Europe (in the colder months of the year).

The results of soil examination for helminth ova provide some information on the extent and intensity of the environmental pollution: in a focus in Sumatra, Indonesia, *Ascaris* eggs were found in 45% of 55 samples (2 g) of soil collected around nine farmers' houses (*52*); in a focus of ascariasis in Poland, 71% of 935 samples (100 g) of soil had *Ascaris* eggs with a mean of 1.8 or 2.8 eggs per 1 g of soil (*53*). The infrastructure of the soil and the mechanical action of rain cause a patchy concentration of *Ascaris* eggs in the ground and may be responsible for massive infections occurring from time to time instead of regular, small-dose infections. However, our knowledge of infection pressure in human ascariasis (number of possible human exposures in a certain area in a given time) remains unsatisfactory. The biology of *Ascaris* eggs in the soil is not well known in endemic areas, nor is the role of coprophagic animals in this context.

3.3.1.3 *Transmission of ascariasis to man.* The level of transmission of the infection from soil to man depends more on socioeconomic factors than on physical ones. The main factors seem to be a high

density of the human population, its involvement in agricultural production (especially with extensive use of human nightsoil as a fertilizer), illiteracy, and poor sanitation. Only a few community profile studies have been carried out—these would be helpful in understanding the local ways of transmission and in enabling some more generalizations to be made (e.g., the prevalence and intensity of ascariasis are highest in the younger age groups (*28*), and the prevalence of ascariasis is very high in some families (*25*)). There is increasing evidence that dust may also play an important role in the transmission of ascariasis in arid areas.

The world distribution of ascariasis mostly follows an endemic pattern. The factors responsible for either high endemicity (over 60% of the human population infected) or low endemicity (less than 20% of the population infected, mostly children) have seldom been studied. A pattern of low endemicity is characteristic of seasonal types of transmission, as in Europe and Saudi Arabia. In areas of low endemicity, a patchy distribution of ascariasis (e.g., in some localities or in some families only) is frequently observed, especially in areas where ascariasis is disappearing spontaneously as a result of improved sanitation. However, epidemics of ascariasis have been observed, e.g., in 1947–48 around the vast irrigated fields at Griesheim in the Federal Republic of Germany.

The various endemic or epidemic patterns of ascariasis not only depend on the infection pressure in the environment but are also regulated by the level of immunity of the local human population.

3.3.1.4 *Classification of host response to* Ascaris *infection. A. lumbricoides* seems to be well adapted to crossing several barriers when invading man. For a successful invasion, the small intestinal mucosa, the hepatic tissue and the lung alveolar walls must be penetrated, passages have to be made through the lymphatics, the blood vessels, the upper respiratory tract and the stomach (twice), and several rapid changes in the quality of the biotype as well as in the protective mechanisms of the host have to be withstood. Even after finally settling in the small intestine, the pre-adult and adult worms cannot always resist the increased body temperature and peristalsis of the human host.

The host response to *Ascaris* invasion of the tissue and the intestines includes a variety of specific and nonspecific reactions—to the mechanical tissue damage (during the migration phase and from irritation of the intestinal mucosa during the later stages of invasion)

and to chemical substances (secreted or excreted by the living parasites or released from the dead larvae and having toxic or immunogenic properties). The immune response of man against *Ascaris* infection is expressed by a number of humoral and cellular phenomena, of both specific and nonspecific character. Ascariasis may also exert an immunosuppressive influence (*29*). The character and range of the host reactions differ widely—from hypersensitivity to larval infection and the *Ascaris* allergen, to a great deal of tolerance to the presence of the largest intestinal nematode parasite, which may even parasitize without being noticed.

In general, there are four different types of host response:
—against *Ascaris* migrating at the larval stage;
—against preadults and adults in the small intestine;
—against the *Ascaris* allergen; and
—against migrating or crowded adult *Ascaris* worms.

Host response to Ascaris *in the larval stages.* The response against the larval stages of *Ascaris* may be strong and follows a typical pattern for tissue parasites, in which cellular immune reactions play a major role. Besides the nonspecific inflammatory reactions, some hypersensitive reactions also develop, such as the Splendore-Hoeppli phenomenon, high eosinophilia, eosinophilic granulomas and Löffler's syndrome. A hypersensitive reaction, such as Löffler's syndrome, is frequent in human populations exposed to *Ascaris* infection sporadically or seasonally, but it is rare where the transmission is year-round (*30*). On the other hand, more or less continuous exposure throughout the year makes the host more resistant to reinfection, as shown by a lower intensity of ascariasis.

The mechanisms responsible for sensitization or desensitization and susceptibility or resistance to reinfection are still not very clear, although some interesting observations have been published recently. Analysis of the protein/polysaccharide antigens of *A. suum* indicates the presence of multiple determinants; some are immunogenic—stimulating the production of precipitating antibodies; others are allergenic—responsible for reaginic IgE production and local or systemic hypersensitive reactions such as bronchial asthma, angioneurotic oedema and urticaria. In the case of ascariasis, allergic reactions and reactions influencing the immunity of the host seem to be separate phenomena.

An increase in circulating IgE globulins is known to be common in human ascariasis but it was found that this was not necessarily

related to the atopic changes present. *Ascaris* infection causes a non-specific potentiation of IgE because only a small part of the total IgE has specific *Ascaris* antibodies (*31*). It has been suggested that the high level of serum IgE, caused by a high infection rate with helminths, suppresses the prevalence of asthma in children in tropical countries; if true, this would be one beneficial aspect of human ascariasis.

Mechanisms of the host regulating the intensity of invasion. In man a sterilizing immunity against *Ascaris* infection seems to be a rare phenomenon; as a rule, immunity is incomplete and manifests itself by a reduction in the number of parasites. The mechanisms responsible for partial resistance to the challenge infections with *A. suum* are active during the early intestinal stage (interfering with the process of hatching and the larval penetration of the small intestine mucosa), during the tissue stage (immobilizing and destroying the *Ascaris* larvae in the lung tissue), and probably in the small intestine (by acting on the immature worms soon after the third moulting). Recently an *Ascaris* culture-fluid antigen was obtained by *in vitro* cultivation of third-stage *A. suum* to the fourth stage, which induced significant protection in guinea-pigs against a challenge infection (*32*).

In clinical observations of human patients infected with *A. suum,* the high level of precipitating antibody titres with IgM mobility was correlated with the reduction in the worm burden. In experimental animals, a significant level of protective immunity against *A. suum* in pigs is achieved only after feeding them with multiple, large doses of infective eggs. Whether the resistance to ascariasis observed in older people in endemic areas is a result of multiple exposures (and, if so, which mechanisms are involved) remains unclear.

Host response to Ascaris *adult stages.* The host response to intestinal infection with adult *Ascaris* shows a great deal of tolerance, which is typical for intestinal luminal parasites. X-ray studies of ascariasis in man show that 87% of the worms are present in the jejunum.

The worms remain stationary most of the time; as they are braced against the intestinal wall, they are not affected by normal intestinal peristaltic action. The spiral forward movement and a tendency to enter small openings, whether it be the ampulla of Vater or a drainage tube, are characteristic of *Ascaris.*

Observations in pigs, infected with *A. suum,* showed a highly significant hypertrophy of the muscle layers, a corrugated appearance of the mucosa, a shortening of the crypt depth, and a diminished amount of mucus. These changes correspond well to the disordered

small bowel pattern, most commonly a coarsening of the small intestinal mucosal folds, which has been observed in the majority of human patients with ascariasis during X-ray examination. The relationship of these morphological changes to the symptomatology of ascariasis and to the impaired absorption of nutrients has yet to be clarified. Strong host trypsin and chemotrypsin inhibitors were found in extracts of *A. suum* and to be secreted in *in vitro* culture media. It seems that this inhibitory action of *Ascaris* antienzymes does not greatly disturb the digestive processes of the host.

The contribution of *A. lumbricoides* to malnutrition has been studied. To date, not enough attention has been focused either on the intensity of infection or on the proper design of such studies. The metabolism of *Ascaris* has been studied extensively; however, there is still too little information on how the host tolerates *Ascaris* metabolites and whether they interfere with the host metabolism. Some interesting observations have been made on the presence of volatile fatty acids (excreted with the urine of the infected host in easily detectable amounts) on functional pyridoxine deficiency, on the impairment of retinol and ascorbic acid levels in parasitized children (*33*), and on the antimetabolic activity of some *Ascaris* metabolites. There are several observations that ascariasis itself is responsible for a decrease in growth, in the absorption of various nutrients, and in lactase tolerance. These pathological effects seem to be closely related to the intensity of *Ascaris* infection and many other factors, including polyparasitism, which affect the nutritional and immune status of the host.

Host responses to the Ascaris *allergen. Ascaris* allergen is the most potent of all allergens of parasitic origin. It is present in all stages of the *Ascaris* life-cycle and its physicochemical and biological properties are known. The *Ascaris* allergen can cause a hypersensitive reaction in the lungs, skin, conjunctiva and gastrointestinal tract. These reactions have been observed in infected individuals and in laboratory workers who were in contact with the *Ascaris* allergen. A gastrointestinal allergy to the *Ascaris* antigen (observed in an uninfected laboratory worker with repeated episodes of abdominal pain, heartburn and diarrhoea) has been described. Various descriptions of cases of allergy to *Ascaris,* sometimes life-threatening, are an important source of information. However, an evaluation of the health impact of this allergy at the population level is not possible without well-controlled studies which should be performed in areas of low endemicity and on seasonal transmission of the infection.

Serious complications of intestinal ascariasis. The most serious complications of human ascariasis are due to migration of the adult worm or intestinal obstruction by a bolus of crowded adult worms. The migration of *Ascaris* worms outside their natural biotope is promoted in the host by fever, a peppery diet, anaesthesia and improper treatment, and is probably more frequent in infections with a single worm or worms of the same sex (*34*). Such migration may cause, in order of decreasing frequency: hepatic duct obstruction, appendicitis, intestinal perforation including penetration of intestinal operation wounds, and pancreatic duct obstruction. Vomiting with *Ascaris* occurs in up to 4% of cases. On such occasions, penetration may occur in the upper respiratory tract and into the Eustachian tube.

Most commonly, intestinal obstruction occurs at the terminal ileum and is caused not only by an aggregation of crowded worms, but probably also by intestinal spasm, produced by irritation of some receptors in the mucosa. Intestinal obstruction is more frequent in intensive infections. The approximate rate of intestinal obstructions per year in south-eastern USA is 2 per 1000 in children aged 1–5 years infected with *Ascaris*. Intestinal obstruction due to ascariasis and complications due to *Ascaris* migration constitute 10–15% of all acute abdominal emergencies in Cape Town, South Africa, and are second in frequency only to acute appendicitis; the peak incidence is observed in children between 4 and 8 years of age (*54*).

There is still inadequate information about the frequency of life-threatening complications of ascariasis requiring surgery in many endemic areas: thus evaluation of the approximate rate of mortality from *A. lumbricoides* in the world is difficult. Even if the fatality rate were as low as 6 per 100 000, this might constitute thousands of deaths when multiplied by the millions of people who are infected.

3.3.1.5 *Immunology of ascariasis.* Studies on the immunology of ascariasis deserve attention because they are significant for the control of this disease, besides having a large theoretical element. According to recent data, the ascarids stimulate an immune response, the degree of manifestation of which depends on the number and intensity of repeated infections and on the physiological and apparently genetic peculiarities of the host, as suggested by experiments in different mouse strains. The immune response in ascariasis develops in accordance with the general rules for the development of immunity in mammals, but it has its own peculiarities which are conditioned by the physiological and biological properties of the agent. The kinetics

of the immune response correlate with the biological cycle of ascarids, each developmental state of which has a different immunizing activity. The most striking immune response is expressed in the early phase of ascariasis, which is conditioned by the immunogenicity of the secretory and excretory antigens of the migrating larvae, but the 6–7-day-old larvae are much more immunogenic than 3–4-day-old ones.

The immune response is expressed by a number of humoral and cellular phenomena, both of specific and of nonspecific character, as described below.

Humoral phenomena. A change of the protein content in the blood (characterized by an increase in the gammaglobulin level during the first 15 days to two months after inoculation) illustrates one of the humoral phenomena. Gammaglobulinaemia reflects the process of antibody formation, which develops very quickly. According to data (*55*) from mice, guinea-pigs, pigs and human beings, the antibodies can be detected already by the fifth to sixth day after inoculation. Fifteen to 25 days later, the antibody levels reach their maximum; thereafter they gradually decrease up to 90–100 days after inoculation. After repeated inoculations, the antibodies can be detected almost immediately. At that time, the IgM antibodies prevail, but later there is a gradual increase of the IgG antibodies, which then reach higher levels than IgM (*35*). This correlates well with the numbers of plasma cells in the spleen and lymph nodes producing them. Heterophilic antibodies, and particularly anti-A and anti-B isoagglutinins, may also be frequently detected and this is conditioned apparently by the presence of group-like antigens A^{37} and $B^{51, 52}$ in the body tissues and excretory-secretory products of ascarids (*36*).

In the blood and other biological fluids of the host, one can detect reagins, homocytotropic antibodies, which belong mainly to the secretory IgE and in significantly smaller quantities to the IgG_1 class. Apparently the IgE reagins are produced by plasma cells in the Peyer's patches of the small intestine—under the influence of antigens of larvae (during their fourth moult) and secretory and excretory antigens of adult ascarids—and also by cells of the lymphoid system of lungs during the migration of larvae.

Cellular phenomena. During the first days after inoculation, there is an increase in the numbers of T cells and in their physiological activity in experimentally infected animals. This activity is expressed by an intensified production of mediators of various types and, in particular, those inducing a delay in migration of lymphocytes and macrophages. An intensified transformation of lymphocytes into lym-

phoblasts reaches its maximum during migration of larvae from the circulating blood into the intestine. On days 5 and 6 after the inoculations, rosettes are formed when lymphocytes are incubated with sheep erythrocytes. A development of delayed hypersensitivity, expressed by the formation of granulomas around larvae in the tissues, is also seen. However, the immune response in ascariasis is formed by B cells and not T cells.

Protective action. Not all the phenomena of the immune response are active in protective reactions; in a number of cases, they act as witnesses of the immune reorganization of the body. This does not exclude, however, their protective significance. Numerous data on the immunization of recipients through sera of infected donors provide evidence of the participation of humoral antibodies in protective reactions. Successful immunization depends on the intensity of infection levels in donors, the quantity of sera introduced into the recipient and the time of its receipt.

The macroglobulin antibodies have the main protective action. Studies carried out in experimental animals by inoculating separate fractions and by transmitting the immunity from mother to offspring (both through the milk and through the placenta) indicate the participation of IgG_1 and IgG_2 classes of antibodies in the protective reactions (*37*). The antibodies may directly influence the migrating larvae, inducing obstruction of their excretory pores with precipitates which deters the normal course of the biochemical processes in the parasite. They may intensify the activity of macrophages, on whose surfaces they are adsorbed, and also may induce (together with the complement) the adherence to the tegument of the *Ascaris* larvae of other cells, including eosinophils, which are now recognized to have a cytophilic activity.

The protective significance of reagins has not been well studied. Considering, however, their ability to adsorb on mast cells and in the presence of antigens to cause the destruction of the mast cells with subsequent discharge of active amines, it seems that they participate in a protective reaction of a local character which is connected with the development of inflammatory allergic reactions.

The protective role of cellular immunity is shown by the fact that the T cell system can be depressed by neonatal thymectomy, radiation, or drainage of the thoracic duct, thereby leading to a decrease in resistance of the host to ascarid infections. Injection of antilymphocytic sera has an analogous effect. Trials in immunization of intact recipients with lymphoid cells from donors infected with *Ascaris* larvae were

also indicative. Self-cure phenomena are protective and are universal for many helminthiases. Their mechanisms are apparently different in various infections. In ascariasis, the protective effect of the self-cure phenomenon occurs in the small intestine both during penetration of the intestinal mucosa by first-stage larvae and at the end of migration when the larvae re-enter the digestive tract.

Expression of protective immunity. The results of experimental investigations have demonstrated that, in laboratory animals inoculated with infective ascarid eggs, a resistance is formed which shows itself by a decrease in numbers and length of life of larvae developed from the challenge infection. In guinea-pigs, inoculated on two occasions with 600 *A. lumbricoides* eggs, the number of larvae in the liver and lungs after the second inoculation was half that following the first inoculation. After four or five inoculations at eight-day intervals, no larvae were detected in the organs, but tissue damage of an allergic character occurred with marked proliferative-resorptive processes in the macrophage system. These experimental studies were confirmed by observations in human patients. In an intensive focus of ascariasis, the eosinophilic infiltrates of ascarid etiology in the lungs were registered in semi-immune local inhabitants ten times less frequently than in newcomers who had lived in the focus for a year.

Immunological and parasitological examinations, and anthelmintic treatment of the representative groups of children (carried out monthly in foci of ascariasis) demonstrated that in only a small percentage of persons who had antiascarid antibodies in their blood did the intestinal phase of ascariasis develop later. Therefore, one may consider that the population in an endemic area, subjected to frequent repeated reinfection, will develop an immunity and will only suffer a low level of ascarid infection in the intestine.

Immunosuppressive action of ascarids. The ability of ascarids to suppress the host's immune response to a large number of antigens has been established in trials on mice and confirmed by observations in human patients. In mice inoculated with *A. suum* larvae or immunized with the body fluid of adult ascarids, antibody formation against paratyphoid B bacteria was depressed (*29*). Injection of ascarid extract into mice caused a suppression of production of reaginic and haemagglutinating antibodies to the lysozyme of ovalbumin. During immunization of mice, infected with *A. suum* larvae, with sheep erythrocytes, the immune response was suppressed during the acute phase of the disease. This was shown by a decrease in the numbers of plasma cells synthesizing the macroglobulins and in the level

of IgM antibodies, as well as by depression of the pad oedema reaction, indicating the participation of macrophages connected with a delayed type of hypersensitivity (*38*).

Observations on patients demonstrated depression of the formation of antibodies to diphtheria and polioviruses in children infected with ascarids and of antibodies to vaccines against typhoid and paratyphoid fevers in adults.

The mechanisms of the various phenomena of suppression in ascariasis are apparently different. They may be connected with the development of true immunological tolerance conditioned by the community of antigens in ascarids and other agents, by the phenomenon of competition between antigens, and also by the influence of the host immunocompetent system of suppressive materials discharged by ascarids, though the last-mentioned has not yet been detected. The possible effect on the immune process by *Ascaris* infections must be taken into account when assessing the morbidity of other infections and also during the drawing-up of a calendar of immunization for children.

Immunological diagnosis. Among various phenomena of the immune response, detection of humoral antibodies has a diagnostic significance. Intracutaneous allergic reactions appeared to be insufficiently specific and the diagnostic significance of the cellular immune response has not yet been studied. A number of serological reactions have been developed and most of them, in accordance with the kinetics of antibody formation, are effective in the early phase of the disease. Cross-reactions are possible in connexion with the heterogeneity of helminth antigens. Ascariasis and larval toxocariasis are especially difficult to differentiate. Nonetheless, serological reactions with ascarid antigens have a place in the assessment of lung damage of parasitic etiology and also in mass surveys of the population for studies on epidemiological processes by seroepidemiology and evaluation of the effectiveness of control measures against ascariasis.

When a survey is adequately planned and organized, when use is made of the most effective available serological tests (the IHA test, the immunoenzyme method, or microprecipitation on larvae), and when the results are adequately evaluated and analysed, seroepidemiological surveys should provide comprehensive information on the endemic status of the foci.

A direct correlative dependence was found between the mean geometrical values of antibody titres and the levels of infection in the population. The percentage of reinfections in the total number of

cases of infection can be determined by repeated examination of a representative group and is indicated by the percentage of patients with periodic increases in antibody titres. Repeated examination of the same elements allows the determination of the time periods and duration of seasonal epidemics and, in the case of examinations after the fulfilment of the control programme, the determination of its epidemiological effectiveness.

* * *

In conclusion, contrary to many other human parasitic infections, ascariasis is characterized by a high host specificity and by a simple life-cycle. The most important factors responsible for the wide spread of ascariasis in the world lie within the spheres of human behaviour and economy. In theory, ascariasis is preventable and, in practice, it is on its way to disappearing in developed societies with a rising standard of sanitation.

