CHAPTER-I

1 INTRODUCTION

Gastroenteritis is one of the most important public health problems in the world having cosmopolitan distribution. Both bacterial and parasitic diseases are known to cause the intestinal infections in man with respect to multiple social, economical, cultural, physiological and behavioral parameters. More than half of the human population in the globe live in misery pain and suffer vast economic loss due to parasites. About one fourth of the world populations are estimated to be infected by one or more species of intestinal parasites (Rai *et al,* 1998). Enteric pathogens, reportedly affect 3.5 billion people globally (Rai *et al*, 2004).

World Health Organisation has estimated that *Ascaris lumbricoides*, Hookworm and *Trichuris trichiura* infect 1.4 billion, 1.3 billion, 1.0 billion people worldwide, respectively. It is estimated that the global burden of the diseases caused by these three major intestinal nematodes is 22.1 million disability adjusted life years lost from Hookworm, 10.5 million from *A. lumbricoides*, 6.4 million from *T. trichiura* and 39.0 million from the three infections combined, in comparison to malaria at 35.7 million and Schistosomiasis at 4.5 million. The protozoan parasites although being less common are associated with the highest number of mortalities (Chan *et al,* 1994). WHO (1995) has reported diarrhoeal diseases as one of the five biggest killers resulting 3.1 million deaths. Similarly, it has reported intestinal worm infections as one among ten biggest killer resulting 135000 deaths.

Intestinal parasites are endemic in most tropical and sub-tropical countries, particularly in developing countries and are one of the important causes of diarrhoeal diseases. At least 750 million episodes of diarrhoea occur per year in developing countries resulting in five million deaths (Bansal *et al,* 2004). Gastroenteritis is a major killer disease in Nepal. Every year 30-40 thousand people die of gastroenteritis (Bista *et al*, 1993).

Although the intestinal parasitosis and the diarrhoeal diseases have been found to bear wider prevalence among children, their significant occurrence among other age groups, especially geriatric population cannot be underestimated. Gastrointestinal infections may not readily spring to mind as a major cause of gastrointestinal disease in the elderly. Nevertheless, a special consideration of gastrointestinal infections in this group is warranted because their response to such infections may be different from that of younger population.

In developed countries, outbreaks of infective gastroenteritis are known to occur among elderly patients in nursing homes, with considerable mortality. In these countries, infectious diarrhoea is fourth most common infectious disease in elderly patients confined to chronic care facilities. Prospective studies report an incidence of approximately 33 cases per 100 patients per year. Risk factors for infectious diarrhoea in these countries include residence in nursing homes, recent antibiotic therapy and travel abroad. Outbreaks of diarrhoea confined to elderly individuals are rare in developing countries where the elderly are rarely confined to nursing homes for chronic care. In such countries, many gastrointestinal infections are endemic in the community, and people are exposed to these in childhood, with resultant immunity. It is rare to find adults being symptomatic from infection with these pathogens. On the other hand, if a pathogenic agent newly enters the community, adults are affected equally with children. Under such circumstances, the elderly may be more often affected.

The elderly people have a predisposition to infectious diarrhoea. This predisposition could result from several factors including age related immune system dysfunction, achlorhydria, altered intestinal or colonic motility and changes in fecal flora. Although gross measures of immune function are normal in the elderly, more sophisticated tests of leukocyte function have identified differences in the immune response of older and younger adults. Hypochlorhydria or achlorhydria may occur in elderly patients secondary to atrophic gastritis or to the use of long term histamine H_2 antagonists or proton pump inhibitors. Gastric acid is one of the recognized barriers that protect against gastrointestinal infection, and its absence may increase the probability of developing infectious diarrhoea. Disturbed intestinal motility due to neuromuscular diseases such as stroke, diabetes or microvascular atherosclerosis is common in the elderly and may predispose to colonization by enteropathogens.

Institutionalization significantly increases the risk of infection from common source outbreaks such as food-borne epidemics and by person to person spread. A shared toilet facility with patients with infective diarrhoea (especially those with faecal incontinence) increases the risk of infection (Ratnaike, 1999). This is more probable in elderly homes.

In developed countries, pathogens isolated from elderly patients with diarrhoea include *Shigella* spp. and *Salmonella* spp. while *Campylobacter jejuni*, *Giardia lamblia* and Rota virus infection are less frequent. Even in developing countries where infection with these organisms is endemic, common enteropathogens must be considered first in elderly patients with diarrhoea. *G. lamblia*, *Entamoeba histolytica* and *Strongyloides stercoralis* must all be considered as causes of diarrhoeal disease in elderly, although no special predilection is reported. Giardiasis causes diarrhoea in children, but is not usually considered as a cause of adult diarrhoea. However, in the elderly, with depressed intestinal immune function, it is quite possible that it may cause diarrhoeal illness. Strongyloidiasis leads to chronic diarrhoea and malabsorption. Besides these, other intestinal parasites like *A. lumbricoides*, *T. trichiura*, Hookworm, *Cyclospora* spp., *Cryptosporidium* spp, *E. coli*, *Blastocystis hominis* etc may be recovered from the elderly people as community acquired parasites and their possible susceptibility to these parasites is remained to be proved (Ramakrishna, 1999).

1.1 Statement of the problem

Children being the major victim of the infection, most of the researches in intestinal infections are concerned with pediatric age group. The condition is not different in Nepal too. In the country like Nepal, there is hardly any research done in the field of health situation of elderly people. The condition of elderly people in any other field is getting vulnerable especially due to rapid and unplanned urbanization and modernization. A global research has shown 89.6% of elderly population has multiple morbidities including gastrointestinal problem (Seshubabu, 1996).

The common predisposing factors for the intestinal infections in elderly people include age related immune system dysfunction, achlorhydria, altered intestinal or colonic motility and change in fecal flora. The development of diarrhoeal disease in elderly carries a particularly high mortality, which increases up to 400 fold in 75+ patients compared to young adult (WHO, 1975). As in the children, gastrointestinal infection has potential to worsen nutritional status in elderly people further worsening the immune system leading to various morbidities and mortalities (Ramakrishan, 1999). Porter *et al* (1995) has reported undernutrition common among elderly people and stated association with increased risk of infection. He has mentioned elderly people both in their own home and in institutional care are at risk from nutritional deficiencies.

Owing to poor sanitary and other living condition of majority of Nepalese families, the elderly people/members also could be the major victim of these infections. Studies carried out by Dhungana *et al* (2004) and Luitel (2003) on geriatric people in Nepal have revealed gastrointestinal complain as the major health complain of old people. The physical disability and less effective self care during old age lead to insufficient sanitary practice and poor personal hygiene which makes old people more susceptible to gastrointestinal infections.

Residents of long-term care facilities are both representative of elderly people generally and reasonably approachable, and therefore constitute a suitable sample for investigation (Lee and Liao, 2004). There are number of elderly homes in different parts of the country that accommodate socially abandoned, childless and helpless old people from different parts of the country. Almost all of them represent the poor socioeconomic background that of an average Nepali citizen. Thus, their lifestyle and health conditions are not much different from an average Nepali old man. However, the diverse socioeconomic status of the Nepalese old people should also not be undermined. So the study tends to consider the elderly residing in the elderly home as the representative of an average Nepali old man despite of the better socioeconomic status of some Nepalese elderly people. It is a notable fact that the sharing of common mess and room, sanitary conditions and other environmental factors in elderly home may significantly affect their health. These conditions also may vary depending upon the ownership and management of the elderly home i.e. government or private.

Thus, a research was needed to reveal the prevalence and pattern of intestinal infections among elderly people and correlate the condition with different social and clinical variables. To assess a portion of geriatric health in Nepal and its condition in relation to elderly homes, a study was desired. Elderly people being honorable property of nation, identification of their problems and development of their solutions become everyone's responsibility.

1.2 Rationale/justification of the research topic

The stated problem is a current and happening problem, getting intensified with the marked increasement in the number of elderly people in the country and with an ever-increasing proportion of homeless, destitute, incapacitated and poor elderly people as well. The lack of studies in the above mentioned sector may have serious consequences in terms of mortality, morbidity and quality of life of the elderly people along with the deficiency of complete information on geriatric health. Obviously, the problem has broad social, economical, epidemiological and health implications. Care of elderly is one of the priority areas as published by MoH in 1999. Similarly, the Government of Nepal has adopted National Policy on Aging since its 9^{th} long term plan. After all, there are very few researches done on elderly health in Nepal and the published data on the prevalence of intestinal infections among aged people is lacking globally. Therefore, this study is believed to be helpful in providing efficient geriatric care, revealing the health problems of geriatrics and eliminating the scarcity of data on some aspects of elderly health. This study may guide the researchers for further studies on different aspects of geriatric health and help planners in launching appropriate plans and policies on geriatric health care.

CHAPTER-II

2 OBJECTIVES OF THE STUDY

2.1 General objective

To determine the prevalence of intestinal infections by parasite and some bacteria among elderly people in Kathmandu Valley.

2.2 Specific objectives

- To assess the prevalence of intestinal parasitosis among elderly people.
- To assess the prevalence of bacterial infections (*Salmonella* spp., *Shigella* spp., *Vibrio cholerae* and C*ampylobacter* spp.) among elderly people.
- To correlate the parasitic and bacterial infections with different social and clinical parameters.
- To correlate the parasitic and bacterial infections with the nutritional behaviours.
- To assess the pattern of parasitic infections among elderly people.
- To compare the pattern of intestinal infections among the elderly people residing in elderly homes with those residing in their own homes.
- To compare the pattern of intestinal infections among the elderly people residing in government run elderly homes with private/community run elderly homes.
- To determine the antibiotic susceptibility pattern of the bacterial isolates

CHAPTER-III

3 LITERATURE REVIEW

Diarrhoea and gastroenteritis are the major health problems in developing countries like Nepal due to unsafe and inadequate water supplies and sanitation, little or no health education, illiteracy, undernutrition, incorrect feeding practice, wide spread fecal contamination of environment, underlying diseases, dense population, gastric hyperacidity, poverty, immunodeficiencies, superstitions etc. (Chand *et al*, 2001). Bacterial pathogens such as *V. cholerae*, *Salmonella* spp., *Shigella* spp., EPEC and others are implicated in various morbidity and mortality and exist together with various kinds of intestinal parasites.

The studies show that along with the individuals of other age group, especially children, the elderly people are not free from the wide spread risk of gastroenteritis. The poor living standard and the raised clinical vulnerability of the elderly people in the countries like Nepal have increased their susceptibility towards such infections.

Ageing is growing old or maturing, progressive changes related to the passage of time (Taber's cyclopedia medical dictionary, 1999). Despite the universality, ageing is difficult to define. Shakespeare probably characterized it best in his elegant description of the seven stages of man. It begins at the moment of conception, involves the differentiations and maturation of the organism and its cells at some variable point in time, leads to the progressive loss of functional capacity of senescence, and ends in death (Robbins, 1998). With age, there are physiological and structural alternations in almost all organ system. Ageing in individuals is affected to a great extent by genetic factors, social conditions and the occurrence of age related diseases.

In Nepal different ages are used as the starting of ageing. At government level, the retirement of civil servant is fixed at 58 years. So for them ageing is supposed to start after the completion of 58 years. But in universities, the retiring age for teachers and administrators is 63 years. But for the purpose of legal activities and granting of pensions to general mass, the age fixed for a person to be old is 75 years. Also old age is classified into two broad groups: 60-74 years ages as young old and 75+ years as old old (Singh, 2003).

The old people above 65 years occupy 7.0% of world's and 6.0% of Asia's total population. In South Central Asia, 4.0% people are above 65 years (2005 World population data sheet of the population reference bureau). In developing countries, the process of population ageing in contrast to developed societies has been much faster and out of pace with all round development in other aspects of the society. The process of achieving life expectancy beyond 70 years and aged population of 10.0% has taken more than 100 years in Europe and North America. On the contrary, the same rate has been achieved in Asia and Latin America in 25 to 30 years. In 2025, three quarters of the 1.2 billion world elderly will be living in the developing countries (Sharma and Dey, 1999).

The population ageing is emerging issue in Nepal eventhough the pace of process is slow. The remarkable improvement in mortality rate over the last 3 decades and clear sign of decline in total fertility rate in the 1990s have brought notable change in the structure of population in Nepal (Luitel, 2003). The proportion of aged people in Nepal is at increasing trend. During first population census in 1911, 4.3% of total populations were people above 60 years, which reached 7.5% by the time of 10^{th} population census in 2001 through 5.3% in 1941 and 5.9% in 1971. It has been found that, though the proportion of young children under 15 years has remained virtually constant around 40% for all the years, the proportion of aged people by all major aged groups is found increasing steadily (Singh, 2003). The growth rate of aged population (60+) in 1961 was 1.8%, where as, it became 2.4% in 1971 and 3.3% in 1981. This is the clear indicator of increasing trend of growth rate of elderly population (Luitel, 2003).

The regional distribution of 60+ people according to 2001 census was: Mountain region-7.2%, Hilly region-7.0% and Terai region-7.9%. According to the census, the literacy rate (who can read and write) for aged 65+ years was 27.0% for male and 4.1% for female. Among 65+ people, 47.1% were found economically active with sex differential of 59.7% for male and 34.3% for female (Singh, 2003).

Normal ageing is not a disease but eventually leads to structural and functional decline and involves increased susceptibility to diseases. Ageing seems not to affect all physiological functions to the same degree, so that the total ageing rate of different organism will differ. Factors related to ageing changes can be determined as intrinsic and extrinsic. The intrinsic factors are related to normal ageing such as genetic, while extrinsic factors include the environment and life style. The physiological changes occur in all body systems as musculoskeletal, cardiovascular, respiratory, neurological and gastrointestinal systems. Significantly, these changes lead to diseases (WHO, 2004).

Diseases often manifest at an earlier stage because of the impaired physiological reserve in older patients. Symptoms always reflect an imbalance between severity of disease and intrinsic compensatory mechanisms. Since preexisting diseases or physiological decline impairs these mechanisms, mild disease often tips the balance. Similarly, drug related side effects could appear with drugs with doses safer for younger individuals (Sharma and Dey, 1999).

Clinical signs considered abnormal in younger patients, are often common and normal in older people and may not be associated with a particular disease. In older patients, multiple symptoms are usually due to multiple diseases and drug use. The usual practice of explaining all symptoms and signs with one pathology as in a younger patient does not hold true in geriatric practice. Many symptoms such as falls, syncope, dizziness, hip fracture, incontinence, delirium etc are caused by multiple pathological states and extensive investigation for a single diagnosis is often non-productive. Even when a diagnosis is made, adequate treatment of a single disorder is often not curative due to the presence other not so prominent pathological states (Sharma and Dey, 1999).

