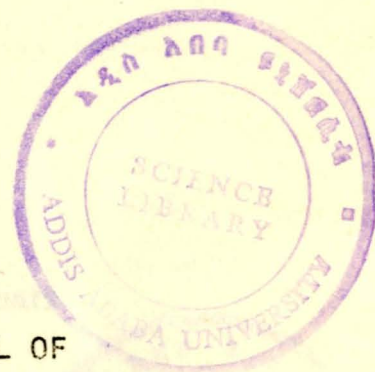


GASTROINTESTINAL INFECTIONS IN PATIENTS  
REPORTING TO MUNICIPALITY HEALTH CENTERS  
IN ADDIS ABABA



A THESIS PRESENTED TO THE SCHOOL OF  
GRADUATE STUDIES  
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By

TESFAYE KINESELASSIE

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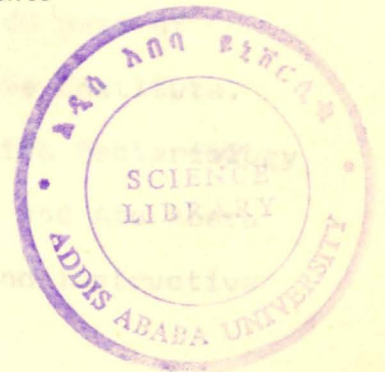
ADDIS ABABA

ADDIS ABABA UNIVERSITY  
SCHOOL OF GRADUATE STUDIES

Gastrointestinal Infections in Patients  
Reporting to Municipality Health  
Centers in Addis Ababa

by

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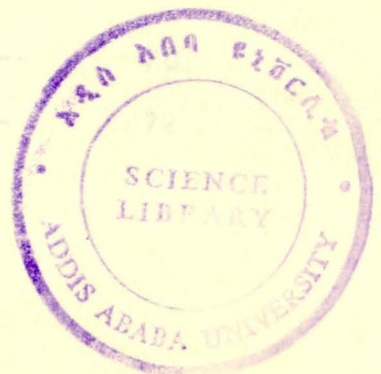


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Out of 552 patients investigated 416 (74.2%) harboured one or more gastrointestinal infectious agents. Among the 61.2% had single infections.

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In all multiple infections are more common than single infections. Out of the 235 patients with diarrhoea, 234 had parasitic and/or bacterial agents diagnosed; Among 56% had single infections while 34.6% had double, 8.1% triple and 1.3% had quadruple infections. In the single infections *E. histolytica* accounted for 51.8% and *G. lamblia* for 8.4%. Similarly, *A. lumbricoides* constituted 11.5%, *I. trichiura* 5.3% and *S. stercoralis* 4.6%. Likewise *Shigella* was associated with 5.9% and *Salmonella* with 1.3%.

Abstract

Out of 552 patients investigated 410 (74.2%) harboured one or more gastrointestinal infectious agents. Among these 61.2% had single infections. Of the single infections, the parasites were dominant i.e. E. histolytica accounted for 53.8%, Ascaris lumbricoides for 19.1%, T. trichiura for 19%, G. lamblia for 5.2%, Strongyloides stercoralis for 4% and hookworm for 3.5%. Bacterial infections constituted small proportions i.e. Shigella sp 3.5% and Salmonella typhi 0.8%. Estimation of intensity of infection involving the intestinal helminths showed A. lumbricoides and T. trichiura to be light infections, while hookworm infections were heavy. Among the multiple infections 31.5% were double, 6.6% triple and 0.7% quadruple. Most frequent among the double infections were those with E. histolytica and A. lumbricoides (30.2%), T. trichiura and E. histolytica (17.1%), A. lumbricoides and T. trichiura (14.7%) and E. histolytica and Shigella sp. (12.4%). Similarly, 70.4% of the triple infections were combinations of E. histolytica, A. lumbricoides and T. trichiura. In all, multiple infections are more common than single infections. Out of the 285 patients with diarrhea, 234 (82.1%) had parasitic and/or bacterial agents diagnosed. Among these 56% had single infections while 34.6% had double, 8.1% had triple and 1.3% had quadruple infections. In the single infections E. histolytica accounted for 61.8% and G. lamblia for 8.4%. Similarly, A. lumbricoides constituted 11.5%, T. trichiura 5.3% and S. stercoralis 4.6%. Likewise Shigella was associated with 6.9% and Salmonella with 1.5%

Introduction

of the cases. Most common combinations of the double infections were E. histolytica and A. lumbricoides (30.9%), E. histolytica and Shigella (19.8%) and E. histolytica and T. trichiura (16%). Furthermore, in 84% of the diarrheal cases with multiple infections, E. histolytica occurred in combination with one or more agents. A general tendency for the proportion of patients with below normal levels of haematocrit and total serum protein appeared to increase as the number of types of infectious agents in the same individual increased.

Reduced economic development, where there also exist a greater reservoir of enteropathogens and a larger susceptible population with nutritional deficiencies, wherever there exists a high prevalence of gastrointestinal infections, living conditions are characteristically poor. These gastrointestinal infections represent a large and serious public health problem in the developing countries, particularly in the tropical regions (WHO, 1982). The impact of these infections on health conditions, particularly on economic development, as well as on the working capacity, is reflected on the social costs of medical care, which are considerable setbacks to social welfare and economic development.

The study of parasitic and bacterial infections of the gastrointestinal system includes the study of the impact of these infections on health conditions, particularly on economic development, as well as on the working capacity, is reflected on the social costs of medical care, which are considerable setbacks to social welfare and economic development.

## Introduction

Gastrointestinal infections constitute an important public health problem to the majority of the world's population. They primarily affect children, the aged and the malnourished. The global prevalence and intensity of human gastrointestinal infections show considerable variations in distribution and seasonal occurrence, because of geographical and climatic factors and human activities. The magnitude of the problem is profound in areas of the world with reduced economic development, where there also exist a greater reservoir of enteropathogens and a larger susceptible population with nutritional deficiencies. Wherever there exists a high prevalence of gastrointestinal infections, living conditions are characteristically poor. Thus gastrointestinal infections represent a large and serious medical and public health problem in the developing countries, particularly in the tropical regions (WHO, 1987a). The impact of these infections on health conditions, particularly on children's growth and development, as well as on the working capacity of adults and on the social costs of medical assistance create great set backs to social welfare and progress in these areas.

most  
The important parasitic and bacterial infections of the gastrointestinal system include Entamoeba histolytica, Giardia lamblia, Ascaris lumbricoides, Trichuris trichiura,

Strongyloides stercoralis and hookworms among the parasites (WHO, 1987a) and Shigella and Salmonella species (WHO, 1980b) among the bacteria.

Human infections with E. histolytica have a world wide distribution (Martinez-Palomo, 1987) although the prevalence is highest in the tropics and subtropics. Humans are the main reservoir and source of infection, although some other primates can be infected (DuPont, & Pickering 1980a). The prevalence of human infection varies greatly, ranging from as low as 2% to as high as 60% in many areas (WHO 1980a). Amoebiasis is a major health problem in certain parts of Africa, Asia and Latin America, where inadequate sanitary conditions and the presence of highly virulent strains of E. histolytica may combine to sustain a high incidence of intestinal and hepatic infections. In Ethiopia a wide distribution of E. histolytica was reported by several investigators (Torrey, 1966; Lemma et al., 1968; McConnel and Armstrong, 1976), who recorded prevalence rates ranging from 3% to 55%.

On a global scale E. histolytica infections are the third commonest parasitic cause of death (Martinez-Palomo, 1987). Walsh, (see, Martinez-Palomo, 1987) estimated that in 1981 probably 480 million people globally, carried E. histolytica in their intestinal tracts, 34-50 million developed invasive forms severe enough to disable them for

several days and 40,000-100,000 died that year as a consequence of the infection. In countries where amebiasis is an important health problem, the majority (approximately 90%) of individuals with colonic E. histolytica infections are carriers, while the remainder have invasive intestinal amebiasis (WHO, 1987a).

Transmission of E. histolytica is by the faecaloral route. Food or drink contaminated with faeces containing cysts of E. histolytica is a common source of infection (Beaver, et al., 1984). Food or drink may become contaminated with cysts of E. histolytica due to a polluted water supply, unclean handling by infected individuals, droppings of flies and use of human excrement as fertilizer in vegetable gardens.

Excystation of the ingested cyst occurs in the lower ileum and colon. The motile trophozoite of E. histolytica lives in the lumen of the large intestine, where it multiplies and eventually differentiates into cysts which are in the faeces and are responsible for transmission of infection. Two forms of amebiasis are recognized: luminal amebiasis, where no clinical signs and symptoms are apparent, and invasive amebiasis where the trophozoites invade the intestinal mucosa to produce dysentery or ameboma, and can spread in the blood to give extra-intestinal lesions such as liver abscess (Martinez-Palomo,

1987). Invasive amebiasis frequently causes an exudative type of diarrhea, due to inflammation and ulceration (WHO, 1980a), the result of which is an outpouring of serum proteins, blood and mucus into the lumen of the bowel. Invasive amebiasis is thus clinically characterized by acute dysentery with bloody mucoid stools, Colicky pain and rectal tenesmus. Although all ages are susceptible, including infants and the elderly, the age groups with the highest incidence of infection and clinical symptomatology are the third through the fifth decades (DuPont & Pickering 1980).

Giardiasis is one of the most commonly diagnosed intestinal protozoan infections. Although the percentage of asymptomatic infections is high, giardiasis is a frequent cause of diarrhea.

The causative agent, Giardia lamblia, has a worldwide distribution (DuPont and Pickering 1980b). Globally, prevalence rates range from less than 1% to more than 50%, depending on the geographical location and the prevailing mode of transmission (WHO, 1987a). It has been estimated that about 200 million infections occur per year in Africa, Asia and Latin America (WHO 1987a). In Ethiopia, prevalence rates ranging from 3% to 23% have been reported (McConnell and Armstrong 1976). The infection is, typically, more prevalent in children than in adults and more frequently affects children of large families.

Transmission of G. lamblia is by viable cysts, that are swallowed together with contaminated food and water (WHO, 1987a). However, in some circumstances it is more likely that intimate contact of an infected individual with an uninfected individual is the usual mechanism (Beaver et al., 1984). Giardia lamblia cysts survive for two months in water and are resistant to routine chlorination. After ingestion, excystation takes place in the upper small intestine and trophozoites are released. In the infected person, the trophozoites repeatedly multiply and line the small intestinal mucosa. Trophozoites do not invade the tissues but feed on mucus secretions. The intimate contact of the sucking disc with enterocytes can damage the microvilli and fuzzy coat and reduce the activity of the brush border enzymes (WHO, 1980a). The most severe pathological conditions in patients with giardiasis is malabsorption, which results from direct toxic effects of the parasite on the small intestine enhanced bacterial colonization and general protein-energy and folate deficiency (WHO, 1981). Disaccharidase deficiency and defects of vitamin B<sub>12</sub> have also been described in patients with giardiasis (DuPont and Pickering, 1980b). In developing parts of the world, malnutrition contributes to the pathogenesis of giardiasis, and improvement of the nutritional status usually corrects the associated pathogenesis.

Gastrointestinal helminths constitute one of the most common and important parasitic agents of mankind and are responsible for much of the morbidity and some mortality. Not only are they common and widespread in populations throughout the world, but, they are frequently chronic and occur throughout the life-time of an individual (Wakelin, 1985).

Helminth infections of the human gastrointestinal tract are annually responsible for 70,000 to 100,000 deaths throughout the world (Walsh and Warren, 1979). Of these, the intestinal nematodes, Ascaris lumbricoides, hookworms (Ancylostoma duodenale and Necator americanus) and Trichuris trichiura are the most common. Estimates of the global prevalence of intestinal soil transmitted nematode infections are 1000 million cases for A. lumbricoides, 900 million for hookworms and 500 million for T. trichiura (WHO, 1987a).

Nutritional impairment is often associated with chronic intestinal helminthiasis that cause protein-energy malnutrition, iron-deficiency anaemia and vitamin A deficiency. The direct host food consumption by the parasite is not likely to be of significance nutritionally. However, if the host is feeding on an inadequate diet, any loss of food to the parasite must contribute to malnutrition (Crompton, 1986).

The occurrence of multiple infection in the same individual with different intestinal nematodes is common and individuals who are predisposed to heavy infection with one species of intestinal nematode are also more likely, on average, to harbour heavy burdens of other species present in a community (Haswell-Elkins, et al., 1987). This partly is due to the gastrointestinal helminths exploitation of the feeding behaviour of their hosts.

Ascaris lumbricoides occurs throughout the world with the highest prevalence in the developing parts. As the most prevalent human parasite in the world it is believed to infect 1000 million people globally, of which about 155 million cases are in Africa (Crompton and Tulley, 1987). In Ethiopia, Sioum et al., (1981) reported prevalence rates ranging from 12.1% to 65.1% in children in Addis Ababa, while Lemma et al. (1968) recorded prevalence rates of 52.7% and 23.3% at Addis Ababa and Debrezeit, respectively. Shibru and Teklemariam (1986) reported infection rates of 43.7% in the different administrative regions.

A. lumbricoides is transmitted by ingestion of infective eggs from contaminated food or water (Cook, 1986). An adult female residing in the gut of the infected person produces, on average, about 240 000

eggs per day (WHO, 1981), which counterbalances the heavy losses in viability and infectivity of these eggs in the environment. The eggs develop in the soil within 2-3 weeks under optimal temperature, oxygen and moisture conditions. Eggs in clay or heavy loam soil, are protected against desiccation and freezing and from heat and the direct rays of the sun, remain infective for several months or possibly a few years (Beaver et al., 1984). The level of transmission of A. lumbricoides from the soil to man depends more on socio-economic factors than on physical ones. The main factors seem to be high density of the human population, illiteracy and poor sanitation (WHO, 1981), particularly when people defecate indiscriminately around human settlements.