The host/parasite relationship in ascariasis is characterized by a high degree of tolerance and control; strong reactions against the migrating larval stages protect the host against an uncontrolled intensive intestinal infection. Acute and fatal complications, which are rare, are caused by hypersensitivity to *Ascaris,* by ectopic localization and by high intensity of intestinal infection. The nutritional and immunological mechanisms of the host seem to be most affected in ascariasis. These phenomena are less obvious in well-balanced hosts. But there is somewhat scanty information about the significance of ascariasis in young children, who are frequently undernourished and whose immunological ability is constantly challenged by other infections; this is an area of research which deserves priority.

Studies on the immunology of ascariasis have a great significance for improving programmes for the control of this infection and must be developed both theoretically and in paractice. The following main research approaches are desirable: (1) studies on the mechanisms of the immunostimulating and immunosuppressive actions of ascarids; (2) improvement of immunodiagnostic methods by isolation of species-specific antigens; (3) studies on the diagnostic role of various phenomena of the immune response; and (4) expansion of seroepidemiological examinations.

3.3.2 *Hookworm infections*

The geographical distribution of the two main hookworms, *Ancylostoma duodenale* and *Necator americanus,* used to be regarded as

relatively distinct, the former being more prevalent in Europe and south-western Asia, and the latter in tropical Africa and in the Americas. However, over the past decades both parasites have become widely distributed throughout the tropics and subtropics, and rigid demarcations are no longer tenable. *A. ceylanicum* infections in humans are generally uncommon but are significant in some areas, e.g., China (Province of Taiwan), south-eastern Asia, and Suriname.

3.3.2.1 *Life-cycle.* The life histories of *A. duodenale* and *A. ceylanicum* are identical, except that the latter commonly infects cats and dogs as well as man. The life history of *N. americanus* differs in one important respect—namely, the infection is acquired percutaneously only, whereas *Ancylostoma* infections are acquired by both oral and percutaneous routes. *Necator* apparently lives longer than *Ancylostoma* and is known to persist for 13 years; estimates of *Ancylostoma* persistence are generally much lower.

The adult worms are small (5–13 mm long) and live in the upper part of the small intestine, mainly in the jejunum, where they attach themselves to villi, which are sucked into their buccal cavities.

The egg (about 60 × 40 μm) is passed in the faeces and contains a segmented ovum. When deposited on warm, moist soil, a larva rapidly develops in the egg and hatches after one or two days. The newly hatched rhabditiform larva passes through a 7–10-day free-living cycle in the soil, moulting twice, and becoming a sheathed filariform larva which is infective to man. In a suitable environment—warm, damp soil—these larvae can survive for several months. Human beings are infected by the larvae penetrating their skin; they then migrate by way of the venous system to the right ventricle of the heart and to the lungs into the alveoli. From the alveoli, the larvae are passively carried upwards to the trachea and larynx, and through the oesophagus to the stomach and small intestine, which they reach 3–5 days after having penetrated the skin. After a further 4–5 weeks the worms become sexually mature and may live from 1 to 13 years. It has been shown that whereas migrating larvae of *Necator* grow and develop in the lungs, those of *Ancylostoma* do not; they undergo the same early development in the intestinal mucosa. One female *Ancylostoma duodenale* produces about 30 000 eggs and one female *Necator americanus* about 9000 eggs per day.

3.3.2.2 *Epidemiology.* Although man is the only important source of human hookworm infection, the epidemiology of the disease is de-

pendent upon the interaction of three factors—the suitability of the environment for the eggs or larvae; the mode and extent of faecal pollution of the soil; and the mode and extent of contact between infected soil and skin or mouth.

Thus, survival of hookworm larvae is favoured in a damp, sandy, or friable soil with decaying vegetation, and a temperature of 24–32 °C. Larvae move very little horizontally, but can migrate upwards as much as a metre. *A. duodenale* eggs resist desiccation more than those of *N. americanus,* while the development of hookworm larvae in the eggs and subsequent hatching can be retarded in the absence of oxygen. Insanitary disposal of faeces or the use of human faeces as manure are the chief sources of human infection in countries where individuals are barefooted. Thus, it is to be expected that hookworm infection will have a higher prevalence in agricultural than in town workers—and in many tropical countries it is an occupational disease of the farming community. As mentioned above, *Necator* infection is acquired almost exclusively by the percutaneous route while *Ancylostoma* infection may be contracted either percutaneously or orally—the latter mode of entry gives special point to the reports of contamination of vegetables by these larvae.

The observation that *A. duodenale* larvae may become dormant in man is the most significant recent advance in understanding the biology of hookworms (*39*). This discovery has important implications for the epidemiology, control and treatment of this hookworm.

Self-induced infections, with progeny of a single female, have shown that quiescence lasts for about eight months, whereupon development resumes and produces a patent infection a month later. Nothing is known about within- and between-strain variability in the duration of dormancy. The sequence of symptoms following percutaneous infection indicates that some larvae migrate to the intestine before becoming quiescent. Others probably rest in the musculature before migrating to the lungs and onward to the intestine. Whether the latent larvae occur in muscle fibres, as do those of the related species, *A. caninum,* is unknown. Transmammary transmission, a correlate of arrested development within the host's musculature, probably occurs in the case of *A. duodenale.*

Larval latency complicates the treatment and control of *A. duodenale* infections since dormant nematode larvae resist most anthelmintics and resume development at a later date. These phenomena also complicate the estimation of the life-span of adult worms. Larval dormancy also occurs in the musculature of other mammals, suggesting

that meatborne ancylostomiasis may occur. Evidence for this proposed new route of infection has been obtained by infecting food animals and feeding the resulting larvae to pups, in which the worms matured.

Provided people are equally exposed to hookworm infection, both sexes and all ages are susceptible. In communities in which the parasite has long been endemic, the inhabitants develop a host/parasite balance in which the worm load is limited; thus, although the infection rate in some rural areas of the tropics may be 100%, only a small proportion develop hookworm anaemia. It is not known whether these heavy infections resulting in anaemia are dependent upon repeated exposure to a high intensity of infection, or whether they represent a failure of immunity. There is little direct evidence about the effects of host immunity on hookworm in man.

Almost the entire spectrum of serological and tissue hypersensitivity reactions has been shown to occur in human hookworm infection. Evidence of the functional relationships between these reactions and protection against reinfection is lacking.

3.3.2.3 *Control.* Control of hookworm infection and anaemia involves four approaches: (1) the sanitary disposal of faeces, (2) health education, (3) chemotherapy, and (4) correction of the anaemia.

The provision of latrines and education in their proper use are crucial to the control of hookworm infections. If fresh human faeces are used as fertilizer, this should be treated in order to kill the larvae, either by composting before use, or by the addition of chemicals such as sodium nitrate, calcium superphosphate or ammonium sulfate. The wearing of protective footwear is an essential complementary measure.

3.3.2.4 *Clinical aspects.* When a parasite is universally distributed it is not always easy to be sure to what extent it is responsible for disease in the community, or what significance to attach to its presence in an individual. Failure to appreciate this point has in some instances led, on the one hand, to an uncritical diagnosis of hookworm anaemia and, on the other, to a reluctance to accept a causal relationship between hookworm infection and anaemia.

Infection is first acquired in infancy or later childhood, or, in the case of newcomers to endemic areas, in adult life. The site of entrance of the filariform larvae through the skin is often characterized by a dermatitis known as "ground itch" or "coolie itch". There is intense itching, oedema, and erythema, and later a papulovesicular

eruption which lasts up to two weeks. In endemic areas, these symptoms either pass unnoticed or are rare. Differences also occur between first infections acquired in young children, born of mothers living in endemic areas, and expatriates entering the endemic area for the first time.

The pathogenicity of the migratory stages of hookworms is mild compared with that of *Ascaris,* although pulmonary reactions to *Ancyclostoma* infection have been noted. In the stage of migration through the lungs, minute haemorrhages may occur with eosinophilic and leukocytic infiltration, but once again these seem rare in the tropics.

In the stages of maturation of the worm and sometimes even before eggs appear in the stools, there is abdominal pain, steatorrhoea, or sometimes diarrhoea with blood and mucus, and a blood eosinophilia of 0.3–0.6 (30–60%). A subject who had been repeatedly infected with *N. americanus* over a period of 5 years showed a rise in serum immunoglobulin IgE from an initial figure of 0.12 mg/l to 0.735 mg/l (120 ng/ml to 735 ng/ml). There was little correlation between skin sensitivity reactions, eosinophil count and serum IgE level. Many workers have reported that in their experience the heaviest worm loads are seen at the ages at which infection is first acquired. In many instances, however, a residual innocuous worm load is maintained throughout life, resulting in symptomless infections.

The majority of sick patients seen in the tropics are chronically infected; a marked eosinophilia and specific intestinal symptoms are uncommon. The main pathological features of the disease are due to anaemia and hypoalbuminaemia.

Symptoms and signs. Patients with hookworm disease describe their symptoms as having been present for between a few days and three years. Most of them are farmers who complain that they are unable to work, their commonest spontaneous symptoms being lassitude, shortness of breath, swelling of the legs, loss of their normal skin colour, anorexia, and impotence. Some patients have angina pectoris and many complain of aching in their thighs or calves on walking.

In general, the patients are all severely anaemic, but the accompanying physical signs vary. The majority of them have in increased pulse pressure, peripheral vasodilatation, and a raised venous pressure; these patients are usually relatively well and walk to the clinic with haemoglobin levels as low as 20 g/l (2 g/100 ml). A minority of patients have a small pulse, collapsed veins, severe oedema (often affecting the face and arms), and ascites. They look very ill, continu-

ally feel cold, and are hypothermic (under 36 °C). Koilonychia and retinal haemorrhages are present.

Skin depigmentation. In Negro patients, the skin is initially pale but begins to darken again within a few days, after treatment with iron, often before the haemoglobin level has risen. In hypoalbuminaemic patients, the depigmentation is much more severe and, in its distribution, resembles that seen in children with kwashiorkor. There are thus two kinds of depigmentation that can be seen in hookworm anaemia: one is attributable to iron deficiency, is nearly always present, and recovery begins before the anaemia is corrected; the other accompanies hypoalbuminaemia and improves only slowly with treatment. Changes in the colour and texture of the hair, as well as parotid enlargement, also occur.

Anaemia. The main feature of the established adult infection is the presence of anaemia. The pathogenesis of the anaemia caused by hookworm is dependent upon three factors: (1) the iron content of the human diet, (2) the state of the iron reserves, and (3) the intensity and duration of infection. These factors will vary in different tropical countries but must always be taken into account for a proper evaluation of a particular situation. Thus, in Nigeria, where the iron intake is high (21–30 mg daily), people whose only pathological source of bleeding is hookworm infection show no evidence of iron depletion, as evinced by a low serum iron concentration or an iron-deficiency anaemia, unless they harbour more than 800 worms (*40*). On the other hand, in Mauritius, where the total iron content of the food is only 5–10 mg daily, it was found that even moderate hookworm loads could cause sufficient blood loss to precipitate anaemia (*41*).

In human hookworm infection, the loss of red cells into the gut is proportional to the worm load and has variously been reported as between 0.03 and 0.05 ml of blood per worm per day for *N. americanus* and between 0.16 and 0.34 ml for *A. duodenale.* The volume of blood sucked does not alter significantly with the development of anaemia, though the quantity of red cells of course decreases. The concept of persistent bleeding ulcers, left behind by migrating worms, has little histological support in human infections and bleeding usually stops immediately after complete deworming. Nor is any concrete proof available, to date, in support of the toxic theory of the causation of hookworm anaemia. It is important to recognize that part of the haemoglobin iron that the hookworm ingests and excretes while in the duodenum and upper jejunum is reabsorbed from the gut. This pro-

portion increases as the patient becomes depleted of iron and amounts to between 40% and 60%, as measured by double isotope studies.

In some parts of the tropics, e.g., Colombia and India, there occurs a superadded folic-acid megaloblastic anaemia which is often masked by the severe iron-deficiency anaemia and which only becomes overt after a partial haematological response to iron therapy. The pathogenesis of folic-acid deficiency in severe hookworm infection may be due to a variety of factors: defective folic-acid absorption, deficiency of folic-acid in the diet, and increased demands. The classical anaemia of uncomplicated hookworm disease is, however, a hypochromic microcytic anaemia. In Nigeria, the distribution of haemoglobin phenotypes is similar in patients with hookworm anaemia and in the general population; thus the hypothesis that haemoglobin S protects against the development of hookworm anaemia is refuted.

Hypoalbuminaemia. In addition to anaemia, loss of protein is another important manifestation of hookworm infection. When hypoalbuminaemia occurs, it is due to a combined loss of blood and lymph, and the protein loss is well in excess of the loss of red blood cells. There is also nearly always a limited capacity for albumin synthesis, the latter being brought about by a variety of factors such as anaemia, which affects liver-cell function; coincidental disease of the liver, e.g., tuberculosis; loss of appetite; and possibly failure to reabsorb amino acids from the albumin passing into the gut. Serum albumin concentration is often low in hookworm anaemia. The oedema does not respond to mercurial diuretics, even after the anaemia has been corrected, but subsides rapidly when patients are dewormed. In conclusion, hookworm disease can be added to the list of causes leading to a protein-losing enteropathy.

Gastrointestinal function. Various types of digestive disorder have been attributed to hookworm disease, which may result from eating dirt; duodenitis and peptic ulceration have both been suspected of being the result of hookworm infection. Gastric acid secretion is low, both at the basal state and after maximal stimulation with histamine, as compared with normal controls; moreover, fibrogastroscopy does not reveal any gross abnormality. Barium meal X-rays reveal nonspecific changes of disordered motor function, and any appearances consistent with a diagnosis of duodenitis are unrelated to hookworm disease.

There is some disagreement in published reports about the effects of hookworm on the mucosa of the duodenum and jejunum. The experience of the majority of workers in uncomplicated hookworm

anaemia is that it is generally not associated with gross malabsorption, and that intestinal morphology, as determined by peroral mucosal biopsies, is within normal range. More recently, no clinical evidence of malabsorption could be found in Jamaican patients, nor was there any significant difference in the rate of small-intestinal DNA loss when compared with normal controls. It is more than likely that some investigators reporting abnormalities have in fact studied patients with hookworm infection and pre-existing intestinal disease, especially when anaemia and the presence of hookworm ova in the stools were the criteria of selection, without consideration of the number of worms present. It is possible, however, that there may be true differences between patients, depending upon such factors as duration of infection, race, and presence of other parasites (e.g., *Strongyloides stercoralis* or *Giardia intestinalis*).

3.3.2.5 *Treatment.* The approach to treatment requires orientation in the context of intensity of infection, probability of reinfection, and economic considerations. Thus, whereas in nonendemic areas it is justifiable to treat all infections, however light, in endemic areas, where reinfection is likely to occur, only heavy or moderation infections are worth treating, unless simultaneous attempts are also made to improve environmental hygiene. In the endemic areas, therefore, the main aim of anthelmintic treatment should be to reduce the load of infection below the level of clinical significance; complete parasitological cure is unnecessary except within the context mentioned above. Nevertheless, in patients who are severely ill from other causes (such as protein-energy malnutrition, marasmus, tuberculosis, or sickle-cell anaemia), hookworm infections, however light, should be treated even though reinfection is certain to occur.

The recommended anthelmintics are relatively nontoxic and in most instances can be given straight away even to debilitated patients. In the past, laxatives before and after treatment were routinely given; they are now usually considered unnecessary except in the presence of constipation. When the anaemia is very severe (less than 50 g/l, or 5 g/100 ml), some practitioners prefer to raise the haemoglobin level to about 70–80 g/l before dealing with the worm infection specifically. Patients with severe hypoalbuminaemia should be adequately and quickly dewormed.

Several drugs are available for treating hookworm infections; their efficacy varies according to the species in question. In many developing countries, economic considerations must determine the choice of drug

that is given; tetrachloroethylene is the cheapest drug. Repeated treatments are usually necessary except in light infections, and this generalization is valid for all the drugs mentioned below.

Broad-spectrum anthelmintics. Multiple intestinal helminth infections are common in the rural tropics. In these cases anthelmintics that are effective against more than one parasite may be useful. Even so, their activity against one species may be selectively lower than against another. Mebendazole, pyrantel embonate, oxantel, oxantel/pyrantel suspension, and tiabendazole are good examples of broad-spectrum anthelmintics that have been used both for individual and mass treatment. When the multiple infections are generally light, the convenience, range and economic advantages of using such compounds are well worth considering. With heavy infections, however, selective treatment for the appropriate species of helminthic infection is preferable.

Iron. The response to iron therapy is usually rapid. A cheap and very effective treatment is ferrous sulfate, 200 mg three times a day, given by mouth and continued for three months after the haemoglobin concentration has risen to 120 g/l (12 g/100 ml). Even without deworming, this regimen will rectify the anaemia and a rise in haemoglobin of 10 g/l per week occurs; unless the worms have been removed, however, the haemoglobin will drop as soon as iron therapy is discontinued and anthelmintics are therefore mandatory in heavy infections. When indicated (e.g., if regular oral administration cannot be guaranteed), intramuscular or intravenous total-dose iron preparations are very successful. If concomitant folic acid deficiency exists, this is treated in the conventional manner.

3.3.3 *Strongyloidiasis*

In contrast to *Ascaris* and hookworm, where the diagnosis of light human infections is made by the presence of eggs in the stool, the excretion of *Strongyloides stercoralis* larvae is intermittent and may be impossible to detect, even by using special procedures. For this reason, prevalence figures are difficult to establish. The infection occurs in all tropical regions; it has a widespread distribution in eastern Europe. Often high endemicity is recorded from circumscribed areas.

Strongyloides is a faecally-transmitted rather than a soil-transmitted helminth. The larvae are infective shortly after passage of the faeces, which remain the focus of contamination in a humid environment for

the natural life of the filariform larva. The establishment of a free-living adult cycle implies that transmission continues. There is some evidence that transmission could occur to the neonate via the mother's milk in the case of *S. fuelleborni* infections of nonhuman primates. Human infections by this parasite of Old World monkeys are diagnosed by finding characteristic eggs in the stool and have been the subject of recent reports. A similar parasite in the Fly river area of Papua New Guinea has also been reported. The clinical and public health importance of such infections is not known at present; nor is the possible value of *S. fuelleborni* in animal models.

Of the human nematode infections, only strongyloidiasis and capillariasis are capable of perpetuation in man by producing new generations of infective larvae from parthenogenic females embedded in the upper small intestinal mucosa. Thus infections of 20–30 years' duration are well documented. The larval penetration into the bowel wall in their passage down the gut must vary with the condition of the host. Possibly host immune factors also play a role. Determinants like these may give rise to the condition known as the hyperinfective syndrome. This syndrome can be precipitated by immunosuppressive therapy, and any procedure implying immunosuppression (e.g., transplantation) must be preceded by exclusion of occult strongyloidiasis and the treatment of apparent infection.

Some patients with *Strongyloides* may be asymptomatic at the time the infection is detected. However, gastrointestinal symptoms, particularly diarrhoea and central epigastric pain, are common. The diarrhoea is due to malabsorption but the cause of the epigastric pain is not clear. Severe small-bowel strongyloidiasis may give rise to thickening and oedema of the bowel wall. This is often visible on contrast radiography.

The severe form of strongyloidiasis has a poor prognosis and a high mortality rate. It occurs either in patients with a basic debilitating condition (i.e., cancer, malnutrition, or the use of immunosuppressives) or in patients with a long-standing infection who build up their parasitic load by internal autoinfection. Gram-negative septicaemia associated with shock is a common cause of death in this form of the disease.

The discovery of unexplained eosinophilia in a patient may be a clue to search for *Strongyloides* although this sign is usually not present in the severe form of the disease. The application of repeated concentration techniques (Baermann funnel, coal culture, etc.) usually leads to detection of the infection. If this fails and diagnosis is still

suspected, Beale's string test, duodenal aspiration, and small-bowel biopsy are employed, in this order, to find evidence of the parasite in the upper part of the small intestine. Only in intensive disseminated strongyloidiasis are larvae found in the sputum or urine. Serology may be useful in nonfilarial areas.

Although one can only speculate at this stage, it may be that strain differences in *Strongyloides stercoralis* account for variations in the infection's severity in man, the presence or absence of larva currens, and the variation in response to tiabendazole therapy. So far tiabendazole is the only drug highly effective in strongyloidiasis.

3.4 Other nematode infections

3.4.1 *Trichuriasis*

Trichuriasis is a common infection, especially in warm, wet parts of the world. In the populations of some tropical countries, the prevalence rates are over 90%; in others they are frequently between 30% and 60%. Trichuriasis is by no means rare in the temperate zones. The persons most frequently affected are between 5 and 15 years of age.