Ageing is probably the commonest cause of an immunocompromised state. As a result, there is an increase in incidence of infection, cancer and autoimmunity. There are several indicators of immunodeficiency in old age, namely decreased production of the specific antibody following immunization and exposure to foreign antigens, involution of thymus and bursa fabricus in birds, decline in T cell proliferation and decreased production of interleukins (IL-2). However, some usual markers of immune deficiency don't always accompany immune senescence. The number of blood lymphocytes and serum concentration of immunoglobulin does not decline with ageing, neither is there any evidence to suggest decrease in the total activity of the immune system with age. In contrast, the secretion of certain interleukins (IL-4, IL-5 and IL-6) increases in old age along with an increase in the number and competence of subsets of lymphocytes that secrete them. Changes in the repertoire of lymphocytes for antigens and regulation of monocyte-macrophage function also have been observed in old age. For these reasons, immune senescence is best described as dysregulation and not deficiency (Sharma and Dey, 1999).

Many concepts of caring for the elderly have emerged through time. Basically, they fall into two categories: "Home for the aged" and "Housing for the Elderly". The former includes nursing homes and long-term care facilities where clinical care is provided to ill and aged people, usually for the rest of their life. The latter includes community residential care, assisted living facilities, adult family home, family care home, planned care development, community ageing-in-place etc. In Nepal, elderly population has always been considered passive recipients of support. The only housing scheme in Nepal is the simple concept of housing for the elderly that was conceived centuries ago. There has been no formal and thorough research into the different schemes for housing and caring for the elderly that can be feasible in Nepal nor is there any systematic inquiry into the quality of life in the existing housing facilities and the problems they are facing (Dhungana *et al*, 2005).

Various types of pathogens are responsible for causing gastrointestinal infections in elderly people. Among bacteria, the common ones are *Helicobacter pylori*, *Shigella* spp., *Salmonella* spp., *Vibrio cholerae*, *Aeromonas* spp., *Plesiomonas* spp., *Campylobacter* spp., *Yersinia enterocolitica*, *Clostridium perfringens*, *Clostridium difficile*, Enterotoxigenic *E. coli*, Enterohemorrhagic *E. coli,* Enteroaggregative *E.coli,* Enteroinvasive *E. coli* etc. Similarly, Rota virus, Norovirus, Astrovirus, Calcivirus and Enterovirus are the major viruses responsible for causing gastrointestinal infections in elderly people. Among the intestinal parasites, the common ones are *Cryptosporidium* *parvum*, *Cyclospora cayetanensis*, *Entamoeba histolytica*, *Giardia lamblia*, *Ascaris lumbricoides*, Hookworm, *Trichuris trichiura*, *Strongyloides stercoralis* etc. The fungi like *Candida albicans* are also found to cause the intestinal infections.

Infection with the organism *Helicobacter pylori* is the most common infection with any organism worldwide. It is responsible for a large number of gastrointestinal diseases throughout the world. *H. pylori* infection causes chronic gastritis and is responsible for most cases of duodenal ulcer and gastric ulcer disease. Strong associations of this infection with gastric cancer and lymphoma have also been noted. The prevalence of *H. pylori* infection increases with age. A substantial proportion of the population (depending on the area of residence) acquires the infection by age 15, and subsequent rate of acquisition is between 1.0-3.0% per year of adult life. Serological studies indicate that 80.0-90.0% of persons over the age of sixty in developing countries are infected with this organism. In western countries, infection is less frequent with about 50.0% of persons over sixty harboring *H. pylori.*

Vibrio cholerae is responsible for causing a devastating disease, cholera due to the rapidity with which severe dysfunction occurs. In epidemics in Southern India caused by *V. cholerae* O139, and in the recent cholera epidemics in the Americas, the elderly were affected disproportionately, with an excess of mortality in this age group. In both situations, an organism was introduced to which the population had not been exposed earlier.

Shigella species are commonly isolated from elderly patients with diarrhoea in developed countries. Several different subgroups, *S. dysenteriae, S. flexeneri*, *S. boydii* and *S. sonnei* are known. Shigellosis is essentially an infection of the colon, and symptoms are caused by invasion of the mucosa causing inflammation and ulceration. This results in diarrhoea with blood and mucus (dysentery). Shiga toxin, an additional pathogenic factor, is produced only by *S. dysenteriae* and by limited strains of *S. flexeneri* and *S. sonnei*. Shiga toxin is a serotoxin, causing cell death due to suppression of protein synthesis and may be responsible for manifestations such as HUS described in children. It appears that some species are more likely to affect the elderly people.

Thus, *S. flexeneri* infection is reported more commonly in elderly patients, as compared to *S. sonnei*. Elderly patients present usually with watery diarrhoea, fever, and abdominal pain. 3-5 days after onset, tenesmus (pain on defecation) and small volume bloody stools may commence. Bloody stools are less frequent in the elderly than in children.

Shigellosis in the elderly is characterized by a prolonged clinical course, often lasting over a week if untreated. HUS as a complication of shigellosis has not been reported in the elderly. A reactive arthritis involving large joints asymmetrically may occur occasionally two to three weeks after the onset of acute dysentery. Laboratory abnormalities are often noted in elderly patients and include abnormalities of serum electrolytes and hepatic enzymes.

Salmonellae (non-typhoidal) are a common cause of gastroenteritis in the elderly in developed countries. Transmission is food-borne, and flies, food, fingers, feaces and fomites are all implicated. Non-human reservoirs play a major role in transmission of the disease. Infection can cause a variety of clinical syndromes including acute gastroenteritis, bacteremias, focal non-intestinal infections, typhoid and asymptomatic carrier state. Gastrointestinal symptoms are due both to invasion of the mucosa by the organism, and to net fluid and electrolyte secretion induced by the organism. The elderly are more liable than younger patients to become symptomatic when infected with non-typhoidal *Salmonella* species.

C. jejuni infection is very common in developing countries. The most common route of transmission is from infected animals to human beings, though consumption of contaminated or improperly cooked food is also causal. The organism has also been isolated from water. In developing countries, exposure is common during childhood and adults are unlikely to develop diarrhoea due to the organism. The organism may colonize either the small or large bowel or produce diarrhoea, which may be either watery or bloody.

C. perfingens produces an enterotoxin that is a common cause of food poisoning. Characteristics of this organism that contribute to its ability to cause food-borne illness include the formation of heat resistant spores that survive normal cooking/heating temperatures, a rapid growth rate in warm food, and the production of enterotoxin (CPE) in the human gut. However, CPE-induced diarrhoea has been reported in the absence of defined food vehicle. These cases typically occur in elderly persons without the history of exposure to contaminated food, and usually occur following a course of antibiotic therapy.

Clostridium difficile was originally implicated in the causation of antibiotic associated colitis. The organism elaborates two cytotoxins, which probably induce secretion as well as epithelial cell death. The organism is found primarily in the colon, where it induces inflammation and ulceration with formation of a 'pseudomembrane'. It is responsible for the colitis seen occasionally following antibiotic therapy. Age is now considered to be a risk factor for infection with the organism. Thus, *C. difficile* has been described as a significant cause of diarrhoea in the elderly in developing countries. This occurs even in the absence of antibiotic use and carries a high mortality. In some elderly patients, infection with *C. difficile* may induce protein losing enteropathy (secondary to colonic exudation) in the absence of diarrhoea.

Rotaviruses, usually thought as a cause of gastroenteritis in children, is also considered as a cause of non-bacterial diarrhoea in elderly patients. Rotavirus gastroenteritis has been noted mainly in chronic care and rehabilitation facilities. The virus appears to spread by fecal-oral route and is highly contagious, but appropriate measures can limit its spread. Unlike infection in children, rotavirus infection in adults produces a milder illness. Symptoms may be severe in elderly patients with underlying malnutrition. Diarrhoeal outbreaks due to other viruses (Norovirus, Astrovirus, Calcivirus and Enteroviruses) are not uncommon in chronic care facilities for the aged. These agents are probably also responsible for sporadic diarrhoea in the community.

Small intestinal bacterial overgrowth has been described in elderly patients even without predisposing structural abnormalities of the small intestine (such as stricture or blind loops). Bacterial overgrowth occurs in these patients probably because of altered motility secondary to ageing. Fasting hypochlorhydria, or changes in mucosal immunity related to age, do not appear to be major contributory factors. The syndrome is reported in patients aged 75 years or more, who present with chronic diarrhoea, anorexia or nausea. Features of vitamin B12 insufficiency may be present. Bacterial overgrowth may also be seen in patients with small intestinal diverticulosis, in whom the incidence of small bowel bacterial overgrowth increases with age (Ramakrishna, 1999).

Global scenario of intestinal infections

Walsh (1986) has stated global prevalence of *E. histolytica* to be 400 million. Warren and Mahmond (1984) have stated the global prevalence of *A. lumbricoides*, Hookworm and *T. trichiura* to be 1000 million, 900 million and 500 million respectively. According to Chan *et al* (1994), it has been estimated that the global burden of the diseases caused by these three major intestinal nematodes is 22.1 million disability adjusted life years lost from Hookworm, 10.5 million from *A. lumbricoides*, 6.4 million from *T. trichiura* and 39.0 million from the three infections combined, in comparison to malaria at 35.7 million and Schistosomiasis at 4.5 million. The protozoan parasites although being less common are associated with the highest number of mortalities.

In Finland, a survey on intestinal parasites was done among 243 inmates of institution for the mentally retarded, 537 hospital patients from 4 areas, 100 military servicemen, and 65 prisoners. Among the mentally retarded, the prevalence of intestinal parasites was high, 41.3% among children and 51.5% among adults. The commonest parasite was *E. coli*. In Helsinki, the prevalence among children in hospital was only 7.0%, but among adult out patients, 18.9%. Among hospital patients in other areas, military servicemen, and prisoners in Helsinki, the prevalence of intestinal parasites was low. The high prevalence of *E.coli*, 14.8% was among adult out-patients in Helsinki. Four cases of *Trichuris* infection were detected among the mentally retarded (Kyronseppa and Pettersson, 1976).

In a survey conducted in Kar Kar island, Madang province, three helminth species were recorded: *A. lumbricoides* infections were highest and heaviest in childhood, and fell to low levels in adult life, the overall infection rate being 57.0%. More than 70.0% of the adult population was infected with *N. americanus*, though almost all infections were light. 21.0% were infected with *T. trichiura*, and all infections with this species were light (Jones, 1976).

A survey of 1,548 stools samples done in Gabon showed parasitism ratio of about 100.0% in the adult population. Among the protozoa, *D. fragilis* was found in 0.3% of the cases. In contrary to the usual observations in tropical Africa, *A. lumbricoides* and *T. trichiura* infestation was very high in the villages (Garin *et al*, 1978).

G. lamblia (15.2%) and *H. nana* (20.4%) were the two most frequently reported species in a study of intestinal parasites from 1683 aboriginal people in Western Australia. Concurrent infection with the two species was statistically significant in the 0 to3 years age group only (P<0.01), and it was suggested that in older age groups the presence of one of these parasites may in some way inhibit the development of the other. *H. nana* infection was more common in males than in females (P<0.001). Hookworm and *S. stercoralis* infections were confined to the tropical north of the State. The total or near-total absence of infection with *A. lumbricoides*, *T. trichiura* and *Entamoeba* spp. (all frequent in Eastern Australian aboriginal communities) was noted (Jones, 1980).

Studies in Namibia revealed prevalence rates of 63.0% for Hookworm and 35.0% for *T. trichiura* in 31 Bushmen, 4-65 years of age, who were encamped in the Kaudom Game Reserve. The study also revealed prevalence rates of 85.0% for Hookworm, 25.0% for *S. stercoralis*, and 1.0% for *T. trichiura* in 103 children, 6-17 years of age, attending 5 schools in Bushmanland. The 25.0% Strongyloides infection rate was one of the highest prevalences recorded in Southern Africa (Evans *et al*, 1990).

The importance of diarrhoeal illnesses among older persons is being increasingly recognized. According to CDC, Out of total diarrhoeal deaths in US from 1979-1987, one-half of deaths occurred in elderly over 74 years (Lew *et al*, 1991). Likewise Out of total diarrhoeal deaths among hospitalized patients, four-fifth of the deaths occurred in 60 and 60+ age group (Gangarosa *et al*, 1992).

In a study conducted in Bendal state of Nigeria among people of age 1-90 years, the overall percentage incidence of six parasites encountered were as follows: *E. coli* (19.7%), *E. histolytica* (3.9%), G. lamblia (1.4%), Hookworm (29.4%), *A. lumbricoides* (38.2%) and *T. trichiura* (7.3%). The age groups in the first two decades of life had 61.7% infection and this stabilized to a low level of 2.8% in the eighth and ninth decades. Males had higher protozoan and helminthic infections than females (Obiamiwe and Nmorsi, 1991).

Roughly, 1.5 billion individuals are infected with *A. lumbricoides* in the world (Crompton, 1999). In Guatemala, 41.0% of the population in a rural village was infected with *A. lumbricoides* and 60.0% with *T. trichiura* (Anderson *et al*, 1993). In a study done among 100 elderly residents of Central America, 48.0% helminth infection rate was assessed which consisted of Hookworm, *Trichuris* and *Ascaris* infection. The prevalence of each of the individual parasites was considered light to moderate and the intensity of infection was generally low in this population (Elias *et al,* 1997).

Other studies have found that there is a positive association between *A. lumbricoides* and *T. trichiura* infections (Udonsi *et al,* 1996). Kightlinger *et al* (1995) and Needham *et al* (1998) have found a dual species intensity correlation for *A. lumbricoides* and *T. trichiura* infections; high intensity infections of *A. lumbricoides* were associated with high intensity infections of *T. trichiura*. The reasons for this were unclear, but it may be explained by the similar route of infection, the faeco-oral, for both parasites. The prevalence of intestinal helminthiasis, ascariasis (73.7%), trichuriasis (73.7%) and necatoriasis have been measured in a town of Guadalupe (Ripert *et al*, 1996). In a study of an urban slum in the state of Ceara´ in Northeastern Brazil, it was found that 10.6% of the sample population was colonized with *E. histolytica* (Braga *et al,* 1998).

A study by Smith *et al* (2001) in the four rural communities of Hondurus revealed the overall prevalence of *A. lumbricoides* and *T. trichiura* 45.0% and 38.0% respectively. Over a quarter (25.8%) of the 240 stool specimens examined had double infection of both *A. lumbricoides* and *T. trichiura*. Children aged 2-4 had the heaviest infections while children aged 5 to 12 were most frequently infected with moderate and light infections compared to other age groups. Overall, there were more intense infections (heavy and/or moderate) of *Ascaris* than *Trichuris* in all age groups. Approximately 45.0% of males and 45.0% of females of all ages surveyed were infected with *A. lumbricoides*. Approximately 41.0% of males and 35.0% of females of all ages were positive for infections with *T. trichiura*.