The host/parasite relationship in ascariasis is characterized by a high degree of tolerance, unless the infection is heavy or the intake of nutrients by the host is inadequate. The pathogenic effects are mainly due to immune reactions of the host, mechanical effects of the adult worms and the effects of adult worms on the host's nutrition.

Ascariasis is associated with significant nutritional impairment, which is more or less proportional to the worm burden. Infections with less than 5000 eggs per

gram (epg) of faeces is known to be light, while infections with over 50,000 epg represent heavy infections (WHO, 1987a). The presence of the adult stages of A. lumbricoides in the human small intestine is associated with impaired nitrogen balance, accelerated mouth to caecum transit time, lactose maldigestion and reduced absorption of vitamin A (Crompton, 1986; WHO, 1987a).

Trichuris trichiura is an intestinal nematode and belongs to the group of soil-transmitted helminths. T. trichiura is present throughout the world, but is more abundant in the humid tropics. In some tropical countries the prevalence rate is over 90%. In others it is frequently between 30% and 60% (WHO, 1981). It is found almost in every community in Ethiopia (McConnell & Armstrong, 1976); Sioum et al., 1981; Tesfamichael, 1983).

T. trichiura has a simple life-cycle, with the egg serving as the infective stage. The adult worms survive firmly attached to the epithelial lining of the large intestine, with the caecum being the most affected region. Each female worm is estimated to produce from about 2000 to 14000 eggs per day (WHO, 1987a) and these leave the host in the stools and contaminate the human environment. Under suitable conditions, infective larvae develop inside the eggs in about 3 weeks and some may retain their viability

for months. T. trichiura most frequently infects people between 5 and 15 years of age (Cook, 1986).

The morbidity associated with trichuriasis is due to the worm's mode of attachment to the wall of the large intestine. Each worm has a thin anterior part with which it burrows into the intestinal wall, where it feeds on the intestinal tissues. This feeding activity of the adult worms is known to cause faecal blood loss from the host at the rate of about 0.005ml per worm per day (Crompton, 1986).

Pathology due to Trichuris is a function of the intensity and chronicity of infection. With heavy infections bloody diarrhea with hypochromic anaemia, hypoalbuminaemia, anorexia, abdominal discomfort and weight loss are reported (Crompton, 1986). Diarrhea is assumed to be due to impaired water reabsorption in the colon, resulting from the lesions caused by parasite attachment. Chronic impairment of the host's nutrition status is suggested when diarrhea, hypoalbuminaemia and iron deficiency are observed in association with the presence of the parasite (WHO, 1987a).

Trichuriasis is frequently found to occur concurrently with Ascaris and hookworm (Buck et al., 1978b). The association of dysentery with trichuriasis does not of itself show that trichuriasis directly causes the colitis. Associated amebiasis and bacterial dysentery may be

important too (Gilman et al., 1983).

The adult stages of the blood sucking nematodes, Ancylostoma duodenale and Necator americanus, are found attached to the mucosa of the small intestine of people living in the tropical and subtropical countries. Both species are found in Ethiopia (Armstrong and Tadesse, 1975). Shibru and Leykun (1985) studied the distribution of hookworm in various regions of Ethiopia. Both species were found in localities with sandy clay loam, or sandy loam soils, but only Necator was found on more sandy soils. Infection rates tended to decrease with decreased altitude.

The life cycle of hook worm is direct and begins with the eggs being released by the female worms into the lumen of the small intestine and passed outside in the faeces. One female A. duodenale produces about 3000 eggs, and one female N. americanus about 9000 eggs, per day (WHO, 1981). The embryo within the eggs develop rapidly under optimal moisture, warmth and oxygen. Skin penetrating third-stage larvae are formed within 5-10 days after the deposition of the eggs (WHO, 1987a). Infection occurs when larvae enter the body through the skin, most commonly through the feet.

The pathogenicity of hookworms is closely related to their mode of feeding (WHO, 1987a). They attach

themselves to the villi, which are sucked into the worm's buccal cavity, causing microscopic blood and serum protein loss, but not significant enterocyte damage (Cook, 1986). Thus the clinical picture in advanced disease is dominated by iron-deficiency microcytic anaemia and hypoalbuminaemia (WHO, 1981). Rate of blood loss for N. americanus and A. duodenale is of the order of 0.03 to 0.05ml and 0.16 to 0.34 ml per worm per day respectively (WHO, 1981). Clearly, the degree of anaemia is dependent on dietary iron content, body iron reserves and intensity and duration of infection (Cook, 1986). Hookworm infection must, therefore, be considered an important factor in the aetiology of tropical iron deficiency anaemia and this has implications for young children, pregnant women and the health and productivity of adults whose livelihood and contribution to the economy depend on hard work.

Strongyloides stercoralis is a soil-transmitted intestinal nematode causing infection in the human duodenum and jejunum. Man is the main host of S. stercoralis, but the parasite has also been reported from dogs and apes (WHO, 1980a). S. stercoralis is widely distributed in the tropics and subtropics in areas of poor sanitation. In Ethiopia it is commonly found in the same geographic areas as hookworms. S. stercoralis infections were reported

from 41 out of 50 study communities in the central plateau and prevalence ranged from 3% to 44% (McConnell and Armstrong, 1976).

Infection occurs when third-stage larvae, which have developed in the soil contaminated by human faeces, penetrate the skin. Parthenogenetic adult females develop and live in the epithelium of the jejunal mucosa. The females release eggs from which larvae emerge while still in the small intestine. Larvae reach the external environment and develop into infective third-stage larvae. Sometimes the larvae become infective before they are passed out, leading to autoinfection (Beaver et al., 1984).

Disease symptoms due to S. stercoralis include diarrhea alternating with constipation, weight loss, anorexia and vomiting (Onile et al., 1985). Malabsorption and associated lesions in the mucosa of the small intestine may also occur (WHO, 1987a).

The endemicity of Schistosoma mansoni in Ethiopia is now well established. All administrative regions except Bale, have one or more endemic foci of S. mansoni (Teklemariam, 1982); the four northern regions of Eritrea, Tigray, Gonder and Wello being the most affected (Lo et al., 1988), with prevalence rates of 10% and higher.

The transmission of S. mansoni depends on the sanitary disposal of faeces, the availability of the appropriate intermediate hosts and the exposure of man to the infective stage. The adult worms live in the veins of the small intestine and lay eggs. The extruded ova and the effects of the young and adult worms are responsible for the pathological changes associated with schistosomiasis.

Gastrointestinal helminthic infections also include cestodes - Taenia saginata, Taenia solium, Diphyllobothrium latum, acquired by ingesting raw or uncooked meat or fish and Hymenolepis nana, transmitted by ingesting eggs eliminated in the faeces of the infected person.

Among the bacterial gastrointestinal pathogens are members of the genus Shigella. This genus is composed of gram-negative, non-motile bacteria that belong to the family Enterobacteriaceae and the tribe Escherichiae. The genus is subdivided into four subgenera or subgroup, S. dysenteriae, S. flexneri, S. boydii and S. sonnei, according to their biochemical reactions (Edwards and Ewing, 1972) Shigellae are aerobic, or facultatively anaerobic, and their optimal temperature for growth is 37°C. With the exception of certain biotypes of S. flexneri, visible gas is not formed from fermentable carbohydrate

According to Edwards and Ewing (1972), members of the genus Shigella do not produce hydrogen sulfide in triple sugar iron (TSI) agar nor in media that possess a similar level of sensitivity. They do not grow<sup>on</sup> Simmon's citrate agar, do not hydrolyze urea and do not decarboxylate lysine. They fail to produce acid from salicin, adonitol or inositol. Only S. sonnei, and cultures of S. boydii 13, are known to possess an ornithine decarboxylase system. Strains of S. sonnei ferment lactose upon extended incubation, but other species do not utilize this substrate in conventional media.

According to WHO (1987b) dysentery and diarrhea caused by Shigella have a global distribution and are major public health problems, with high morbidity and substantial mortality in most developing countries. Shigellosis has been reported from hot and humid tropics to the arctic climates (Rahama, 1984)

In Ethiopia a number of studies on the genus Shigella have been made. The common serogroups were identified (Afeworki and Yetnebersh, 1980; Mesele and Alebachew, 1982). These studies found S. flexneri to be the most frequently isolated species. S. dysenteriae is second in frequency of isolation, followed by S. boydii and S. sonnei. Serogroup distribution in urban and rural areas is comparable for S. flexneri, S. dysenteriae

and S. sonnei, while S. boydii is more common in urban centers (Afeworki and Eyasu, 1984). The predominance of S. flexneri in developing countries is well known (WHO, 1980b; Rahama, 1984). Likewise, the prominence of S. flexneri in Ethiopia has been confirmed (Afeworki and Yetnebersh, 1980; Mesele and Alebachew, 1982; Afeworki and Eyasu, 1984) and this indicates that S. flexneri is the predominant aetiological agent of Shigellosis in Addis Ababa and in rural areas.

Infection is by the faecal-oral route and the most common mode of spread is by person-to-person transmission, owing to the low infection dose, 10-100 organisms (WHO, 1980b). Endemic Shigellosis is closely associated with poverty, overcrowding, poor sanitation, water sources inadequate in quantity and quality, and malnutrition. The incidence of infection will, therefore, vary with the level of sanitation.

With the advent of economic prosperity, and the consequent improvement of water supplies and sanitation, Shigellosis has ceased to be a threat in industrialized countries. However, even in these countries, occasional outbreaks still occur, mostly caused by S. sonnei. Shigellosis caused by S. flexneri is often encountered in less-developed countries with unclean water supplies and poor sanitation (Rahama, 1984).

Probably the most important property of Shigella species that determines their virulence is their ability to penetrate epithelial cells (Dupont et al., 1972). This process leads to epithelial cell death, mucosal inflammation and epithelial ulceration and haemorrhage (WHO, 1987). Enterotoxin is now believed to be a second factor in virulence (WHO, 1980b), causing secretory diarrhea without histological damage and marked inflammatory cell migration in the lamina propria, epithelial cell death, microulcer formation and exudation of leucocytes into the bowel lumen.

The clinical manifestations of Shigellosis generally include frequent passage of stools containing blood and mucus, fever, abdominal pain and tenesmus (WHO 1987b). The illness usually starts with watery diarrhea followed after 24-48 hours, by the appearance of blood and mucus in the stools. Loss of plasma protein through the gut, leading to hypoproteinaemia, is a common feature of acute shigellosis (Rahama, 1984). Association of malnutrition and shigellosis is frequently seen in developing countries.

The genus Salmonella is composed of motile, gram-negative bacteria that belong to the family Enterobacteriaceae and tribe Salmonellae. Salmonellae grow readily on ordinary media, since they are not fastidious, but do not ferment lactose, sucrose or salicin. Lysine, arginine and ornithine are decarboxylated. They form acid, and usually gas, from glucose, maltose and mannitol (Edwards and Ewing, 1972).

In general, salmonellae can be differentiated from other Enterobacteriaceae by virtue of their indole negativity, inability to ferment lactose and urea and production of  $H_2S$  in triple sugar iron agar or Kligler's iron agar. All salmonellae produce an alkaline slant and acid butt. Salmonella species other than S. typhi produce gas in triple sugar iron agar and are much more likely to produce  $H_2S$  than S. typhi.

The three species recognized in the genus Salmonella are S. choleraesuis, S. typhi and S. enteritidis (Edwards and Ewing, 1972). About 2000 serotypes are known (WHO, 1980b). These serotypes can infect a wide range of warm-and cold-blooded animals. The primary reservoir for salmonellae are the intestinal tracts of many animals, including birds, farm animals and reptiles (Volk, 1978). Humans become infected through the ingestion of contaminated water or food. Water becomes polluted by the introduction of faeces from any animal excreting Salmonellae. Infection via food usually results either from ingestion of contaminated meat or via hands which act as intermediates in the transfer of salmonellae from an infected source. Because humans can become asymptomatic carriers of salmonellae, infected food handlers are responsible for the spread of these organisms.

The general types of infections which may be caused by salmonellae usually are grouped into three categories: enteric fever, septicaemia and gastroenteritis. Gastroenteritis is the most common type of Salmonella infection. Salmonella gastroenteritis is a common problem in all areas of the world, regardless of economic development. It has been an even greater problem in developed countries, where, mass production and distribution of food products has occurred. Among the non-typhi Salmonella species S. concord and S. typhimurium have been reported in Ethiopia (Afeworki, 1985). Gastroenteritis may be caused by any one of the thousand serotypes of Salmonella (Volk, 1978) and it is characterized by the fact that organisms remain localized in the gut. On average, symptoms occur 10 to 28 hours after ingesting contaminated food and headache, abdominal pain, nausea, vomiting and diarrhea may continue for two or four days (Volk, 1978).