The prevalence and the intensity of infection are important in understanding the local epidemiological situation and the clinical picture of trichuriasis infection. In Colombia the prevalence rate is 50%; and 50% of those infected have symptoms and 20% have diarrhoea or dysentery (*56*). In Poland the prevalence rate among schoolchildren is 36%; 5% of these have symptoms but diarrhoea is exceptional (*56*). The intensity of infection depends on the exposure to *Trichuris trichiura* eggs that are present in soil contaminated with human faecal material. In studies in Colombia, infections with over 5000 *T. trichiura* eggs per gram of faeces are usually symptomatic and most of those with more than 20 000 eggs per gram of faeces develop severe diarrhoea or a dysenteric syndrome. Although it has been shown in a volunteer that *T. suis,* a parasite of pigs, can infect man, infections of zoonotic origin probably play a minor role, if any, in the epidemiology of human trichuriasis.

Light infections with *T. trichiura* do not produce significant lesions in the large intestine. Heavy infections with loads of hundreds of adult worms produce necrosis of mucosal cells around the parasite, intensive cellular infiltation, and inflammatory reaction of the mucosa. Diarrhoea is assumed to be due to impaired water reabsorption in the colon resulting from these lesions. Rectal prolapse is a complication

of extensive colitis and proctitis in undernourished children. Anaemia, cachexia, and bacteraemia may be other serious complications of severe trichuriasis.

Eggs usually appear in the faeces about two months after ingestion. Finding the characteristic eggs in the stool during a microscopic examination is easy. A concentration technique is needed only for the diagnosis of light infections. The most practical method for evaluating the intensity of infection is the quantitative Kato thick smear. Eggs of *T. trichiura* in man are similar to those of *Trichuris* found in animals.

In symptomatic cases two drugs are commonly used, mebendazole and oxantel, and they seem to be very effective. Although immunity has been observed in *T. muris* experimental infections in mice, it is not clear whether immunity can be acquired against *T. trichiura* in man.

3.4.2 *Intestinal capillariasis*

The first case of intestinal capillariasis in man was reported in 1963. The distribution of the infection is restricted to some coastal areas of the Philippines and certain areas in Thailand, where the consumption of raw fish is a common custom, particularly among fishermen. Invasion of the jejunal mucosa by the adult nematodes causes enteritis, which is frequently complicated by malabsorption and a protein-losing enteropathy.

Diarrhoea and sometimes steatorrhoea with abdominal pain are the main symptoms of intestinal capillariasis, which is not infrequently fatal. A diagnosis of acute *Capillaria philippinensis* infection can usually be made by coproscopic examination. Antigens for serological testing are not yet specific enough for routine use. Prolonged treatment with mebendazole is so far the only therapy known to be effective.

3.4.3 *Trichostrongyliasis*

Human trichostrongyliasis is most commonly produced by *Trichostrongylus orientalis,* for which man seems to be a natural host. Herbivorous mammals have many other species of *Trichostrongyloides,* such as *T. axei, T. colubriformis, T. vitrinus, T. probolurus* and *T. lerouxi,* which may occasionally and sporadically infect man. Most of the cases of human trichostrongyliasis are reported from Asia.

Man acquires the infection mainly by eating plants contaminated with infected larvae; rarely the larvae may invade man through skin

penetration. Adult worms, 5–10 mm long, if numerous, may cause enteritis; however, most of the infections in man are symptomless. *Trichostrongylus* eggs that are excreted in faeces must be differentiated from those of hookworm; *Trichostrongylus* larvae grown in coprocultures differ from filariform larvae of other nematodes parasitizing man.

Bephenium and pyrantel are effective drugs against human trichostrongyliasis.

3.4.4 *Enterobiasis*

The distribution of *Enterobius vermicularis* is cosmopolitan but human infections are most common in the developed countries of the northern hemisphere. The highest prevalence is in preschool children and schoolchildren, enterobiasis being more frequent in some families and institutions because crowding is an important factor in transmission. In most cases it is a benign infection, which may continue for years owing to easy reinfection. There is inadequate information on its distribution in developing countries.

The pathology in enterobiasis is mostly related to the migration of the worms. Nocturnal migration of the female worms out of the anus may cause changes in the perianal and perineal regions. Ectopic migration may cause appendicitis, vaginitis, endometritis, salpingitis and granuloma formations in the peritoneal cavity. *Enterobius* may play a role as a vector of *D. fragilis* (see section 3.2.4.2).

Enterobiasis is readily diagnosed by finding female worms or eggs on anal swabs. Pyrantel, mebendazole, pyrvinium and piperazine are widely used in the therapy of enterobiasis; some infections need repeated treatments.

3.4.5 *Nonhuman nematode infections*

Several nonhuman nematodes are associated with the formation of eosinophilic granulomas in the gut wall. Human anisakiasis, presenting as a tumour in the stomach or small bowel, is the result of larval penetration of *Anisakis* or *Phocanema* species after the ingestion of raw fish. Infected larvae of *Oesophagostomum* may form similar tumours in the caecum or colon. A migratory granuloma of the bowel wall occurs with gnathostomiasis, which can be acquired not only from ingestion of raw fish, frog, chicken or snake meat, but also from drinking water contaminated with the primary intermediate host, a

copepod. *Angiostrongylus costaricensis,* maturing in the radicals of the mesenteric arteries of the caecum or colon, may produce eggs or larvae in the tissues. Like oesophagostomiasis, a tumour in the right iliac fossa is a common presentation. The true identity of the above-mentioned eosinophilic granulomas is usually established, if parasites are present, only by examination of tissue resected at surgery.

* * *

The Scientific Group considered a background paper by Dr M. D. Little (see Acknowledgements), giving guidelines for the differentiation of nematode larvae in coprocultures. The Group agreed to annex these to the present report in order to provide practical guidance for medical laboratories (Annex 1).

3.5 Cestode infections

3.5.1 *Human taeniasis*

Taeniasis, as a zoonotic infection, was widely discussed by the WHO Expert Committee on Parasitic Zoonoses in 1978 (*42*). The clinical pathology and symptomatology of human taeniasis are insufficiently studied, although lowered gastric secretion and increased production of serum IgE occur commonly and are very interesting phenomena. Human cysticercosis caused by *Taenia solium* is a far more important public health problem than human taeniasis. However, as man is the main disseminator of *T. solium* and *T. saginata* eggs, the control of taeniasis in man is an essential part of the control of the taeniasis/cysticercosis complex.

Man is the definitive host for only two species, *T. solium* and *T. saginata,* but has been reported to be an intermediate host for six species: *T. solium, T. saginata, T. multiceps, T. hydatigena, T. ovis,* and *T. taeniaeformis.*

The bladder worms recorded from man as *Cysticercus cellulosae* may represent more than one species. The taxonomic examination of cysticerci in man should be made, whenever possible, by using the criteria and techniques proposed by Šlais (*43*).

In practice, the species diagnosis of *Taenia* in taeniasis may not be easy. A scolex is rarely found after treatment with niclosamide, tin compounds or praziquantel, which usually cause the disintegration of the anterior part of the strobila. The value of the routine differential

diagnosis between *T. solium* and *T. saginata* (performed by counting the lateral uterine branches in gravid proglottids) has been questioned by the taxonomists. However, this is the only practical method that can be carried out in a basic laboratory. In cases of doubt, the proglottids should be sent to a specialized helminthological laboratory.

The differentiation of taeniid cestodes by enzyme electrophoresis is possible, but it is still a matter for research rather than general practice. The same applies to the serological diagnosis of taeniasis, which may be very useful when the proglottids are not discharged, i.e., in the early stage of an infection or after treatment. A combination of questioning to get an adequate history and examination of anal swabs and faecal material, and where possible serological tests, is suggested as providing the most effective methods for field studies required in mass diagnosis.

T. saginata and *T. solium* taeniasis cause changes in motility and secretion of the gastrointestinal tract more frequently than local pathological changes of the intestinal mucosa. Straying *T. saginata* proglottids may sporadically cause appendicitis or cholangitis. The most serious risk comes from *T. solium* infections that can be complicated by cysticercosis, i.e., the invasion of the host's organism by larval stages (onchospheres) liberated in the intestinal lumen. These larvae can develop in different organs to become cysticerci, particularly in the central nervous system and in the eyes; as a result of mechanical pressure, obstruction or inflammation, a variety of pathological changes are produced, leading to epilepsy, intracranial hypertensive syndromes, hydrocephalus, psychiatric diseases, or death.

The highest prevalences of *T. solium* cysticercosis are now found in some areas in Africa and Asia. In the Americas, the highest rates of cases are in Brazil, Chile, El Salvador, Guatemala, Mexico and Peru; the frequency of cysticercosis at autopsies varies from 0.12% to 3.6%; psychiatric hospitals give the highest percentages of infections.

3.5.1.1 *Planning of control programmes of taeniasis.* The objectives of a control programme are likely to differ in different countries or regions, depending on (1) the health and economic priorities, (2) the funds and personnel available, and (3) the realistic possibilities to carry out a successful programme.

The primary objective—eradication of the infection in a selected area—is usually unrealistic.

The second objective is the reduction of the existing high level of transmission in both human and animal populations. To this category

of control activities, at the national level and over a long-term period, belong the anthelmintic campaigns in the USSR which have significantly reduced the incidence of taeniasis/cysticercosis in many areas.

The third objective includes the protection of selected groups of individuals against high risks of infection. The epidemic situation among the Kapadoku people in Irian Jaya, Indonesia, may serve as an example to justify a special campaign against *T. solium* infection in that area. To this category also belongs to some extent the protection of people exposed to infection with *T. solium* eggs disseminated by carriers. The control measures in those cases are through early detection and early treatment of all *T. solium* cases and improvement of the general sanitary level.

The fourth objective includes the active prevention of the creation of new, usually man-made conditions favouring transmission. To this important category of objectives belongs the prophylactic chemotherapy of workers in feed-lots and ranches, the safe treatment of sewage used for farming, and the prospective analysis of dangers connected with migration of infected man and animals, changed animal husbandry, and increased consumption of meat.

The decision to introduce a control programme following the abovementioned objectives, with full control activities, needs a preparatory stage. During this stage, basic surveillance (notification, pilot studies, and topographic analysis) should be carried out, administrative arrangements (reference centres, trained personnel, and the ensuring of adequate funds) should be made, and the required constitutional and legal authority should be settled to secure full cooperation between the medical, veterinary and environmental protection authorities. So far, few countries seem to be prepared to plan an effective control programme.

3.5.2 *Diphyllobothriasis*

Infection with *Diphyllobothrium latum* in man is due to the consumption of raw or insufficiently cooked fish, meat, liver or hard roe. Pike, burbot, perch and ruff may contain plerocercoids of the worm. These four species, particularly pike and burbot, are the most important sources of infection in Asia and Europe. Plerocercoids have also been found in salmonids. Infections with *Diphyllobothrium pacificum* are recognized in South America.

3.5.2.1 *Epidemiology. D. latum* is the species of diphyllobothriids that has been most fully described and is most easily recognized. Human infection with other kinds of *Diphyllobothrium* (especially *D. dendriticum*) has, however, proved to be possible, both experimentally and in nature. Further investigations are required in this connexion. It has not been satisfactorily enough clarified which diphyllobothriids are involved in human infection in the Arctic, eastern parts of Siberia, USSR, and in the arctic parts of North America. The lack of reliable taxonomic criteria constitutes a problem. The definition of such criteria is an important task for helminthological research today.

In Finland, where fish tapeworm was endemic, a rapid decrease in the prevalence has occurred, so that today it is one-tenth of what it was some 20–30 years ago. The parasite has also been reported to occur in regions of northern Sweden and in certain northern parts of the USSR.

3.5.2.2 *Control.* Preventing infection of the stock of fish is a problem. Lakes and rivers are frequently contaminated by sewage. The construction of efficient purification plants is a complicated and expensive undertaking, and 100% elimination of tapeworm eggs can hardly be guaranteed. In addition, faeces containing eggs may end up in the water from latrines and from ships traversing the lakes, as well as from infected domestic or wild animals. The life-cycle of the broad tapeworm may be maintained without the intervention of man. On the other hand, it is generally accepted that man is the most important natural host for *D. latum.*

In most cases the broad tapeworm causes its host very little discomfort and is therefore often ignored. From a medical standpoint, diphyllobothriasis is interesting chiefly as a potential cause of pernicious anaemia (PA) (also called cyanocobalamin deficiency, tapeworm-PA). About half the carriers of *D. latum* have a decreased cyanocobalamin serum value (below 74 pmol/l, or 100 pg/ml). The incidence of manifest anaemia in *D. latum* carriers is about 2%. Tapeworm-PA can be cured simply by expulsion of the worm. It differs from genuine PA in one respect only: the gastric secretion contains intrinsic factor and often free hydrochloric acid (*44*).

The worm is readily killed by modern drugs (e.g., niclosamide, praziquantel).

3.5.3 *Hymenolepiasis*

The two species of the genus infecting man—namely, *Hymenolepis nana* and *H. diminuta*—are rarely associated with symptoms. Only in heavy infections have diarrhoea and abdominal discomfort been noted. *H. nana* is by far the commonest of the two parasites but both species have a cosmopolitan distribution. *H. nana* is easily transmitted directly from person to person. The life-span of *H. nana* is a few weeks only, but the population of tapeworms is renewed easily by new generations as *H. nana* can complete its cycle in the human intestine. *H. nana* may cause epidemics in institutions for children. Recently it has been shown that the course of *H. nana* infection in mice is profoundly influenced by immunosuppression. This immunosuppression, caused by T-cell deprivation or by induced steroid treatment, resulted in masses of abnormal cysticercoids multiplying in the viscera. A bizarre disseminated parasitic infection in a patient with Hodgkin's disease, who had received immunosuppression, showed a similar histology. Thus hymenolepiasis could be another parasitic condition which should be eliminated before initiating immunosuppressive therapy in man. Niclosamide and praziquantel are the drugs of choice; repeated courses of treatment and treatment of all infected family members or inmates of institutions are sometimes necessary.

3.6 Trematode infections

Intestinal trematodes are one of the least known groups of parasites in medical parasitology. The diversity of the incriminated species, the relative mildness of their pathogenicity and their endemicity among isolated populations seem to be the main factors contributing to our lack of knowledge of this group. These trematodes are, in general, zoonotic. There are many reservoir hosts for each species. In endemic areas, human infection is contracted by people becoming involved in the zoonotic life cycle either through traditional eating habits or through accidental infection.

It is too early to weigh the public health importance of these infections, because only limited information on their distribution, epidemiological characteristics and pathogenic effects is available. More local surveys are needed to reveal the real extent of morbidity caused by these trematodes. Simple diagnostic techniques are required for this purpose. Much further screening of possible anthelmintics for these infections is still necessary.

Infections may be fishborne (as in the case of heterophyiasis, and echinostomiasis). Fasciolopsiasis and gastrodiscoidiasis are contracted by eating raw water plants with metacercaria attached.

The Group decided that liver-fluke infections such as fascioliasis, clonorchiasis and opisthorchiasis should be dealt with separately. They still cause important health problems, especially in south-eastern Asia.

3.6.1 *Infections with Heterophyidae*

Heterophyes heterophyes infections are distributed widely in many parts of the world: in China, Japan and the Republic of Korea in eastern Asia, the Balkan countries in Europe, and Egypt in Africa.

Metagonimus yokogawai is distributed in north-eastern Asia, including the Amur river basin, and in the Balkans. The main source of infection is the freshwater fish *Plecoglossus altivelis.* There are many kinds of reservoir, such as dogs, cats, rodents and sometimes bird.

Stellantchasmus falcatus, Pygidiopsis summa, Haplorchis tachiui and *Centrocestus armatus* are species known to infect man. They are distributed in eastern and south-eastern Asia. *Cryptocotyle lingua* occurs among Eskimos. The zoonotic nature of these trematodes is basically the same.

The amount of worm burden generally determines the degree of clinical manifestation in all the infections. For example, in intensive heterophyiasis acute catarrhal enteritis occurs in the first infection. Worms enter the intervillous spaces, erode the intestinal epithelium with their tegumental spines, and cause mucosal inflammation. High eosinophilia (up to 40%) is associated with malaise, fatigue, loss of appetite, abdominal pain, watery diarrhoea and dehydration. Repeated infection seems to immunize the patient and following reinfection at this stage there are minimal clinical symptoms. Eggs have been reported in the heart, brain and spinal cord.

Stool examination is the best diagnostic method for these infections. Differentiation of these ova from those of opisthorchids is not easy. A diagnostic serological technique has not yet been developed.

3.6.2 *Fasciolopsiasis*

Fasciolopsis buski is a large trematode found in China, India and south-eastern Asia. Pigs are important reservoir hosts in many endemic areas. The worms cause enteritis, the degree of inflammation

depending on the number of infecting worms. Heavy infection is accompanied by diarrhoea, abdominal pain and a characteristic facial oedema. Examination of the stools for eggs is the only diagnostic method, but differentiation from *Fasciola* eggs and some of the *Echinostoma* is difficult.

3.6.3 *Gastrodiscoidiasis*

Gastrodiscoides hominis is an amphistome trematode which occurs mainly in the Ganges river basin of India. The exact mode of infection is not known, although many workers believe it to be through water plants. The extent of its endemicity has not been fully defined. Lightly infected cases are usually asymptomatic. Mucosal inflammation of the caecum and colon occur. This infection is diagnosed by finding the large rhomboidal ova in the stool.

3.6.4 *Infections with Echinostomatidae*

Echinostoma cinetorchis, E. macrorchis, E. hortense, and *Echinochasmus perfoliatus* have all been identified in eastern Asia. In Indonesia, Malaysia, the Philippines, and Thailand, *Echinostoma revolutum, E. recurvatum, Euparyphium ilocanum, E. malayanum,* and *Hypoderaeum conoideum* are the species causing human infection. Only sporadic cases have been reported in parts of the world other than eastern and south-eastern Asia. According to the species, birds, dogs or rodents are the reservoir hosts.

Intestinal pathology is similar to that of heterophyid infections. Diagnosis depends on the recovery of ova in the stool. Differentiation from eggs of *Fasciola* or *Fasciolopsis* is not easy.

Bithionol, niclosamide, niclofolan and praziquantel have been used successfully for treatment of intestinal trematodiasis. Tetrachloroethylene has been found to be effective against fasciolopsiasis and some types of echinostomiasis, and bithionol against heterophyiasis and fasciolopsiasis.

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4. PRINCIPLES OF SURVEILLANCE, PREVENTION AND CONTROL

4.1 Principles of surveillance

Surveillance, as it relates to tropical diseases, has been defined as "the continuous scrutiny of the factors that determine the occurrence and distribution of disease and other conditions of ill health. Surveillance is essential for effective control and prevention, and includes the collection, analysis, interpretation, and distribution of relevant data for action." (*1*)

Surveillance for intestinal parasitic diseases has not been defined because there has been very little planned, comprehensive surveillance of intestinal parasitic diseases in practice.

The specific requirements of surveillance programmes designed for intestinal parasitic infections have to be adapted to local conditions and may be identified as follows:

(1) Defined objectives
(2) Data collection, processing and analysis
(3) Logistics
(4) Communications
(5) Statistical sampling
(6) Disease notification systems
(7) Field manuals
(8) Training.

For the sake of brevity, these general requirements will not be examined in detail.

4.1.1 *Objectives of surveillance*

For a surveillance activity to be meaningful it should be part of a programme that has set specific, realistic, and achievable goals. Sur-

veillance data should not be collected for their own sake—they should be collected and used for a worthwhile purpose.

Eradication of intestinal parasitic diseases is not a realistic goal in most communities and countries at the present time. The available methods for the control of most intestinal parasitic diseases in large populations are either technically inadequate or too costly, or there is insufficient trained manpower to put them into effect. More limited goals should therefore be defined and surveillance programmes should be designed and integrated to monitor progress towards these limited goals.

Most of the intestinal parasitic infections of man lack clinical features that are sufficiently characteristic to permit differential diagnosis, by simple clinical examination. These diseases are chronic entities with poorly defined borders between clinically inapparent infections and detectable disease. Yet, a case definition is essential for any kind of surveillance activity. Clear-cut definitions of the terms "infection", "prevalence", and "morbidity" are needed for these intestinal parasitic infections of man.