Magambo *et al* (1998) found that the infection rate of several intestinal pathogens including *Trichuris* was higher in males compared to females in Sudan, however, it was not reported if this difference was statistically significant. An epidemiological study from Madagascar found that girls had a significantly higher prevalence and intensity of ascariasis (Kightlinger *et al,* 1995) while another study from Guatemala did not find any gender differences with respect to parasitosis (Anderson *et al,* 1993). These results may indicate that gender may or may not play a role in parasitosis, depending on the region and other environmental or behavioral factors.

Amoebiasis due to the infection with *E. histolytica* is one of the most important parasitic diseases worldwide. It has been estimated that *E. histolytica* causes 50.0 million cases of amoebic colitis and liver abscess, resulting in 100000 deaths annually. In developed countries, *E. histolytica*/*E. dispar* infection has been observed in homosexual man and institutionalized persons in addition to overseas travelers. The outbreaks have been also reported in the institutions for mentally retarded people (Tachibana *et al,* 2000).

In a study conducted by Oliveira *et al* (2003) among children and adults from a landless camping in the rural area of Uberlândia, State of Minas Gerais, Brazil, 65.4% individuals were found to be infected with intestinal parasites; 45.1% children and 54.9% adults, of whom 66.7% were mono-infected, 17.6% bi-infected, and 15.7% poly-infected. 47.0% individuals were infected with protozoa, 29.4% with helminths and 23.6% with both infections. According to sex, the positivity rate for intestinal parasites and commensals in the studied population was 41.0% for male and 24.4% for female ($P < 0.05$). Regarding the age groups, positivity rate was 29.5% in 1-15 years, 20.6% in

16-30 years, 5.1% in 31-45 years, 6.4% in 46-60 years and 3.8% in 60+ people. Among parasites, *H. nana* was the most frequent helminth (14.1%) and *G. lamblia* (11.5%) the major pathogenic protozoa identified. Positivity rate of 6.4% was detected both for Hookworm and *S. stercoralis*. Positivity rate of 5.1% was detected for *T. trichiura* and 2.6% for *T. solium*. In the people above 60 years, infection rate of *E. coli* and *Endolimax nana* were 25.0% and 50.0% respectively.

In the United States in 1997, giardiasis cases per 100,000 state populations ranged from 1.0 to 42.3 with a national average of 9.5 cases per 100,000 population (Furness *et al*, 2000). A study conducted by Laupland and Church (2005) during 1999-2002 in Canada found that *Giardia* spp. infection occurred at a rate of 19.6 per 100,000 populations per year. Although the yearly incidence was stable, a significant seasonal variation was observed with a peak in late summer to early fall. Males were at significantly higher risk for development of this infection as compared to females (21.2 Vs.17.9 per 100,000/yr) and there was a significant decrease in risk associated with an increasing age. The incidence rate were 24.6/100000, 19.4/100000 and 6.2/100000 for the age group below 20 years, 20-64 years and above 64 years respectively. Similarly they found that *Cryptosporidium* spp. infection occurred at an overall rate of 6.0 per 100,000 populations per year although a large outbreak of *Cryptosporidium* spp. infections occurred in the second half of the summer of 2001. During August and September of 2001, the incidence of cryptosporidiosis was 55.1 per 100,000 per year as compared to 3.1 per 100,000 per year for the remainder of the surveillance period ($P<0.0001$). Cryptosporidiosis was largely a disease of children with an incidence of 17.8 per 100,000 per year occurring among those aged < 20 years of age compared to 1.25 per 100,000 per year for adults ≥ 20 years of age. The incidence rate was slightly higher among male, however it was not significant.

In a study conducted by Araujoi and Fernandezi (2005) in the city of Eirunepe, Amazon, samples of 413 patients were analyzed and positivity was found in 64.4% of the total samples. The intestinal parasites most prevalent were: *A. lumbricoides* (35.6%); *T. trichiura* (18.6%); Ancylostomides (9.9%); *S. stercoralis* (1%); *E. vermicularis* (0.5%); *E. coli* (24.9%), *E. histolytica* (13.3%) and *G. lamblia* (1%).

In a study conducted by Aimpun and Hshieh (2004) in Belize, Central America 66.0% of the population was found to have one or more intestinal parasites. The most common infection was Hookworm (55.0%) followed by *A. lumbricoides* (30.0%), *E. coli* (21.0%), *T. trichiura* (19.0%), *G. lamblia* (12.0%), *E. histolytica/dispar* (6.0%) and *I. butschlii* (9.0%). Other parasites found were *E. hartmani*, *S. stercoralis*, *E. nana, Isospora belli*, and *C. mesnili*. Children were more often infected than adults were and more females had hookworm infections.

A study in a rural community of Brazil showed prevalence of *A. lumbricoides* above 39.0% in all age groups, except for the group with oldest subjects (50-82 years). Prevalence of *T. trichiura* ranged from 25.0%, in the group with the oldest subjects (50-82 years), to 65.9%, in the 7-14 year-old group. Prevalence of Hookworm was above 43.0% in all age groups, except for the youngest one (0-6 years). The highest values of prevalence were found in the 7-29 year old range (Zani *et al*, 2004).

A study conducted in a community of rural Cote d'Ivoire found the prevalence of Hookworm, *E. histolytica/E. dispar* and *S. mansoni* to be 45.0%, 42.2%, and 39.8% respectively. Three-quarters of the population harboured multiparasites (Raso *et al*, 2005).

Tzipori *et al* (1983) observed cryptosporidial oocysts in 4% (36) of patients with acute gastroenteritis. Five patients had mixed infections while none without gastroenteritis had cryptosporidiosis. Prevalence was 1.6% in adults and was higher in summer.

A study conducted by Naumova *et al* (2003) among gastroenteritis hospitalizations before and during the 1993 Milwaukee outbreak found that the rate of gastroenteritis related emergency room visit increased with age in the elderly, suggesting that the elderly were at an increased risk. It suggested that the elderly had an increased risk of severe disease due to *Cryptosporidium* infection, with a shorter incubation period than has been previously reported in all adults and with a high risk for secondary person-to-person transmission.

In Egypt, the incidence of cryptosporidiosis in immunocompromised patients was 13.3% and 18.2% in Alexandria as reported by Hammouda *et al* (1996) and AbouEl-

Naga et *al* (1998) respectively, while it was 8.0% in Ismailia (Abaza *et al*, 1995) and 7.0% in Cairo (Khalil *et al*, 1991).

6.1% of diarrhoeal patients in developing countries and 2.1% of diarrhoeal patients in developed countries were determined to have been infected with *Cryptosporidium*, whereas the infection rates among HIV-positive diarrhoeal patients were significantly elevated, at 24.0% in developing countries and 14.0% in developed countries (Adal *et al,* 1995).

Neill *et al* (1996) in a community-based longitudinal study over 5 years in USA, observed cryptosporidiosis in stools of 13 chronically ill elderly patients with diarrhoea with the cause attributed to a hospital setting in nine patients and to co-infection with *C. difficile* in two patients.

In a study conducted by Pratdesaba *et al* (2001) *C. cayetanensis* was observed in samples of only 7 of 474 (1.5%) subjects, distributed as follows: 6 of the 157 HIV or AIDS patients (3.8%) and 1 of the 111 malnourished children (0.9%). No *C. cayetanensis* oocysts were observed in any of the samples from the raspberry farm workers. For the 474 subjects based on wet preparation only and Modified AF stains for coccidia, the most commonly observed parasites were *E. coli* (19.6%), *A. lumbricoides* (14.8%), *E. nana* (13.3%), *T. trichiura* (12.0%) and *B. hominis* (11.4%). *C. parvum* was observed in samples from 15 (3.2%) subjects, of which 7 (6.3%) were malnourished children and 8 (5.1%) were HIV or AIDS patients. Svenungsson *et al,* (2002) detected *Cryptosporidium* in 2.0% of faecal samples of adult Swedish patients with diarrhoea.

A study has shown that the incidence of parasitic infections was found to be 48.0% among the immunocompromised patients whereas it was 20.0% in normal control. In immunocompromised patients, the parasitic findings were intestinal 46.0%, pulmonary 6.0%, intranasal 2.0% and mixed 6.0%. *C. parvum* oocysts in 30.0%, *Microspora* spores in 12.0%, *Cyclospora* oocysts in 8.0% and *G. lamblia* cyst in 14.0% were the intestinal parasites detected. Diarrhoea was found in 13 cases out of 15 harboring *cryptosporidia* (El-Diffrawy *et al,* 2002).

Burstein (2005) has reported the prevalence of *C. cayetanensis* among apparently healthy persons in Peru. One group included those consulting private physician and next included people from marginal area. They had common complain of abdominal pain and diarrhoea. The incidence rate has been found to be 41.6% and 7.3% respectively among the two groups. He reported higher rate among young and elder adults up to 60 year-old.

Urasa *et al* (2000) reported significant increase in the proportion of *V. cholerae* 01 isolates resistant to tetracycline, ampicillin, nalidixic acid and erythromycin during two cholera outbreaks in Dar es Salam, Tanzania in between 1997 and 1999.

In a study done among the patient with enteric illness in Ontario, Canada by Lee and Middletone (2003) from 1997-2001, it was found that *Campylobacter* spp. accounted for the highest annual average incidence rate at 42.3 cases per 100,000 persons, with *Salmonella* spp. following at 22.6 and *Shigella* spp. at 2.7. A recent study of seroprevalence in Danish adults looked at IgG antibodies in a sample of 1112 people from Copenhagen. It was found that the percentage of people with *C. jejuni*-specific IgG antibodies increased with age, from 20.6% in the 15–34 years age group to 32.4% in the 50 – 69 years age group (Linneberg *et al*, 2003).

The overall incidence of *Campylobacter* infection by five-year age groups showed a large number of cases in the 0–4 years age group (241 cases per 100,000). There was a drop off, before a second peak found in the 25–29 years age group (205 cases per 100,000). From 30 years of age onwards there was a gradual reduction in cases down to the minimum of less than 60 cases per 100,000 among the patients 75 years and older (Miller *et al*, 2005).

Asian scenario of intestinal infections

In the Northern Sumatra of Indonesia, the most common intestinal helminthes were *T. trichiura* (87.0%), *A. lumbricoides* (75.0%) and Hookworm (58.0%). Other helminthes found in low numbers were *E. vermicularis*, *S. stercoralis*, *Taenia* spp. and *Echinostoma* spp. eggs. *E. coli* (25.0%) was the most common intestinal protozoa followed by *E. nana* (8.0%), *E. histolytica* (7.0%), *G. lamblia* (6.0%), *I. butschlii* (5.0%), *E. hartmanni* (1.0%) and *C. mesnili* (1.0%). The amoeba prevalence rate was 31.0% (Cross *et al,* 1976).

In a study conducted at South Kalimantan Province of Indonesia, 1 to 8 different intestinal parasitic infections were detected in 97.0% of the people. Those parasites most frequently found were *T. trichiura* (83.0%), *A. lumbricoides* (79.0%), and Hookworm (65.0%), followed by *E. coli* (37.0%), *E. nana* (12.0%), *E. histolytica* (12.0%), *I. butschlii* (11.0%), *G. lamblia* (5.0%), *E. hartmanni* (2.0.0%), *C. mesnili* (2%). Other parasites found were *E. vermicularis*, *S. stercoralis*, *Capillaria* spp., *Echinostoma* spp., *H. diminuta*, and *T. hominis*. *G. lamblia* was found more often in younger people and *E. coli*, *I. butschlii* and Hookworm in the older age group. Hookworm occurred more frequently in males and *A. lumbricoides* in females (Cross *et al,* 1976).

A biomedical survey for intestinal parasites was conducted in Napsan on the island of Palawan, Philippines in September 1986. A total of 353 stool specimens were obtained from 155 males and 198 females ranging in age from one and half months to 70 years. *A. lumbricoides* (34.8%), Hookworm (34.8%) and *T. trichiura* (25.2%) were the most common helminthes encountered, while *E. coli* (27.7%) and *E. nana* (22.2%) were the common protozoans (Oberst and Alquiza, 1987).

Hookworm prevalence rate was found to be 29.0% among 50+ people in two villages of Malayasia (Cheghani *et al,* 1989). A study conducted in some semiurban region of Bangladesh inhabited by the fisherman found the parasite positive rate of 50.0% in 50+ people (Rahman, 1993).

Among urban slum dwellers, Kan (1993) found the infection rate of 40.1%, 36.7% and 0.3% respectively for *A. lumbricoides*, *T. trichiura* and Hookworm. The single infection with one helminth was 60.5%, while with double infection was 39.5% but the triple infection was not reported. The parasite positive rate for 60+ people was 28.6% (helminth only).

In a study conducted by De silva *et al* (1994) in Srilanka, a total of 192 stool samples from the adult outpatients (mean age 51.4 years) and 354 samples from the pre-school children (mean age 30 months) were examined. *E. histolytica* was not seen in any of the samples; *Giardia* cysts and *Cryptosporidium* oocysts were seen in 3 and 1 sample respectively from the pre-school children. The overall prevalence of geohelminth infections was 21.3% among the adults and 24.5% among the children. *A. lumbricoides* was the predominant species in both populations. Comparison of the rate of intestinal parasite infection among 37 adult patients with non-specific abdominal complaints, with the rate among 37 matched controls with no abdominal complaints showed no significant difference (16.0% and 19.0% respectively). This suggested that the presence of abdominal pain or diarrhoea was unrelated to the presence of intestinal parasites in the adult study population.

The first nationwide survey on human intestinal parasites in China found that the overall infection rate of human intestinal parasite and the infection rate of most species of parasites were higher in females than that in males. The highest infection rate was found in the group aged 5-14 years (Xu *et al*, 1995). In a study in China, *E. vermicularis* (47.0%), *T. trichiura* (18.8%) and *T. saginata* (17.2%) were the most frequent causes of intestinal parasitic infections (Xu, 1995).

Out of 4222 samples observed in Malayasia, 43.2% were positive for parasite. The prevalence rate was *T. trichiura*-31.9%, Hookworm-8.0%, *A. lumbricoides*-21.2%. The rate of infection for female was 43.9% and that for male was 42.4%. The prevalence rate was 28.4% among 50+ people (Sinniah and Rajeswar, 1998).

In a study by Feng *et al* (2001b), the infection rate of STH in Fujian Province was found to be 55.5%. Among them 55.1% were *E. vermicularis*, 19.2% were *T. trichiura*, 36.5% were *A. lumbricoides*, and 11.3% were Hookworm. In the people above 50 years the infection rates were 75.9% in 1988 and 51.3% in 1999. In the general population, the infection rates were found to be 74.4% and 83.2% for male and female respectively in

1988. Similarly, the infection rates were found to be 56.2% and 54.7% for male and female respectively in 1999. In 1998 the single, double and multiple infection rates were 44.2%, 47.0% and 8.8% respectively. In 1999 the single, double and multiple infection rates were 76.4%, 22.2% and 1.5% respectively.