In general, gastrointestinal infections are often associated with poverty, illiteracy, poor sanitation and high risks of exposure to environmental hazards. These factors are also essential determinants in the epidemiology of a variety of infections, with quite different aetiologies. Such conditions are conducive for the occurrence of multiple infections in people. In many regions of the world, individuals are found

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In general, gastrointestinal infections are often associated with poverty, illiteracy, poor sanitation and high risks of exposure to environmental hazards. These factors are also essential determinants in the epidemiology of a variety of infections, with quite different aetiologies. Such conditions are conducive for the occurrence of multiple infections in people. In many regions of the world, individuals are found

to harbour different species of helminth parasites concomitantly. This is especially true for the intestinal nematodes, Ascaris, Trichuris, hookworms and Enterobius (Haswell Elkins et al., 1987). Ascaris, hookworm infections and trichuriasis commonly occur as multiple species infections in the "Third World" (Buck et al., 1978b; Cook, 1986). For example, about 85% of the population of two Somali communities were found to harbour soil-transmitted intestinal nematodes and/or protozoa (Iladi et al., 1987), and of these 74% had mixed infections; the most common combination being Trichuris trichiura and hookworm.

The occurrence of multiple infections in the same individual is not restricted only to intestinal helminths. Several workers reported the occurrence of concomitant intestinal protozoal infections with nematodes and/or enteropathogenic bacteria. Giardia lamblia, E. histolytica T. trichiura, hookworm and Strongyloides are protozoan and helminth parasites most commonly found in association with Ascaris lumbricoides infections in African people (Crompton and Tulley, 1987). Concurrent infections with T. trichiura and E. histolytica, and coinfection with Shigella and Salmonella, appear to significantly increase in patients with heavy T. trichiura infection (Gilman et al., 1976). Up to four pathogens (including

intestinal protozoa, nematodes and enteropathogenic bacteria) were isolated from diarrheal patients in Bangladesh (Moyenudin et al., 1987).

Evidence indicating interactions between the effects of the pathogenic agents also exist. Some of the interaction between the infectious agents are found to lessen the effects of particular infectious agents, while others tend to enhance its pathogenicity. In ascariasis and malaria-endemic areas, children heavily infected with A. lumbricoides were found to be free from malaria, whereas children treated for ascariasis developed attacks of malaria (Murray et al., 1978). Coexistence of amoebic infection and Shigellosis frequently occurs in the tropics (WHO, 1981) and in some patients, conditions aggravate each other to produce a mixed colitis characterized by copious diarrhea, high fever and pronounced dehydration. A direct role for Salmonella in aggravating the pathogenesis of glomerulonephritis in patients with concomitant Salmonella and Schistosoma mansoni infections has been described (Lamberticci et al., 1988).

Evidence for interactions between the infectious agents in multiple infections also exist from experimental studies in animal models. Laboratory animal studies have revealed complex interactions between parasites and also

between parasitic protozoa and bacteria. In general, infections with many parasites is accompanied by a period of immunodepression during which superimposed infections are favored (Cox, 1987). On the other hand, organisms that activate macrophages may protect the host against subsequent infection with other agents. For example, Trypanosoma musculi infections are enhanced in mice concomitantly infected with Trichinella spiralis, as measured by reduced expulsion of adult worms from the gut and increased larval worm burdens in the muscles (Bell et al., 1984). T. musculi infections are also considerably enhanced in mice with concurrent infections of the malaria parasite, Plasmodium yoelli. It has also been shown that immunodepression during P. yoelli infections coincides with peak parasitaemia and lasts for about 10 days (Cox, 1975). Concomitant infections in mice with P. berghei and Salmonella typhimurium have been found more fatal than either infection alone (Kaye et al., 1965) and it appeared that the decreased survival time was related to the enhancement of malaria by the bacterial infection.

Multiple infection as a widespread and frequent phenomenon also appears to occur in Ethiopia. Shibru et al., (1982) found up to five intestinal helminth parasites per individual in age groups below 20 years.

Similarly, Kloos et al., (1980) reported multiple infections mostly with two and three, but occasionally with four parasites, concurrently occurring in the same individual in farm labour populations of the Awash Valley irrigation schemes. Infections with hookworm and Schistosoma mansoni, Ascaris lumbricoides and Trichuris trichiura were the most common combinations. Survey of intestinal helminthiasis in Zway, Central Ethiopia, indicated double and triple infections and most of these multiple infections consisted of A. lumbricoides and T. trichiura (Tesfamichael, 1983).

One apparent manifestation of gastrointestinal infections is diarrhea. Because of its geographic ubiquity and multiplicity of aetiologies, diarrhea has been recognized as one of the major causes of morbidity and mortality in the developing countries. Diarrhea can be caused by a number of bacterial, viral and/or parasitic pathogens (Sen et al.; 1983). Among the bacterial and viral agents Shigella, Salmonella, Escherichia coli, Campylobacter jejuni and Rotavirus are the most important (WHO, 1987b).

Parasite-related diarrheas of public health importance are primarily amebiasis, giardiasis trichuriasis, Strongyloidiasis, balantidiasis, schistosomiasis and capillariasis. Among these amebiasis, giardiasis,

trichuriasis and strongyloidiasis are infections with a global distribution, in which diarrhea is a common symptom (WHO, 1980a). In addition, although it is not a characteristic symptom, diarrhea may occur in severe hookworm disease, ascariasis and some other intestinal nematodes and cestodes,

There is not adequate information on the aetiology of diarrheal infections in Ethiopia, especially in the adult population. In a hospital-based study Mesele and Alebachew (1981) isolated Salmonella typhi from a few stool specimens. Similarly Afeworki (1985), in his study to determine the prevalent Salmonella species in Ethiopia, found S. typhi the predominant species and among the non-typhi salmonellae, S. concord and S. typhimurium predominated.

The frequency of isolation of Shigella species in Addis Ababa was reported by several workers (Afeworki and Yetnebersh, 1980. Mesele and Alebachew, 1982). These studies found S. flexneri to be the most frequent isolate. S. dysenteriae was second in frequency of isolation, followed by S. boydii and S. sonnei. Mogesie (1983) isolated thirty-five strains of Shigella (9%) and Salmonella (4.5%) from one thousand diarrheal specimens from adult out-patients in Addis Ababa hospitals and clinics. Based on this, he concluded

that Shigella and Salmonella obviously are not the only aetiologic agents of diarrhea in the adult population, as these organisms were not involved in 86% of the cases.

Among the several species of intestinal tract protozoa only Entameba histolytica, Giardia lamblia and Balantidium coli are incriminated as pathogens causing diarrhea. The first two species are commonly found in Ethiopia and the third only rarely encountered (McConnell and Armstrong, 1976).

In Ethiopia, a wide distribution of E. histolytica is recorded. In one report, it was encountered in 55% of the Saysay people in the Blue Nile Gorge in Wellega (Torrey, 1966). In a survey of 50 communities in five administrative regions in the central plateau, the parasite was found in 94% of the communities (McConnell and Armstrong, 1976), whereas it was found in only 0.5% among 468 Addis Ababa school children and among 2.2% of 90 Debre Zeit School children (Lemma et al., 1968).

The agent of giardiasis, G. lamblia, is also widespread in Ethiopia, although with varying levels of prevalence. In a survey of 50 rural communities on the central plateau, giardiasis was encountered in 98% of the communities investigated, with prevalence

rates ranging from 3% to 23% (McConnell and Armstrong, 1976). In Addis Ababa out of 468 school age children 8.9% were found harbouring the parasite (Lemma et al., 1968).

Infectious agents in which diarrhea is a major symptom are, therefore, widely distributed in Ethiopia. However, whether infectious diarrhea in the adult population is predominantly of parasitic, bacterial or of joint origin, is not well established.

Although a few reports are available, especially on intestinal helminths, multiple infections by gastrointestinal infectious agents has not been dealt in any detail in Ethiopia.

In addition to their disease effects on the health of people, multiple infections also enhance diagnostic problems which are responsible for gross errors of reporting, leaving many of the infectious agents unrecognized and lumped together with other ill-defined conditions. This may also entail problems of prescribing toxic drugs that do not serve useful purposes.

The purpose of this study, therefore is:

1. to define the types of combinations of infectious agents that may be concurrently present in the adult

population in Addis Ababa.

2. to elucidate the effects of some of the interactions of infectious agents on the health of the people.
3. to determine the extent of involvement of parasitic and bacterial agents in infectious diarrhea in the adult population of Addis Ababa.

The study was restricted to the most frequently implicated parasitic and bacterial agents. According to Mogesie (1983): no Yersinia enterocolitica was found in one thousand stool specimens from adult diarrheal patients in Addis Ababa. With this information in mind, no attempt was made to detect Yersinia enterocolitica infections in the present study. Viral agents and Cryptosporidium sp. infection could not be considered in the study because of technical difficulties.

## Materials and Methods

### 1. Collection of Specimens

Stool and blood specimens were collected in sterile containers from adult patients (age 15 and above), reporting for medical help to the Arada and Teklehaimanot health centers in Addis Ababa. The study was conducted between August, 1988 and April, 1989. These health centers render free medical service to patients with low or no income.

### 2. Parasitological Examination

Both the direct thin-smear and Kato thick-smear techniques were used to detect intestinal parasitic infections.

Direct thin-smear - Direct saline-iodine smears were used to detect intestinal protozoal infections. In this, one drop of saline (0.85% NaCl) and aqueous iodine solution (1% aqueous solution of KI saturated with iodine crystals) were placed separately in the same slide. With an applicator, approximately 2mg faeces each was placed in the saline and aqueous iodine drops, and stirred until completely suspended and covered with 22 x 22 mm glass coverslips. The preparations were then microscopically examined under the high power magnification (10 x 40).

Kato thick-smear - The kato thick-smear technique (Martin and Beaver, 1968) was used for the diagnosis of intestinal helminth infections. The procedure briefly involved transfer of about 50mg faeces to a microscope slide from nonfibrous specimens. For fibrous specimens, faeces were placed on a clean sheet of paper and a square of stainless-steel bolting cloth placed over it. A sample of screened faeces was removed with an applicator stick by pressing the cloth into the faeces and scrapping the applicator across the surface of the cloth

Faecal samples thus prepared were covered with a cellophane coverslip previously soaked in glycerine-malachite green solution. The preparation was inverted and pressed against soft paper on a table top. The preparation was then allowed to stand face up for about 1 hour at room temperature. The entire film was examined under low power and helminth eggs quantified.

### 3. Bacteriological Examination

#### Collection of specimens

Stool specimens were collected by using "Cary and Blair" semisolid transport medium. This medium was inoculated with specimens collected on a swab and the swab was left in the tube. The medium is known to hold the bacterial

population in the specimen more or less at stationary state, and to prevent overgrowth of any particular micro-organism (Edwards and Ewing, 1972).

### Culture Media

MacConkey and Shigella-Salmonella (SS) agar (Oxoid, Basingstoke, Hants, England) plates and Kauffmann enrichment broth (Oxoid, Basingstoke, Hants England) were used for primary isolation. Similarly, Nutrient broth, Kligler's iron (KI) agar slants, Lysine iron (LI) agar slants, Urea agar slants, Simmon's citrate agar slants, semisolid motility medium, mannitol broth and glucose broth (Oxoid, Basingstoke, Hants, England) were used for biochemical tests.

### Bacterial Isolation

The stool specimens were brought to the laboratory and plated on two primary media. MacConkey and Shigella-Salmonella agar plates were inoculated with the swab, and streaked using a sterile loop. The swab was then dipped and left in Kauffman enrichment broth. All inoculated plates and the enrichment broth were incubated at 37°C for 24 hours. The MacConkey and SS agar plates were examined for non-lactose fermenting colonies. By using a straight wire, a single colony was picked out and inoculated into about 3ml of nutrient broth.

Nutrient broth cultures were incubated at 37<sup>0</sup>c for about two hours, until growth was ascertained by turbidity. Kauffmann enrichment broth cultures were streaked on the same type of agar plates used for primary isolation, and incubated at 37<sup>0</sup>C for 24 hours.

### Biochemical Reactions of Bacterial Isolates

By using a sterile straight wire, the broth cultures were inoculated in the following manner:-

- Kligler's iron agar slant, to determine the fermentation of glucose or lactose and the production of hydrogen sulfide. The butt was stabbed and the slant streaked.
- lysine iron agar slant, to detect oxidative deamination of lysine on the slant and decarboxylation of lysine in the butt. In this process, the butt was stabbed and the slant streaked.
- Urea agar slant, to determine hydrolysis of urea.
- Simmon's citrate agar slant, to check the utilization of citrate as a sole source of carbon.
- Semisolid motility, medium to determine motility
- Mannitol broth, to determine fermentation of mannitol.
- Glucose broth with inverted Durham tube, to detect fermentation and gas production from glucose. All biochemical test culture tubes were incubated at

37°C for 24 hours.

To check the purity of broth inocula, and the reliability of biochemical tests, each broth inoculum was subcultured on MacConkey check-plates and incubated at 37°C for 24 hours.

Serogrouping - The slide agglutination test was used, with "Difco" polyvalent and group antisera for serogrouping and confirmation of biochemically identified Salmonella sp. or Shigella sp., respectively.

Haematocrit and Total Serum Protein Determinations - At the same time the stool samples were collected, 5ml of venous blood was obtained from each patient by use of a sterile syringe and the blood used for total serum protein quantification and haematocrit determination.

Haematocrit determinations were made by drawing blood samples into heparinized capillary tubes (75mm, 75µl, Hirschmann, Laborgerate, Germany) sealed with plasticine, followed by centrifugation of the packed red cell volume by haematocrit reader (Hawksley, England). Centrifugation was performed for 5 minutes in a micro-haematocrit centrifuge (Hawksley, England).