There are many ways to define surveillance and surveillance can encompass many functions, but it achieves its full potential when it accurately describes the incidence of disease. Yet, prevalence is a much more relevant measurement of parasitic diseases than is incidence. Surveillance is best suited to diseases that are undergoing change, preferably declining, where the detection of new cases is of greater importance and more easily accomplished. Intestinal parasitic diseases are generally stable diseases that are characterized by high prevalences in endemic areas. It would be difficult to perceive and describe the dynamics of transmission where infection is abundant and stable, and this is an impediment to the surveillance of these infections.

There should be new strategies for surveillance with the aim of obtaining and using the best information currently available and achieving a steady upgrading of the quantity and quality of epidemiological data. It is a challenge to epidemiologists to develop usable surveillance techniques for intestinal parasitic diseases despite numerous obstacles and the lack of precedents.

All surveillance programmes must contain three basic components: a system for data collection, a system for data analysis, and a system of response. Surveillance programmes should also be sufficiently informative to guide decision-makers; they should be suitable for a variety of situations, and they should require a minimum of time and

resources. Beyond these basic requirements, surveillance programmes can assume any character and dimension. Because parasitic diseases are varied and the same disease may even have major regional differences, it is not possible to lay down specific formulae for the conduct of surveillance that will fit various situations. A guide to the opportunities for surveillance of intestinal parasitic infections may be seen by reviewing the elements of surveillance. All comprehensive surveillance programmes are based on one or more of the following elements. Programmes for the surveillance of intestinal parasites should therefore adapt one or more of these elements to their own purposes.

4.1.2 *Data collection, processing and analysis*

4.1.2.1 *Mortality registration.* Although carried out in man countries and although especially useful for the surveillance of some diseases (e.g., influenza), mortality registration has limited value in chronic parasitic infections, especially the intestinal parasitic infections. However, the records of postmortem examination should be used whenever possible to obtain information on the frequency of fatalities due to certain parasitic infections, i.e., amoebiasis, strongyloidiasis, ascariasis.

4.1.2.2 *Morbidity reporting.* Most of the intestinal parasitic infections remain undiagnosed; nor are they notifiable, and they are not even reported when recognized. Because reporting of chronic parasitic infections is minimal, special reporting systems would have to be established; however, it would be essential to define the morbid features of each of the intestinal parasitic infections that would constitute a case, in order to have useful morbidity reporting. Hospital records are a potential source of morbidity data on the intestinal parasitic infections; they may not reflect the morbidity in the population at large, but they are useful for comparing the frequency of hospitalization for various diseases.

4.1.2.3 *Epidemic reporting.* Some intestinal parasitic infections may be responsible for epidemics, e.g., giardiasis, hymenolepiasis, and taeniasis. Early reporting is essential for carrying out epidemiological studies and control.

4.1.3 *Epidemiological methodology*

4.1.3.1 *Individual case investigation.* Individual case investigation has value as a possible indication of a cluster; however, it is more useful in acute diseases than in chronic diseases. It can also be important in special circumstances—for example, when a new pathogenic species is suspected, e.g., *Capillaria philippinensis, Angiostrongylus costaricensis.* Individual case investigations are less practical in surveillance when the disease is abundant, which is often the case with intestinal parasites.

4.1.3.2 *Epidemic field investigations.* Investigations of epidemics are necessary to determine both the cause and the mechanisms of transmission, which are essential prerequisites to control. Epidemic field investigations have been very useful in controlling some intestinal parasite outbreaks, e.g., waterborne giardiasis in the USA.

4.1.3.3 *Epidemiological surveys.* Epidemiological surveys are the principal means that have been used to study the prevalence of intestinal parasites in communities. They may be used to study changes in levels of endemicity based on control schemes and other man-made alterations of the environment. Epidemiological surveys of intestinal parasitic infections should be carried out in a programmed, systematic manner to ensure comparability in time and place, and maximum use of information.

4.1.3.4 *Animal reservoir surveys.* Animal reservoirs play an important role in some intestinal parasitic infections of man, e.g., taeniasis. Surveys of the animal reservoir population are essential to gain a complete understanding of these diseases.

4.1.3.5 *Demographic and environmental data.* Age, sex and other demographic data, as well as information about population movements and changing environmental factors, should be included in epidemiological studies of intestinal parasitic infections.

4.1.4 *Laboratory investigations*

Laboratory investigations are well suited to the surveillance of intestinal parasites since the organisms are identifiable in the stool by

conventional laboratory techniques. Laboratory support is also essential for confirming the diagnoses that were based on clinical criteria in the surveys. Field laboratories, dispensary and hospital laboratories, as well as reference laboratories in distant places, are all essential to provide diagnostic support for surveillance. In addition, periodic summaries and reporting of laboratory findings, as is done in the USA, can provide useful surveillance data (*2*).

4.2 Principles of prevention

The transmission of intestinal protozoan and helminthic infections follows different mechanisms depending on the species of parasite and the local ecological conditions. These is a major difference between the monoxenous species, with a single host in the life-cycle, and the heteroxenous species, with definitive and intermediate hosts.

Monoxenous parasites can be transmitted:

(1) directly through the polluted air, as in enterobiasis;

(2) by direct faecal–oral transmission, through unclean hands and contamination of food by food handlers or by kitchen personnel who are parasite carriers, as in amoebiasis, giardiasis, etc.;

(3) indirectly through the passive infective stages (eggs or cysts) maturing in the soil, as in the case of *Ascaris, Trichuris* and the coccidial parasites that must be ingested by the host, after maturation outside; and

(4) through infective larval stages produced in the soil and actively penetrating the skin, as in strongyloid and hookworm infections.

The last two modes of transmission characterize the geohelminthic infections.

The heteroxenous intestinal parasites have larval stages that are ingested with the food by man, the definitive host. This is the case with the cestodes, *T. solium, T. saginata* and *D. latum,* each causing foodborne infections.

Parasites may reach the host in different ways: *A. duodenale* can infect man by penetration of the skin or through ingestion of vegetables contaminated with filarioid larvae; embryonated eggs of *H. nana* usually follow the faecal–oral route but can also produce infections when man accidentally ingests arthropods such as *Tenebrio* or fleas containing cysticercoid larvae. Epidemic waterborne infections can occur in giardiasis and amoebiasis.

Prevention, to be effective, must take into consideration the natural cycles of parasites and the peculiar ecological, social and cultural circumstances that prevail in a community or social group.

4.2.1 *Focal points of control*

The focal points for the control of intestinal parasitic infections should take into consideration the routes of dissemination of these parasites. Most of the parasites are excreted with the faeces into the external environment; this is why great emphasis has always been put on proper faecal and sewage disposal.

But a closer analysis of the problem shows various ways adopted by different parasites to enable them to reach their target; some are simply disseminated with faeces, and their infective stages mature on the soil, as hookworm, roundworm, and whipworm; others are transmitted directly from the faeces to the mouth through dirty hands or food contaminated by parasite carriers; and another group has the infective stages disseminated, not with faeces but by active migration through the anus, as in the case of the female *Enterobius* and gravid proglottis of *T. saginata.* To reach the new host, most of the infective worms find their way to the mouth through dirty hands and contaminated food or water.

These particular circumstances show that faecal disposal can contribute most to the control of intestinal parasites because faeces are the principal vehicle of dissemination of the infective stages. In other cases, hand-washing and general hygiene may assume a relevant significance in control methodology, making adequate water provision as important as, or more important than, latrine construction.

The mechanisms of transmission of soilborne infections are dependent on two kinds of circumstances: the biology of each species of parasite and human behaviour. Infective hookworm larvae are concentrated in limited and discreet places where adolescents and adults go for defaecation. This habit makes these persons more exposed to the risk of hookworm infection or reinfection. *Ascaris* eggs are disseminated around the house by children, who have no regular habits and widely pollute the house and surrounding area; infective eggs can then easily reach other children who play on the ground and contaminate their hands and food. So the use of latrines can produce greater benefits against hookworm than against *Ascaris* or *Trichuris* infections. As human behaviour depends on cultural traits and edu-

cation, there may be complex reactions to the proposed sanitary measures of parasitic control.

4.2.2 *Sanitation*

Sanitation has been a major factor in controlling faecally transmitted diseases in the urban and rural areas of many developed countries. On both theoretical and practical grounds, sanitation has been systematically recommended and adopted to control faecally transmitted protozoan and helminthic infections throughout the world.

In the rural areas of developing countries, and particularly in tropical regions, control through sanitary faecal disposal has generally not been successful. Even in urban areas, the geohelminths remain prevalent in the poor, crowded quarters of large and small cities. This situation tends to be aggravated by squatter settlements at the periphery of the cities, which have been continuously and rapidly increasing in recent years.

Many reasons can be presented to explain the difficulties in obtaining good results from sanitation:

(1) the cost of a latrine in areas where income is low may be prohibitive; but more important is the refusal to use cheaper types of latrines because they produce repulsive odours and their platforms are often polluted by faeces and covered with flies, and because rainwater transforms the area into a vast breeding place for culicine mosquitos;

(2) rejection of use may be because of cultural reasons and longstanding habits of the adult population who find more comfortable and discreet places to go to in the bush;

(3) children tend to imitate their parents and have still more irregular habits. The younger children are more inclined to cause soil pollution even when the adults have accepted the use of latrines; they are very often the most important disseminators of intestinal parasites;

(4) the failure to use latrines by adults and children considerably reduces the efficacy of sanitation as a method of control; and

(5) the non-use of latrines is very often a consequence of parasitism. Some of the intestinal protozoan and helminthic infections, and particularly heavy infections, are characterized by diarrhoea with liquid stools, accompanied by colic or tenesmus. Infected patients make numerous evacuations per day, sometimes in places far from

latrines; hence pollution of the soil cannot be avoided under such pressing conditions.

Sanitation has a greater impact when, owing to the better economic circumstances of the people, well-built latrines can be constructed and high housing standards, water facilities and general education are usual. Hand-washing must be considered as important as the disposal of faeces, as has been noted.

4.2.3 *Protection against infective stages*

4.2.3.1 *Physical methods.* Water filtration and boiling are effective measures against waterborne infections, although acceptance of these methods as a routine practice in endemic areas is often hindered by many obstacles. Thorough cooking is the most efficient method to prevent foodborne parasitic infections. Nevertheless, ignorance and cultural habits generate quite hard resistance to change from traditional methods for the preparation of food.

Some important parasites, such as hookworms and *Strongyloides,* can be controlled by wearing shoes. In reality this has been the most important factor that permitted the control of ancylostomiasis in Europe and the USA in the past, and it is the first effective link between the improved economic status of a population and the reduction of prevalence and morbidity.

In general, sandy soil is more favourable to hookworms than to roundworms, while clay and silt soils are more favourable to *Ascaris* eggs than to the larval stages of hookworms. Hookworm prevalence and parasite load in man are closely related to the percentage of sand in the soil, if other conditions are constant. A suitable mixture of sand and clay, with a little cement, can produce a compact soil surface unfavourable to helminthic eggs and larvae.

4.2.3.2 *Chemical methods.* Chemical substances, with ovicidal or larvicidal properties, have been used in the control of soilborne helminthic infections to compensate for the absence of widespread acceptance of sanitary facilities.

More than 50 natural and synthetic products have shown larvicidal activity against the rhabditoid stages of *Strongyloides stercoralis* and hookworms, after the first 24 h of contact. Some insecticides used in agriculture or in malaria control have also been proved to have an activity against nematode larvae, and contributed to the reduction of the prevalence of human infections. In the Republic of Korea, en-

couraging results were obtained with dimpylate, benzene hexachloride and sodium pentachlorophenoxide monohydrate. But they have been little used in control, in places where diffuse promiscuous defaecation is the rule. Some compounds have been used in the treatment of nightsoil.

An aqueous solution of iodine has been recommended for the destruction of protozoan cysts in drinking-water and on fresh vegetables.

4.2.3.3 *Biological methods.* Plants with a parasitic inhibitory effect were tested in Brazil. The lemon-grass, *Cymbopogon citratus,* when at an average density of one clump per 10 m^2, seems to contribute to the reduction of hookworm and *Strongyloides* prevalence if associated with chemotherapy. In control areas, with chemotherapy alone, this reduction was not so marked (*3*). Among animals that are known to destroy the eggs of helminths are mites, springtails, dung beetles and cockroaches, but no practical use has been made of these ecological agents. The ovicidal and larvicidal action of fungi has been tried for hookworm control in mines and in some other situations. In large areas, however, the seeding with the controlling agent would have to be extensive, which does not make this a very practical method.

4.2.3.4 *Meat inspection and processing.* Human intestinal taeniids can be controlled by the association of two methods: meat inspection and adequate cooking. While inspection cannot detect all infected caracasses, those with heavy infections are identified and condemned or sent for compulsory processing. The others must be submitted to thorough cooking to ensure effective protection of the consumer. This is an important field for health education. The same can be said about tapeworms transmitted by raw or undercooked fish.

4.2.3.5 *Health education.* The obstacles found in introducing simple and efficient preventive measures against intestinal protozoan and helminthic infections are of many kinds:

(1) ignorance or lack of adequate information about the parasites, and about the link between cause and effect;

(2) difficulty in understanding how the infection can be acquired is sometimes enhanced by the mental habit of interpreting facts in a nonlogical way (as magic, fetish, etc.);

(3) health education may fail in many cases because its message is inadequate—e.g., when the change of behaviour that is proposed to a community implies certain practical or economic difficulties, or

when it is proposed to change deep cultural habits, or when no alternatives are offered to compensate for abandoning certain traditional resources (such as drinking water from polluted sources); and

(4) failure is often due to a communication barrier between health educators and the community—e.g., language or comprehension difficulties, cultural differences between foreigners and nationals or between urban educators and the rural population, and so on.

Two important rules should be observed in health education: the first is that the recommended change in behaviour must be compatible with the practical, economic, cultural and social circumstances of a given community; the second is that the best educators are always members of that community who have been adequately prepared to transmit the message to their compatriots or neighbours.

Health education has a limited impact when divorced from education in general. That is why most of the needed information must be integrated into the educational system (by way of books, classroom material, etc.) and must have the young population as the principal target. But it must also become a mass phenomenon to have the impact of a collective social practice or constraint. The mass media and social organizations must also be mobilized to help introduce these new habits and new attitudes without conflicting with the mores and the collective reaction to particular changes. In specific situations, legislation can be used to reinforce the pressure to change collective behaviour.

4.2.4 *Community participation*

The fight against intestinal parasitic diseases will be difficult to manage and to sustain for the long periods required for control if the population is not involved in this struggle. Because they are the most relevant part of the ecosystem where the human intestinal parasites circulate and the agent of most of the actions that assure the transmission of these parasites, the members of the community must be aware of the problem and must participate in its solution. Through health education the population can become conscious of the subject and can be motivated to take partial or full responsibility in control measures. But the control methodology must also be selected and adapted for application by local personnel, using local resources whenever possible.

The conditions for successful community participation change from place to place, and according to the social or the political or-

ganization. It depends largely on local leadership, and on political and administrative support. In fact, community participation cannot be proposed for a single and exclusive project unless it is considered by the population to be of the utmost priority. In general, each project must be incorporated into a wider programme of community development where health and preventive medicine are important elements, and must be linked to primary health care activities.

4.2.5 *Concluding remarks on prevention*

There are a number of techniques and procedures that could contribute to the prevention and control of intestinal protozoan and helminthic infections. These methods are sometimes of more or less general use and valuable against different species of parasites, but others are directed to particular parasitic agents or to specific modes of transmission. The efficient use of these preventive methods requires a sound knowledge about the natural history of the parasites and local ways of transmission. For practical purposes they must be presented as a global strategy, including the more general and reliable methods, chosen according to the local or regional pathology and the priorities adopted by health planners.

Although only the principles of prevention—through sanitation, health education and community participation—are being discussed, it is obvious that an integrated approach must be used to control intestinal parasitic infections if efficacy and low-cost/high-benefit programmes are expected. Such programmes must be comprehensive and cover the fundamental aspects of sanitation and prevention, such as water supply of acceptable quality, latrine construction, waste disposal, the general use of shoes, personal hygiene (with a great emphasis on hand-washing), and adequate cooking of meat or fish.

Past and present experience has shown that periodic mass chemotherapy (or concomitant treatment of all infected inhabitants) is an essential aspect of the control of intestinal parasites, because it is the only way to reduce drastically and rapidly the human sources of infection in any community. The value of other methods of control will increase after this reduction in the prevalence and in the global parasitic load has been achieved, contributing finally to cut transmission when the reduced number of infective stages of parasites will have a low probability of reaching new hosts. Community participation must be promoted and sustained by the engagement of their own leaders

and the local authorities and by a well-adapted system of health education, using local agents.

4.3 Principles of control

Most of the available data and experience deal with the control of soil-transmitted helminthic infections, and only these are therefore presented here. Efforts to control hookworm infection date back to the early period of this century. Based on knowledge of the worms' life-cycle, the concept of interrupting transmission by sanitary disposal of faeces was introduced; also certain drugs were shown to expel the hookworms. Thymol was generally the drug of choice and was highly regarded for its efficacy, notwithstanding the necessity for multiple treatments; in one series of cases, 97.8% of those who expelled all their hookworms received thymol five times or less (*4*). The reported rates of cure, when the drug was given only once, ranged from 20% to about 40%.

Extensive control programmes, initiated in south-eastern USA and elsewhere, have been set up with three objectives: to offer faecal examination to all, and treat all who are infected; to educate the population on facts regarding the hookworm and to encourage community participation; and to assist in building latrines which—if the educational part of the programme was successful—the people would be eager to have and willing to use. In more recent programmes for the control of soil-transmitted helminths, the basic objective of interrupting transmission has not changed.

4.3.1 *Population-based chemotherapy*

In the economic production of livestock the scheduled use of anthelmintics is effective, but it has been shown that total control—the elimination of all worm parasites from all animals—is not a reasonable goal. What is desired, and is now known to be attainable, is the development of schemes that can be used to hold worm burdens down to low levels in particular areas or in given circumstances (*5*).

It has been strongly recommended that community-based intestinal helminth control programmes be set up within the organization responsible for the general health services. The extent to which that recommendation has been accepted is unknown. Countrywide successes were reported previously in Japan and parts of the USSR; it was expected that China (Province of Taiwan) would become a third

such example but no recent progress report has appeared. The results of control programmes involving smaller communities and with different approaches have been reported. Examples are summarized below.

4.3.1.1 *Periodic mass treatment.* In the Philippines, an entire village of about 600 people, living in approximately 100 houses, most of which had water-sealed toilets, was surveyed by examination of faeces. Irrespective of the findings in the faecal examination, all residents were given pyrantel in a single dose (5 mg/kg). A follow-up faecal examination was done 7–10 days after treatment. The treatments and follow-up stool examinations were repeated after 3, 6, 9 and 12 months, and they were scheduled to be continued at 4-month intervals for an additional year. Before the first treatment was given, 84.4% of 606 people were positive for *Ascaris.* The first treatment reduced the positives to about 34%, the second to 9%, the third to 8%, and the fourth to less than 1% of the 561 persons examined. No medical or logistical problems were reported and the results were expected to encourage implementation of this trial on a wider scale (*6*).

4.3.1.2 *Treatment of selected samples.* In a Zaire project, three communities of 500–600 people were selected and the families within each were allocated at random to either a treatment or a control group in ratios of 9:1, 3:2 and 2:3, respectively (*7*).

The treatment groups were given a single dose of levamisole (2.5 mg/kg) at the start and a placebo after 3, 6 and 9 months; the control groups were given placebo tablets initially and at each 3-month interval. At the beginning of the study, 54–60% of the people in the three villages were positive for *Ascaris,* 42–61% for hookworm, 12–18% for *Strongyloides,* and 4–17% for *Trichuris.* Only the *Ascaris* infections were measurably affected by the levamisole.

The results showed that with more extensive levamisole coverage, the difference between treated and control subjects was greater. At 3 months after treatment, the *Ascaris*-positive percentages of treated and control groups in the three villages were respectively 5% and 33%, 17% and 35%, and 22% and 33%; and after 9 months the percentages for the village with 9:1 ratios were 19% and 32%. It was concluded that mass treatment with a single oral dose of levamisole, repeated at 3-month intervals, might help control ascariasis, that population coverages between 60% and 90% might be appropriate, and that indiscriminate mass treatment with levamisole was feasible.

Trimestrial administration of levamisole to heavily infected schoolchildren in Kimwenza, Zaire, led to almost complete eradication of *Ascaris,* whereas the egg outpout of hookworm and the output of *Strongyloides* larvae at the end of one year was reduced by 77% and 87%, respectively (*8*).