A study conducted by Changhua *et al* (1999) in Sichuan Province of China found that among 310 residents of Lugao Village (Hejiang County), 87.0%, 63.0% and 60.0% residents were infected with Hookworm, *A. lumbricoides* and *T. trichiura* respectively. The prevalence of hookworm was found to rise linearly with age. The majority of these individuals harbored mixed infection with *N. americanus* and *A. duodenale,* although the former predominated. It was found that despite economic development which was occurring in some parts of China, significant Hookworm infections and clinical Hookworm anemia existed in areas of Sichuan Province. In Hejiang County, it was found that the intensity of Hookworm infection had actually risen within the last 10 years. Hookworm is a medical problem among the elderly in Sichuan.

Ascariasis is the most common parasitic disease in China. According to a nationwide sampling survey in 1988-1992 the average infection rate was 47.0% i.e. around 531 million people were infected (Feng *et al*, 2001a). A study by Chai *et al* (2001a) in Jiangxi Province of China has reported 72.5% prevalence rate. The positive rate of different parasites was *A. lumbricoides*-50.9%, *T. trichiura*-33.4%, Hookworm-11.4%, *G. lamblia*-2.8%, *E. coli*-1.2% and *E. histolytica*-0.8%

In a study done among people in Coastal areas of Korea, the prevalence rate was found to be 4.2% and 0.4% respectively for *T. trichiura* and *A. lumbricoides* respectively. Similarly, the infection rates for other parasites were *C. parvum* (3.5%), *E. coli* (2.2%), *G. lamblia* (1.8%), *E. nana* (1.4%) and *T. trichiura* (0.2%). Among people above 60 years, *T. trichiura* (4.2%) was the commonest parasite followed by *A. lumbricoides* (1.1%) (Chai *et al,* 2001b).

A study of helminthic infection in Vientiance Province of Lao PDR found the positive rate 63.3%. The single, double, triple and multiple infection rates were 64.0%, 28.0%, 7.0% and 1.0% respectively. *A. lumbricoides* (40.0%) was the commonest helminth followed by *T. trichiura* (28.0%) and Hookworm (8.0%). The study has found that the women have 1.25 times more chance of being infected with *A. lumbricoides* than their counter parts ($P<0.005$). The monoinfection rate of *T. trichiura* was found to be only 8.6% while its infection along with *A. lumbricoides* was 23.5%. Similarly its infection with other parasites was found to be 25.6% (Phetsouvannh *et al*, 2001).

In a study by Phompida *et al* (2001) in Vientiane Municipality, the prevalence of Hookworm, *T. trichiura* and *A. lumbricoides* was found to be 9.8%, 5.3% and 4.3% respectively has been reported. Majority of infected were healthy adults.

Gastrointestinal problems have been reported as the most common health problems among elderly people in Bangladesh and Myanmar and a leading cause of death over there. Diarrhoea has been recognized as the commonest cause of hospitalization among the elderly people in Thailand. In India too, it is one among top ten causes of hospitalization and death (WHO, 2004).

In a national survey of intestinal parasitosis in Iran, intestinal parasitic infection rate of 19.3% was in the study population (19.7% male, 19.1% female). In the study, *G. lamblia* (10.9%), *A. lumbricoides* (1.5%), *E. histolytica* (1.0%) and *E. vermicularis* (0.5%) were the most common parasites. The infection rate was highest in the 2–14 years age group (25.5%) and in rural residents (23.7%). Only *G. lamblia* was more frequent in males than females, while the prevalence of other parasites was higher in females than males (with the exception of *Trichostrongylus colubriformis*, which had a similar frequency in both sexes). The prevalence rate of *T. saginata*, *T. colubriformis*, *T. trichiura* and *A. duodenale* were 0.2%, 0.2%, 0.1% and less than 0.1% respectively. The total prevalence of intestinal parasite among people of age group 40-69 was 15.0% and greater than 69 years was 11.6%. The prevalence of individual parasites in 40-69 years age group was *G. lamblia* (7.3%), *A. lumbricoides* (1.5%), *E. histolytica* (1.1%) and *E. vermicularis* (0.2%). The prevalence of individual parasites was *G. lamblia* (5.0%), *A. lumbricoides* (1.2%), *E. vermicularis* (0.1%) and *E. histolytica* (0.7%) in 69+ age group. (Sayyari *et al,* 2005).

In Asia, *Cryptosporidium* has been isolated in 2.0–20.0% of cases of acute diarrhoea (Aye *et al*, 1994; Jirapinyo *et al*, 1993). Acute infectious diarrhoea caused by Cryptosporidium has been reported in 7.9% of Indian adults (49–60 years) (Nath *et al*, 1999).

Katsumata *et al* (1998), in a hospital-based study in Indonesia, observed 2.8% of patients with diarrhoea having *C. parvum* oocysts, the prevalence being higher during the rainy season. Chai *et al* (2001c) found a higher prevalence of cryptosporidial oocysts in faecal samples of elderly people (57.0%), with household clustering, higher rates in spring and a weak positive correlation with rainfall.

In a study by Gambhir *et al* (2003) during 1997-2000 in India, *Cryptosporidium* was isolated in 22 older patients with diarrhoea (18.3%) and was highly significant (P< 0.01) compared with healthy age-matched controls. Of these patients, 66.0% had a history of close contact with animals. Among the elderly patients, 17.0% suffered from vomiting and abdominal pain, 31.0% were febrile; none were severely dehydrated. Stools numbered 3 to 9 per day with duration of 5-17 days. Stool leucocytes were <6/hpf and no RBCs were seen. Isolation of *Cryptosporidium* in older persons was associated with diabetes mellitus (22.7%), tuberculosis (9.0%), malignancy (4.5%) and coronary artery disease (4.5%). *Cryptosporidium* infections were most often detected in older patients in the rainy season (15 patients, 68.2%), followed by summer (4 patients, 18.2%) and winter (3 patients, 13.6%). 2.5% were infected with *E. histolytica*. Among healthy elderly controls 1.8% had *G. lamblia*, 5.3% had *E. histolytica* and 1.8% had *Cryptosporidium* spp. infection. The study concluded that *Cryptosporidium* is an important cause of acute infectious diarrhoea in elderly Indians and should be looked for routinely, although more studies were required to establish its impact on nutrition and morbidity.

A study conducted in rural population in Jordan among 180 patient with acute or persistent diarrhoea and 100 nondiarrhoeal people revealed single infection by enteropathogen 77.8% and infection by multiple enteropathogens 32.7%. Potentially pathogenic parasites were observed in 90 (50.0%) patients; those that were associated significantly with diarrhoea were *G. lamblia*, *B. hominis*, *Cryptosporidium* spp., *E. histolytica* and *C. cayetanensis*. Pathogenic bacteria were isolated from 72 (40.0%) patients, and, of these, 62.5% were resistant to at least one antibiotic, and 30.6% of these were multiresistant. The most common enteropathogenic bacteria found were *Shigella* spp., *C. jejuni* and *Y. enterocolitica*. Unusual bacterial species were the predominant organisms recovered in a few cases and could represent a possible cause of diarrhoea. Overall, there was a high endemicity of diarrhoeal disease in the area studied. Risk factors that correlated significantly with contracting diarrhoea were socio-economic status, education, use of unchlorinated well or tank water, and a low level of personal hygiene (Nimri and Meqdam, 2004).

A study has reported 8.2% of inhabitants in Jeollanam-do in South Korea were infected with *C. parvum* (Yu *et al*, 2004). Another epidemiological study conducted in the same region revealed that 10.6% of residents were infected with *C. parvum* (Chai *et al*,1996).

1.0% of the stools among the 942 diarrhoeal patients were positive for *C. parvum*. The positive rate in the males was 1.1% and the positive rate of the females was 0.7%. Age distribution revealed that the highest positive rates were in patients in their 60s, with a positive rate of 2.5%. The highest positive age group in males was patients in their sixties, at 4.1%, and the highest positive age group among females was patients in their 70s, at 3.8%. *Cryptosporidium* diarrhoea was found to occur primarily between March and April (2.1-2.8%) and between July and September (2.9-4.3%). The peak incidence rate was in September (4.3%). Diarrhoea also occurred in January also with incidence rate of 1.3%. The medical records of the 9 *Cryptosporidium* positive patients were reviewed, in order to detect a possible specific clinical correlation with *C. parvum* infection. One of the nine positive patients suffered from pulmonary tuberculosis (TB), and two of the patients were hypertension. The others had acute duodenal ulcers or gall stones. Only one of the nine patients had a history of food poisoning, after visiting a neighbour’s house one week before the onset of diarrhoea. The majority of the patients complained of nausea, vomiting, and abdominal pain, in addition to their diarrhoeal symptoms, and received negative results on stool occult blood and parasite ova tests. The duration of the diarrhoea in these patients was 6 to 18 days, with some individual differences (Lee *et al*, 2005).

Agtini *et al* (2005) has reported the incidence of diarrhoea 50 per 1000 population per year in Jakarta. They found the overall incidence of shigellosis 4/1000/year. Shigellosis was most common among children and the elderly with the highest incidence at 32/1000/year in those 1 to 2 years of age. Of the 1203 *Shigella* isolates, 866 (72.0%) were *S. flexneri*, 277 (23.0%) were *S. sonnei*, 21 (2.0%) were *S. dysenteriae* (none of which were *S. dysenteriae* type 1) and 39 (3.0%) were *S. boydii*. Incidence by type of species was 2.6/1000/year for *S. flexneri*, 0.8 for *S. sonnei*, 0.1 for *S. dysenteriae*, and 0.1 for *S. boydii*. The most frequently encountered *S. flexneri* serotypes were 2a and 3a, followed by 1b and 1c. In all, 73.0% to 95.0% of *S. flexneri* isolates were resistant to ampicillin, trimethoprim-sulfamethoxazole, chloramphenicol, and tetracycline. None showed resistance to ciprofloxacin and only a single isolate (of *S. flexneri*) was not susceptible to ceftriaxone and nalidixic acid. During the surveillance, other *Vibrio* organisms were also detected. The incidence of diarrhoea due to *V. cholerae* non-O1 was 0.5/1000/year and that due to *V. parahaemolyticus* was 0.4/1000/year. *V. cholerae* non-O1 diarrhoea was most common among children and the elderly, whereas the incidence of *V. parahaemolyticus* diarrhoea gradually increased with age . In one of the outbreaks of cholera in and around Nagpur, India during June to October 2003, the isolates were multidrug resistant (Mishra *et al*, 2004).

National scenario of intestinal infections

In the fiscal year 2004/2005, 3.7% of patients visiting OPD had diarrhoeal diseases and 2.4% had one or more intestinal worms. The hospital inpatient morbidity rate among the people above 50 years for different gastrointestinal infections in the year was as shown in the table below.

Table 1 Hospital inpatient morbidity rate among 50+ people in 2004/2005

S.N.	Type of Infection	Morbidity Rate
1.	Cholera	14.3% (4/28)
2.	Typhoid and Paratyphoid	13.2% (1296/9798)
3.	Other Salmonella infection	11.6% (5/43)
4.	Shigellosis	14.0% (116/830)
5.	Other bacterial intestinal infections	20.0% (1/5)
6.	Other bacterial food borne intoxication	50.0% (1/2)
7.	Amoebiasis	23.9% (44/184)
8.	Other protozoal intestinal diseases	16.0% (12/75)
9.	Viral and other specified intestinal infections	12.5% (1/8)
10.	Diarrhoea and gastroenteritis of presumed infectious origin	11.3% (1831/16178)
11.	Taeniasis	100.0% (1/1)
12.	Cysticercosis	22.2% (2/9)
13.	Other cestodes	7.7% (1/13)
14.	Hookworm	32.1% (9/28)
15.	Ascariasis	0.0% (0/26)
16.	Trichuriasis	0.0% (0/1)
17.	Enterobiasis	0.0% (0/1)
18.	Other intestinal helminthes(not else where classified)	15.4% (2/13)
19.	Unspecified intestinal parasitism	50.0% (1/2)
20.	Other helminthiasis	7.5% (18/239)

(Annual report, Department of Health Service 2004/2005, Kathmandu, Nepal)

The annual prevalence rate of helminthiases during 1985 to 1992 ranged from 18.1 to 36.6% with a marginal decrease in successive years. The incidence however showed an increase after the year 1993. The positive rate of intestinal parasitosis was found to be 29.1-44.2%. *Ascaris* was the commonest parasite and showed static incidence through out the study period. Incidence of intestinal parasitosis in adult male was 29.0-41.6% and in female it was 27.0-48.5%. The incidence of parasitosis among adult and children was seen to be almost same in the year 1987, 1992 and1993. Among intestinal protozoans, *G. lamblia* topped the list (5.2-26.2%). The infection rate for *E. histolytica* was 1.9-14.6 % (Rai *et al*, 1995). The annual rate of protozoal parasitosis has been found to range from 3.3% to 13.6% increasing trend every year (Rai *et al*, 1994a). Among the protozoal parasitosis in the intestine, *G. lamblia* has been found to contribute major portion in majority of studies followed by *E. histolytica* and *B. hominis* respectively. Similarly, among the helminthes, *A. lumbricoides* has been found to be the commonest one followed by *T. trichuria* and hookworm (Ishiyama *et al*, 2003; Rai *et al,* 1994b, 1995, 2000b, 2002, 2004a).

Similarly, genderwise studies have shown the intestinal parasitosis to be common among female, which appear to be due to their role in child care and low educational level attributed to male child preference (Rai *et al,* 1995; Rai *et al,* 2004b). Children are found to be the major victim of diarrhoeal disease in Nepal, around 50.0% of which is caused by protozoal and helminthic parasitosis. About 25.0% of child death in Nepal occurs due to diarrhoea (Bista *et al*, 1993; Rai *et al,* 2004a). The higher prevalence rate in children may be due to the lack of natural or acquired resistance along with their poor personnel hygiene.

A study conducted in Jiri revealed that the prevalence of Whipworm, Roundworm and Hookworm among Jirel of age group 45 above were 18.1%, 25.3% and 73.5% respectively. Similarly, prevalence of Whipworm, Roundworm and Hookworm among Sherpa of age group 45 above were 11.2%, 23.6% and 46.1% respectively. Similarly, the prevalence of Whipworm, Roundworm and Hookworm among Hindu of age group 45 above were 7.1%, 26.2% and 59.5% respectively. The study found the increasement in multiple helminthic infections with increasement in age (Blangero *et al*, 1993).

The parasitic infection rate of 50.0% has been reported by the studies conducted in the Nepalese communities from 1979-1995 by different organizations. *A. lumbricoides* and *G. lamblia* topped the list respectively among helminthes and protozoans. Similarly, the hospital records showed the infection rate of 30.0-40.0% (Chhetri, 1997).

In a study conducted in Boya VDC and Okharpauwa VDC, the mixed infection rate of parasites was found 5.0 %. (Rai *et al,* 1998). Another study in rural hilly region of Nepal has reported the prevalence rate of parasites to be 76.4%. The parasitic infection rates for male and female were 76.5% and 76.3% respectively. The predominant parasite was *A. lumbricoides* followed by Hookworm. The polyparasitism was found significant among male (Rai *et al,* 2001).