Total serum protein was determined by the Biuret method (Kaplan & Szabo 1983). Briefly, 5ml of venous blood obtained from the patients was transferred into

optically clear glass vials and incubated at 37°C for about two hours to extract the serum. The serum was drawn out with pasteur pipets and the remaining coagulate centrifuged at 1000 rpm for ten minutes, using an Angle Head centrifuge (Gallenkamp, England) to extract as much serum as possible. The serum was stored at 20°C until assayed, or the assay was conducted immediately to determine the total serum protein. In the assay, 2.5 ml of biuret reagent (3.0g  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) dissolved in 500 ml freshly distilled water to which 9.0g KNa tartrate.  $4\text{H}_2\text{O}$ , 5.0g KI and 100ml of 6M NaOH was added, and diluted to 1L) was pipeted into Test series of tubes and a 2.5 ml biuret blank (9.0 g KNa tartrate.  $4\text{H}_2\text{O}$  and 5.0g KI dissolved in 800 ml water, to which 100ml 6 M NaOH was added and diluted to 1L) into Blank series of tubes, each in triplicate. 50 $\mu$ l of specimen (serum or protein standard) was pipeted into one of each series and thoroughly mixed. Reagent blanks were prepared by pipeting 50 $\mu$ l of distilled water into 2.5ml of biuret reagent. All test and blank series tubes were incubated at 37°C for 10 minutes. Absorbance was read at 540 nm by using a Spectronic 21 spectrophotometer (Bausch and Lomb, USA) following zeroing with biuret blank and absorbance reading of the blank series.

To obtain the absorbance values of the test series, absorbance values of both reagent blanks (Arb) and

sample blanks ( $A_{sb}$ ) were subtracted from the corresponding absorbances obtained for the test series. That is,  

$$A_{net} = A_{test} - (A_{rb} + A_{sb})$$
 protein concentration in grams per deciliter was thus calculated as,  $g/cl = \frac{A_{net}}{A_{snet}} \times C$

$A_{u net}$  and  $A_{snet}$  absorbance of specimen and standard, respectively,  $C$  = concentration of standard  $g/dl$ .

### Statistical Analysis

$\chi^2$  analysis was used to test the association of diarrhea with infection, and possible disease implications in multiple infections. Similarly Spearman's rank correlation coefficient was used to compare patterns of double infections in the two sexes.

Classification of intensity of infection with the gastrointestinal helminths was based on the amount of eggs in the stool. Egg counts for *A. lumbricoides*, *J. trichiura* and hookworm ranged from 80 to 7280, 40 to 160 to 2240 per gram of stool, respectively. It was observed that *A. lumbricoides* and *J. trichiura* were light in all positive cases, while hookworm infections appeared to be heavy.

Among the pathogenic *Enterobacteriaceae*, *Shigella* and *Salmonella* were the two genera considered in the

## Results

Based on the parasitological and bacteriological examinations of the stool specimens, 9 types of parasitic or bacterial agents that are implicated in causing gastrointestinal diseases were detected, in 410 (74.2%) of the patients. The most common gastrointestinal infectious agents diagnosed, and the frequency of their infection rates, is shown in Figure 2. Among the 410 positive cases, 251 (61.2%) had single infections. The parasites involved as single infections were primarily Entameba histolytica (53.8%), Ascaris lumbricoides (19.1%), Trichuris trichiura (10%), Giardia lamblia (5.2%), Strongyloides stercoralis (4%) and Hookworm (3.5%). Among the bacteria, Shigella sp (3.5%) and Salmonella typhi (0.8%) were identified as single infections.

Estimation of intensity of infection with the gastrointestinal helminths was based on the amount of egg load in the stool. Egg counts for A. lumbricoides, T. trichiura and hookworms ranged from 80 to 7280, 40 to 880 and 160 to 2240 per gram of stool, respectively. This showed that A. lumbricoides and T. trichiura infections were light in all positive cases, while hookworm infections appeared to be heavy.

Among the pathogenic Enterobacteriaceae, Shigella and Salmonella were the two genera considered in the

study, as they most commonly are implicated in causing diarrhea in the adult population. It was determined that infection rates with Shigella were higher when compared with Salmonella rates of infection (Table 1). Among the Shigella sp., S. flexneri was the most common isolate followed by S. dysenteriae and then by S. boydii. However, no S. sonnei infections were encountered. S. typhi was the only species encountered among the Salmonella isolates.

Among the 410 individuals found positive, 39% were found to harbour more than one infectious agent. Most prevalent were double infections consisting of more than one parasite and bacteria. Among the multiple infections 31.5% were double, 6.6% triple and 0.7% were quadruple infections (Table 1 and 2).

Most frequent among the double infections are those with E. histolytica and A. lumbricoides, E. histolytica and T. trichiura; A. lumbricoides and T. trichiura; and E. histolytica and Shigella sp. representing 23.4% of the total infections (Table 1). Similarly, 70.4% of the triple infections consisted of a combination of E. histolytica, A. lumbricoides and T. trichiura, which were the three most frequently encountered parasites (Table 2). Cases of Schistosoma mansoni in combination with E. histolytica and hookworm were also detected. No significant difference

was seen in intensity of infection between single and multiple infections involving the intestinal helminths. Multiply infected individuals had overall egg counts below 7280 for Ascaris and 880 for Trichuris: both egg loads falling within the range of light infections.

Consistently: the percentage frequency of occurrence of each infectious agent was found greater in combination with others, rather than alone (Table 7).

Out of 285 patients with diarrhea, 234 (82.1%) had single or multiple infections (Table 3). Among the diarrhea cases 56% of the infections were single, 34.6% double, 8.1% triple and 1.3% quadruple. Figure 3 shows frequency of infection rates by 7 infectious agents in the diarrheal patients. In the single infections, E. histolytica accounted for 61.8% and G. lamblia for 8.4%. E. histolytica and G. lamblia infections represented 34.6% and 4.7% of the total diarrheal cases respectively. About 21% of these diarrheal patients were found harbouring 3 helminth parasites; that is: A. lumbricoides (11.5%), T. trichiura (5.3%), and S. stercoralis (4.6%). Shigella sp. (6.9%) and Salmonella typhi (1.5%) together accounted for 8.4% of the total diarrheal cases. The most common combination of the double infections were E. histolytica and A. lumbricoides (30.9%) and E. histolytica and

Shigella sp. (19.8%). Furthermore, in about 84% of the multiple infections, E. histolytica occurs in combination with one or more of 6 gastrointestinal parasites and Shigella. Likewise, G. lamblia was found to occur in concomitant infections with one or more of the parasites encountered, in 15% of the cases. The two intestinal protozoan parasites were also found to be the most frequently implicated parasitic infections in the diarrheal patients. Among the bacteria, Shigella appeared to be an important cause of diarrhea in the adult population.

Results of the study also revealed that in diarrheal patients with concomitant E. histolytica and Shigella sp infections, the patients were clinically characterized by higher fever, severe rectal tenesmus, bloody mucoid stools and urgency for frequent defecation.

The  $X^2$  test showed diarrhea to be significantly associated with infection. This was significant at the 0.5% level (Table 4). Similarly  $X^2$  tests indicated a noticeable association of the percentage occurrence of diarrhea with increasing number of infectious agents concurrently occurring in the same individual (significant at 0.5% level), (Table 6). The proportion of the incidence of diarrhea also consistently increased with an increase in the number of infectious agents concomitantly occurring

Table 1. Single and Double Infections  
in Total Sample

Single Infections	No. infected	% of Single infection	% of total infection	PRCV* less than normal	TSP** less than normal	% low PRVC	% low TSP
1. <u>Entameba histolytica</u>	135	53.8	32.9	46	6	34.0	4.4
2. <u>Giardia lamblia</u>	13	5.2	3.2	2	-	15.4	-
3. <u>Ascaris lumbricoides</u>	48	19.1	11.7	10	2	20.8	4.1
4. <u>Trichuris trichiura</u>	25	10.0	6.1	4	1	16.0	4.0
5. <u>Strongyloides stercoralis</u>	10	4.0	2.4	4	-	40.0	-
6. Hook worm sp.	9	3.5	2.24	9	-	100.0	-
7. <u>Shigella</u> sp.	9	3.5	2.24	-	-	-	-
8. <u>Salmonella typhi</u>	2	0.8	0.5	-	-	-	-
Total -----	251		61.2				

Table 1 contd...

Double Infections	No. infected	% of double infection	% of total infection	PRCV* less than normal	TSP** less than normal	% low PRCV	% low TSP
1. <u>E. histolytica</u> and <u>G. lamblia</u>	8	6.2	1.95	2	-	25	-
2. <u>E. histolytica</u> and <u>Shigella</u> sp.	16	12.4	3.9	3	7	18.8	43.8
3. <u>E. histolytica</u> and <u>A. lumbricoides</u>	39	30.2	9.5	11	1	28.2	2.6
4. <u>E. histolytica</u> and <u>T. trichiura</u>	22	17.1	5.4	10	2	45.5	4.5
5. <u>E. histolytica</u> and <u>S. stercoralis</u>	4	3.1	1.0	1	1	25	25
6. <u>E. histolytica</u> and <u>S. typhi</u>	1	0.8	0.24	-	-	-	-
7. <u>E. histolytica</u> and <u>Schistosoma mansoni</u>	1	0.8	0.24	-	-	-	-
8. <u>G. lamblia</u> and <u>A. lumbricoides</u>	9	6.9	2.24	1	1	11.0	11.0
9. <u>G. lamblia</u> and <u>T. trichiura</u>	4	3.1	1.0	-	-	-	-
10. <u>A. lumbricoides</u> and <u>T. trichiura</u>	19	14.7	4.61	1	-	5.3	-
11. <u>A. lumbricoides</u> and Hookworm	3	2.3	0.7	3	2	100	66.7
12. <u>T. trichiura</u> and Hookworm	2	1.6	0.5	1	-	50	-
13. <u>T. trichiura</u> and <u>S. stercoralis</u>	1	0.8	0.24	-	-	-	-
	129		31.5				

\* packed Red cell volume

\*\* Total serum protein

in the same individual (36%, 52%, 62%, 70%, 100% for uninfected, single, double, triple and quadruple infections respectively). Likewise, comparison of the frequency of diarrheal cases versus protozoal and helminthic infections also showed the protozoa to be more frequently involved in causing diarrhea than the helminth parasites, in the adult population.

Comparisons of the levels of haematocrit and total serum protein, when the infectious agents occur singly and in combinations, showed a general tendency for the proportion of patients with decreased haematocrit and total serum protein values to increase as the number of infectious agents in the same individual increased, except for combinations involving A. lumbricoides and any one other parasite. For example, out of the E. histolytica infected individuals 34% and 4.4% had low haematocrit and total serum protein respectively. Similarly, from the patients harbouring A. lumbricoides alone, 20.8% showed low haematocrit and 4.2 had low total serum protein levels, while 28.2% and 2.6% of the patients concurrently infected with E. histolytica and A. lumbricoides had haematocrit and total serum protein below normal levels respectively. Patients with Shigellosis alone had no indication of anaemia or protein deficiency. However, out of the patients with mixed E. histolytica and Shigella

Table 2. Triple and Quadruple Infections in Total Sample

Triple Infection	No Infected	% of Infection	% of total Infection	PRCV* less than normal	TS P** less than normal	% low PRCV	% low TSP
1. <u>E. histolytica</u> , <u>A. lumbricoides</u> and <u>T. trichiura</u>	19	70.4	4.6	12	1	63.2	5.3
2. <u>E. histolytica</u> , <u>A. lumbricoides</u> and <u>S. mansoni</u>	1	3.7	0.24	-	-	-	-
3. <u>E. histolytica</u> , <u>G. lamblia</u> and <u>S. mansoni</u>	1	3.7	0.24	1	-	100	-
4. <u>E. histolytica</u> , Hookworm and <u>S. mansoni</u>	1	3.7	0.24	1	-	100	-
5. <u>E. histolytica</u> , <u>G. lamblia</u> and <u>T. trichiura</u>	1	3.7	0.24	-	-	-	-
6. <u>E. histolytica</u> , <u>A. lumbricoides</u> and <u>S. stercoralis</u>	1	3.7	0.24	-	-	-	-
7. <u>G. lamblia</u> , <u>A. lumbricoides</u> and <u>T. trichiura</u>	1	3.7	0.24	-	-	-	-
8. <u>G. lamblia</u> , <u>T. trichiura</u> and <u>S. stercoralis</u>	1	3.7	0.24	1	-	100	-
9. <u>A. lumbricoides</u> , <u>T. trichiura</u> and <u>S. stercoralis</u>	1	3.7	0.24	1	-	100	-
Total	27		6.6				
Quadruple Infection							
1. <u>E. histolytica</u> , <u>G. lamblia</u> , <u>A. lumbricoides</u> & <u>T. trichiura</u>	1	33.3	0.24	1	-	100	-
2. <u>E. histolytica</u> , <u>A. lumbricoides</u> , <u>T. trichiura</u> & <u>S. typhi</u>	1	33.3	0.24	-	-	-	-
3. <u>E. histolytica</u> , <u>A. lumbricoides</u> , <u>T. trichiura</u> & Hookworm	1	33.3	0.24	1	-	100	-
	3		0.72				

Table 3. Single and Multiple Infections in Diarrheal Patients

Single Infections	No of positive cases	% of single infection	% of all diarrheal cases	PRCV* less than normal	TSP** less than normal	% low PRCB	% low TSP
1. <u>E. histolytica</u>	81	61.8	34.6	26	6	32.1	7.4
2. <u>Giardia lamblia</u>	11	8.4	4.7	2	-	18.2	-
3. <u>A. lumbricoides</u>	15	11.5	6.4	5	-	33.3	--
4. <u>T. trichiura</u>	7	5.3	3.0	2	-	28.6	-
5. <u>S.stercoralis</u>	6	4.6	2.6	3	-	50.0	-
6. <u>Shigella</u> sp	9	6.9	3.8	-	-	-	-
7. <u>Salmonella typhi</u>	2	1.5	0.85	-	-	-	-
<u>Total</u>	131		56.0				
<u>E. histolytica and Giardia lamblia</u>	2	1.5	0.9				
<u>G. lamblia and A. lumbricoides</u>	1	0.8	0.5				
<u>G. lamblia and T. trichiura</u>	1	0.8	0.5				
<u>A. lumbricoides and T. trichiura</u>	2	1.5	0.9				

Table 3. contd... Single and Multiple Infections in Diarrheal patients

Double Infections	No of positive cases	% of double infection	% of all diarrheal cases	PRCV* less than normal	TSP** less than normal	% low PRCV	% low TSP
1 <u>E. histolytica</u> and <u>G. lamblia</u>	8	9.9	3.4	2	-	25.0	-
2 <u>E. histolytica</u> and <u>A. lumbricoides</u>	25	30.9	10.7	7	1	28.0	4.0
3 <u>E. histolytica</u> and <u>T. trichiura</u>	13	16.0	5.6	6	1	46.2	7.7
4 <u>E. histolytica</u> and <u>Shigella</u> sp	16	19.8	6.8	3	7	18.8	43.8
5 <u>E. histolytica</u> and <u>S. typhi</u>	1	1.2	0.4	-	-	-	-
6 <u>E. histolytica</u> and <u>S. stercoralis</u>	4	4.9	1.7	1	-	25	-
7 <u>G. lamblia</u> and <u>A. lumbricoides</u>	8	9.9	3.4	3	-	37.5	-
8 <u>G. lamblia</u> and <u>T. trichiura</u>	1	1.2	0.4	-	-	-	-
9 <u>A. lumbricoides</u> and <u>T. trichiura</u>	5	6.2	2.1	2	-	40.0	-
	81		34.6				

Table 3 contd.....