4.3.1.3 *Treatment for assessment of relationship to nutrition.* In Kenya, the relationship between ascariasis and growth of malnourished preschool-age children was examined (*9*). In two villages with a high prevalence of *Ascaris* infection, children between 1 and 6 years of age were examined three times at 14-week intervals between December and July. The first examination included about 80% of the preschool-age population, and 62% attended all three examinations, which included anthropometric measurements, clinical examination, and faecal examination. At the second and third examination each child, infected or not, was given levamisole in a single dose (40 mg and 80 mg, respectively, to those children under or over 5 years of age). Children who were not infected with *Ascaris* were defined as the control group, whereas the test group included all children who were positive for *Ascaris* at the first or second examination, or had passed worms after treatment. Of 186 children who were examined three times, 61 fell into the *Ascaris*-infected group and 125 were controls. The *Ascaris* group had passed 0–53 worms (mean: 7). Egg-counts were not done. In the 14 weeks before deworming, the *Ascaris* and control groups did not differ in the percentage of expected weight gains. At 14 weeks after deworming, the previously infected children showed a higher percentage of expected weight gain that did the controls. Likewise, skinfold thickness showed a favourable response to the deworming. The authors concluded that even a light *Ascaris* infection may adversely influence a child's nutritional status, and deworming may enhance growth.

A study in the United Republic of Tanzania also seemed to show that growth of malnourished preschool-age children was accelerated by the removal of light *Ascaris* infections (*10*). About half of 341 children, 6 months to 7.5 years of age, randomly selected, were given levamisole treatment (2.5 mg/kg), which was repeated at 3, 6, 9 and 12 months. The rate of weight gain in the treated group was 8% greater than that in the control group. In 78 children, known to have been infected with *Ascaris* (1–30 eggs per mg of faeces; mean, 6/mg), the weight gain exceeded that of controls by 21%. The weight gain in the levamisole-treated *Ascaris*-negative group of 68 children

was 13%. The results were interpreted to be consistent with a causal association between ascariasis and malnutrition. At a cost of less than US$ 0.25 per year per child, periodic anthelmintic treatment was recommended as a practical adjunct to nutrition programmes in areas of high ascariasis prevalence.

4.3.1.4 *Treatment supplementary to sanitation.* In eight Liberian communities, after a 10-year programme of environmental hygiene and health education, the prevalence rates of soil-transmitted helminths remained high (hookworm, 42–76%; *Ascaris,* 10–22%; *Trichuris,* 10–23%). Populations of 768 and 787 persons (87% of the target population) were treated with ciclobendazole and pyrantel, respectively (*11*). Cure rates for *Ascaris* and *Trichuris* with ciclobendazole were 88% and 90% respectively, and with pyrantel, 95% and 96% respectively. Neither drug was satisfactory for hookworm; but with mebendazole the cure rate was 81% for hookworm infection. About 75% of the cost of the treatment programme (US$ 4 per person treated) was for the anthelmintics. It was observed that hookworm is a particularly resistant parasite and that poorly maintained latrines increase the risk of infection.

4.3.1.5 *Selective treatment for disease control.* In an attempt to develop a sound programme of hookworm control that could be carried on by the local health agencies, a plan for disease control was set up in Georgia, USA, in 1940 (*12*). With attention focused primarily on schoolchildren in the areas where hookworm anaemia was the only parasitic disease of major concern, a programme was initiated—consisting of selective surveys, followed by investigation of family contacts, and treatment of all persons found to be anaemic or to be carrying a hookworm burden sufficient to cause anaemia (5000 or more eggs per gram of faeces).

Thirty years later (in 1972), 3729 elementary schoolchildren in a random sample from the area were examined for intestinal helminths, and 5016 were tested for anaemia (*13*). Also, about 800 members of 500 rural households were randomly selected and examined for intestinal helminths (*14*). In the meantime, the threshold level of significant egg output for hookworm was confirmed for that population, and the feasibility of selective surveys (based on haemoglobin determination) in hookworm disease case-finding was established (*15*). These practices had been tried out in communities where the infection rates remained high. The 1972 surveys showed that in communities

with the highest endemicity (where the soil and habits of the people were most favourable for transmission, where in 1950 over 50% of schoolchildren were infected with hookworms, and 43% of these had moderate to heavy infections), only 12% (125 out of 1039) had hookworm infections and only 27% of the infections were classified as moderate to heavy (*13*). Among the rural families, 16% of white and 8% of black members had hookworm infections; for *Ascaris* the rates were markedly reversed, being 1% and 12%, respectively (*14*). Disease control in communities where the control of transmission is not feasible has apparently not been attempted in any other region.

4.3.2 *Available drugs*

The drugs for the treatment of intestinal parasites can be grouped into three different categories:

(1) Therapeutic agents of recent origin, widely used or with good potential for future use, and well described in the literature, such as dichloroacetamide derivatives, metronidazole, tinidazole, mebendazole, pyrantel and praziquantel. For these drugs the following aspects are considered: the chemical composition and mode of action, their toxicity, side-effects and contraindications, the individual indications for therapy, the dosages, and their use in large-scale treatment.

(2) Well-known drugs, both old and new, whose use in large-scale practice is variable. This group consists of emetine and dehydroemetine, ornidazole, nimorazole, flubendazole, tetramisole-levamisole, oxantel, tiabendazole and niclosamide.

(3) Long-standing antiparasitic drugs, still found on the market, which are only mentioned but are not described: furazolidone, mepacrine, paromomycin, piperazine, bephenium hydroxynaphthoate, pyrvinium chloride and tetrachloroethylene.

4.3.2.1 *Antiprotozoan drugs.* These include the most commonly used luminal amoebicides, tissue amoebicides, and drugs against giardiasis.

(i)–(iv) *Dichloroacetamide derivatives*

These luminal amoebicides are poorly absorbed from the intestine and are presented as white or yellowish powders, tasteless and almost insoluble in water. Four compounds are currently in use: (i) clefamide or 2,2-dichloro-*N*-(2-hydroxyethyl)-*N*-[[4-(4-nitrophenoxy) phenyl]methyl]acetamide (250 mg tablets); (ii) diloxanide furoate

or 2,2-dichloro-*N*-(4-hydroxyphenyl)-*N*-methylacetamide 2-furancarboxylate (500 mg tablets); (iii) etofamide—a derivative of clefamide, closely related in chemical structure (200 mg tablets); and (iv) teclozan or *N,N*-[1,4-phenylenebis(methylene)]bis[2,2-dichloro-*N*-(2-ethoxyethyl)acetamide] (100 mg tablets).

These compounds are amoebicides which act by contact in the intestinal lumen. Like all antiamoebic drugs, they act against the trophozoites and do not affect the cysts. The amides are effective in high dilutions, over 1:80 000. The mode of action against the parasites is not completely known.

Toxicity is low; the LD_{50} by the oral route is over 5000 mg/kg in experimental animals. At therapeutic dosages, no major toxic side-effects have been reported. No contraindications are known. A frequent side-effect is flatulence, which disappears after completion of treatment.

Indications for these drugs are treatment both of asymptomatic cyst passers (since the cysts must originate from trophozoites in the lumen) and of symptomatic intestinal amoebiasis, in the latter case combined with an amoebicide that acts in the tissues. The dosage for adults is 3–6 tablets per day (depending on the drug used) for 5–10 days. For children, lower dosages of a suspension can be used.

Mass treatment for amoebiasis is rarely used and there is little experience with chemoprophylaxis.

(v)–(viii) *Nitroimidazole derivatives*

These tissue amoebicides are administered by mouth only; they are absorbed from the small intestine and act against amoebic trophozoites in the intestinal wall and other body tissues. They have very little activity against the parasites in the intestinal lumen. For this reason it is necessary to add a luminal amoebicide. These drugs are also active against giardiasis. There are a good number of compounds of the 5-nitroimidazole group, all with antiprotozoan activity; the four that are more commonly used are described below.

(v) *Metronidazole.* This was the first of the nitroimidazole derivatives used clinically in protozoan diseases. Since 1959, it has been used for *Trichomonas vaginalis* infections with excellent results. Several years later, its use was expanded to include treatment of amoebiasis and giardiasis. Metronidazole (2-methyl-5-nitro-1*H*-imidazole-1-ethanol) is a crystalline powder, soluble in water and readily absorbed from the small intestine. The plasma levels rise rapidly, reach effective concentrations in 2–3 h, and maintain these levels for 12 h after a single oral

dose. It is excreted mainly through the urine, which sometimes presents a reddish colour; it is also eliminated through the bile in the form of a metabolite that, added to the small amount of the unabsorbed drug, is responsible for some activity against the luminal amoebic trophozoites. Other secretions in which the metabolite may be found are the saliva, semen, vaginal secretions and milk; the drug crosses the placenta, a fact that restricts its use in pregnant women.

The drug has a bitter taste and produces side-effects, usually of low intensity, in 15–30% of the treated cases. The most common symptoms are gastrointestinal, such as nausea, vomiting, abdominal pain, a metallic taste and diarrhoea. Symptoms of the nervous system, such as dizziness, headache, and numbness of the extremities, are seen less frequently. Joint and muscle pains occur rarely. The inhibition of several enzymes concerned with the metabolism of alcohol is responsible for the frequency of symptoms that appear when alcohol is ingested during or shortly after the treatment with metronidazole. This disulfiram-like effect is characterized by a confusional state, flushing, headache, nausea, vomiting, drowsiness, and a fall in blood pressure. Contraindications are pregnancy during the first trimester, blood dyscrasias, and diseases of the central nervous system.

The question of carcinogenicity of metronidazole was reviewed in depth by the International Agency for Research on Cancer (*16*). It was found to be carcinogenic in mice after oral administration; the incidence of lung tumours in both sexes and of lymphomas in females was significantly increased. Its oral administration to rats increased the incidence and multiplicity of mammary fibroadenomas. The drug has been widely used throughout the world for the last 20 years. No case reports or epidemiological studies in man are available for assessing the human risk of teratogenicity or cancer, which is almost certainly not large and may be negligible (*17*).

For the treatment of symptomatic intestinal amoebiasis the dosage used is 30 mg/kg/day, divided into 3 doses after meals, for 8–10 days. For giardiasis, 15 mg/kg/day is given in divided doses for 5 days. The liquid preparations are recommended for children. For balantidiasis, a treatment similar to that for amoebiasis can be used.

Mass treatment or mass chemoprophylaxis with this drug is not recommended for amoebiasis. For giardiasis, group treatment can be used in children's institutions in which the inmates show high prevalence rates.

(vi) *Tinidazole.* Chemically it is 1-[2-(ethylsulfonyl)ethyl]-2-methyl-5-nitro-1*H*-imidazole. This drug has absorption and pharmacokin-

etic properties similar to those of metronidazole, but the blood concentration after 24 h is twice as high as that for metronidazole. The median half-life of tinidazole is 10–14 h, which is longer than that for metronidazole. Unlike metronidazole, which is eliminated as a metabolite, tinidazole is eliminated as the drug itself and in a smaller proportion through the urine.

Experiments on acute toxicity in rodents showed that the oral and intraperitoneal LD_{50} was higher than 2000 mg/kg. Studies on subacute toxicity revealed that dosages of 150 mg/kg twice daily for 30 days did not produce clinical or necropsy changes in rats and monkeys. Toxic effects have not been observed in patients receiving therapeutic dosages. Side-effects are infrequent; when present, they are mainly those of the gastrointestinal tract (such as nausea and vomiting) and are usually transient. Although no teratogenicity has been demonstrated, the drug crosses the placenta and is also excreted in the milk; for these reasons it is a wise precaution not to give this drug during the first trimester of pregnancy or to nursing mothers. Disulfiram-like reactions, when alcohol is taken during treatment, have been observed.

The most common regimens used in the treatment of intestinal amoebiasis with tinidazole are 2 g daily, as a single dose after a meal, for 2 days for adults; and 40–60 mg/kg in a similar way for children, during 2–3 consecutive days. These short treatments have shown results that are as good as the longer treatments with metronidazole. The efficacy of both drugs in eliminating the parasites from amoebic carriers is low, since the effect on the luminal trophozoites is small or nonexistent. This is why a luminal amoebicide should be added when treating intestinal symptomatic cases.

For giardiasis a single dose of 2 g for adults or 60 mg/kg for children is effective.

(vii) *Ornidazole.* It is α-(chloromethyl)-2-methyl-5-nitro-1*H*-imidazole-1-ethanol. In most pharmacological aspects it is similar to the two compounds already mentioned. Some special characteristics of ornidazole are its high plasma concentration within 1–2 h after oral administration, an excretion of 85% of the ingested amount in the first 5 days (with 63% elimination through the urine and 22% through the faeces), no incompatibility with alcohol, and little neurotoxicity and no teratogenicity.

Ornidazole is used to treat intestinal amoebiasis and giardiasis at a dosage of 500 mg, twice a day for 5–10 days for adults, and proportionally lower dosages for children. The results in treating symp-

tomatic intestinal amoebiasis are similar for ornidazole and metronidazole, with clinical and parasitological cure rates of over 90%. Tolerance is also similar for both drugs: 15% had side-effects usually of low intensity, consisting of dizziness, muscle and joint pains, nausea and vomiting.

(viii) *Nimorazole.* This drug, also called nitrimidazine, is 4-[2-(5-nitro-1*H*-imidazol-1-yl)ethyl]morpholine. The *in vitro* activity against *E. histolytica* is similar to that of metronidazole, but the blood and urine levels are higher. The recommended dosage for treatment of amoebiasis and giardiasis is 40 mg/kg/day for 5–10 days. In cases of balantidiasis, similar dosages have been effective. The results of treatment and the side-effects seem to be similar to those of the other imidazoles mentioned.

(ix)–(x) *Emetine and dehydroemetine*

(ix) Emetine has been in use for more than 50 years against *E. histolytica* in the tissues. The active ingredient is the methyl ester of cephaline, which is derived from the plant ipecac and can also be synthesized. Emetine is freely soluble in water and is given by injection. Oral presentations are poorly tolerated and not very effective. It is stored in the tissues and eliminated slowly. For this reason the toxic action is cumulative. At therapeutic doses, side-effects are not severe; they comprise pain in the area of injection, diarrhoea, and nausea. The more important toxic effects are cardiovascular and neuromuscular, when the drug is used for long periods or when there are predisposing factors. The symptoms are hypotension, tachycardia, and changes in cardiac rhythm and in the electrocardiogram. Occasional deaths due to cardiac complications have been reported. The neurological symptoms are mainly muscular weakness. Bed rest and medical supervision are recommended during treatment. In very old and debilitated patients the dose should be reduced, and it is advisable not to use emetine in patients who are pregnant or who have cardiac, renal or neuromuscular diseases.

(x) Dehydroemetine is a recent synthetic substance, racemic 2-dehydroemetine dihydrochloride, which is considered to be equally effective and less toxic. This drug is excreted more rapidly. The side-effects and toxicity appear to be similar to those of emetine but probably occur with less frequency and severity.

The dosage of both emetine and dehydroemetine is 1 mg/kg/day for 4–6 days.

4.3.2.2 *Anthelmintic drugs.* The following eight drugs are commonly used.

(i) *Mebendazole.* This synthetic compound belongs to the benzimidazole group. Chemically it is methyl (5-benzoyl-1*H*-benzimidazol-2-yl)carbamate. It is presented as a white to yellowish powder, very slightly soluble in water, and tasteless. Absorption from the intestine is minimal and 90% of the drug is excreted unchanged in the faeces, within 24 h after oral administration. Its mode of action is through inhibition of glucose uptake by the helminths, resulting in a depletion of the glycogen and adenosine triphosphate contents, which are necessary for parasite survival, thus leading to the slow death of the worms.

It is generally accepted that this drug does not show toxic effects at therapeutic dosages. The LD_{50} values in mice and rats is 1280 mg/kg, and in dogs and guinea-pigs 640 mg/kg. It has been observed in children who have been heavily parasitized by *Ascaris* that some of these worms are expelled through the mouth and nose during treatment with mebendazole. This side-effect may be linked with starvation of the parasites (as a consequence of the inhibition in the use of exogenous glucose) and their subsequent slow death. When the drug was administered during the period of embryonic organogenesis in pregnant rodents, abnormalities of the fetuses were observed, consisting in skeletal deformities in the ribs and tail. Neither teratogenic nor embryotoxic effects were observed in dogs, sheep or horses. As a precaution, mebendazole should not be used during the early months of pregnancy.

Individual treatment with mebendazole is effective against trichuriasis, ascariasis, enterobiasis and hookworm infections. The usual dosage is 100 mg, twice daily for 3 days, irrespective of the patient's age. In the case of ascariasis and enterobiasis, even shorter treatments are effective; but intensive trichuriasis may need double the above doses. Other indications for this drug are in the treatment of *Capillaria philippinensis* infections, for which it is considered to be the drug of choice, at a dosage of 400 mg daily for 10–30 days.

Mass treatment with mebendazole, aiming to reduce the prevalence rates of the four common nematode infections for which it is effective, has been successful. Repeated mass treatment (at 1–3 months' interval) has shown good effects and produced drastic reductions in the prevalence rates. It is necessary to have in mind that the ideal for mass therapy is the use of a single dose, which is effective only against *Enterobius* and *Ascaris.* For enterobiasis a single dose of 100 mg

cures from 87% to 100% of cases. The possible teratogenic effects and the occasional *Ascaris* migrations are drawbacks and a warning against indiscriminate mass treatment with this anthelmintic.

(ii) *Flubendazole.* This is a parafluor analogue of mebendazole and shows activity against animal and human nematodes. The LD_{50} for flubendazole in acute toxicity tests is 2560 mg/kg for several animal species. No teratogenic effects have been observed in rats and rabbits, which gives it an advantage over mebendazole. Flubendazole is effective in the treatment of enterobiasis in a single dose of 200 mg; and of ascariasis, trichuriasis and necatoriasis at a dose of 200 mg daily for 3 days or 300 mg twice in one day (*18*). The results are similar, but not superior, to those found with mebendazole. Tolerance is equally good for both drugs.

(iii) *Tetramisole/levamisole.* Tetramisole and its isomer levamisole have been used against a broad range of nematodal infections in animals. These two benzimidazoles are soluble in water and readily absorbed from the intestine. They act on the neuromuscular system of the worms, producing paralysis. Owing to the good tolerance and low single dosage, levamisole has been successfully used in the mass treatment of ascariasis. At present, levamisole is under clinical investigation for use in immunodeficiency diseases and some malignancies; several cases of agranulocytosis have occurred after prolonged use of this drug.

(iv) *Tiabendazole.* This is one of the oldest benzimidazoles and is absorbed from the intestine, with good activity against the adult and larval forms of some tissue nematodes. It is still the drug of choice for strongyloidiasis and cutaneous larva migrans. Side-effects are present in 50% or more of the treated patients, dizziness being the most common symptom. Other symptoms are nausea, vomiting, abdominal pain, anorexia and diarrhoea. Headache, drowsiness, lethargy, crystalluria, erythema multiforme and Stevens-Johnson syndrome, with two fatalities, have been reported. The recommended dosage is 25 mg/kg/day, divided into 3 doses and administered with meals, for 3 or more days.

(v) *Pyrantel.* This compound of the amidine group is a tetrahydropyrimidine, with the chemical name (*E*)-1,4,5,6-tetrahydro-1-methyl-2-[2-(2-thienyl)ethenyl]pyrimidine 4,4′-methylenebis[3-hydroxy-2-naphthalenecarboxylate]. It is a crystalline powder, insoluble in water and very slightly absorbed from the intestine. It has no special taste and is stable to moisture, light, and temperature. Its mode of action is by inhibiting neuromuscular transmission, thus producing spastic paralysis of the worms.

The oral LD_{50} for mice, rats, and dogs is in the range 2–5 g/kg. No toxic effects at therapeutic dosages have been reported and the side-effects are mild and transient; some reports have shown that from 4% to 20% of the treated patients presented with gastrointestinal symptoms such as abdominal cramps, diarrhoea, nausea and vomiting. Less frequent were headache, dizziness and drowsiness. No teratogenicity has been found. There are no specific contraindications.

Pyrantel is effective in the treatment of ascariasis and enterobiasis with a single dose of 10 mg/kg. Against hookworm, the same daily dosage should be repeated for 3 consecutive days (*19*).

Mass treatment with pyrantel has been effective in reducing *Ascaris* infection to very low prevalence rates. Repeated treatments, every 3 months, have maintained large groups of populations under control against ascariasis and reduced the prevalence rate from 80% to less than 1%.