Ishiyama *et al* (2001) has reported the parasite prevalence of 72.4% among school children in Kathmandu. *Indoaryans* were found to have marginally high rate of infection. 46.9% had multiple infections. *T. trichiura* (30.4%) was found to be the commonest helminth and *G. lamblia* (17.0%), the commonest protozoan. No bacterial enteropathogens were reported in the study population.

Researchers have found over 60.0% people infected with one or more intestinal parasites in Nepal. The prevalence rate of even 100.0% has been observed in some backward areas (Rai, 2004).The hospital based study conducted by Takemasha *et al* (2004) over one decade in Kathmandu illustrated that intestinal parasitosis rate ranged from 29.0-44.0%, with a static prevalence of *A. lumbricoides*, the most common parasite in Nepal. Few studies have shown *T. trichuria* as the commonest parasite detected in faecal samples. It is said to be due to longer life span of parasite and difficulty in removing all parasite by single dose of antihelminth therapy (Rai, 2004 and Rai *et al,* 2005). Among the Tapeworms, *H. nana* was the commonest in Nepal with incidence rate less than 5.0% (1985-1994 A.D). The low prevalence of *Taenia spp.* may be due to no raw pork eating and prohibition of beef consumption in Nepal (Rai, 2004).

A study done by Rai *et al* (2004a) showed that ethnic group wise prevalence of bacterial enteropathogens was marginally higher in *Tibetoburman*s and lowest in *Dalit*, a so called untouchable group with low socio-economic profile. No significant difference was seen in children consuming treated and untreated water. In another study, the highest prevalence of intestinal parasitosis has been found among *Dalit*. Then the *Tibetoburman* and *Indoaryan* respectively follow them in prevalence rate (Ishiyama *et al*, 2003; Rai, 2004, Rai *et al,* 2004b). Rai *et al* (2002) has reported the parasitic prevalence rate of 60.0% among school children of rural hilly area of Nepal with the highest prevalence of *A. lumbricoides* followed by Hookworm. Ethnically the prevalence rates were 74.1%, 65.7% and 38.5% among *Dalits, Tibetoburmans* and *Indoaryans* respectively.

In Nepal the prevalence of Cyclospora has been documented 29.8% in the patients with clinical symptoms by Sherchand *et al* (1999). The prevalence of *C. cyatenensis* has been found to be 16.0% in Kathmandu, 0-15 years age group being the most vulnerable group. The prevalence was found very less after 30 years of age due to immunity developed after repeated attack (Sharma and Sherchand, 2003).

In a study conducted by Tamang *et al* (2005) in 2004 in Kavre district, out of the 148 stool samples, 46 cases (31.0%) were found to be positive for *V. cholerae* serogroup O1, biotype El Tor, serotype Ogawa. Both sexes were equally affected. Young age group of less than 30 years were mostly affected. Brahmin was the most affected ethnic group. The isolates were sensitive to all the antibiotics tested except cotrimoxazole i.e. sensitive to ampicillin, chloramphenicol, tetracycline, doxycycline and ciprofloxacin. Among the laboratory confirmed cholera cases, 30.0% exhibited co-infection with other parasites among which *G. lamblia* and *A. lumbricoides* were the most common. Out of 148 total stool specimens processed, parasitic infection was found in 37 samples. In 3 cases multiparasitic infestation was found. Among the protozoa *E. histolytica* was the commonest and among helminthes, *A. lumbricoides* was the commonest. *Salmonella* spp. and *Shigella* spp. were recovered from one stool sample each. 3 cases with cholera were of 60+ age group. In one of the recent studies, Kansakar *et al* (2004) reported *V. cholerae* 01 El Tor Ogawa was responsible for cholera outbreak in Kathmandu Valley. Out of 89 cases of the age above 60 years, 6 were found positive. 100.0% of the isolates were sensitive to ciprofloxacin and tetracycline. 100.0% isolates were resistant to cotrimoxazole. Similarly 2.0% and 92.0% of the isolates were resistant to erythromycin and furazolidone respectively. It has been documented that the same strain of *V. cholerae* has been propagated in and across the Kathmandu Valley and neighbouring districts causing most of the cholera outbreaks in summer-monsoon season.

CHAPTER-IV

4 MATERIALS AND METHODS

A list of materials, chemicals, equipments, media and reagents required for the study is presented in Appendix 1.

4.1 Subject and site of the study

The laboratory investigation part of this dissertation was carried out in National Institute of Tropical Medicine and Public Health Research, Kathmandu. The study period was from August 2005 to July 2006. The stool samples were collected from the people of 60 to 93 years from the following places.

- Social Welfare Center, Pashupati Briddhashram, Kathmandu (a government organization)
- Tapasthali Nepal Briddhashram, Kathmandu (a non-government organization)
- Old Age Care Center, Kathmandu (a non-government organization)
- Matatirtha Senior Citizens Residence Committee, Kathmandu (a non-government organization)
- Mother Teresa Home, Kathmandu (a non-government organization)
- Nisahaya Sewa Kendra, Kathmandu (a non-government organization)
- Lubhu V.D.C, Lalitpur

4.2 Sample collection

Each subject was provided with a clean, dry, disinfectant free, wide mouthed container and asked to collect about 20 gm or 20 ml of stool specimen into the container. They were cautioned not to contaminate the stool with water and urine. The containers were labelled with patients' name, code number, date and time of collection. Questionnaires accompanying the queries about their clinical history, hygienic practice and nutritional behaviour were also filled.

The collected stool samples were immediately brought to the laboratory and processed according to standard methods.

4.3 Sample processing

Each stool sample was processed to examines in three steps.

a. Macroscopic examination.

b. Stool culture for *Salmonella* spp., *Shigella* spp., *V. cholerae* and *Campylobacter* spp.

c. Microscopic examination:

I) Formal-ether concentration method followed by Saline/Iodine wet mount

II) Sheather's sucrose floatation technique followed by Modified Acid-Fast staining

4.3.1 Macroscopic examination

The direct visual examination of each sample was done for the colour, consistency, presence of blood, presence of mucus and presence of adult worm or worm segments.

- Colour- The colour of normal stool is yellowish brown. The colour may vary during different infectious and noninfectious conditions, such as pale during giardiasis, black during upper intestinal bleeding, red during lower intestinal bleeding, green during consumption of specific antibiotic etc.
- Consistency- Stool may be hard, formed, mushy formed, diarrhoeal or watery. Normal stool is generally formed. Protozoal cysts are found in formed and mushy formed stool. The trophozoites are usually found in diarrhoeal and watery specimens. Helminthic eggs and larvae are found in any types of specimen.
- Blood and mucus- Presence of blood and/or mucus in stool indicates a pathological condition. Stool mixed with fresh blood indicates bleeding from lower intestinal tract where as dark stool indicates bleeding from upper intestinal tract. Presence of both blood and mucus in the stool is highly suggestive of amoebic dysentery. Blood and mucus may be found in stool during bacillary dysentery, Campylobacter enteritis, ulcerative colitis, intestinal tumor and haemorrhoids.
- Adult worms or worm segments- The adult intestinal helminthes may be occasionally seen in stool with unaided eyes. *A. lumbricoides* and

E. vermicularis adult worms are sometimes found. Tapeworm segments are also occasionally seen in stool.

4.3.2 Isolation of *Salmonella* spp., *Shigella* spp., *V. cholerae* and *Campylobacter* spp. from stool specimen

I) Isolation of *Salmonella* spp. and *Shigella* spp. from stool specimen

i) Several loopful of sample was inoculated into Selenite F. Broth medium and incubated at 37ºC overnight.

ii) A loopful of sample from enrichment broth was inoculated on Salmonella-Shigella (S-S) agar plate and incubated at 37ºC overnight.

iii) The overnight incubated culture plates were observed for their characteristic colony morphology, especially the nonlactose fermenting pale colonies on the S-S agar plate.

iv) The non-lactose fermenting colonies from S-S agar plate were Gram stained and microscopic morphology of the bacterial isolates was observed. The isolated colonies were subcultured on Nutrient agar (NA) plate and incubated overnight at 37ºC.

v) The isolated colonies on NA plate were subjected to biochemical tests.

vi) Identification of the isolate was done as per the identification scheme shown in Appendix 4 and 5.

II) Isolation of *V. cholerae* from stool specimen

i) Several loopful of sample was inoculated into Alkaline Peptone Water and incubated overnight at 37ºC.

ii) A loopful of sample from the enrichment broth was subcultured on Thiosulphate Citrate Bile salt Sucrose Agar (TCBS) and incubated overnight at 37ºC.

iii) The incubated culture plates were observed for the characteristic colony morphology of *V. cholerae* i.e. sucrose fermenting yellow colonies 2-3 mm in diameter.

iv) The isolated colony was subcultured on NA plate and incubated overnight at 37ºC.

v) The isolated colony was subjected to gram's staining to see gram morphology and hanging drop motility test to observe typical darting type motility of the desired organism.

vi) The biochemical tests were performed for the confirmation of the *V. cholerae*. (Appendix 4)

III) Isolation of *Campylobacter* spp. from stool specimen

i) A loopful of stool sample was inoculated onto Campylobacter Blood Free Selective Agar Base (Modified CCDA-Preston), consisting CCDA Selective Supplement.

ii) The plate was incubated for 48 hours at 42ºC in the Anaeropack system "Micro Aero" that created the incubating environment of 8-9% O_2 and 7-8% CO_2.

iii) The plate was observed for the grey to pinkish or yellowish grey moist, slightly mucoid looking colonies.

iv) The isolated colony was subjected to gram's staining and motility test.

v) The biochemical tests were performed for the confirmation of the *Campylobacter* spp. The positive test results for the Catalase, Oxidase and Nitrate reduction and lack of H_2S production were the biochemical basis for its identification.

IV) Antibiotic susceptibility test

The antibiotic susceptibility test was done by Kirby-Bauer method to determine which antibiotic was likely to be effective in curing of the infections. 2-3 isolated colonies of the test organism were inoculated into Nutrient broth and incubated at 37ºC for 4 hours. The turbidity of the Nutrient broth containing test organism was maintained as per the turbidity of McFarland 0.5 standard solution.

The Mueller Hilton Agar (MHA) plate was dried and with the help of sterile swab stick the test organism was swabbed on MHA plate. Then with the help of sterile forceps appropriate antibiotic discs were placed on the MHA plate at the distance more than 15 mm from the edge of the plate and no closer than about 25 mm from disc to disc. After placing antibiotic discs, plates were kept at room temperature for 30 minutes and then incubated at 37ºC for 24 hours. After incubation, the size of the zone of inhibition developed were measured and compared with interpretative chart provided by Hi-media laboratories .

4.3.3 Microscopic examination

This is required for the detection and identification of protozoal cysts, oocysts, trophozoites and helminthic eggs or larvae. It also detects the presence of RBCs and WBCs in the stool specimen. Specimen should be examined as soon as possible after collection. Ideally, watery and diarrhoeal stool should be examined within 30 minutes and semiformed and mushy stool specimen should be examined within 60 minutes of passage; formed stool may be examined within the day of passage.

Microscopic examination was done by Saline wet mount, Iodine wet mount and Modified Acid-Fast staining technique. The mounted slides were examined under low power (10X) followed by (40X) and in case of Acid-Fast stained smears, they were examined using oil immersion (100X). Parasites were identified by their morphology, motility and staining characteristics.

Prior to wet mount performance, all the samples were subjected to concentration method. It concentrates the eggs, larvae and cysts when they are present in small number and increases the sensitivity of microscopic examination. Trophozoites are destroyed in the process. There are various floatation and sedimentation techniques of concentration.

I) Formal-ether sedimentation method leading to saline/iodine wet mount

This is the most sensitive method of concentrating cysts, eggs and larvae without distortion of their morphology. It takes short time and the chances of error are minimum. The technique was applied as follows:

1. About 1gm of stool sample was emulsified in about 4ml of 10% formal saline solution, shaken well and the suspension was allowed to stand for 30 minutes for adequate fixation.
2. A further of 3-4ml of 10% formal saline was added and then shaken well.

3. The suspension was sieved through the double cotton gauze in a funnel into a 15 ml centrifuge tube
4. 3-4 ml of ether was added and shaken vigorously for 5 minutes.
5. The tube was immediately centrifuged at 1000 rpm for 10 minutes.
6. Four layers of suspension were obtained in the tube after the centrifugation.

a. A small amount of sediment at the bottom of the tube containing the parasite.
b. A layer of formalin on the top of the sediment
c. A plug of fecal debris on the top of formalin layer.
d. A layer of diethyl ether at the top.

7. The plug of debris formed between diethyl ether and formalin was removed by rotating the tip of the applicator along the inner wall of the tube.
8. The supernatant layers of suspension were discarded and the sediment was examined by saline and iodine wet mount.

Saline wet mount

It was used to detect helminth eggs and larvae and the protozoal cysts. A drop of normal saline was taken on a clean glass slide; a drop of sediment from the above process was mixed with it and observed under microscope after covering with a cover slip.

Iodine wet mount

This was mainly used for detecting protozoal cysts, however helminthic eggs were also stained and could be detected. Iodine stained cysts showed pale refractile nuclei, yellowish cytoplasm and brown glycogen material. A drop of 5 times diluted Lugol's iodine was taken on a slide and a drop of sediment from above process was mixed with it. The preparation was covered with a cover slip and observed under microscope.

The diarrhoeal stool samples were undertaken Sheather's sucrose floatation technique followed by Modified Acid Fast staining for the detection of the oocysts of *C. parvum*, *I. belli* and *C. cayetanensis.*

II) Sheather's sucrose floatation technique

1) About one gram of stool sample was mixed with 5ml of normal saline in a test tube.

2) The suspension was filtered through 3 layers of cotton gauze to give 2 ml in a test tube.
3) The tube was filled up to its brim with the sucrose solution. The tube was centrifuged at 1000 rpm for 10 minutes.
4) With the help of a bent inoculating loop of shorter length the particles floating on the top surface of the sucrose solution were picked up used to prepare smears on the glass slide
5) The smear was stained with Modified AF stain.

Modified AF staining (Ziehl-Neelsen)

It is required for the accurate identification of the oocysts of *C. parvum*, *I. belli* and *C. cayetanensis* and the spores of *Microsporidia* spp.. The oocysts are acid-fast and stained red or pink against green background stained with Malachite green. Both hot and cold methods of staining can be used with equal sensitivity. The Cold Kinyoun method was followed in this study.