## Single and Multiple Infections in Diarrheal Patients

Triple Infections		No. of positive cases	% of Triple infect- ion	% of all diarrheal cases	PRCV* less than normal	TSP** less than normal	% low PRCV	% low TSP
1	<u>E. histolytica</u> , <u>A. lumbricoides</u> and <u>T. trichiura</u>	11	57.9	4.7	7	1	63.6	9.0
2	<u>E. histolytica</u> , <u>A. lumbricoides</u> and <u>S. mansoni</u>	1	5.26	0.4	-	-	-	-
3	<u>E. histolytica</u> , <u>G. lamblia</u> and <u>S. mansoni</u>	1	5.26	0.4	1	-	100	-
4	<u>E. histolytica</u> , Hookworm and <u>S. mansoni</u>	1	5.26	0.4	1	-	100	-
5	<u>E. histolytica</u> , <u>G. lamblia</u> and <u>T. trichiura</u>	1	5.26	0.4	-	-	-	-
6	<u>E. histolytica</u> , <u>A. lumbricoides</u> and <u>S. stercoralis</u>	1	5.26	0.4	-	-	-	-
7	<u>G. lamblia</u> , <u>A. lumbricoides</u> and <u>T. trichiura</u>	1	5.26	0.4	1	-	100	-
8	<u>G. lamblia</u> , <u>T. trichiura</u> and <u>S. stercoralis</u>	1	5.26	0.4	1	-	100	-
9	<u>A. lumbricoides</u> , <u>T. trichiura</u> and <u>S. stercoralis</u>	1	5.26	0.4	1	-	100	-
		19	% of quadruple infection	8.1				
Quadruple Infection								
1	<u>E. histolytica</u> , <u>G. lamblia</u> , <u>A. lumbricoides</u> & <u>T. Trichiura</u>	1	33.3	0.4	1	1	100	100
2	<u>E. histolytica</u> , <u>A. lumbricoides</u> , <u>T. trichiura</u> & <u>S. typhi</u>	1	33.3	0.4	-	-	-	-
3	<u>E. histolytica</u> , <u>A. lumbricoides</u> , <u>T. trichiura</u> & Hookworm	1	33.3	0.4	1	-	100	-

\* packed red cell volume

\*\* total serum protein

Fig. 1. Frequency of single and multiple infections

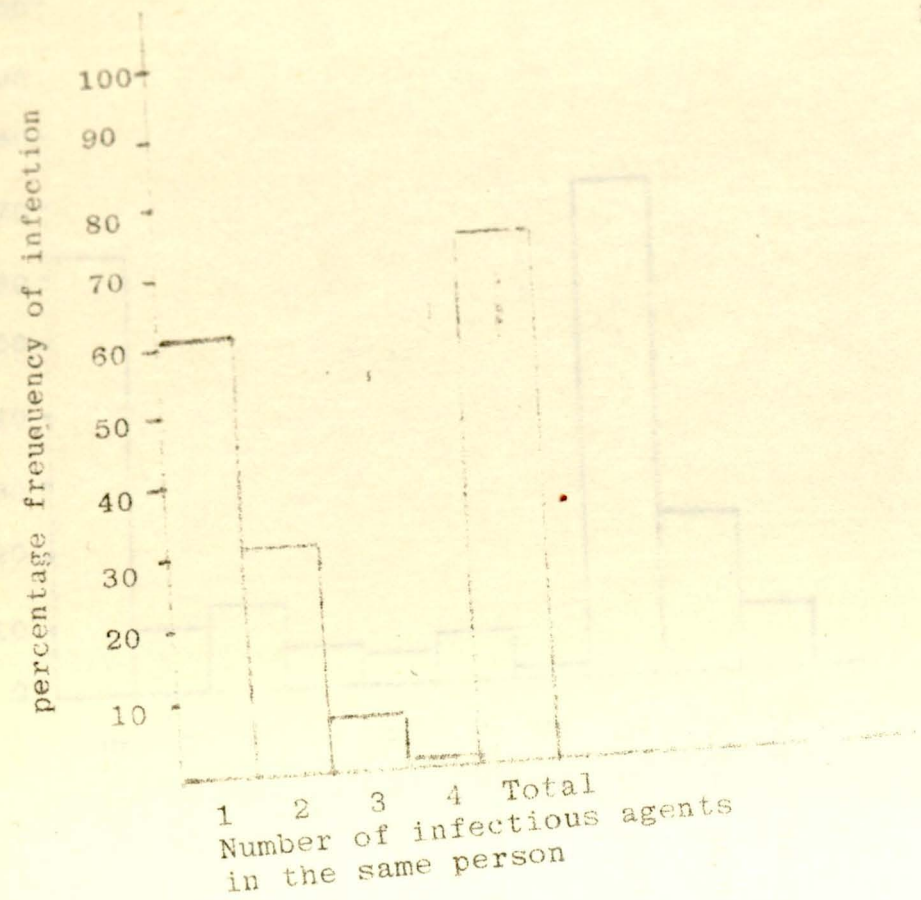


Fig. 2. Frequency of infection by 8 infectious agents

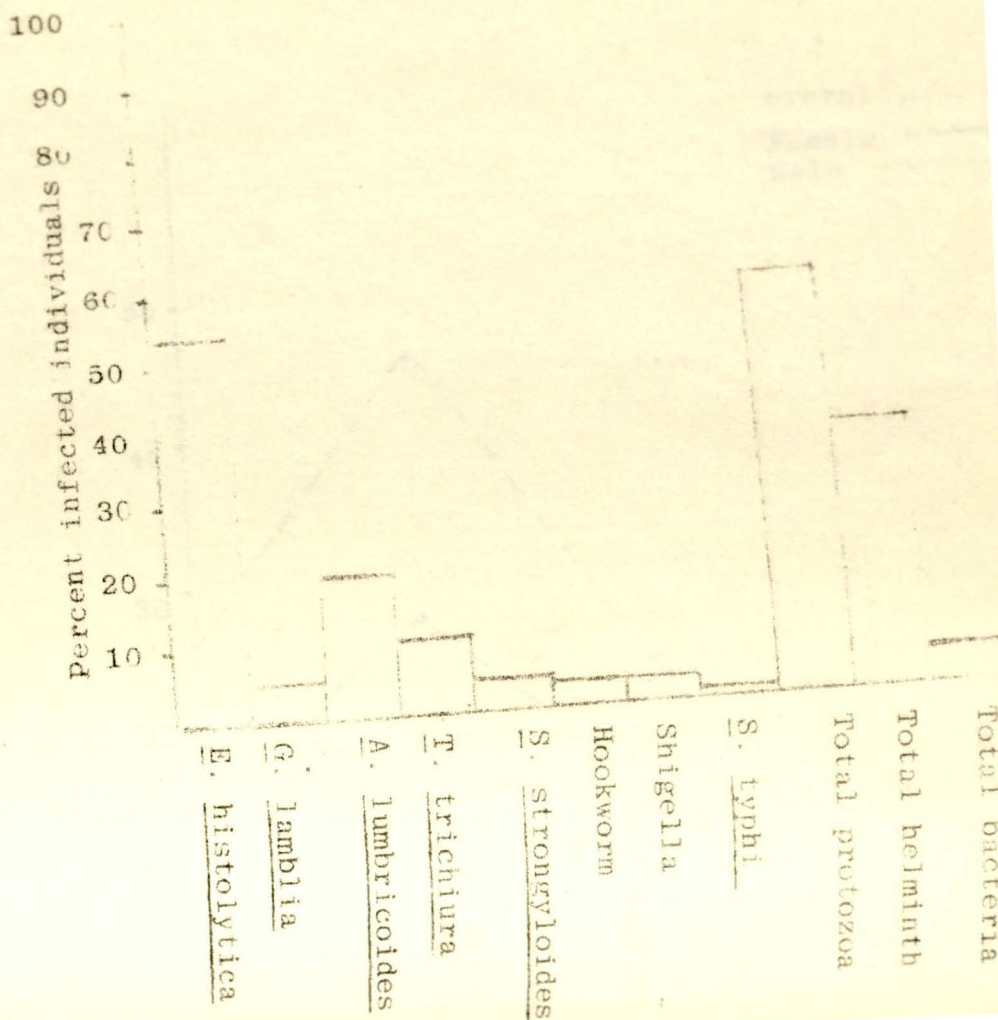


Fig. 3. Frequency of infection by 7 infectious agents in diarrheal cases.

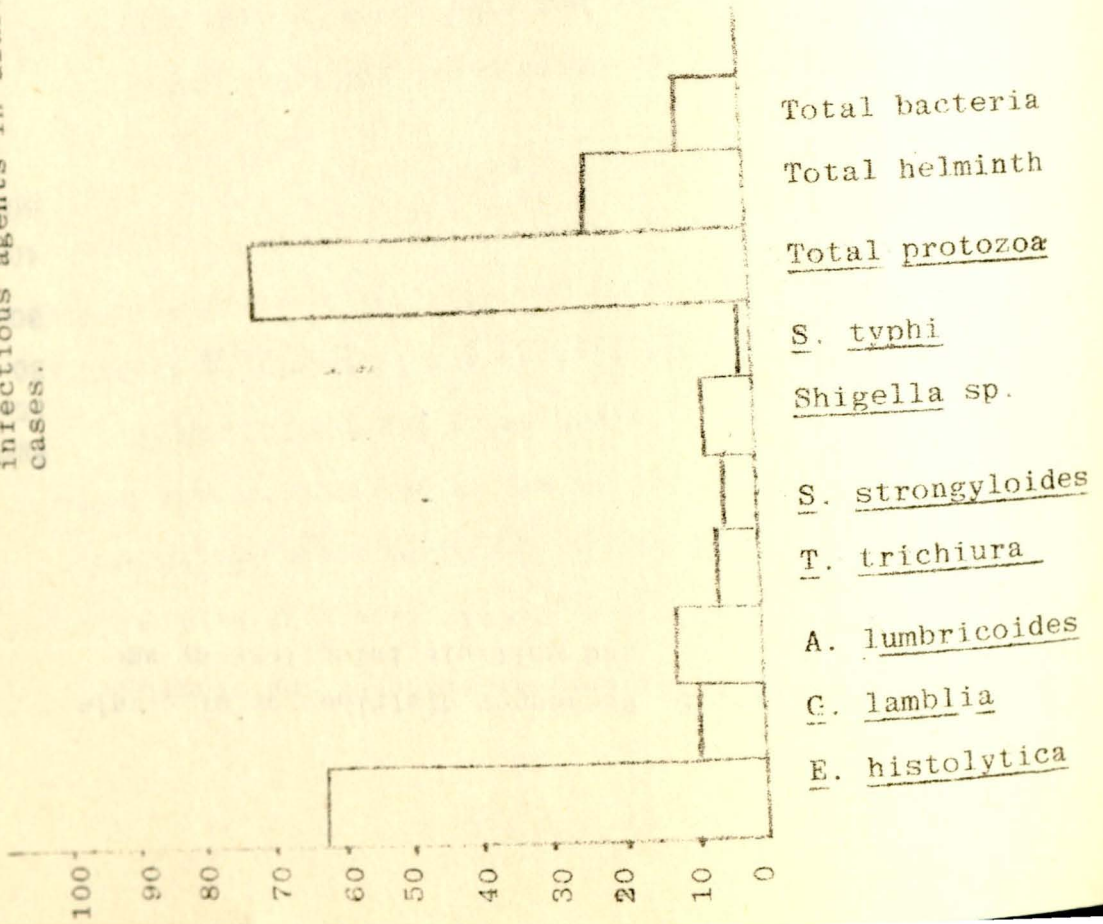


Fig. 4. Frequency distribution of single and multiple infections by sex.