This drug is also applicable to groups of children infected with *Enterobius* and has the advantage of being effective against both parasites in a single dose. The good tolerance and the lack of teratogenicity are advantages, favouring the use of this drug for mass therapy, especially on a large scale. Against hookworms, it is necessary to give the treatment for 3 days in order to achieve appropriate worm reductions.

(vi) *Oxantel.* Oxantel is (*E*)-[2-(1,4,5,6-tetrahydro-1-methyl-2-pyrimidinyl)ethenyl]phenol 4,4′-methylenebis[3-hydroxy-2-naphthalenecarboxylate]. It is a crystalline salt, yellow in colour, and practically insoluble in water; this drug is well tolerated and does not cause toxic effects. It is used in the treatment of *T. trichiura* infections (*20*). Unlike its analogue, pyrantel, oxantel is not effective in cases of ascariasis. The possible effect on other helminthiases has not yet been defined.

For the treatment of trichuriasis a dosage of 10–15 mg/kg/day for 2–3 days is recommended. Single doses are effective only in light infections. The combination of pyrantel and oxantel has been successfully used as a wide-spectrum anthelmintic, being effective against *Ascaris, Enterobius, Trichuris* and hookworm. The efficacy of this combination is promising for use in mass therapy.

(vii) *Praziquantel.* Chemically this drug is 2-(cyclohexylcarbonyl)-1,2,3,6,7,11b-hexahydro-4*H*-pyrazino(2,1-*a*)isoquinolin-4-one, a new isoquinolinepyrazine derivative. It is a colourless crystalline powder with a bitter taste and is insoluble in water. After oral administration the drug is rapidly absorbed from the gastrointestinal tract; maximum

serum concentrations appear after 2 h; it is metabolized in the liver and excretion is completed after 24 h, mainly through the urine in the form of several metabolites. The detailed mode of action of this drug is still under investigation, but initial studies have shown that the compound acts on the carbohydrate metabolism of the parasites.

Acute toxicity tests in rats and mice showed that the LD_{50} was over 2000 mg/kg when administered orally. Tolerance is good in experimental animals and in human beings. Very few transient side-effects (of the gastrointestinal tract, as well as dizziness, headache and drowsiness) have been mentioned by some authors, while others refer to complete absence of side-effects. No embryotoxic, teratogenic or mutagenic effects were found experimentally.

Therapeutic efficacy has been demonstrated against *T. solium* and *T. saginata,* using a single dose of 10 mg/kg. Cure rates have been very near 100% (*21*).

Against *H. nana,* the single dose used is 25 mg/kg with around 80% of cures; probably repetition of the treatment, once or twice, would produce higher cure rates. In diphyllobothriasis a single dose of 25 mg/kg is effective. The drug is also effective against cestode larval forms in different animals in experimental conditions. The efficacy in human cysticercosis seems to be promising, although clinical trials are still few. Praziquantel has been widely used in the treatment of animal cestode infections with good results. This drug is effective against all the schistosomes infecting man and animals (*22*). Some trials have shown its effectiveness in the treatment of human trematodiasis.

Owing to its efficacy in single doses, the good tolerance and the lack of toxic effects, this drug promises well for mass treatment of many trematodiases and cestodiases in humans.

(viii) *Niclosamide.* Niclosamide, still a widely used anticestodal drug, acts against the parasites after direct contact by inhibiting the oxidative phosphorylation process in the mitochondria, causing release of the parasites from the intestinal mucosa. This drug is well tolerated and because it is not absorbed, no toxic effects are produced. The usual dosage against *T. solium* and *T. saginata* is 2 g as a single dose on an empty stomach; it should be thoroughly chewed and swallowed with a little water. In the treatment of hymenolepiasis, the dosage should be repeated daily for 5 days and a second treatment after 3 weeks is necessary.

* * *

It can be seen that there is a wide spectrum of drugs available for the treatment of the numerous intestinal protozoan and helminthic infections. Yet surprisingly in numerous developing countries, their use in both urban and rural areas is minimal. Feasible programmes to control the prices and quality of these drugs and to encourage local production facilities are needed in order to make them available to the people in the rural areas and to schoolchildren in the tropics, the groups commonly at risk of infection.

4.3.3 *Environmental control*

4.3.3.1 *Community hygiene.* Measures to prevent environmental contamination with human faeces have been successful among the urban and suburban communities of highly developed regions. In many other areas, direct faecal contamination of soil is uncommon except in areas occupied by rural people of all ages, and by urban children of preschool ages (*32*). In a number of regions of the world, the practice of using human excreta for the fertilization of crops persists. Thus, programmes designed to interrupt the soil-transmission of helminths will be required, depending on local circumstances. For example, in a large community of eight settlements in Liberia, it was recently found that, although over the past decade the environmental hygiene had been improved by piped water supplies, latrines, and basic health education, the prevalence rates had remained high for all of the common soil-transmitted helminths (*11*). Hookworm infection was found to be particularly persistent and it was noted that inappropriately maintained latrines actually increased the risk of hookworm infection.

4.3.3.2 *Dissemination and unequal distribution of eggs in the soil.* Observations on the acquisition of massive numbers of infective eggs of *Ascaris* and *Trichuris* were reviewed recently and indicate that, in children of dirt-eating age, massive infections are more likely to be acquired by ingesting large numbers of infective eggs within a brief period (1 or 2 days) than by the ingestion of small numbers repeatedly over a longer period of weeks or months (*23*). As a result of the spreading and filtering action of rain, the eggs of helminths are concentrated in puddles at the surface and sedimented in a layer of fine silt between a thin protective blanket of colloidal clay and a deeper layer of coarser particles of silt and sand. As the eggs of zoonotic

helminths are disseminated and concentrated in the same way, endemic and zoonotic helminth infections are frequently acquired simultaneously.

This pattern of dispersion by rain and other factors was illustrated by an experiment in Australia (*24*). By having lambs graze in grassy plots at various distances from a fixed site where for 50 days a tethered dog had been passing proglottids and eggs of *T. hydatigena* in the faeces, it was found that the eggs were distributed mainly in clusters within a radius of 25 m, but the nonrandom dispersion of eggs extended over a radius of 80 m. The number of eggs ingested by the lambs was determined by counting the cysticerci at slaughter 3 months after the experimental grazing.

4.3.3.3 *Persistence of viable eggs in soil.* Over a 15-year period at Samarkand, USSR, periodic observations on eggs of *Ascaris,* buried near the surface in a garden plot, showed that in 9 years some of the eggs moved to a depth of 20 cm and their viability at that depth was less than 2%, whereas at depths of up to 10 cm, 5.9–7.6% were viable, as demonstrated by inoculation of guinea-pigs. Even at 14 and 15 years some of the eggs were infective for guinea-pigs (*25*). An important factor, among others, to account for the destruction of eggs in the soil is the ovicidal action of soil fungi. This action is accelerated by the high temperatures in tropical areas (*26*).

In England, eggs of *T. trichiura* were recovered from depths of up to 60 cm, but all that were deeper than 20 cm were nonviable. At shallower levels, 20–35% appeared to be viable at 18 months. In an area that had been heavily contaminated for several years, 980 *T. trichiura* eggs were recovered from a 2.5 kg soil sample, taken at a depth of 50–60 cm (*27*).

4.3.3.4 *Disinfection of soil, food, and water.* In the study just referred to (*27*), an attempt was made to control *T. trichiura* infection at an institution for ambulatory patients. The patients in one section were treated with difetarsone, and the soil of selected plots in play areas was treated with methyl bromide, chloropicrin or dazomet, or with a mixture of dazomet and chloropicrin. At the end of an 18-month trial period, most of the patients were again passing large numbers of eggs despite their having been confined to new recreation areas. In both the treated and the untreated soil plots, the number of viable eggs decreased rapidly, but in the plot giving the best results (methyl bromide), roughly 15% of the eggs remained viable.

The insecticides and herbicides commonly used in the Republic of Korea were tested for activity against the eggs and larvae of *A. caninum* and *Trichostrongylus orientalis,* and the unembryonated eggs of *A. lumbricoides* and *T. trichiura* (*28*). The results indicated no significantly lethal effect on the eggs of any of the four species, but the larval forms (especially the rhabditoid larvae) were destroyed in 6–24 h at ordinary concentrations by the insecticides dimpylate and benzene hexachloride, and by the herbicides simazine (6-chloro-*N,N'*-diethyl-1,3,5-triazine-2,4-diamine) and the sodium salt of pentachlorophenol.

A saturated aqueous solution of iodine (prepared from iodine crystals), when added in the amount of 12.5 ml per litre of drinking-water, was reported to kill pathogenic bacteria, protozoan cysts, and viruses in less than 15 minutes at 25 °C (*29*). With excystation as the test for viability, the suitability of aqueous iodine (13 ml saturated solution per litre) for killing *Giardia* cysts in drinking-water (taken from polluted lakes and streams) was confirmed (*30*). This recalls earlier reports of the feasibility of using aqueous iodine solutions to kill helminth eggs and larvae on fresh vegetables, and the lethal action of natural food-preservation substances on the infective eggs of *A. lumbricoides* and larvae of *A. duodenale.*

4.3.3.5 *Sanitation.* The use of modern methods for the disposal of human and animal wastes do not immediately interrupt disease transmission. There is first the problem of educating the people in the appropriate individual utilization of such facilities, which may require a decade or so, and then the problem of achieving appropriate community utilization of the products of sewage disposal—i.e., the effluents and sludges. Both of these products, as used or disposed of at present, present health risks to people and domestic animals. Possible solutions to these problems are at present being considered. The infections of chief concern are those caused by species of *Ascaris, Toxocara, Trichuris* and *Taenia,* because the eggs of these helminths are not destroyed by any of the usual forms of sewage treatment and they may be abundant in the waste sludges.

4.3.3.6 *Conclusions.* In recent trials of population-based chemotherapy, approaches have been made by periodic mass treatment of the whole population, treatment of sample groups, treatment for assessment of relationship of ascariasis to malnutrition, and selective treatment for disease control. Single-dose treatments with pyrantel (5 mg/kg) or with levamisole (2.5 mg/kg) have been used satis-

factorily for ascariasis, being almost 100% effective in groups treated at 3-month intervals for a year—less so for other worms. Removal of light *Ascaris* infection appears to have accelerated the growth of preschool-age children.

In a population whose health services carried out selective case-finding and provided treatment for hookworm disease, the prevalence and morbidity rates after 30 years were markedly reduced. Sanitation and education programmes, unaccompanied by treatment, have had little short-term effect on disease transmission. Because of the unequal distribution of eggs in the soil, it is possible for children to acquire massive infections of soil-transmitted helminths after a short period of exposure. Infective *Ascaris* eggs may persist for many years in the soil; *Trichuris* eggs perish more quickly. Chemical disinfection of soil is not feasible. Infective stages of protozoa and helminths on vegetables and in drinking-water can be killed with aqueous iodine. Modern sewage systems have not solved the problem of how safely to utilize or to dispose of sewage effluents and sludges.

4.4 Implementation of preventive and control measures in existing national health care programmes

It is well known that the level of public health is related to how far a population has the means and the responsibility for coping with illness. This ability to cope can be supported (but not totally replaced) by medical intervention. A society where such professional intervention can be reduced to a minimum may be regarded as one that has the best conditions for health, i.e., by autonomous adaptation to the individual, the society and the environment. Illich (*31*) observed that:

> "Healthy people are those who live in healthy homes on a healthy diet: in an environment equally fit for birth, growth, work, healing, dying: sustained by a culture which enhances the conscious acceptance of limits to population, of aging, of incomplete recovery and even imminent death. Healthy people need no bureaucratic interference to mate, give birth, share the human condition and die."

This approach should be considered when discussing methodologies for implementing prevention and control measures within existing national health care programmes of developing countries. While it is true that most policy-makers have undoubtedly realized the importance of preventive and control measures, it is well known that in most developing countries the processes adopted and the measures taken to strengthen preventive control measures are extremely weak and inadequate.

From the point of view of cost/benefit it is necessary that higher priority should be given to the development of preventive and control measures in all developing countries. Such a development in the first instance would require its incorporation into an existing national health care programme. The compulsion to pay attention to preventive and control measures is basically economic but it is also multidimensional. The most important factor, however, is the economic cost to society because of the high morbidity of the population. It has to be realized that the prevalence rate of parasitic diseases in many developing countries is well over 50%.

4.4.1 *Reasons for incorporation*

There are several reasons why preventive and control programmes should be treated as part of a national health programme. Some of the most important are the following:

—the high incidence of communicable diseases;
—the economic and social losses due to morbidity;
—the multidisciplinary nature of the work involved;
—the high degree of popular participation required for its success; and
—the need for policy and budgetary coordination.

The first step, therefore, is to ensure that institutional facilities are built into the existing national health institutional framework, and in such a manner as to make it possible to deal with the subject at all levels and to facilitate the appropriation of budgetary provisions for such work programmes at different levels of the institutional framework. The application of prevention and control measures calls for direct contact with the community at the level of the individual (as a member of a family), of the village, and of society as a whole. Secondly, the approach must, of necessity, be multidisciplinary. While, therefore, the recognized institutional framework is of importance, the relationship between such institutions and the community, through appropriate decentralization, must be given even more importance.

The nature of the work is such that institutionalization by itself would not be the best form of organization. At different levels of the health programme, whether it be for research, treatment or other objectives, special facilities should be provided for building up capabilities to enable contact and consultation to be established and maintained with other sociocultural organizations of society. Problems, such as those pertaining to the environmental impact of development,

have to be dealt with in close consultation with active workers in other disciplines that are connected with the establishment of preventive and control measures.

Yet another aspect, which has perhaps not received adequate consideration, is that legislative authority for such consultation should be granted to institutions that are responsible for dealing with these measures. For example, this applies to the collection of data. Many countries have departments that are responsible for the collection of statistical data in various areas of the economy. These departments are set up statutorily and possess legal authority for such collection. It may, however, be necessary that they should be equipped with powers through by-laws to implement certain preventive and control measures in cases where the data-collecting agents are private entrepreneurs.

It need hardly be stressed that, in order to introduce and implement such programmes, social and economic statistical services are required not only to monitor and evaluate the impact of existing measures, but also to identify critical areas which are requiring increasing attention. The organization of a system to deal with this problem will vary from country to country, but the basic factor would be the capability of the research and administrative departments to obtain up-to-date information on current situations.

4.4.2 *Forms and methodologies of implementation*

In order to achieve the objectives of a national plan for health which includes the components of prevention and control, attention must be paid to the parallel development of specific institutions with existing facilities in other disciplines within the medical system. It is a *sine qua non* that every country should have its department of communicable diseases control; there may also be separate divisions for various parasitic diseases.

What is more important, however, is the establishment of departments or units that are properly equipped to undertake research, training, treatment and consultation. Adequacies and inadequacies may be identified only through a closer examination of the situation in each country. The programme has potential for success which could be limited only in terms of the inputs required for its full implementation. From the point of view of cost, there should not be a heavy burden on the national budget because of the built-in capacity

for mutual collaboration between programmes, resulting in efficient use of manpower and management.

Nevertheless, for dealing with the parasitic diseases there are a number of important disadvantages that detract from the value of the programme itself and tend to limit its applicability, thereby diminishing the chances of its success in the long run. The major problem, undoubtedly, is the limited coverage of ascariasis and hookworm infections. There is an obvious lacuna from the point of view of health education and community participation, which are of primary importance. This is evident from the fact that the reinfection rate is still too high, often requiring more than three consecutive years of treatment for control of these helminths.

The implications of prevention and control must, of necessity, be looked at from various angles. First, the extent of voluntary participation must be of the highest order, official connexions being kept to a minimum.

Secondly, different procedures for the rural, urban and semiurban communities are necessary. There is a clear need to undertake extensive studies pertaining to the ways and means of introducing prevention and control measures in the different sectors of society as indicated above. The priorities in the urban and semiurban centres would obviously differ from those of the rural areas.

Thirdly, health education as a factor must itself be given priority. The communicable diseases centres must, through the departments of education and labour and other organized centres of activity, provide for suitable schemes which will enable the government, through its health officers, to introduce programmes of health education in the prevention and control of communicable diseases on a more or less compulsory basis.

Fourthly, it is important that voluntary activities should become an essential part of the programme. This can only occur through imparting a sense of responsibility to the citizens. Cooperation with the mass media (radio, newspapers and television) should be established on a regular basis to create and maintain the necessary interest and sense of responsibility.

All the above are needed to permit full community participation in the implementation of this public health programme, particularly in the area of prevention and control of communicable diseases. An important element is the infrastructure in terms of manpower and institutions. It is absolutely essential that every family should be fully aware of the implications of the programme, and of their responsi-

bilities in its implementation. This is undoubtedly a difficult task for the developing countries, particularly in view of the rather low levels of literacy. Nonetheless, there are ways and means of making even the most complicated programme available and understandable through current mass media techniques.

4.4.3 *Evaluation and monitoring*

If a comprehensive and clear-cut programme is worked out and incorporated into the ongoing activities of the ministries of health, it is easier to incorporate changes whenever necessary. Monitoring exercises should be undertaken at regular, but reasonable, intervals. The objective of the programme should be related to the targets, so that by a given time the benefits would be appreciated by all; this achievement would have a good effect on the people, so that the implementation of future programmes would be undertaken with enthusiasm.

4.5 Relationship of preventive and control measures to primary health care

In order to make preventive and control measures effective, it is necessary that special attention should be paid to primary health care. The interrelationship between preventive and control measures and primary health care is therefore not only logical but essential. Preventive and control measures are fundamentally directed at making the environment suitable for healthy living. Successful implementation of a programme concerned with the latter is impossible without its being closely coordinated with primary health care.

The objective of primary health care is to maximize the extent of community participation at all levels of society in the acquisition of elementary health knowledge and basic skills, so as to avoid conditions that create and spread communicable diseases. It also envisages the development of a sense of judgement among people to take action with regard to their own health problems. The two prerequisites are, therefore, availability of basic knowledge and ability to take action when necessary. This implies that primary health care is the concern of the people, whose fullest participation in this activity must be promoted, since healthy living is dependent on adequate preparation of the environment.

A considerable number of diseases are prevalent in the tropical countries, where geographical conditions, overpopulation, and poverty are basic factors that promote their spread in communities. It has often been stated that the morbidity caused by communicable diseases in developing countries is one of the major hindrances to economic development. The most prevalent form of such morbidity among tropical populations is caused by the intestinal parasitic diseases, which can easily be brought under control by properly conceived health care systems with a minimum of institutional support and financial resources. It cannot be assumed that the high prevalence of these diseases is due to poverty alone. Conditions of poverty could certainly be brought about by the morbidity caused by these diseases.

In the case of the most prevalent intestinal parasitic diseases, it is possible to conceive the adoption of preventive and control measures which could be integrated into the primary health care system with the minimum of adjustment and financing.

Thailand is one of the few countries where attempts have been made to implement the control and prevention of intestinal parasitic infections within the primary health care system. Therefore, the plans originating from Thailand are presented below as an example of the conceptual ideas aiming to solve the problem of the relationship between the prevention of such infections and primary health care.

In order to make preventive and control measures successful, it is necessary that the government should plan schemes for introducing effective primary health care programmes at all levels of society.

This implies that:

(1) Such a programme (to be successful) must be addressed to all people at all levels to ensure mass participation. A comprehensive programme to make people conscious of their responsibilities must be undertaken.

(2) The national government must provide the initiative to use existing socioeconomic relationships as the basis upon which such a programme could be built. Mass participation calls for identification of leadership among the rural and urban communities and the training of such leaders not only to undertake this work but also to train other members of the community.

(3) The government must provide a minimum of intervention to ensure the activation of voluntary work and, where necessary, to back the intervention with legislation. It is well understood that government intervention should not in any way become a burden on the capabilities and capacities of the people to accept and adopt and

implement the necessary measures in their community. As far as possible, it is essential that existing practices and sociocultural habits should be utilized in order to promote primary health care.

(4) Schools should be provided with materials necessary for teaching subjects related to primary health care.

(5) Incentive schemes must be developed among various communities which would provide, on a competitive basis, for improvement of the environment and conditions of living.

(6) A continuing training scheme should be established. Primary health care must necessarily rely on a well-founded system or network of connexions. The points of linkage to the hierarchy of the higher echelons of the professional system must be carefully worked out at different levels of the existing institutional framework. It should be a gradual connexion linking the layman to the professional. This can only be achieved by training people at all key levels of society, which has to be undertaken continuously and through the training of trainers.