1) The smear was made with the particles obtained from Sheather's sucrose floatation method and dried on air
2) The smear was fixed in absolute methanol for 3 minutes.
3) The slide was flooded with Carbol fuchsin for 15-20 minutes and washed with tap water.
4) The smear was decolourised with 1% acid alcohol for 10-15 seconds
5) The smear was washed with tap water and then counter stained with 0.5% Malachite Green for 30 seconds.
6) The slide was washed with tap water, air dried and examined under 40x followed by oil immersion (100x)

Sporulation of *C. cayetanensis* oocysts

C. cayetanensis oocysts are excreted unsporulated in the feaces. Specific identification of this coccidian parasite can be established by stimulating its sporulation and subsequent finding of two sporocysts within each oocysts of the parasite. For the enhancement of sporulation, about 2 gm of stool sample was mixed with about 5ml of

2.5% Potassium dichromate solution and incubated at room temperature for 15 days. Assessment of sporulation was confirmed in light microscopy by observing two sporocysts in each oocyst.

4.4 Statistical analysis

Chi-square test was used to evaluate apparent differences for significance. Association of intestinal infections with different variables was tested. Results were considered significant if P values were less than 0.05.

4.5 Distribution of medicine

A complete dose of antiparasitic drugs was distributed to the elderly people with the parasitic infections after the investigation.

CHAPTER V

5 RESULTS

Within the study period from August 2005 to July 2006, a total of 235 samples were collected from the elderly people above 60 years of age. Out of them 122 stool samples were collected from a government elderly home at Kathmandu and a total of 66 samples was collected from five different private elderly homes in Kathmandu run by NGOs. The remaining 47 samples were collected from elderly people in Lubhu V.D.C., Lalitpur, who did not belong to any elderly home; they were rather living at their own houses in the community.

Out of 235 stool samples, 98 (41.7%) samples had one or more intestinal parasites (Fig. 1). Out of 98 positive samples, 68 (69.4%) samples were found to contain single parasite, where as 30 (30.6%) samples were found to contain multiparasites (Table 2 and 3).

Among three study groups of the elderly people, the highest parasitic infection rate was found in government run elderly home (50.8%) followed by 46.8% in the rural community of Lubhu and 21.2% in private elderly homes. The result was found to be statistically significant at the 5.0% level of significance (Fig. 1). The average positive rate in elderly homes was 40.4%.

The overall positive rate of the parasites among male and female was found to be 43.8% and 40.4% respectively, but the difference was not statistically significant (Fig. 1). Both the multiparasitic infection and monoparasitic infection rates were not found to vary in two sexes. The multiparasitosis among infected male and female were 30.8% and 30.5% respectively. Similarly, the monoparasitosis among them were 69.2% and 69.5% respectively (Table 2 and 3).

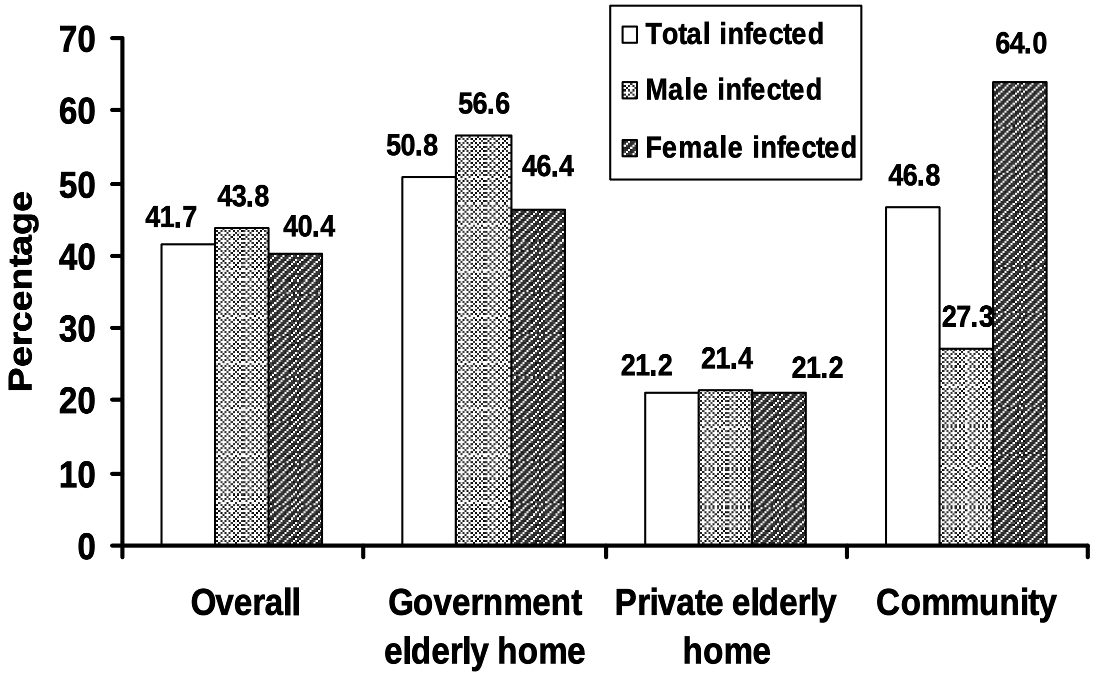

Figure 1 Distribution of parasites in two genders of different study groups (n=235)

The rate of monoparasitosis and multiparasitosis among total male were 30.3% and 13.5% respectively. Likewise, among total female the rate of monoparasitosis and multiparasitosis were 28.1% and 12.3% respectively. The difference was not statistically significant (P>0.05). Out of 235 elderly people under the study, 28.9% had monoparasitosis and 12.8% had multiparasitosis (Fig. 2).

Table 2 Rate of multiparasitism in total positive cases

Gender	Total positive	Multiparasites	Percentage	P-value
Male	39	12	30.8	P>0.05
Female	59	18	30.5	
Total	98	30	30.6	

Table 3 Rate of monoparasitism in total positive cases

Gender	Total positive	Monoparasite	Percentage	P-value
Male	39	27	69.2	P>0.05
Female	59	41	69.5	
Total	98	68	69.4	

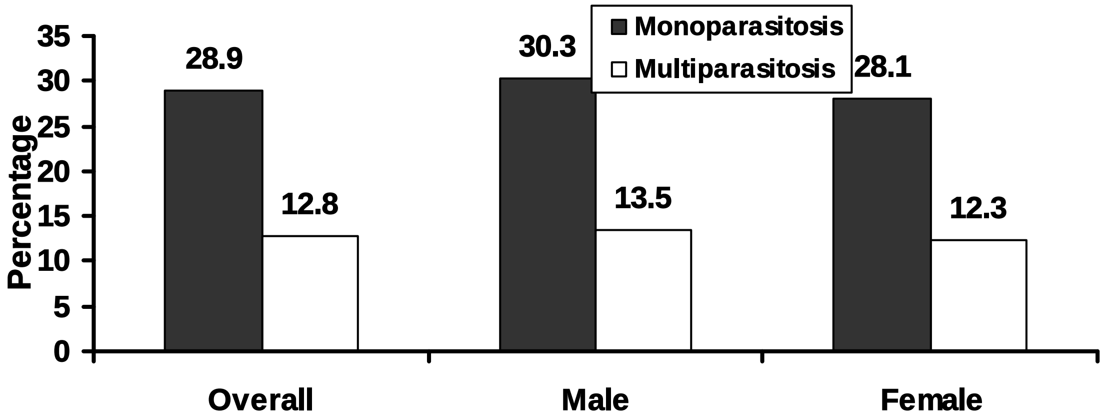

Figure 2 Pattern of parasitic infections in two genders

Among 89 males under study, 24 (27.0%) had helminthic infection and 23 (25.8%) had protozoal infection. Similarly among 146 females under study, 35 (24.0%) had helminthic infection and 32 (21.9%) had protozoal infection. Both the differences were not statistically significant (P>0.05) (Fig. 3).

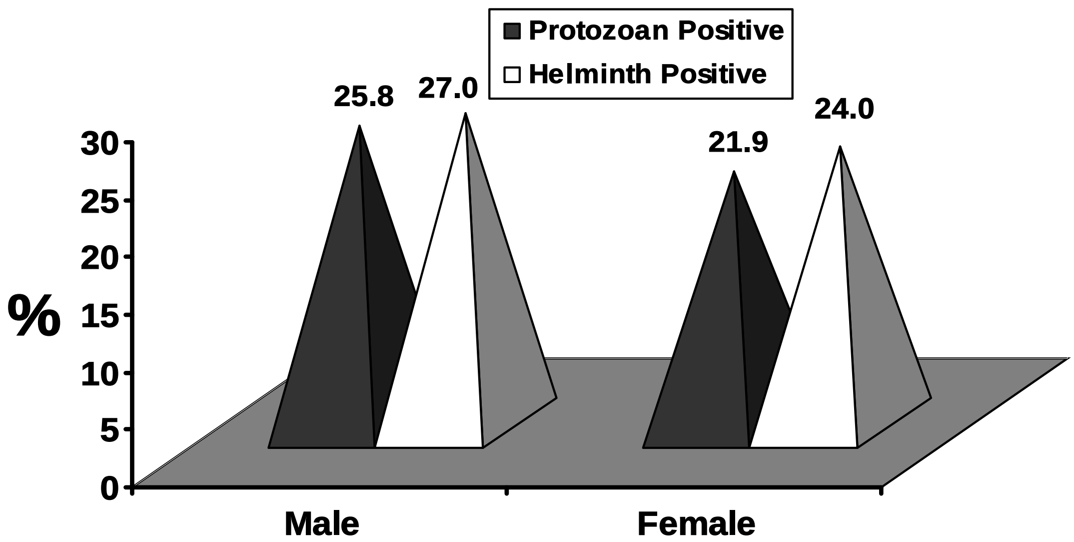

Figure 3 Distribution of protozoal and helminthic infections in two genders

A. lumbricoides was found in association with at least *T. trichiura* in 6 cases. *T. trichiura* was found in association with at least Hookworm in 6 cases. Likewise, *T. trichiura* was found in association with at least *E. histolytica* in 7 cases. *T. trichiura* was found in association with at least *E. coli* in 6 cases (Appendix 2).

Table 4 Pattern of parasitic infections in elderly people

Type of infection	Total	Percentage
Single parasite	**68**	**69.4**
Protozoa	34	34.7
Helminth	34	34.7
Multiparasites	**30**	**30.6**
Protozoans	5	5.1
Helminthes	9	9.2
Protozoans + Helminthes	16	16.3
Total	**98**	**100.0**

Out of 132 total parasites obtained from 235 stool samples (98 positive samples), 54.5% were helminthes and 45.5% were protozoans. Among individual study groups, the government run elderly home had helminthic and protozoal infection rates 51.8% and 48.2% respectively. Similarly, at community, the helminthic and protozoal infection rates were 74.2% and 25.8% respectively. At private elderly homes, the helminthic and protozoal infection rates were 31.3% and 68.8 % respectively (Table 5).

Out of 235 study population, 111 were *Indo-Aryans* and 124 were *Tibeto-Burmans*. Among *Indo-Aryans*, 53 (46.9%) were found to be infected with parasites where as among *Tibeto-Burmans*, only 46 (37.1%) were found to be infected with the parasites; however, the difference was statistically insignificant (Table 6).

The illiterate elderly people were found to be significantly more infected with the intestinal parasites as compared to literate people ($P<0.05$). Out of 42 literate people only 11 were infected, where as 87 people were infected out of 193 illiterate people (Table 7).

The age wise distribution of the parasites showed that the people of age group 70 to 79 years were mostly infected with the parasites (47.3%).The people of age group 60 to 69 years were least affected (36.3%). Likewise 41.2% of the people above 80 years were infected with the parasites. This difference was also not statistically significant (P>0.05) (Fig. 4).

Table 5 Frequency of the parasites detected in different study groups.

Parasites	Elderly people at			Total
	Government elderly home	Private elderly home	Community	
T. trichiura	32 (37.6%)	4 (25.0%)	16 (51.6%)	52 (39.4%)
A. lumbricoides	5 (5.9%)	-	3 (9.7%)	8 (6.1%)
Hookworm	6 (7.1%)	1 (6.3%)	4 (12.9%)	11 (8.3%)
S. stercoralis	1 (1.2%)	-	-	1 (0.8%)
Total Helminthes	**44 (51.8%)**	**5 (31.3%)**	**23 (74.2%)**	**72 (54.5%)**
E. histolytica	16 (18.8%)	5 (31.3%)	5 (16.1%)	26 (19.7%)
E. coli	15 (17.6%)	4 (25.0%)	2 (6.5%)	21 (15.9%)
B. hominis	5 (5.9%)	1 (6.3%)	1 (3.2%)	7 (5.3%)
G. lamblia	2 (2.4%)	-	-	2 (1.5%)
E. hartmani	-	1 (6.3%)	-	1 (0.8%)
C. cayetanensis	1 (1.2%)	-	-	1 (0.8%)
C. parvum	2 (2.4%)	-	-	2 (1.5%)
Total Protozoans	**41 (48.2%)**	**11 (68.8%)**	**8 (25.8%)**	**60 (45.5%)**
Total Parasites	**85**	**16**	**31**	**132**

Table 6 Distribution of parasitic infection in different ethnic groups

Ethnic group	Total	Positive	Percentage	P-value
Indo-Aryan	111	52	46.9	P>0.05
Tibeto-Burman	124	46	37.1	
Total	235	98	41.7	

Table 7 Parasitic infection in relation to the literacy of the elderly people

Educational Status	Total	Positive	Percentage	P-value
Literate	42	11	26.2	P<0.05
Illiterate	193	87	45.1	
Total	235	98	41.7	

Out of 235 study population, 83 were vegetarian including 15 male and 68 female and 152 were non-vegetarian including 73 male and 79 female. Among 83 vegetarians, 25 (30.1%) had parasitic infections, and among 152 non-vegetarians, 73 (48.0%) had parasitic infections. Thus the increased infection rate among the non-vegetarians was found to be statistically significant (P<0.05) (Fig. 5).