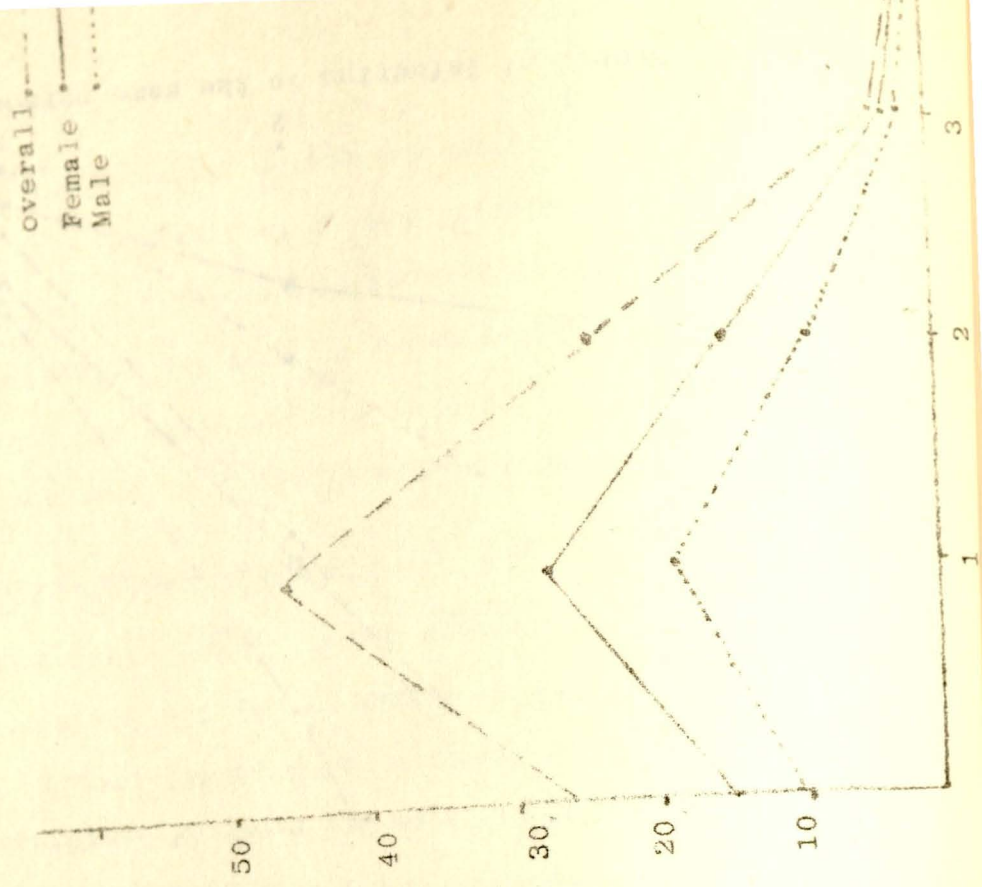
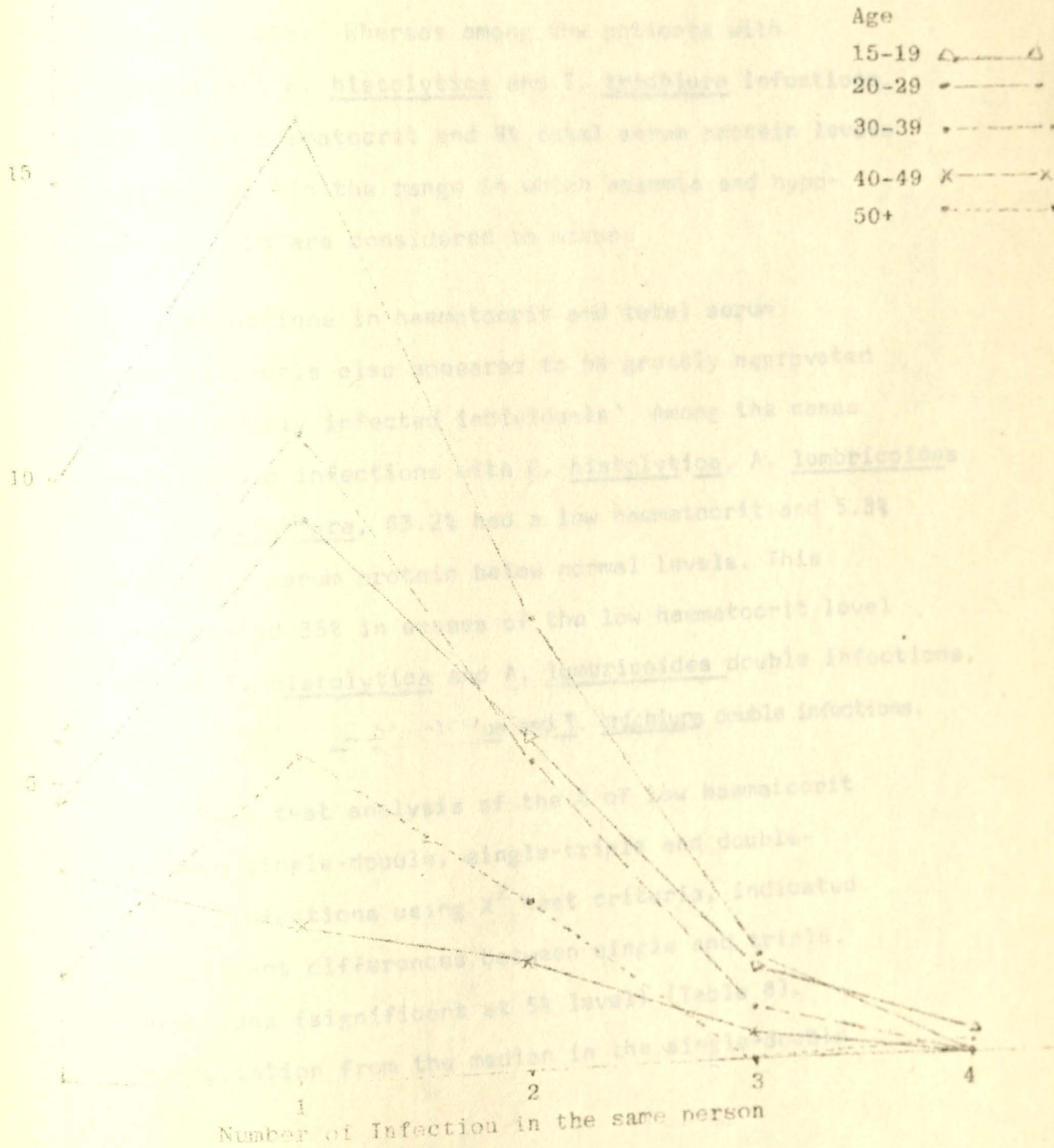


Fig. 5. Frequency distribution of single and multiple infections by age



infections 18.8% had low haematocrit and 43.8% were found with total serum protein levels below normal. Likewise, out of the T. trichiura cases 16% showed low haematocrit and 4% had total serum protein below normal levels. Whereas among the patients with concomitant E. histolytica and T. trichiura infections, 45.5% had haematocrit and 9% total serum protein levels falling within the range in which anaemia and hypo-proteinemia are considered to occur.

Reductions in haematocrit and total serum protein levels also appeared to be grossly aggravated in the triply infected individuals. Among the cases with triple infections with E. histolytica, A. lumbricoides and T. trichiura, 63.2% had a low haematocrit and 5.3% had total serum protein below normal levels. This represented 35% in excess of the low haematocrit level of the E. histolytica and A. lumbricoides double infections, and E. histolytica and T. trichiura double infections.

Sign test analysis of the % of low haematocrit between single-double, single-triple and double-triple infections using  $\chi^2$  test criteria, indicated significant differences between single and triple infections (significant at 5% level) (Table 8). The deviation from the median in the single-double

Table 4. Frequency of Association of Diarrhea with Infection with parasite/bacteria (N = 552)

Diarrheal status	Infection status		
	Infected	Not Infected	Total
Diarrhea	234	51	285
No diarrhea	176	91	267
Total	410	142	552

$$\chi^2 = 18.8936 (P < 0.005)$$

Table 5. Frequency of Diarrhea cases versus protozoal and Helminth Infections (N = 240)

Diarrheal status	Infection status	
	Protozoal	Helminth
Diarrhea	92	28
No diarrhea	56	64
Total	148	92

$$\chi^2 = 19.886 (P < 0.005)$$

Table 6. Frequency of Diarrhea versus Number of Types of Agents/individual

Diarrheal status	No. of Individuals with different status of infection					
	None	Single	double	Triple	Quadruple	Total
Diarrhea	51	131	81	19	3	285
No diarrhea	91	120	48	8	0	267
Total	142	251	129	27	3	552

$$\chi^2 = 28.999 \quad (P < 0.005)$$

Table 7. Percentage frequency of occurrence of Infectious Agents Alone or with others (N=410)

Ag. t	% frequency of occurrence	
	alone	with others
<u>E. histolytica</u>	48	52
<u>L. lamblia</u>	33	63
<u>A. lumbricoides</u>	28	72
<u>T. trichiura</u>	19	81
<u>S. Stercoralis</u>	48	52
Hookworm	47	53
<u>S. mansoni</u>	0	100
<u>S. typhi</u>	33	67
<u>Shigella</u>	36	64

Sign test (2-sided)

$$X^2 = 9.0(P < 0.05)$$

Table 8. Percentage low PRCV in individuals with single, double or triple infectious agents

Infection	% individuals with low PRCV		
	Single Infection	+ one other (double)	+ 2 others (triple)
<i>E. histolytica</i>	34	29.7	58.3
<i>G. lamblia</i>	15.4	14.3	50
<i>A. lumbricoides</i>	20.8	26.7	56.5
<i>T. trichiura</i>	16	25.5	60.9
<i>T. trichiura</i>	40	20	66.7
Hookworm	100	80	100
<i>S. mansoni</i>	-	0	66.7
<i>S. typhi</i>	0	0	-
Shigella	0	18.8	0

Sign test (2-sided)

Single-double:  $\chi^2 = 0.14$  ( $P > 0.05$ )

Single-triple:  $\chi^2 = 0.5$  ( $P < 0.05$ )

Double-triple:  $\chi^2 = 3.6$  ( $P > 0.05$ )

Table 9: Spearman's rank correlation coefficient for double infections by sex.

Double Infections	Males (n = 50) rank	Females (n = 79) rank
<u>E. histolytica</u> a . . <u>lumbricoides</u>	1	1
<u>E. histolytica</u> - <u>T. trichiura</u>	2	3
<u>E. histolytica</u> - <u>Shigella</u> sp	3	4
<u>A. lumbricoides</u> - <u>T. trichiura</u>	4	2
<u>G. lamblia</u> - <u>A. lumbricoides</u>	5	6
<u>T. trichiura</u> - Hookworm	7	5
<u>E. histolytica</u> - <u>S. stercoralis</u>	7	10
<u>A. lumbricoides</u> - Hookworm	9	8.5
<u>G. lamblia</u> - <u>T. trichiura</u>	10	8.5

Spearman's rank correlation coefficient,  $r_s = .806$  ( $Z = 2.42$ )

(Significant at 0.1%)

and double-triple infections was not large enough to show significant differences in the haematocrit and total serum protein levels.

Comparison of infections in the males and females, by using the Spearman's rank correlation coefficient for the corresponding infectious agent combinations showed general patterns of double infections to be similar for the two sexes. (Table 9). This is significant at the 0.1% level. Comparison of infection rates in the males and females is also shown in Figure 4.

It also appeared that the chances of harbouring more than one infectious agent concomitantly by an individual is greater in the young adults (age 15-30) than in the older age groups (Figure 5). For example, well over 50% of the single and multiple infections occurred in the age group below 30 years.

### Discussion

The prevalence of gastrointestinal parasitic and bacterial infections is closely linked with conditions predisposing to infection and reinfection. The quality of the environment plays an important role in the transmission of gastrointestinal infections. Unsanitary environmental conditions greatly facilitate the spread of infectious agents by faecal-oral and person-to-person contact mechanisms. Individuals with gastrointestinal problems reporting to Arada and Teklehaimanot health centers for medical assistance can be expected to be more exposed to gastrointestinal infections, as they live in inadequate housing conditions i.e. houses without proper water supplies and sanitary facilities. The food intake of the patients is also presumably marginal, both in quantity and quality, by virtue of the fact that they have a low income or no income at all.

The results of the present study support the prevalence of such situations in the population sampled. A high prevalence of infection with parasitic and/or bacterial infectious agents was indicated by the fact that 74.2% of the patients were found harbouring one or more agents. Among the intestinal parasites E. histolytica, is the most commonly encountered with an infection prevalence

of 53.8% of the single infections and 32.9% of the total infections. Previous reports indicated a wide distribution of E. histolytica in Ethiopia. For example, a prevalence rate of 55% was reported in the Saysay people in the Blue Nile Gorge in Wollega (Torrey, 1966), with prevalence rates ranging from 3% to 55% in 50 communities in the central plateau of the country (McConnell and Armstrong, 1976). The results of this study more or less support the previous reports on the level of prevalence of E. histolytica in the country. The extent of harm caused by parasitic infections to the health of individuals depends on the parasite species (WHO, 1987a), E. histolytica is known to cause inflammation and ulceration due to its invasive action on the intestinal mucosa, which usually results in the outpouring of serum protein, blood and mucus into the bowel (WHO, 1980a). Also, chronic amebiasis often leads to anaemia (DuPont and Pickering, 1980). The low haematocrit and total serum protein in 34% and 4.4% of the E. histolytica infected individuals, respectively, can be explained in terms of the invasive property of the parasite and the associated malnutrition.