It is easy to envisage the necessary conditions for promotion of primary health care. However, what is more important is to undertake such a programme on the basis of given population needs. The responsibility for introducing these schemes must be designated within the programmes of preventive and control measures, and medical officers and other officials responsible for implementing these programmes should also be responsible for dealing with primary health care.

In a general analysis of the health situation made in 1976, it was ascertained that more than 50% of people in Thailand were accustomed to buy drugs for self-treatment (*33*). On the other hand, around 40% had no treatment, which was not entirely due to the shortage of medical facilities. Self-treatment can be extremely dangerous. In societies where such practices are common, primary health care would enable the people to utilize their knowledge and have ready to hand basic medical facilities which could cope with situations of this kind.

To take steps to introduce preventive and control measures there must be a fairly comprehensive administrative and technical institutional framework within the national health plan. At each of the vital points of decision-making, it is necessary that facilities should be made available for providing information on health care (based on information distributed to the preventive and control centres) which could

be used to inform the people of measures that should be taken in order to eliminate the causes of the spread of these diseases.

Fig. 1 illustrates diagrammatically the Lampang health development project for primary health care in Thailand; it shows the flow of responsibilities and the levels to which the district health community could have access through the health communicators. Fig. 2 shows the multidisciplinary framework of the Samerng project in the same coun-

Fig. 1. Schema illustrating primary health care at various levels in the Lampang health development project, Thailand.

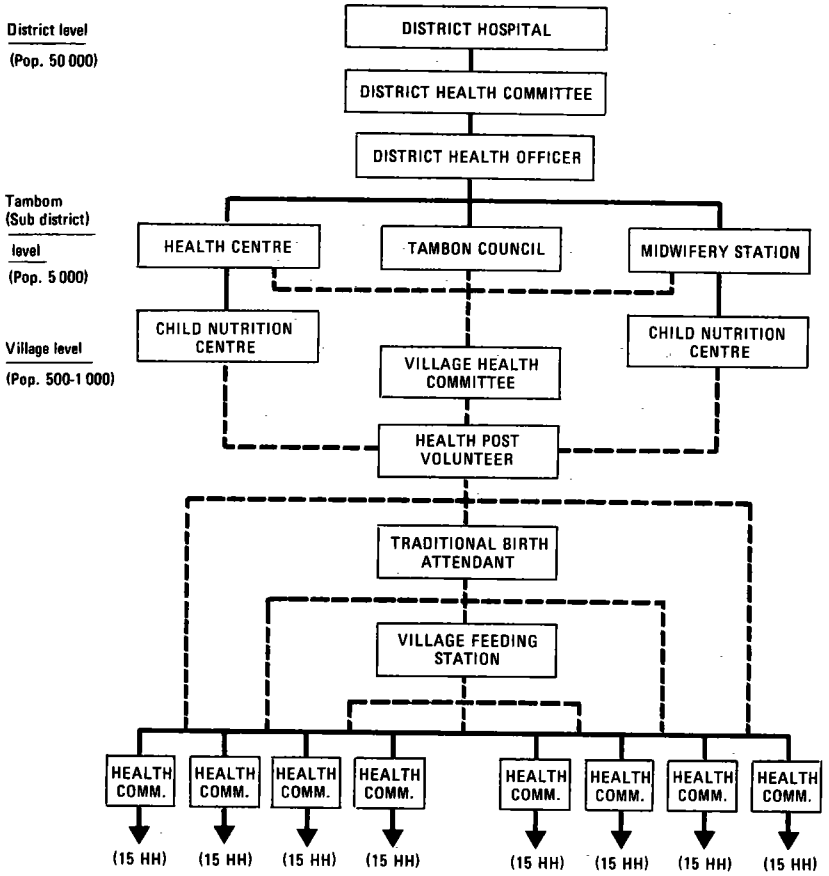
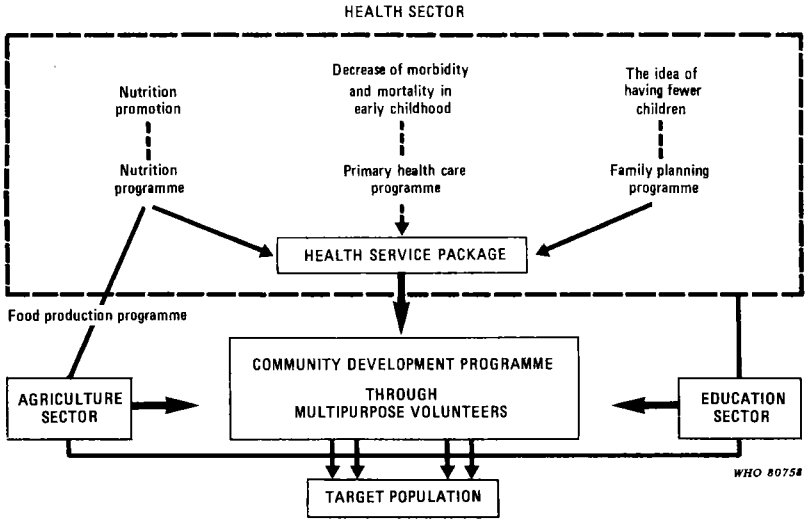


Fig. 2. The conceptual framework of the Samerng project, Thailand.



try, in which the different disciplines in primary health care involved include family planning, nutrition and education. In the national health plan (the fourth five-year plan, 1977–1981), the planners envisage a number of measures which are basically directed through primary health care, including the development of specialized programmes to cope with certain communicable diseases. Throughout the plan, care has been taken to indicate the extent of mass participation and its vital importance. In order to make the preventive and control measures effective, it is therefore absolutely essential that steps should be taken to develop programmes of primary health care and implement them on a realistic basis.

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5. TRAINING

One of the most important factors in the successful control of intestinal parasitic diseases at the level of the community is the human factor, i.e., the community involvement, the expertise of those formulating the programme, and the motivation and training of both medical and paramedical personnel working in the field. Many examples of the positive involvement of some communities in their health problems are apparent in the results of the action undertaken by dedicated health and/or social workers. For promoting the interest of people (from all the key levels) in the health problems of their community and for building up links between the layman and the medical profession there is need for both experience and training, especially on the part of health administrators, public health workers, and educators (not necessarily in the medical field). Population-based treatment of some intestinal parasitic infections (e.g., ascariasis), which offers the community direct and visible results with children, a most sensitive

sector of the population, is frequently a good starting-point for integrated public schemes (including, for example, maternal and child care, family planning and nutrition). Therefore, the most effective ways of promoting community involvement in active integrated programmes, including control of intestinal parasitic infections, should be among the subjects taught during medical and paramedical studies and discussed during postgraduate courses.

In endemic areas where active programmes are not likely to be organized, the incidence of many diseases could be reduced if the people themselves were to apply the preventive measures that are available for most of the intestinal infections. Health and sanitary education of the people plays a fundamental role in population-based prevention. However, as the number of professional health educators is usually inadequate for the local needs, members of the medical and paramedical professions should be trained and encouraged to deliver health education in the communities where they are working (*1, 2*). This means that the medical and paramedical student should be well aware of the preventive aspects of parasitic infections and the basic techniques of effective sanitary education.

The control of intestinal parasitic infections could be carried out by implementing active programmes or by improving the diagnosis, treatment and prevention of these diseases in everyday curative or public health practice. There is, however, a lack of personnel interested and skilled in the diagnosis and epidemiology of intestinal parasitic infections.

Diagnosis of the majority of intestinal parasitic infections is still based on the microscopical examination of faecal material. Whereas it is relatively easy to diagnose the most common soil-transmitted helminthiases, such as ascariasis, trichuriasis and hookworm infections, the diagnosis of protozoan and some helminthic infections needs considerable skill on the part of the technical assistants and frequently consultation with a parasitologist. There is a tendency to develop serological techniques for more widespread use in nonparasitological laboratories, but this trend is expensive and the specificity and simplicity of the tests are still not satisfactory. On the other hand, studies on the development of simpler diagnostic techniques either with a microscope (for protozoan infections) or without (for coprocultures of nematode larvae) are inadequate.

There are two ways to train the required personnel for diagnosis of intestinal parasitic infections: either by using the existing systems of medical education or by organizing special training in diagnosis.

In general, parasitology is not represented adequately (considering the local or international needs) in the curricula of medical faculties, undergraduate studies of biological sciences, and technical medical schools (*3*). The reasons for this are that medical parasitology has less tradition than, for example, bacteriology, and it requires a good knowledge of the zoological sciences—protozoology and helminthology, at least. In traditional medical studies the various elements of parasitology are expected to be taught at three or four levels: in the course of general biology (zoological aspects), within microbiology and public-health-related disciplines (laboratory diagnosis, epidemiology and prevention), and in various clinical disciplines (clinical parasitology). The fact that most medical graduates have a negligible practical knowledge of parasitology is due mainly to the scarcity of teachers of parasitology and to inadequate availability and quality of teaching-learning materials. Therefore any prospective plans aiming at the control of intestinal parasitic infections on a national or on a international scale should start with building up a cadre of teachers and trainers as well as the establishment of a material basis for the training activity (microscopes, teaching aids, textbooks). Improvement of knowledge of parasitology using existing systems of education is a slow process involving undergraduates only. Postgraduate or accelerated training can be achieved only by having special training projects. For many reasons WHO is predisposed to assist with the introduction of more practical subjects into the curricula of medical and paramedical studies and to promote, on an international scale, the postgraduate training of parasitologists and epidemiologists in the effective control of intestinal parasitic infections.

The accuracy of parasitological diagnosis in health service laboratories in several developed countries is unsatisfactory and could be improved in a relatively short time both by special in-house courses and by postal distribution of specially prepared didactic material (slides, tapes, booklets) for self-education, as has been recently demonstrated in the USA by the Center for Disease Control. Periodic checking of the quality of laboratory diagnosis of intestinal infections on a national scale is an initiative worth encouraging in all countries; it may not only improve the standard of laboratory services but may also provide justification for better training in parasitology (*4, 5*).

The training of cadres in parasitology, epidemiology and public health in the organization of effective control of intestinal parasitic infections needs a different approach. As the priorities and possibilities for control differ widely in many countries, only some general prin-

ciples may be taught *ex cathedra,* but all the details of active programmes must be elaborated through discussion on how to achieve the best results with the available resources in a particular epidemiological situation. Therefore the best way to develop optimum strategies and the most useful implementation of the control programmes is through working seminars at which both knowledge and experience are exchanged between scientists, local public health workers, and practitioners.

There are two key problems that are important for the effective control of intestinal parasitic infections in endemic areas: adaptation of technical tools to local needs and training local people in their use. Interregional working seminars are expected to be most helpful in solving these problems, because they give an opportunity to exchange experience, to evaluate the practical value of existing methodologies, and to adapt old ones or develop new ones according to the local needs. The Asian Parasitic Control Organization has organized working meetings in south-eastern Asia each year since 1974 and has been successful in promoting regional control efforts both in research and in field work. For the other regions of the world there is no organizational framework other than WHO which could organize interregional working seminars on intestinal parasitic infections.

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6. RESEARCH NEEDS

During the course of its consideration of intestinal protozoan and helminthic diseases of man, the Group noted the inadequate or sometimes complete lack of research into matters of considerable importance for the elucidation of a number of problems. The research needs were classified under the headings of biology, pathology, immunology, epidemiology, and diagnosis.

6.1 Biology

The following needs were identified:

(1) In considering strain differentiation of *Entamoeba histolytica,* the Group believed that further studies were required on the DNA base composition and on isoenzyme electrophoretic patterns. It would be of interest to examine noninvasive strains isolated from *E. histolytica* carriers for viruses.

(2) Better methods of isolation of *E. histolytica* from faeces and continuous cultivation *in vitro* are needed for the detection of infection, and for characterization of strains of *E. histolytica* or other species of *Entamoeba* (including their antigenic and biochemical characteristics).

(3) Acceptable yields of *E. histolytica* for most research purposes and for production of antigens can now be attained with currently available media for axenic cultivation. Future research should be directed to: (*a*) development of a defined medium to permit elucidation of the nutritional requirements of the parasite and other special studies, such as the chemistry of membrane biolipids; (*b*) development of a technique for inducing encystation under axenic conditions, to enable this much neglected but important process to be studied.

(4) Special efforts should be made to cultivate axenically the strains of *E. histolytica* that have been isolated from asymptomatic individuals; no method is at present available for this study.

(5) In order to interpret prevalence and accurately to assess published and future observations on the pathogenesis of *E. histolytica,* further information should be obtained on the morphology, taxonomy, and prevalence of the common species and strains of amoebae that are capable of growing *in vitro* at relatively low temperatures, e.g.,

the so-called *E. histolytica* Laredo and Huff strains; these amoebae are morphologically indistinguishable from *E. histolytica* but differ with respect to pathogenicity.

(6) For the recognition and characterization of the species and strains of *Giardia* that infect and may cause disease in man, there is need for further development of animal models suitable for experimental infections with *G. intestinalis* from man and with species or strains of zoonotic origin.

(7) A problem demanding attention is the isolation and *in vitro* cultivation of *Giardia;* investigations on the pathogenicity and physiology of *Giardia* would be enhanced by the ability to cultivate and produce encystation and excystation of *G. intestinalis* and other species of the genus.

(8) The biological characteristics of *Balantidium* species should be identified to ascertain whether there are differences between the species parasitizing man and those affecting pigs.

(9) More attention should be paid to *E. polecki, D. fragilis,* and *Sarcocystis* species with a view to clarifying their biological characteristics and importance in human disease.

(10) Little work has been carried out on the biology of *Ascaris* eggs in the soil and more is required, especially in highly endemic regions.

(11) The nutrition of children and that of *Ascaris* are, presumably, interrelated; to reach conclusions regarding how (or whether) the worms affect a child's nutrition, there is need for knowledge of the metabolic substrates used by the worms and an answer to the question of what *Ascaris* uses as food and where and how that nutrient is obtained.

(12) Methods using tissue and organ culture should be developed for study of metabolic interactions between the host and intestinal parasites.

(13) For a better understanding of hookworm infection there is a need for further study of the location and behaviour of the larvae and developing worms of *Ancylostoma* species, including *A. duodenale, A. ceylanicum, A. caninum, A. brasiliense* and other species of *Ancylostoma* during the prepatent period. The period between entry to the body and the appearance of eggs in the faeces may be only a few weeks or several months; the reason for this wide variability is not understood for *A. duodenale.*

(14) Studies are needed to explain the apparent strain differences in *Strongyloides stercoralis* and their adaptation to different ecological

conditions, as well as their varying effects on man and on the response to therapy.

(15) The exact classification of *Diphyllobothrium* species found in man is still not clear and taxonomic criteria of these diphyllobothrids are needed; morphological differences are apparent but chemotaxonomy (through isoenzyme patterns) may offer further means of species identification.

(16) The taxonomy and life-cycles of many of the human intestinal trematodes should be studied, including the egg morphology related to the adult worm and the pathology caused by these flukes.

6.2 Pathology

The following needs were identified:

(1) Certain symptoms in parasitic disease (such as anorexia, abdominal pain, change in bowel habit, etc.) are not explained; because of their importance, further studies on them are needed.

(2) The role of iron and lectins in the pathogenesis of amoebiasis and of T lymphocytes in protecting the liver from amoebic invasion require further research.

(3) Studies are required on the morphological changes that take place in the intestinal mucosa as a result of *Ascaris* infection and how these may impair the absorption of nutrients, and also on how much ascariasis contributes to malnutrition. Research is needed on whether the metabolites of *Ascaris* interfere with the host metabolism.

(4) Field research should be carried out on the ways in which the nutritional and immunological mechanisms of the host are affected by ascariasis, particularly in already undernourished young children.

(5) Similarly, the health impact of *Ascaris* allergies should form the subject of well-controlled field research.

(6) Details on blood loss in hookworm infection and on the nature and sources of metabolic substrates utilized by hookworms are needed to assess the significance of light infections and to resolve conflicting views regarding the causes of blood loss in hookworm disease.

(7) A neglected but much needed field of research is on the clinical pathology of taeniasis.

6.3 Immunology

The following needs were identified:

(1) The harmful effect of immunosuppression on disease expression in certain intestinal parasitoses is well documented (e.g., stron-

gyloidiasis, hymenolepiasis). There is, however, a need to clarify the effect of immunosuppression in other intestinal parasitic infections and to institute prophylactic measures. Studies are also recommended on the immunosuppressive action of some intestinal parasitic infections and the possible interference with the results of immunization against various infectious diseases.

(2) In areas where amoebiasis is a major public health problem, a vaccine would be an obvious preventive measure and the Group strongly recommended that a much greater research effort should be made to this end.

(3) The role of the thymus in *Giardia muris* infections in the mouse needs further work and much remains to be done in the mouse model system of *G. muris* and its relativity to the spectrum of immunity in human giardiasis.

(4) The mechanism of protective immunity to ascariasis in man requires study as does the relationship of the high level of IgE due to ascariasis and the apparent infrequency of asthmatic children in particular areas. Laboratory research is required on the protective significance of reagins and on the mechanisms of immunostimulating and immunosuppressive actions of ascarids.

(5) Studies are required on the functional relationships between serological and tissue hypersensitivity reactions and protection against hookworm reinfection.

(6) Studies should be made to ascertain whether the presence of certain host genetic markers (such as blood groups or histocompatibility leukocyte antigens) can be correlated with the presence of parasitic intestinal infection or the expression of disease, e.g., in giardiasis.

6.4 Epidemiology

The following needs were identified:

(1) Further work is required on the seroepidemiology of amoebiasis and the prevalence of invasive amoebiasis in different parts of the world.

(2) There is need for more studies on the routes of transmission of *Giardia intestinalis,* the frequency of waterborne epidemics, and the role of zoonotic strains in human giardiasis.

(3) The mode of transmission of less important protozoan infections (such as those caused by *Dientamoeba fragilis, Entamoeba polecki,* and *Sarcocystis* species) requires more study.

(4) There is need for accurate information on the frequency and significance of pig ascariasis in humans and of human ascariasis in pigs in areas where pigs and people live together.

(5) Information is inadequate about the frequency of life-threatening complications of ascariasis requiring surgery and on the specific mortality rate of the disease.

(6) In the case of *Strongyloides* infection, there is need for detailed studies on the routes of transmission and the relative role of soil, water and faeces as the medium for development of the infective-stage larvae. The factors responsible for the failure of *Strongyloides* to have the same geographical distribution as hookworm are not understood.

(7) It is known that the *Strongyloides* species that parasitize pigs, sheep, cattle, horses and other animals are transferred from the mother to the newborn offspring through the milk. As this has been reported in one human case of *S. fuelleborni* infection, information on the frequency and significance of milk transmission of *S. stercoralis* would be of great value in studies on the epidemiology of the infection.

(8) There is need for studies on the distribution and prevalence of *Enterobius vermicularis* in many developing countries.

(9) Epidemiological investigations should be intensified on human taeniasis and cysticercosis, especially where human cysticercosis is prevalent or occurs in the apparent absence of *Taenia solium* taeniasis in the community.

(10) The information currently available on the distribution of trematodes, other than *Schistosoma,* is limited, as is knowledge of the real extent of morbidity caused by these helminths.

6.5 Diagnosis

The following needs were identified:

(1) Studies on parasite-specific antigens in faecal material of infected persons should be encouraged.

(2) The *Giardia* species complex requires better definition to assist diagnosis.

(3) The immunodiagnostic procedures for ascariasis need improvement through isolation of species-specific antigens, and studies are required on the diagnostic value of various phenomena of the immune response.

(4) Studies on various coprocultural techniques for detection of nematode larvae are required in order to improve their practical

applicability and assess their value in diagnosis of different intestinal infections.

(5) Simple diagnostic techniques need to be developed for the intestinal trematode infections.

7. RECOMMENDATIONS

With the aim of international promotion of research and control of intestinal parasitic infections, the Group agreed upon the following recommendations, dealing with basic science and clinical research, diagnostic and therapeutic tools, field studies and public health practice, and training of cadres.

7.1 Basic science and clinical research

(1) In many intestinal parasitic infections, the gaps in basic biological research greatly inhibit the understanding of the complex host-parasite-environment relationships and the effective control of these infections. The research needs, as identified by the Group in section 6 of the report, all require further promotion and more effective funding from international and national resources. In the control of intestinal parasitic infections, the development and implementation of new and better tools may be less costly than applying some of those at our disposal at the present time.