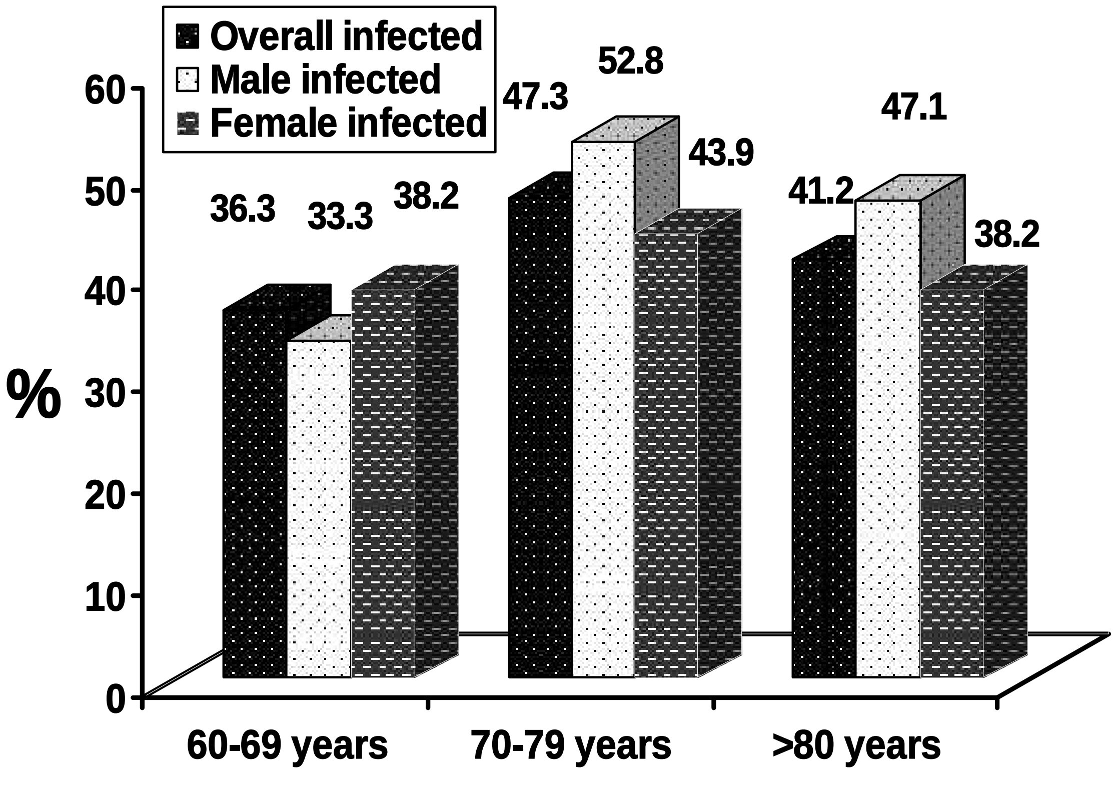

Figure 4 Agewise distribution of parasitic infection

Out of 188 elderly people from different elderly homes, 118 had complains of one or more gastrointestinal symptoms. Here abdominal pain, anorexia, nausea, constipation and diarrhoea were regarded as the gastrointestinal symptoms. Out of them 52 (44.1%) were found to be infected with parasite/s. One the other hand, among 70 people with no any gastrointestinal complains, 24 (34.3%) were found to be infected with parasite/s. The difference was not statistically significant (Table 8).

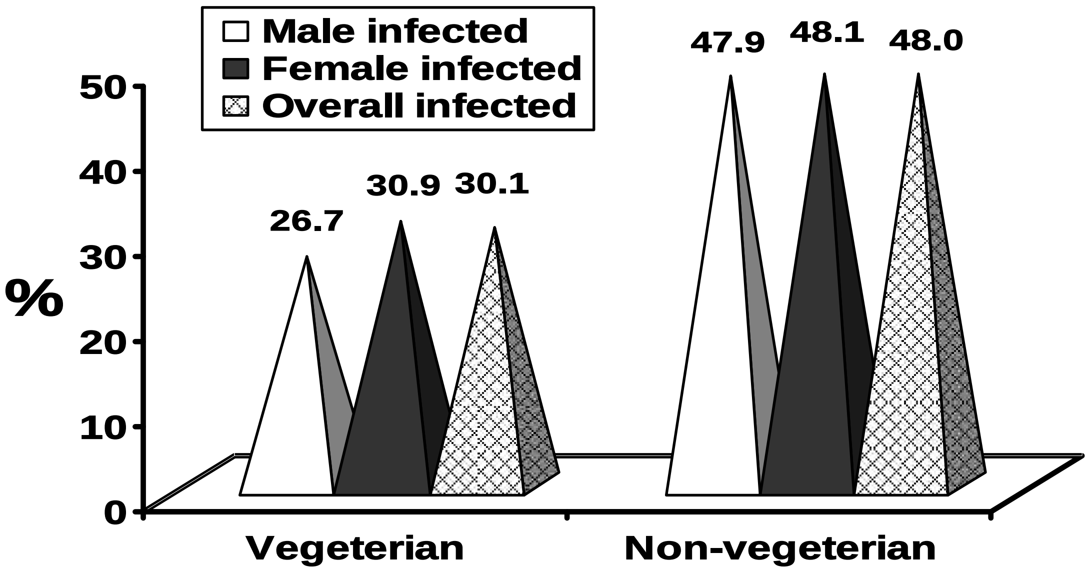

Figure 5 Parasitic infection in relation to type of diet

Similarly, out of 235 elderly people under study, 109 people had one or more type of clinical disease/symptoms other than gastrointestinal. Out of which, 34 (31.2%) were infected with parasite/s. Out of 126 people having no any such disease/symptom, 64 (50.8%) were found infected with the parasite/s. The difference was found to be statistically significant (Table 9). Here the clinical diseases included hypertension, asthma, arthritis, diabetes, oedema, paralysis, filariasis, epilepsy etc.

Table 8 Parasitic infection in relation to the Gastrointestinal (GI) symptoms

GI symptoms	Total	Positive	Percentage	P-value
Present	118	52	44.1	P>0.05
Absent	70	24	34.3	
Total	188	76	40.4	

Out of 188 elderly people living in the elderly home, 110 (58.5%) belonged to Kathmandu Valley, where as 78 (41.5%) of them were from out of the Valley. The parasitic infection rates were 41.0% and 40.0% for those from Kathmandu Valley and those from out of the Valley respectively. The difference was statistically insignificant (P>0.05).

Table 9 Parasitic infection in relation to the clinical diseases other than GI infection

Clinical diseases	Total	Positive	Percentage	P-value
Present	109	34	31.2	P<0.05
Absent	126	64	50.8	
Total	235	98	41.7	

Out of 188 elderly people at different elderly homes, 39 had habit of eating outside the mess of the elderly homes. Remaining 149 did not have such habit. Those eating outside the mess were found to be more infected with parasites as compared to those who did not eat outside the mess; however the difference was not significant statistically (51.3% Vs. 37.6%).

In the elderly homes, 146 people had the habit of washing the fruits and vegetables prior to the raw consumption while 42 used to eat them without washing. The infection rate of 45.2% was seen among the people of latter category where as it was found only 39.0% among those having washing habit. However, the result was not statistically significant.

Out of 188 stool samples collected from the elderly homes during the study period, 35.0% were mucoid and 65.0% were non-mucoid. Similarly the proportion of hard formed, mushy formed, diarrhoeal and watery samples were 15.0%, 64.0%, 14.0%, 4.0% and 3.0% respectively (Fig. 6).

Out of 188 elderly people in the elderly homes, 165 used to drink water, directly from the tap. The parasite positive rate among them was 44.2%. Similarly 9 were well water users and 14 used to have only boiled water. The parasite positive rate among well water user was 33.3%, where as no person using boiled water had any intestinal parasite.

Table 10 Bacteria isolated from stool culture

Bacteria isolated	*Salmonella* spp.	*Shigella* spp.	*V. cholerae*	*Campylobacter* spp.
Frequency	3	Nil	2	Nil

The stool cultures done for the isolation of *Salmonella* spp. *Shigella* spp. *V. cholerae* and *Campylobacter* spp. resulted *Salmonella* spp. in three stool specimens, *V.cholera* in two stool specimens and *Campylobacter* spp. in none (Table 10).

The antibiotic susceptibility pattern of the bacterial isolates found that all three isolates of *Salmonella spp.* were sensitive to ofloxacin, ciprofloxacin and tetracycline. Two of the isolates were sensitive to chloramphenicol and amoxicillin and one was intermediately sensitive to them. Two of the isolates were sensitive to nalidixic acid and one was resistant to it.

Among two isolates of *V. cholerae*, both of them were resistant to tetracycline and ampicillin. Similarly, both of them were sensitive to ofloxacin, amikacin, and gentamicin. Likewise, both of them were intermediately sensitive to erythromycin.

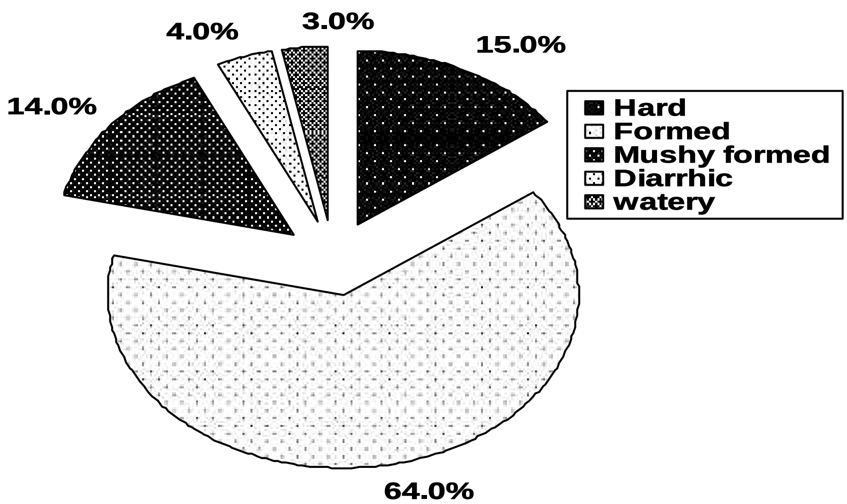

Figure 6 Percentage of stool samples with different consistencies from elderly home

CHAPTER-VI

6 DISCUSSION AND CONCLUSION

6.1 Discussion

In the present study about two-fifth of the elderly people above 60 years were found to be infected with one or more intestinal parasites. The rate of infection was found to be similar to the general trend of parasitic infection among Nepalese population as reported by Chhetri (1997). The result was also within the range reported by Takemasha *et al* (2004) in Kathmandu in a study of over one decade. However, among the 60+ people, the intestinal parasitic infection rate has been observed very low in Brazil as compared to the result of the present study (Oliveira *et al*, *2003*). The lower rates of infection were also reported among 40-69 years age group and 69+ age group people in Iran (Sayyari *et al*, 2005) and 50+ people in Malaysia (Sinniah and Rajeswar, 1998). As compared to the result of the present study, the parasitic infection rates were seen significantly higher in 50+ people in 1988 as reported by Feng *et al* (2001b). The result of the study was marginally lower than the findings reported in Bangladesh among 50+ people (Rahman, 1993). Greater prevalence of intestinal parasites has been reported in general population of Nepal (Rai, 2004) and elsewhere (Phetsouvannh *et al*, 2001). Sharma *et al* (2004) has found about two-third of the school children infected with one or more intestinal parasites in Kathmandu Valley.

The similarity of the study result with the current trends of intestinal infection in the country may be due to the equal susceptibility of the elderly people towards the infections as the other age group. However, illiteracy, poor living standard, poverty, lack of proper hygiene, clean drinking water etc. among the elderly people may be the causes behind the increased rate of the parasitic infection as compared to the infection rate among the people of similar age group elsewhere.

Among three study groups under study, the highest parasitic infection was found in the government elderly home followed by community and private elderly homes. The over crowdedness, poor sanitation and lack of proper care may be the causes behind high infection rate in the government elderly home. On the other hand, the private elderly homes had lower prevalence of intestinal parasitosis. The elderly people in those places were comparatively more facilitated. They were less crowded and the level of sanitation was comparatively better. In one of the elderly home, there was provision of even single room per person and boiled water. The parasitic infection rate seen in community was quite closer to that of government elderly home, which reflects the poor living status of Nepalese elderly people especially in the rural region. The poverty, illiteracy, lack of awareness and lack of proper care of these senior members may be the causes behind this. The disabilities in old age also lead to the lack of personnel care and personnel hygiene. This is also a predisposing factor behind higher infection rate among elderly people.

The average parasite positive rate in elderly homes was found lower than that in the community. It may be because of the system of regular health check up and health care facilities in almost all elderly homes in, where as this could not be expected in the rural community. Beside this, the lesser mobility and no involvement in household activities may be the other reasons behind the lower positive rate among the people at the elderly homes. Most of the people from the rural community under study were involved in agriculture which may be the reason behind high prevalence of the soil transmitted helminth among them.

In the study, the parasitic infection rate was found to be marginally higher among male but the difference was not statistically significant. The result was in agreement with the trend observed by Rai *et al* (1995) in Nepal during 1985-1992, the finding of Sharma *et al* (2004) among school children and that of Rai *et al* (2000) among rural population. This trend of nearly equal infection rate among two genders was also seen elsewhere in the world (Anderson *et al*, 1993; Sayyari *et al*, 2005; Sinnah and Rajeswar, 1998). In contrary to the study result, males have been found to be infected significantly at higher rate in Nigeria, Thailand, Brazil and Sudan as reported by Agi (1995), Nuchpravoon *et al* (2002), Oliveira *et al* (2003) and Magambo *et al* (1998) respectively. Similarly, the significantly higher infection rates have been found among female in Nepal (Rai *et al*, 1995, 2004b) and elsewhere in the world (Feng *et al*, 2001b; Kightlinger *et al*, 1995;

Rajeswari *et al*, 1994; Singh *et al*, 1993; Xu *et al*, 1995). Phetsouvannh *et al* (2001) has reported that the female has 1.25 times more chances of being infected with *A. lumbricoides* than the male. This indicated that the gender may or may not play role in parasitosis depending on the region and other environmental or behavioural factors.

Generally, the increased mobility of the males increases the risk of infection in them, while the involvement of female in child care and their low educational status is responsible for increased risk in them. Females are also supposed to have more soil contact by growing vegetables and eat raw vegetable with prepared food more often than males (Phetsouvannh *et al*, 2001). But in this study, as most of the elderly people in the study belonged to the elderly homes, the above mentioned conditions were rarely applicable to them. This may be the cause of nearly equal infection rate in two genders.

The ratio of monoparsitism and multiparasitism among total infected people was almost 7:3. The finding was consistent with the rate of multiparasitism among general population in Brazil (Oliveira *et al*, 2003) and Lao PDR (Phetsouvannh *et al*, 2001). In the contrary, the multiparasitism rate was higher in Fujian Province in 1998 and lower in the same place in 1999 (Feng *et al*, 2001b). Higher multiparasitism rate was also observed in Northern India by Singh *et al* (1993). Similarly, very low rate of multiparasitism has been reported in rural regions of Nepal (Rai *et al*, 1998). Out of total study population, only about one-eighth had multiparasitism, which was very less in comparison to the three quarter of population harbouring multiparasites as reported by Raso *et al* (2005). The rate of monoparasitism and multiparasitism were found to be independent of the gender of the elderly people; however, Rai *et al* (2000) has reported higher rate of multiparasitism among male in rural region of Nepal. The reason behind the equal infection rate might be as stated above.

Among the multiple infections, *T. trichiura* was found in association with *A. lumbricoides, Hookworm, E. histolytica* and *E. coli* in almost equal cases. This indicated the absence of any special predilection of *T. trichiura* towards any one of the helminth or protozoa during co-infection. In contrast to this finding, the positive association between *A. lumbricoides* and *T. trichiura* has been reported elsewhere in the world (Kightlinger *et al*, 1995; Needham *et al*, 1998; Udonsi *et al*, 1996), though the clear reason behind this association has been reported nowhere. Phetsouvannh *et al* (2001) has suggested their very close lifecycle as a cause behind it. Similar type of association could be expected in the present study with the increased sample size.