G. lamblia is another intestinal protozoan parasite encountered in 5.2% of the single infections and 3.2% of the total infections. According to Lemma et al.,

(1968), G. lamblia was found in 8.9% of 468 school-age children in Addis Ababa. The present findings indicate that the parasite may be more common in children than in adults. The interference of G. lamblia with absorption of digested food (WHO, 1981), added to the poor nutrient intake of the hosts, may have been responsible for development of anaemia in 15.4% of the giardiasis patients.

In conformity with previous reports (Lemma et al., 1968; Sioum et al., 1981). A. lumbricoides and I. trichiura were found to be the dominant intestinal helminths occurring in 19.1% and 10% respectively of the single infections. A. lumbricoides and I. trichiura infections are predominantly prevalent in children. (Cook, 1986). The relatively high infection rates with A. lumbricoides and I. trichiura in the adult population is a reflection of the high transmission level of the two parasites in the population sampled. High population density, low education level and poor sanitary conditions may be implicated in the high prevalence. Estimation of the intensity of infection by the Kato thick-smear technique showed A. lumbricoides and I. trichiura to represent light infections in all positive cases. As reflected in the findings of the study, light infections involving these intestinal nematodes are not known to cause

anaemia. However, the low haematocrit values in 20.8 % of the ascariasis and 16% of the trichuriasis cases may be, perhaps, basically due to nutritional stress in the patients although the infections may have some contributions to the gradual development of anaemia.

Strongyloides stercoralis is commonly found in Ethiopia (McConnell and Armstrong, 1976). It was the third most common intestinal nematode (4%) diagnosed in this study by detecting larvae in the faeces. The excretion of S. stercoralis larvae is intermittent and it may be impossible to detect light infections, even by using special procedures (Beaver et al., 1984). Thus the detection of S. stercoralis larvae with the direct thin-smear method may be an indicator of heavy infections by this parasite in the individuals. It is also possible that the inappropriateness of the diagnostic methods used for the diagnosis of S. stercoralis infections may have under-estimated the prevalence and the load of infection in the patients investigated. S. stercoralis is known to cause diarrhea, anorexia, malabsorption and lesions in the mucosa of the small intestine (Onile et al., 1985; WHO, 1987a). Thus the low haematocrit level in 40% of the patients with S. stercoralis is not unexpected

Hookworm infections in Ethiopia are widespread. The prevalence of infection is highest at intermediate

Altitude (1001-2000m) followed by low altitudes (0-1000m), while the highest altitudinal range (2001-3000m) has the lowest infection rate (Shibru, 1986). Compared to ascariasis and trichuriasis the hookworm prevalence rate in the study population appeared to be relatively lower, although it is comparable with that of *S. stercoralis*. This may be because of the high altitude of Addis Ababa (2400m) and the wearing of shoes by the majority of the population, which minimizes the chance of exposure to larval penetration through the feet, the most common site of entry. Hookworm infection causes blood loss and depletion of the body's iron store leading to iron-deficiency anaemia (WHO, 1981). The severity of anaemia, however, depends both upon the iron content of the diet of the individuals and on the intensity and duration of infection. The intensity of infection, however, differs locally depending on age, sex and the species of hookworm. Thus, heavy hookworm infection is understood as hookworm parasite infection intensive enough to cause anaemia (WHO, 1987a). All hookworm infected individuals in this study showed haematocrit levels within the anaemic range. Inadequate food intake, combined with the blood loss enteropathy of hookworm infections, may have resulted in the precipitation of anaemia in these patients. Although anaemia is not a serious public health problem in Ethiopia, because

of the high iron content of the traditional tef diet (Abraham et al., 1980), hookworm infections can be expected to cause anaemia in individuals with nutritional deficits.

Shigellosis and Salmonellosis are highly infectious diseases of worldwide significance, with the highest incidence in the tropical and subtropical regions, where general standards of living are usually poor (WHO, 1980b). In the present study, infections with Shigella sp constituted 3.5% of the single infections while Salmonella infections represented only 0.8%. This difference in prevalence rate between the two enteropathogens may be due to the close association of Shigella infections with low sanitary conditions and the property of low infection dose in the genus. Reports on Shigellosis in Ethiopia indicate S. flexneri to be the most commonly isolated species, followed by S. dysenteriae and then S. boydii (Afeworki and Yetneberesh, 1980; Mesele and Alebachew, 1982). In conformity with these reports, results of serotyping in the present study showed S. flexneri to be the most common isolate (66.7%), followed by S. dysenteriae (22.2%) and S. boydii (11.1%). No S. sonnei infections were encountered, in conformity with the report that its prevalence in the developing countries is low (WHO, 1980; Rahaman, 1984). The limited

number of studies on Salmonellosis in Ethiopia have shown the predominance of S. typhi (Mesele and Alebachew, 1981; Afeworki, 1985). Similarly, only S. typhi was encountered in this study, again, confirming the endemicity of S. typhi in Addis Ababa.

The public health impact of gastrointestinal infectious diseases on the population can be expected to be aggravated by the occurrence of multiple infections in the same individuals. Multiple infections with intestinal parasites have been reported by some workers in Ethiopia (Kloos et al., 1980; Shibru et al., 1982; Tesfamichael, 1983). In the present study, multiple infections with two agents were the most predominant (31.5%); there were some with three (6.6%) and only few with four (0.71%) parasites and/or bacteria in the same individuals.

The chances of harbouring single and multiple infectious agents appeared to be greater in the young adults (age 15-30) than in the older age groups (Figure 5). Social factors, exposure and acquisition of immunity may be responsible for the observed age difference in the infection prevalence. Also, although not statistically significant, infection prevalence in the females was consistently higher than in the males (Figure 4). This may be attributable to the home

management and food handling responsibilities of the females in Ethiopian society. Such activities are believed to increase the chance of exposure of the individual to the infective stages of the infectious agents.

Most frequent among the multiple infections were infections involving E. histolytica and A. lumbricoides (24.5%), E. histolytica and T. trichiura (13.8%), A. lumbricoides and T. trichiura (11.9%) and E. histolytica and Shigella (10.1%). The type of combinations in the triple and quadruple infections likewise followed similar patterns (Table 2). This shows that in addition to the clinical implications, multiple infections may have some epidemiological bases for co-occurrence. The observed combinations also appeared to indicate the co-existence of some parasitic and bacterial infections in the population

From the gastrointestinal helminth parasites, A. lumbricoides and T. trichiura combinations were most common. In studies elsewhere, A. lumbricoides T. trichiura and hookworm have been found to co-occur in multiple infections (Buck et al., 1978). This pattern has also been reflected in the present study. As in the single infections, A. lumbricoides and T. trichiura egg counts were low, indicating light infections. This may be due to fairly stable level

of endemicity of the intestinal helminths, resulting from repeated infection. In light of the similarities of their life-cycles during the exogenous developmental phases, and their modes of transmission, simultaneous exposure to infectious eggs of both parasites can occur more frequently than with other agents.

Most of the Schistosoma mansoni cases were patients who had once lived in Tigray or Gondar regions, where S. mansoni is widely endemic (Lo et al., 1988). These few cases harbouring S. mansoni in combination with other gastrointestinal parasites may indicate the high possibility of S. mansoni occurring in multiple infections, in endemic areas, with far-reaching public health implications.

Most of the agents involved in combined infections have a well-documented status of pathogenicity. However there is a very poor understanding of their interactions when they co-occur with other agents. The most obvious effect of the combined infections appeared to be the enhancement of anaemic resulting from amebiasis, Shigellosis and hookworm infections. The proportion of individuals with below normal haematocrit levels and total serum protein increased with infections involving combinations of E. histolytica and Shigella

or hookworm and other parasites. A notable exception was seen in double infections involving A. lumbricoides and other parasites aside from hookworm. It appears that infections with A. lumbricoides may have induced the development of a non-specific immune response, that may have reduced the pathological effects of other concomitant infections. This is possible because children heavily infected with A. lumbricoides were shown to be free from malaria, whereas children treated for ascariasis develop attacks of malaria (Murray et al., 1978).

E. histolytica and Shigella infections are known to co-occur frequently in the tropics (WHO, 1981). The disease is characterized by copious diarrhea, high fever and pronounced dehydration. In the present study, patients with E. histolytica and Shigella concomitant infections clinically presented with high fever, severe rectal tenesmus and bloody mucoid diarrhea, as opposed to patients with infections of Shigella or E. histolytica alone. Thus the combined infections may have caused severe colitis, thereby leading to hypo-proteinemia and anaemia in the majority of the patients.

Infectious diseases are important causes of morbidity and mortality (WHO, 1981). They can interfere

with both social and economic aspects of the living conditions of the population. For example, helminthiasis alone accounts for over a quarter of a million annual visits of out-patient cases in Ethiopia, and is known to afflict entire communities (Zein, 1988).

Of the communicable diseases, diarrheal diseases are not only important causes of morbidity and mortality in Ethiopia but also contribute to malnutrition. Recently, it has been reported that dysentery and gastroenteritis constitute 8.9% of the leading cause of out-patient morbidity (Zein, 1988). However, despite their frequent occurrence in the population, the aetiological pattern has not been fully determined.

Parasite-related diarrhea is known to primarily include amoebiasis, giardiasis, trichuriasis and strongyloidosis (WHO, 1980a). E. histolytica and G. lamblia infections represented 61.8% and 8.4% of the single infections, and 34.6% and 4.7% of the total diarrheal cases, respectively. About 12% of the diarrheal cases were associated with the helminth parasites A. lumbricoides, T. trichiura and S. stercoralis. Among the bacteria, Shigella (6.9%) and Salmonella (1.5%) together accounted for 8.4% of the diarrheal cases associated with single infections. The incidence and severity of diarrhea significantly increased with an

increasing number of types of infectious agents. Similarly, statistical comparison of the association of diarrhea with protozoal and helminthic infections showed protozoal infections to be more frequently associated than the helminth parasites (Table 5). The combination of E. histolytica and G. lamblia is most frequently encountered in the double, triple and quadruple infections, and also most frequently with diarrhea.

Carriers of Salmonella represent the most important reservoir of infection, while a long carrier state is exceptional in Shigella (WHO, 1980b). In the light of this information, statistical comparison were not made to see the strength of association of bacterial infection with diarrhea, because stool cultures for bacteria were made only for the diarrheal stools. On the whole, there is a pattern for the protozoal infections to be associated with diarrhea more frequently than the helminth parasites and bacteria. However, this has to be verified by the examination of a larger size sample.

No infectious agents were detected in 17.9% of the diarrheal patients. Rotavirus and Cryptosporidium sp. are among infectious agents known to cause diarrhea (WHO, 1987b). Since detection of these agents was not included in this study, the involvement of these agents

in causing diarrhea cannot be excluded.

Wherever E. histolytica occurred in combination with parasites of even mild pathogenicity, the proportion of patients with low haematocrit levels, and total serum protein showed an increasing pattern from single through quadruple infections. However, only the difference between single and triple infections was found to be statistically significant. On the whole, this appears to be the result of the pathogenic effects of E. histolytica, aggravated by the presence of other concomitantly occurring agents. Furthermore malnutrition, which can be presumed to be prevalent in such a low income population, may contribute to the severity of the clinical outcome of the concomitant infections.

Difficulties of differential diagnosis of parasitic diseases are particularly more pronounced in areas of high prevalence. Diagnostic problems are enhanced by multiple infections (Buck et al., 1978a). These are responsible for gross errors of reporting; leaving many of the infectious diseases unrecognized and lumped together with ill-defined conditions. Such difficulties have been observed in Arada and Teklehaimanot health centers. In these, laboratory technicians were observed to stop exhaustively examining stool specimens as soon as they see one parasite or parasite

products. Even for the diarrheal cases only thin-smear microscopic examinations were made for parasite and bacteria detection. No attempts to culture the stools for bacteria were made. Such practices would lead to prescription of drugs without immediate curative value. For example, detection of just a few A. lumbricoides eggs in a diarrheal stool, with unnoticed E. histolytica trophozoites will not lead to a treatment schedule leading to relief for the patient. This study has revealed varieties of multiple infections. Among these were E. histolytica and Shigella occurring in the same individuals presenting with bloody mucoid stools. Knowledge of the existence of multiple infections are of practical importance, since they affect the accuracy of clinical and laboratory diagnosis and the therapeutic measures that may follow. Leaving parasites and bacterial diseases improperly diagnosed not only leads to maltreatment and the ensuing disease effects on the individual patients, but have important epidemiological implications, such as the persistent existence of reservoirs in the community.

### Conclusion

The prevalence level of gastrointestinal infectious agents in the study population was high. The high prevalence level and the quality of the environment appear to create conditions conducive to the occurrence of multiple infections. Results of the study have shown infectious diarrhea to be more frequently associated with parasitic infections. Of the parasites associated with diarrhea, E. histolytica and G. lamblia were involved over 50% of the time, followed by the helminth parasites A. lumbricoides, T. trichiura and S. stercoralis. Although the overall percentage prevalence of Shigella was much lower than that of the parasites, the association of Shigella with diarrhea in the adult population was relatively high. The high prevalence of infection has a health implication. The high level of infection can interfere with both social and economic aspects of the population. As the population is vulnerable to these infections, the physical and mental efficiency can be affected by malabsorption, blood and protein loss and diarrhea. Gastrointestinal infections have a considerable impact on the working capacity of the adults and may also contribute to the aggravation of the unbalanced nutritional situation in the low income or no income groups.