(2) In particular, clinical and pathological research plays an important role in achieving a better understanding of the mechanisms of the pathological processes responsible for parasitic intestinal diseases, as well as a more objective evaluation of the public health importance of intestinal infections; it may also have a serious impact on determining the priorities and selecting the most effective control measures against intestinal parasites. Since the present activities in clinical research of intestinal parasitic infections are still inadequate for the needs, the Group recommends: actively promoting interest in clinical research among the younger generation of clinicians; strengthening scientific cooperation between various specialists, including gastroenterologists, pathologists and parasitologists; improving the standards of research design and the accuracy of clinical and pathological documentation; and, finally, ensuring adequate funding of clinical research in proportion to basic biological research.

(3) The Group recommended that clinical and pathological studies should concentrate on: estimates of the proportion of persons with symptomatic intestinal infections (especially giardiasis, hymenolepiasis and strongyloidiasis) in different parts of the world; further investigations on physiopathological, immunological and genetic characteristics of patients with intensive, relapsing or repeated infections; clarification of the pathogenesis of the following complications of amoebiasis—post-dysenteric bowel syndromes in amoebiasis, persistent liver abscess, amoeboma, and parasitization of bowel carcinomas by *E. histolytica* trophozoites; and clinical and experimental studies on human pathogenesis of giardiasis, sarcocystosis, intestinal trematodiasis and strongyloidiasis.

(4) The Group also recommended that fatal parasitism should be better documented by clinical and postmortem studies in countries where intestinal parasites are endemic and not infrequently fatal.

7.2 Diagnostic tools

(1) The Group strongly emphasized the need to develop simple and more effective techniques for the diagnosis of intestinal protozoan diseases (e.g., faecally eliminated antigens) so that these can be used in nonparasitological laboratories, as well as to standardize and implement the most sensitive techniques for epidemiological diagnosis, especially that of giardiasis, strongyloidiasis and taeniasis.

(2) Fresh evaluation should be made of the role of serology in both the clinical diagnosis and the epidemiological surveys of several intestinal parasitic infections other than amoebiasis.

(3) The value of serology in the diagnosis of amoebic disease and in epidemiological investigations of amoebiasis has been clearly established, yet application of these techniques is not widespread. Two factors limit their wider use: (*a*) a steady decline in commercial production of *E. histolytica* antigen that has reduced its availability; (*b*) inability of many developing countries, in which the need is greatest, to finance the purchase of a commercial product still available. Therefore the Group recommends that interested governments should be encouraged to develop local centres for the production and distribution of this antigen; for the development of these centres there is a need for trained cadres capable of carrying out mass cultivation of *E. histolytica* under axenic conditions. Training is available at established institutions, located principally in developed countries.

7.3 Therapeutic tools

(1) A wide spectrum of drugs is available for the treatment of intestinal parasites but often their use is minimal. Innovative programmes for pricing, quality control, and local production facilities are needed.

(2) In spite of the considerable progress made in chemotherapy, attempts should be continued in order to develop better synthetic drugs which can be given in a single dose, which reliably eliminate multiple protozoan and helminth parasitic intestinal infections, and which are cheap.

(3) Strategies should be developed by WHO to evaluate in each parasitic infection and in multiple infections the most feasible chemotherapeutic delivery system—namely, mass chemotherapy, selective population chemotherapy, or targeted population chemotherapy.

(4) WHO should stimulate and support research on indigenous drugs against intestinal parasites, their pharmacology including mode of action and the active principles involved, their toxicology, and the possibilities of standardized local growth and production.

(5) The Group recommended studies on whether or not the intestinal parasites that are frequently found in man in the tropics prejudice or modify the bioavailability of certain drugs, or affect the complications of such drug therapy (e.g., by increased migration in the case of *Ascariasis).*

(6) Efforts should be made to determine why, in some patients, conventional therapy is ineffective. Clarification of the mechanisms of drug action may be useful in this respect, as may identification of the strains of parasite or concomitant conditions involved.

7.4 Field studies and public health practice

(1) Since there is no reliable information on the prevalence and incidence of intestinal parasitic infections in many countries, the Group recommended that surveys should be made on a national scale using valid sampling procedures and good parasitological methodology.

(2) Prevalence and incidence studies should be complemented by investigations that could establish to what degree these parasitic infections are causing disease in different areas of the world, and what their factual, medical and public health importance is in different countries. An aspect which needs special elucidation is the effect of parasitic infections on the nutrition of the affected populations.

(3) As information on local parasitic problems rarely appears in international scientific journals, it is recommended that, through the members of the WHO Expert Advisory Panels or by special programmes organized at the national level, appropriate information should be sent for publication in the *Weekly Epidemiological Record* or the *Bulletin of the World Health Organization,* or for issue in the WHO Amoebiasis or WHO Helminth series of documents.

(4) Control programmes for intestinal parasitic infections should be initiated at the local or national level and should be incorporated into the existing health programmes, such as primary health care, family health, diarrhoeal diseases control, essential drugs, and water and sanitation for all. The traditional educational and sanitary measures should be established, as well as community- based chemotherapy programmes that can be applied to selected populations, to specific parasitized groups or sometimes as a mass treatment plan. Control programmes must be permanent and should aim at raising the living standards of the affected populations if positive results are to be obtained.

(5) It is well established that faecal contamination of the soil is the main cause of the widespread distribution of several intestinal parasitic infections throughout the world. Studies should be made on human behavioural patterns and the sociocultural determinants involved which permit this faecal soil contamination, so that countries may be able to introduce more effective sanitation and health education programmes for the control of these infections.

(6) The information indicating the significance of the reservoir of parasitic eggs and larvae in the environment is inadequate in most of the endemic areas of the tropics and subtropics, e.g., there is need for a restudy of the distribution and persistence of infective-stage eggs of *Ascaris* in the soil in regions where the control of ascariasis is undertaken.

(7) Although intestinal parasitic infections are prevalent in the developing tropical countries, other more developed areas of the world are increasingly affected by local or exotic intestinal parasites such as *E. histolytica, Giardia, H. nana* and *Strongyloides.* Surveillance programmes should be established in order to detect these health problems in developed countries.

7.5 Training of cadres

(1) One of the most important aspects of the control of intestinal parasitic infections is the human factor: community involvement as

well as personal involvement and knowledge of intestinal parasitic diseases on the part of medical and paramedical and laboratory personnel. Teaching programmes on parasitology should therefore be included in the regular curricula of all medical schools; tuition should be both theoretical and practical.

(2) Regular training courses or seminars for both medical and paramedical personnel should be conducted in association with WHO, its regional offices, and individual countries.

(3) The production of modern teaching-learning aids should be encouraged because these play a fundamental role in effective training in parasitology; these teaching aids should be distributed at low cost where they are most needed, i.e., in schools and laboratories in the endemic areas.

ACKNOWLEDGEMENTS

The Group acknowledges with gratitude the special contributions made by the following persons, who provided background documents discussed during the meeting:

Dr J. W. Bier, Division of Microbiology, US Food and Drug Administration, Washington, DC, USA

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Professor B. von Bonsdorff, Helsinki, Finland

Dr H. J. Bos, Parasitology Research and Development, Intervet International B.V., Boxmeer, Netherlands

Dr H. Van den Bossche, Janssen Pharmaceutica, Research Laboratories, Beerse, Belgium

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Mr R. P. C. Holland, Medical Information Group, Imperial Chemical Industries Limited, Macclesfield, Cheshire, England

Dr G. J. Jackson, Division of Microbiology, US Food and Drug Administration, Washington, DC, USA

Dr I. G. Kagan, Center for Disease Control, Atlanta, GA, USA

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Annex 1

DIFFERENTIATION OF NEMATODE LARVAE IN COPROCULTURES: GUIDELINES FOR ROUTINE PRACTICE IN MEDICAL LABORATORIES[1]

The use of faecal cultivation for the routine detection of human hookworm infection was introduced more than 60 years ago (*8*); however, coprocultures were not widely used for the diagnosis of human intestinal nematode infections until Harada & Mori (*3, 4*) introduced a simple test-tube culture method. The Harada-Mori type of culture, which consists of faeces smeared on a filter-paper strip placed in a test tube containing a small amount of water, has mostly been used for determining the identity of the hookworm responsible for an infection in a person. Since the eggs of the two human hookworms, *Ancylostoma duodenale* and *Necator americanus,* are indistinguishable as they appear in the faeces, it is necessary to base identification on the differences between the filariform larvae of the two worms. This type of culture has also been used extensively in surveys to detect hookworm infections in human populations (*7, 16*).

Modifications of the Harada-Mori culture method have been described by several workers (*6, 16*). For details of the procedure used in this type of coproculture, the reader should refer to these papers as well as to the original paper of Harada & Mori (*4*).

The usefulness and reliability of any culture method depends upon the ability of the laboratory worker to recover and accurately identify the nematode larvae developing in the culture. Several investigators have published keys for the identification of nematode larvae in coprocultures (*2, 6, 16*) and these have been useful aids. The keys and drawings presented in this annex are partly based on those earlier ones, but they are also based on the studies of individual parasites made by a number of investigators (*1, 5, 9–12, 15, 17*).

Recovery of larvae from cultures

Cultures should be kept for a least 8 days at 24–29 °C to be sure that all larvae have sufficient time to reach the infective stage. If cultures have been kept at lower temperatures, examination should be delayed for 2 or more days.

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[1] Prepared by Dr M. D. Little—see section 3.4.3.

When examining a Harada-Mori type of culture, first withdraw the water in the bottom of the tube with a pipette and transfer it to a clean conical centrifuge tube (15 ml tubes preferably). Then add water (distilled or boiled and filtered to prevent addition of free-living nematodes) to completely immerse the filter-paper strip, since some types of larvae tend to remain on the portion of the filter-paper strip above the water level. After 2 or more hours, the filter-paper strip should be removed with forceps and discarded into a container of disinfectant. The water in this tube should then be transferred to one or more additional centrifuge tubes.

In order to concentrate any larvae present in these tubes, centrifuge and then discard the supernatant, leaving larvae in 0.5 ml or less of water at the bottom. If a centrifuge is not available, larvae may be concentrated by gravity sedimentation. Examine each tube separately.

Examination of larvae

The presence of living larvae in the sediment can be detected by using a hand lens (4× or greater), a dissecting microscope or a special inverted microscope (Ancyloscope of Sasa et al. (*16*)), and bright illumination from the side. If living larvae are observed, they should first be immobilized by immersing the bottom of the tube in water heated to 50–60 °C, or by adding a few millilitres of acetic acid (20 ml of glacial acetic acid in 80 ml of distilled water) to the suspension and mixing. If acetic acid is used, the larvae should again be concentrated by centrifugation. The use of iodine for killing the larvae is not recommended since it tends to overstain them and this obscures their internal structures.

A drop or two of the sediment containing the killed larvae is transferred to a microscope slide (a wide slide is preferred) and an appropriate-sized coverslip added. If the examination is likely to be prolonged, the edges of the coverslip may be sealed with clear fingernail polish or melted paraffin to prevent evaporation.

The slide should first be examined under the low magnification (about 100×) of a compound microscope, with the light reduced by closing the stage diaphragm as much as possible so that the refractile larvae can easily be observed. When a larva is found, it should be examined under high power (about 400×) to observe the distinguishing features indicated in the key presented below. A calibrated ocular micrometer is required for determining measurements of larvae.

Since more than one type of larva may be present in a culture and the larvae of one species may be relatively few in number, all larvae recovered should be examined. The presence of larvae of different types can often be detected when the preparation is examined under low magnification.

Key for the identification of filariform nematode larvae in human coprocultures (Annex Fig. 1 and 2)

1a. Oesophagus about one-half length of body; body slender (14–17 μm), lacking cuticular sheath; tip of tail not pointed, appearing notched ..... *Strongyloides (S. stercoralis* or *S. fuelleborni)*[a]

1b. Oesophagus about one-fourth of body length; cuticular sheath present; body thicker than 20 μm ..... see 2

2a. Intestinal lumen straight ..... see 3

2b. Intestinal lumen not straight but zigzagged ..... see 4

3a. Body (not including sheath) abouth 500–600 μm long; tail (anus to tip) less than 73 μm long (50–72 μm); intestine, at oesophagointestinal junction, as wide as oesophageal bulb; buccal "spears" conspicuous, parallel throughout length, about 15 μm long; conspicuous transverse striations present on sheath in tail region ..... *Necator americanus*

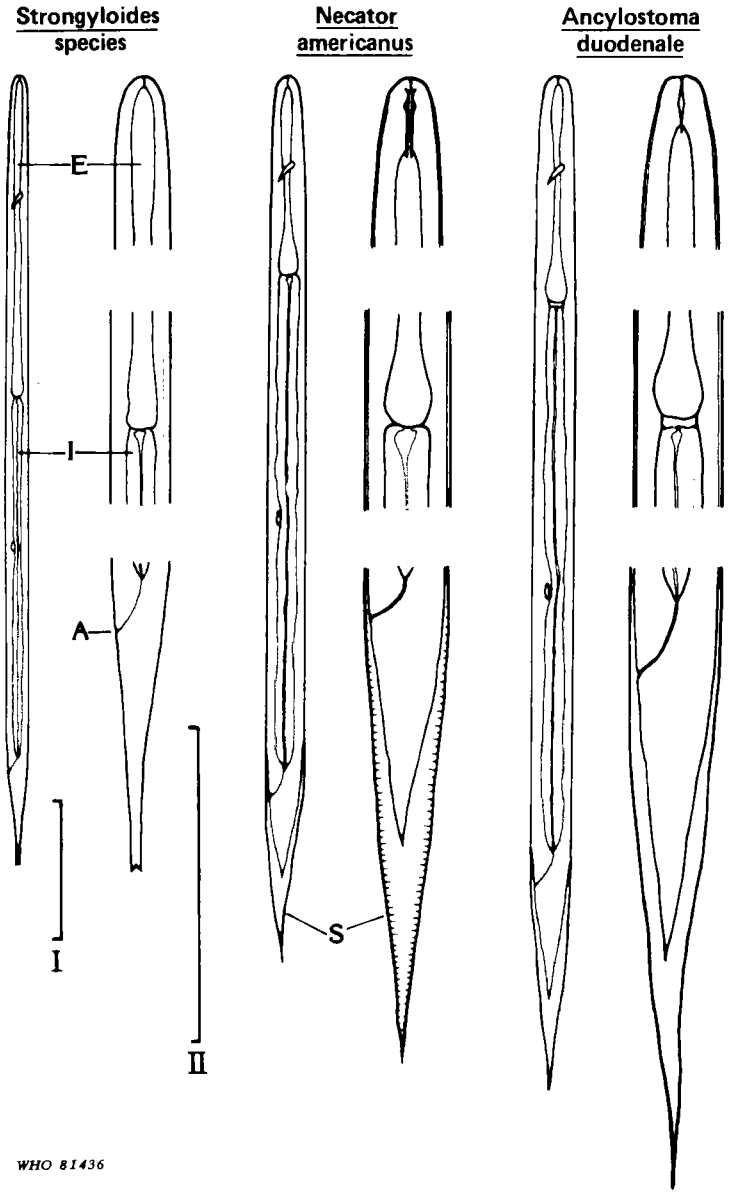
3b. Body (not including sheath) about 600–700 μm long; tail more than 73 μm long (75–94 μm); intestine, at oesophagointestinal junction, narrower than oeseophageal bulb; buccal "spears" inconspicuous, about 10 μm long; transverse striations on sheath in tail region inconspicuous ..... *Ancylostoma duodenale*

4a. Sheath relatively thin (thinner than cuticle of larva); pair of elongate sphincter cells present between oesophagus and first pair of intestinal cells; tip of larva's tail pointed; posterior end of sheath elongate, tapering to thread-like tip; body 630–730 μm long by 29–35 μm wide ..... *Ternidens deminutus*

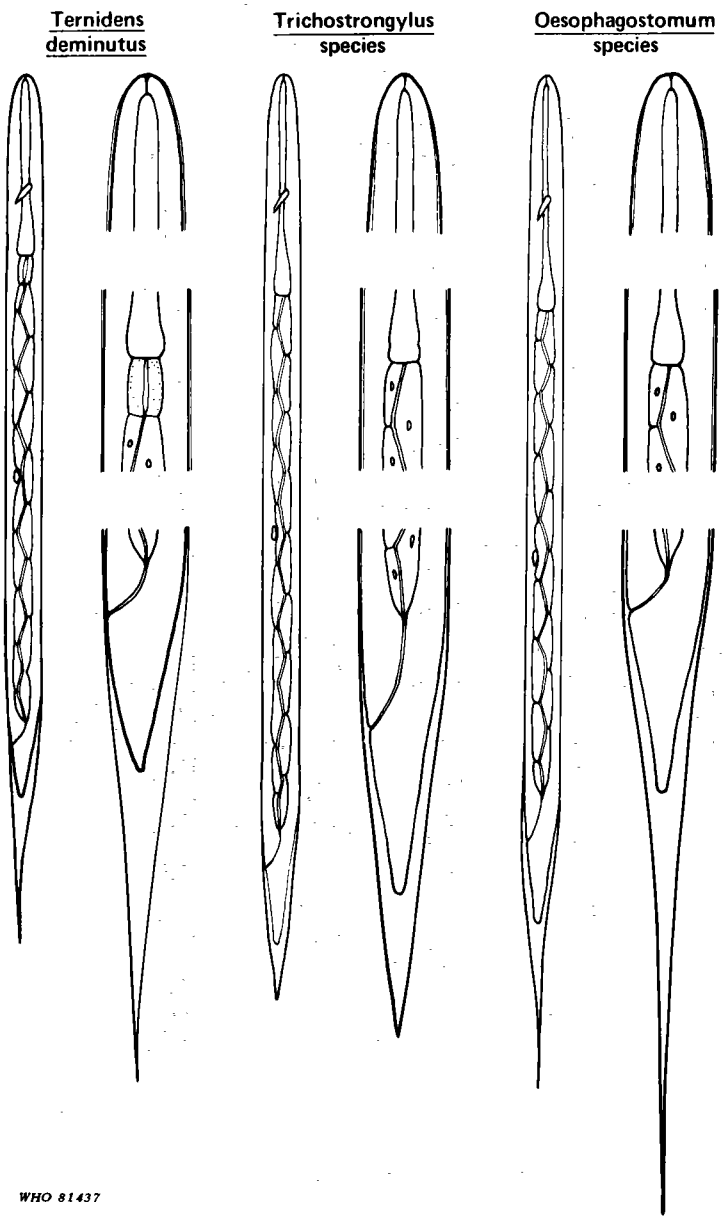
4b. Sheath relatively thick (thicker than cuticle of larva), no sphincter cells between oesophagus and intestine, tip of larva's tail rounded or blunt ..... see 5

[a] Filariform larvae of *Strongyloides stercoralis* and *S. fuelleborni* cannot easily be differentiated (*9*). Diagnosis must be made on the basis of stages occurring in fresh faeces (rhabditoid larvae in the case of *S. stercoralis,* and eggs containing a developing embryo in the case of *S. fuelleborni*), or on morphology of free-living female worms from culture (prominent constriction of body behind vulva in *S. fuelleborni;* body not markedly constricted in *S. stercoralis*) (*9, 14*).

Annex Fig. 1. Diagnostic features of filariform larvae found in human coprocultures (A = anus, E = oesophagus, I = intestine, S = sheath. The scales represent 100 μm: I, for intact larvae; II, for portions of larvae).



Annex Fig. 2. Diagnostic features of filariform larvae found in human coprocultures (same scales as given in Annex Fig. 1).



5a. Posterior end of sheath relatively short, not tapering to fine points (distance from tip of larva's tail to tip of sheath is less than distance from anus to tip of larva's tail) ..... *Trichostrongylus* sp.

5b. Posterior end of sheath relatively long, tapering to a fine point (distance from tip of larva's tail to tip of sheath is greater than distance from anus to tip of larva's tail) ... *Oesophagostomum* sp.

Comments

Adult and larval stages of free-living nematodes (*Rhabditis* spp., *Pelodera* spp., *Rhabditoides* spp., etc.) may occur in cultures of faeces if contaminated with soil, and must be differentiated from larvae of the human parasites.

It is possible that the first-stage larvae of *Angiostrongylus costaricensis* may occur in the faeces of some infected individuals and may appear in coprocultures. These larvae are 260–290 μm long and 14–15 μm wide, much smaller than any of the filariform larvae mentioned in the above key. See the paper of Morera (*13*) for morphological features of this larva.

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