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Considering the damage caused by these infectious agents arising from unsanitary conditions, it seems imperative to improve environmental sanitation in the city of Addis Ababa. In light of the occurrence of multiple infections, it is recommended that more appropriate methods and thorough examination of stools be made for the diagnosis of intestinal parasites. It is also necessary to perform both microscopic and in vitro culture methods

Ethiopia 1. Prevalent Shigella serotypes and Serotypes, J. Hyg. Dis. Rep. 2:79-82.

2. S.Y. 1985. Shigella from Ethiopia: Frequency and their susceptibility to drugs. Ethiop. Med. J. 23:97-101.

Armstrong, J.C. and Tadesse, C. 1975. Identification of hookworm species in Ethiopia. Am. J. Hyg. 101: 13-18.

Beaver, P.C., Jung, R.C. and Cook, J. Parasitology 1978 ed. 2. Philadelphia.

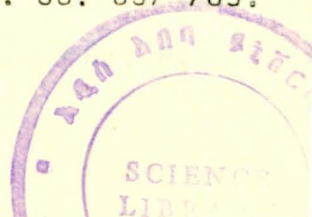
Bell, J.G. Adams

1971

References

- Abraham B., Atnafseged, A. and Mebratu, O. 1980. Critical study of the iron content of tef (Eragrostis tef). Ethiop. Med. J. 18:45-52.
- Afeworki, G.Y. and Yetnebersh, L. 1980. Multiple drug-resistance within Shigella serogroups. Ethiop. Med. J. 18:7-14.
- Afeworki, G.Y. and Eyasu, H. 1984. Shigellosis in Ethiopia 1. Prevalent Shigella serogroups and Serotypes. J. Diar. Dis. Res. 2:79-82.
- Afeworki, G.Y. 1985. Salmonella from Ethiopia: Prevalent species and their susceptibility to drugs. Ethiop. Med. J. 23:97-101.
- Armstrong, J.G. and Tadesse, C. 1975. Identification of hookworm species in Ethiopia. Ethiop. Med. J. 13: 13-18.
- Beaver, P.C., Jung, R.C. and Cupp, E.W. 1984. Clinical Parasitology (9th ed.)\* Lea and Febiger Philadelphia.
- Bell, R.G., Adams, L.S. and Ogaden, R.W. 1984. Trypanosoma musculi with Trichinella spiralis or Heligmosomoides polygyrus: concomitant infection in the mouse. Exp. Parasit. 58: 8-18.

- Buck, A.A., Anderson, R.I., Macrae, A.A. and Fain, A.  
1976a. Epidemiology of Polyparasitism I.  
Occurrence frequency and distribution of multiple  
infections in rural communities in Chad, Peru,  
Afghanistan and Zaire. *Tropenmed. Parasit.*  
29: 137-144.
- Buck, A.A., Anderson, R.I., Macrae, A.A., and Fain, A.  
1978b. Epidemiology of Polyparasitism II.  
Types of combinations, relative frequency and  
association of multiple infections. *Tropenmed.  
Parasit.* 29 61-70.
- Cook, G.G. 1986. The Clinical significance of gastro-  
intestinal helminths, a review. *Trans. Roy.  
Soc. Trop. Med. Hyg.* 80: 675-696.
- Cox, F.E.G. 1975. Enhanced Trypanosoma musculi infections  
in mice with concomitant malaria. *Nature*,  
London, 258:148-149.
- Cox, F.E.G. 1987. Interactions between parasitic protozoa  
of small mammals. *Mammal Review* 17:143 147.
- Crompton, D.W.T. 1986. Nutritional aspects of infection  
*Trans. Roy. Soc. Trop. Med. Hyg.* 80: 697-705.



- Crompton, D.W.T., and Tulley, J.J. 1987. How much ascariasis is there in Africa? *Parasitology Today*. 3:123-126.
- DuPont, H.L., Hornick, R.B. and Synder, M.J. 1972. Immunity in Shigellosis II Protection induced by Oral live vaccine or primary infection. *J. Infect. Dis.*, 125: 12-26.
- DuPont, H.L. and Pickering, L.K. 1980a. Amebiasis. In: Infections of the Gastrointestinal Tract: Microbiology, Pathology, and Clinical Features. Plenum Medical Book Company, New York pp 21-46.
- DuPont, H.L. and Pickering, L.K. 1980b. Giardiasis. In: Infections of the Gastrointestinal Tract: Microbiology, Pathology and Clinical Features. Plenum Medical Book Company, New York pp 47-60.
- Edwards, P.R. and King, W.H. 1972. Identification of Enterobacteriaceae (3rd ed). Minneapolis, Burgess Publishing Company. Minneapolis.
- Gilman, R.H., Davis, C. and Fitzgeralds, F. 1976. Heavy Trichuris infection and amebic dysentery in Orang Asli Children: a comparison of the two diseases. *Trans, Roy. Soc. Trop. Med. Hyg.* 70: 313-316.

Gilman R.H., Chong, Y.H., Davis, C., Greenberg, B., Virik H.K. and Dixon, H.B. 1983. The adverse consequences of heavy Trichuris infection. Trans. Roy.Soc. Trop. Med. Hyg. 77:432-438.

Haswell-Elkins, M.R., Elkins, O.B. and Anderson, R.M., 1987. Evidences for predisposition for humans to infection with Ascaris, hookworm, Enterobius and Trichuris in a South Indian Fishing Community. Parasitology 95:323-337.

Ilradi, I., Shido, S.G., Mohammed, H.H., Musa, G. and Hussien, A.S. 1987. The prevalence and intensity of intestinal parasites in two Somali Communities. Trans. Roy. Soc. Trop. Med. Hyg. 81:336 338.

Kaplan, A. and Szabo, L.L. 1983. Biuret Method. In: Clinical Chemistry: Interpretation and Techniques (2nd ed.) Lea and Febiger, Philadelphia.

Kaye, D.J.G., Mersells, J.G. and Hook, E.W. 1965. Influence of P. bergberi infection on susceptibility to Salmonella infection. Proc. Soc. Exp. Biol. Med. 120: 810-813.

Kloos, H., Lemma, A., Belete, K. Asefa, G. Bahara, M., Getachew, F and Desole, G. 1980. Intestinal Parasitism in migrant farm labour population

in irrigation schemes in the Awash Valley,  
Ethiopia and in major labour source area.  
Ethiop. Med. J. 18: 53-62.

Lamberticci, J.R., Godoy, P., Neves, J., Vambira E.A.  
and Ferreira M.D. 1988. Glomerulonephritis  
in Salmonella - Schistosoma mansoni association  
Am. J. Trop. Med. Hyg. 38: 97-102.

Lemma, A., Mesfin, O., and Mazengia, B. 1968. Parasito-  
logical survey of Addis Ababa and Debrezeit  
School-children with special emphasis on  
bilharziasis. Ethiop. Med. J., 6: 61-70.

Lo, C.T., Kloos, H. and Hailu B. 1988. Schistosomiasis.  
In: The Ecology of Health and Disease in  
Ethiopia. Zein Ahmed Zein and H. Kloos  
(editors). EMPDA, Addis Ababa. pp 196-207.

Martin, L.K. and Beaver, P.A. 1968. Evaluation of Kato  
thick-smear technique for quantitative diagnosis  
of helminth infections. Am. J. Trop. Med. Hyg.,  
17: 382-391.

Martinez-Palomo, A. 1987. The pathogenesis of amebiasis  
Parasitology Today, 3: 111-118.

in irrigation schemes in the Awash Valley,  
Ethiopia and in major labour source area.  
Ethiop. Med. J. 18: 53-62.

Lamberticci, J.R., Godoy, P., Neves, J., Vambira E.A.  
and Ferreira M.D. 1988. Glomerulonephritis  
in Salmonella - Schistosoma mansoni association  
Am. J. Trop. Med. Hyg. 38: 97-102.

Lemma, A., Mesfin, O., and Mazengia, B. 1968. Parasito-  
logical survey of Addis Ababa and Debrezeit  
School-children with special emphasis on  
bilharziasis. Ethiop. Med. J., 6: 61-70.

Lo, C.T., Kloos, H. and Hailu, B. 1988. Schistosomiasis.  
In: The Ecology of Health and Disease in  
Ethiopia. Zein Ahmed Zein and H. Kloos  
(editors). EMPDA, Addis Ababa. pp 196-207.

Martin, L.K. and Beaver, P.A. 1968. Evaluation of Kato  
thick-smear technique for quantitative diagnosis  
of helminth infections. Am. J. Trop. Med. Hyg.,  
17: 382-391.

Martinez-Palomo, A. 1987. The pathogenesis of amebiasis  
Parasitology Today, 3: 111-118.

- McConnell, E. and Armstrong G.C. 1976. Intestinal Parasitism in 50 communities on the central plateau of Ethiopia. *Ethiop. Med. J.* 14: 158-168.
- Mesele, G. and Alebachew, T. 1981. Antimicrobial resistance and R<sub>f</sub> factors of Salmonella isolates from Addis Ababa. *Ethiop. Med. J.* 19: 77-85.
- Mesele, G. and Alebachew T. 1982. Shigella species from Addis Ababa: Frequency of isolation and in vitro drug sensitivity. *J. Hyg.* 88:47.
- Mogessie, A. 1983. The Prevalence of Salmonella, Shigella and Yersinia enterocolitica in Adult Diarrheal out-patients in some Hospitals of Addis Ababa. M.Sc. - Thesis, School of Graduate Studies, Addis Ababa University.
- Moyenudin M. Rohman, K.M. and Sack, D.A. 1987. The aetiology of diarrhea in children at an urban hospital in Bangladesh. *Trans. Roy. Soc. Trop. Med. Hyg.* 81: 229-302.
- Murray, J. Murray, A. Murray, M. and Murray, G. 1978. The Biological suppression of malaria: an

ecological and nutritional interrelationship of a host and two parasites. Am. J. Clin. Nutrit. 31: 1363-1366.

Onile, B., Komolafe, F. and Oladiran, B. 1985. Severe strongyloidiasis as an occult gastrointestinal tract malignancy. Annals. Trop. Med. Parasit. 79: 301-304.

Rahman, M.M. 1984. Shigellosis: an old disease with new faces. J. Diar. Dis. Res. 2:208.

Sen, D., Saha, M.R., Niyogi, S.K., Nair, G.B., De, S.P. Datta, P., Datta, D. Pal, S.C., Bose, R. and Roychowdhury, J. 1983. Aetiological studies on hospital in-patients with acute diarrhea in Calcutta. Trans. Roy. Soc. Trop. Med. Hyg. 77: 212-214.

Shibru, T., Teklemariam, A, Hailu, B. and Lo.C.T. 1982. Intestinal helminthiasis in Ethiopia. In: Schistosomiasis in Ethiopia. Proceedings of a symposium on human schistosomiasis in Ethiopia. Institute of Pathobiology, Addis Ababa University pp 51-58.

Shibru, T. 1986. Hookworm infections in several communities in Ethiopia. East African Medical Journal 63: 134-138.

- Shibru, T. and Teklemariam, A. 1986. Ascariasis distribution in Ethiopia. *Ethiop. Med. J.* 24:79-86.
- Shibru, T. and Leykun, J. 1985. Distribution of Ancylostoma duodenale and Necator americanus in Ethiopia. *Ethiop. Med. J.* 23: 149-158.
- Sioum, T., Yahya, A. and Fisseha H.M. 1981. Intestinal parasitic infection in pre-school children in Addis Ababa. *Ethiop. Med. J.* 19:35-40.
- Teklemariam A., 1982. The distribution of Schistosomiasis in Ethiopia. Results of 1978-82 survey. In: Schistosomiasis in Ethiopia, proceedings of a symposium on human schistosomiasis in Ethiopia, Institute of Pathobiology, Addis Ababa University pp 1-8.
- Tesfamichael, TY. 1983. Intestinal helminthiasis among the out patients of Zway health centre, Central Ethiopia. *Ethiop. Med. J.* 21:155-159.
- Torrey, F.E. 1966. A medical survey of the Saysay people in the Blue Nile Gorge. *Ethion. Med. J.* 4:155-165.
- Volk, W.A. 1978. Essentials of Medical Microbiology J.B. Lippincott Company, Philadelphia, pp 288-3


- Wakelin, D. 1986. Genetic and other constraints on resistance to infection with gastrointestinal nematodes. *Trans, Roy. Soc. Trop. Med. Hyg.* 80:242-247.
- Walsh, J.A. and Warren, K.S. 1979. Selective primary health care: an interim strategy for disease control in developing countries. *New Eng. J. Med.* 301: 967-974.
- WHO, 1981. Intestinal protozoal and helminthic infections. Technical Report. Series No. 666. World Health Organization, Geneva.
- WHO, 1980a. Enteric Infections due to Campylobacter, Yersinia, Salmonella and Shigella. *Bulletin of the World Health Organization* 58: 529-537.
- WHO, 1980b. Parasite-related diarrheas. *Bulletin of the World Health Organization* 58: 819-830.
- WHO, 1987a. Public health significance of intestinal parasitic infections. *Bulletin of the World Health Organization*, 65:575-588.
- WHO. 1987b. Development of vaccine against Shigellosis: Memorandum from WHO meeting. *Bulletin of the World Health Organization* 65: 17-15.

Zein, A. 1988. Health and Health Services in Ethiopia,  
A general survey. In: The Ecology of Health  
and Disease in Ethiopia. Zein Ahmed Zein and  
H. Kloos (editors), EMPDA Addis Ababa, pp 1-17.

DECLARATION

I, the undersigned, declare that this thesis is my work and that all sources of material used for the thesis have been dully acknowledged.

Name Tesfay Kineselassie

Signature  \_\_\_\_\_

Place and date of submission

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