The rate of helminthic infection was almost equal to the rate of protozoal infection in the elderly people with no marked difference in the genderwise distribution. None of them showed any special predilection towards any of the genders. However, other studies in Nepal among general population have found higher prevalence of helminthic infection (Estevez *et al*, 1983; Nepal and Palfy, 1980; Rai and Gurung, 1986; Rai *et al*, 1995 and 2000; Sherchand *et al*, 1996; Sharma *et al*, 2004). Similarly, Oda *et al* (2002) has found that males were more susceptible to protozoa and to be due to genetic and physical factors. Among the individual study groups, the government elderly home had nearly equal protozoal infection rate and helminthic infection rate; however, in the private elderly homes, the protozoal infection rate was higher than the helminthic infection rate and vice-versa in the community. It may be due to the presence of more open land and agriculture as the major occupation of the people the rural community, while the use of contaminated water may be the cause behind higher protozoal infection rate in private elderly home.

In the above study, *T. trichiura* was the commonest parasite observed infecting almost two-fifth of the total infected elderly people. The result was similar to the finding made by Chai *et al* (2001b) among the people of same age group but the rate of infection was lesser. The findings made by Rai *et al* (2005) and Ishiyama *et al* (2001, 2003) among general population in Nepal were also not different from our result. It has been reported as the commonest helminth also among the school children in Kathmandu Valley. *T. trichiura* has been reported as the commonest helminth even in Namibia, Venezuala, Malaysia, Srilanka and China (Amarasinghe and Weenasooriya, 1999; Chacin *et al*, 1992; Evans *et al*, 1990; Lili *et al*, 2000; Sinniah and Rajeswar, 1998). Similar findings have been reported elsewhere by Kasuya *et al* (1989) and Rajeswari *et al* (1994). This might be due to the difficulty in complete removal of this helminth with a single dose of anti-helminthic drug, particularly in those with heavy infection (Albonico *et al*, 1999) and this has indicated *T. trichiura* to cause more chronic infection than other helminthes.

As compared to our finding, very low prevalence of *T. trichiura* infection has been reported elsewhere in the world (Motabar and Montazemi, 1978; Obiamiwe and Nmorsi, 1991; Phetsouvannh *et al*, 2001; Phompida *et al*, 2002; Sayyari *et al*, 2005). Blangero *et al* (1993) has also reported very low infection rate by the helminth among 45 + people. Among the people above 50 years of age the infection rate was one quarter of the total infected people in Brazil (Oliveira *et al*, 2003).

In the study, the infection rate by *A. lumbricoides* was quite low where as, it has been reported as the commonest parasite in Nepal by one decade study (Takemasha *et al*, 2004). It has been reported as the predominant parasite in the country also by Nepal and Palfy (1980), Estevez *et al* (1983), Rai and Gurung (1986), Sherchand *et al* (1996), Rai *et al* (1994, 1995, 1998, 2000, 2001, 2002, 2004b) and Ishiyama *et al* (2003) and in elsewhere by Phetsouvannh *et al* (2001) and Feng *et al* (2001a). The higher prevalence of *A. lumbricoides* has also be been reported by Blangero *et al* (1993) among 45+ people, Lili *et al* (2000), Smith *et al* (2001) and Phetsouvannh *et al* (2001). Similar to our finding, lower prevalence of the helminth has been observed among 40+ people in Iran by Sayyari *et al* (2005) and in Vientiane Municipality by Phompida *et al* (2001).

In the above study, the prevalence of Hookworm infection was marginally higher than that of *A. lumbricoides* but very low as compared to *T. trichiura* infection rate. Similar rate of Hookworm infection has been reported in Vientiane Municipality by Phompida *et al* (2001) and in Lao PDR by Phetsouvannh *et al* (2001). On the other hand, higher prevalence of Hookworm infection has been reported among 45+ people and 50+ people by Blangero *et al* (1993) and Cheghani *et al* (1989) respectively. Lili *et al* (2000) has also reported its higher prevalence in China among adults.

In the study, *E. histolytica* has been found to be the commonest protozoa infecting almost one-fifth of the total infected people. It was seen as the commonest protozoa in Rwanda too (Scaglia *et al*, 1983). The other studies in Nepal among the people of different age group have reported *G. lamblia* as the commonest protozoa (Oda and Sherchand, 2002; Ishiyama *et al*, 2001). *G. lamblia* has been reported as the commonest protozoa in Thailand too (Nuchpravoon *et al*, 2002). On the contrary, *E. nana* has been reported as the commonest protozoa in Brazil among 60+ people (Oliveira *et al*, 2003). Very low prevalence of *E. histolytica* infection has been reported by Sayyari *et al* (2005) among 40+ people, by Gambhir *et al* (2003) among the healthy adults and by Braga *et al* (1998) in Brazil. Infection with *E. histolytica* is common in inhabitants of developing countries; it predominantly affects people with poor socioeconomic conditions, non-hygienic practices, and malnutrition (Braga *et al*, 1998).

In the study, the prevalence rate of *E. coli* was found to be marginally lower than that of *E. histolytica.* Similar rate of *E. coli* infection was observed elsewhere (Obiamiwe and Nmorsi, 1991; Pratdesaba *et al*, 2001). Our finding for *E. coli* was somewhat lower than the finding of Oliveira *et al* (2003) among 60+ people in Brazil. On the other hand, very high *E. coli* prevalences have been reported by Oberst and Alquiza (1987) in Philippines and Cabrera *et al* (2000) in Peru.

The very low prevalence of *G. lamblia* observed in our study was in agreement with the findings of Gambhir *et al* (2003) among healthy Indian elderly people. Risk of infection with *G. lamblia* has been found to decrease with ageing (Laupland and Church, 2005). As a reaction to infection with *Giardia*, both humoral and cellular immune response generated by host secretary IgA and IgM appear to play role in clearance of intestinal infection. This gives some degree of protection against reinfection (Oda *et al*, 2002). So the chronically exposed people, especially elderly group have lower attack rate. However, greater prevalences of *G. lamblia* have been reported among 40+ people in Iran (Sayyari *et al*, 2005). Some studies have reported *G. lamblia* as the commonest protozoa in Nepal (Estevez *et al*, 1983; Ishiyama *et al*, 2001; Rai and Gurung, 1986; Sherchand *et al*, 1996).

In the study very low prevalence of *C. cayetanensis* has been found. It might be due to the immunity developed after repeated attack as reported by Sharma and Sherchand

(2003). However, a higher rate of Cyclosporosis was observed in young and elder adults up to 60-year-old by Burstein (2005) in Peru.

C. parvum is generally found to infect the immunocompromised hosts including the elderly people. The prevalence rate of *C. parvum* found in our study was quite consistent with the findings made by Lee *et al* (2005) among 60+ people and by Tzipori *et al* (1983) among adults. As compared to other age groups, elderly people have been found to be at higher risk of *C. parvum* (Naumova *et al*, 2003). Comparatively, higher prevalences of the protozoa have been reported by Lee *et al* (2005) among 70+ female, Chai *et al* (2001c) among elderly people and Nath *et al* (1999) among Indian adults. Cryptosporidiosis among the elderly people without immunocompromised status may be due to the higher prevalence of the organism in the environment and the individuals' close contact with the animals (Gambhir *et al*, 2003). Commonly, *C. parvum* infection has been reported more among the immunocompromised people (Abaza *et al*, 1995; Abou El-Naga *et al*, 1998; Hammouda *et al*, 1996; Khalil *et al*, 1991). Cryptosporidiosis has been found to be positively associated with diarrhoea (Adal *et al*, 1995; Gambhir *et al*, 2003; Lee *et al*, 2005; Svenungsson *et al*, 2002). In the above study too, around one- fourth of the diarrhoeal elderly people had Cryptosporidiosis.

The study revealed higher infection rate of intestinal parasites among the elderly people of *Indo-Aryan* ethnicity as compared to *Tibeto-Burman* ethnicity, however the difference was not statistically significant. The finding was consistent with the finding of Ishiyama *et al* (2001) among Nepalese students. On the other hand, other studies have shown higher infection rate among *Tibeto-Burman* (Ishiyama *et al*, 2003; Rai *et al*, 2004a; Rai, 2004) while studies made by Rai *et al*, 2002 and Sharma *et al*, 2004 among school children found similar prevalence of intestinal parasites in both ethnic groups. This indicated that ethnic aggregation and predisposition to the parasitic infection is not genetically determined but may be more strongly determined by the environmental and behavioural factors.

The level of literacy was found to determine the occurrence of intestinal parasitic infection. The illiterate people were found to be significantly more infected with the intestinal parasites. The result was in agreement with the finding made by Nimri and Meqdam (2004) among Indians and Smith *et al* (2001) among rural people of Hondurus. Here, those who can read and write were considered as the literate people. The literate people are supposed to be more aware about personnel hygiene, surrounding sanitation, importance of clean drinking water, hygienic food and health. They can easily get benefited from the means of information like newspaper, magazines, radio and television as compared to the illiterate people. This might have raised the level of awareness among them and helped them in getting prevented from the parasitic infection. The information disseminated through the media might have made them more conscious regarding the mode of transmission of the parasitic infections and their preventive measures. Thus, the educational intervention could be an effecting means of reducing parasitic prevalence among elderly people.

The people of age group 70-79 years were found to be more infected with the intestinal parasites as compared to 60-69 years and 80+age groups; however, the difference was not statistically significant. In the study, the people of age group 60-69 years were found to be least affected. The least effect on this age group might be due to their better physical health as compared to that of the people at increased age. They were also supposed to have lesser physical disabilities and stronger immune status as compared to the people of greater age group. With the increasement in the age, the physical disabilities increase, immunity power wanes and there occurs the lack of self-care in terms of hygiene and sanitation and therefore the probabilities of intestinal infection increase. This might have resulted higher infection rate among people above 70 years.

The study had the predominance of non-vegetarians. Most of the vegetarians in the study were female. The non-vegetarians were found to be significantly more infected with the intestinal parasites as compared to the vegetarians. But Rai (2003) has reported higher rate of enteropathogens among vegetarians.

The abdominal complains were found to be associated with the parasitic infection though the association was not statistically significant. The finding was consistent with the finding of De Silva *et al* (1994). The intestinal parasitic infection and the clinical diseases other than the gastrointestinal were found to be negatively associated with each other. The association was statistically significant. It might be due to the reason that the drugs used for the treatment of different diseases might have shown anti-parasitic effect. Other reason might be that during the periodic health check up for the other chronic diseases present, they might have obtained anti-parasitic drugs frequently. However, further study is required for finding specific and accurate reasons.

Those people having the habit of eating outside the mess of the elderly homes were found to be more infected as compared to those without the habit; however, the finding was not statistically significant. The food taken outside might have increased the risk of infection in this case. The food product sold in the market might be the source of infection for them. But Rai (2003) has reported lower infection rate among children consuming street food as compared to those not consuming them.

The people having the habit of washing fruits and vegetables before their raw consumption were found to be more infected as compared to those without the habit; however the difference was not statistically significant. This indicated the unwashed fruits and vegetables as the mode of transmission for the intestinal parasites. In the study very few people were found to drink boiled water and none of them were found to be infected with any kind of intestinal parasite. Lesser infection rate among boiled water users was also reported by Oda *et al* (2002). This indicated the boiling as an efficient means of making drinking water free of intestinal parasites.

In the study, *V. cholerae* isolates were found resistant to tetracycline and ampicillin. Urasa *et al* (2000) has also found *V. cholerae* isolates resistant to tetracycline and ampicilin in Tanzinia. Similarly Mishra *et al* (2004) has reported multi drug resistant *Vibrio* from Nagpur region. The Salmonellae isolates from the study were sensitive to ciprofloxacin, which coincides with the finding of Rai (2003). Similarly, One out of three isolates were resistant to nalidixic acid which also tends to coincide with the finding of Rai (2003)

In the study, *Campylobacter* spp. could not be isolated. This might be due to increasement in resistance towards *Campylobacter* infection with the increasement in age. Linnerberg *et al* (2003) has described the increasement in *C. jejuni* specific IgG antibody with increase in age. Humans are likely to be exposed to *Campylobacter* a number of times during their lifetime, resulting in immune responses that can last several years. Further, as the types/strains of *Campylobacter* are diverse, it is probable that people are more likely to develop immune protection to the more common types to which they are exposed (Miller *et al*, 2005). Similarly, no *Shigella* spp. could be isolated in the study, though it has been reported the commonest in elderly people by Agtini *et al* (2005).

The total absence of *Shigella* spp. and *Campylobacter* spp. and the lower isolation rate of other enteropathogens in the above study may be attributed to the study group being almost healthy and very few of them were diarrhoeal and symptomatic. The isolation rate for the enteropathogens could be higher if the study population were hospitalized patients, as the bacterial enteropathogens can be best studied among hospitalized patients. The findings would be richer if studied during the outbreaks by comparing with other study groups.

To the best of our knowledge, this is the first research of its kind in the country, in terms of study group. As there were no any previous researches found specifically focused on the elderly population and related data published, there has been remarkable scarcity of the relevant literature from the country and even at international level. This has resulted lack of comparison of our findings with the other findings especially regarding different clinical, behavioural and social variables.

Although the study is novel in the country and provides important information, there are some important limitations that merit discussion.

- Due to time factor and other constraints, the study had to be confined over limited sample size. More significant findings would have been resulted if more elderly homes and even urban population were included under study.

- More reliable picture of the parasitic prevalence would have been revealed if stool samples from single individual on three consecutive days were taken.
- Due to lack of resources, the study for other enteropathogens (*H. pylori*, ETEC, EHEC, EAEC, EIEC, *Y. entercolitica*, *C. difficile*, viruses etc.) causing gastroenteritis in elderly people could not be done.
- The findings cannot be extrapolated to the entire elderly population of Nepal due to the confounding variables like socioeconomic strata, however the study population has been tried to be perceived as the representative of average Nepali old people.
- The results from the elderly homes under study should only be cautiously extrapolated to other elderly homes in the country as their modus operandi might differ greatly.

By increasing standards of health and controlling the carriers or intermediate hosts, most industrialized countries have successfully decreased the rates of intestinal parasitic infection. In developing countries, however, geographic and socioeconomic factors as well as unpredictable factors such as natural disasters contribute to the problem. These countries are mainly located in warm or hot and relatively humid areas that, combined with poverty, malnutrition, high population density, unavailability of potable water and low health status, provide optimum conditions for the growth and transmission of intestinal parasites. Insufficient research into infectious and parasitic diseases, lack of attention in developing countries to the problem and lack of follow-up treatment are also barriers to decreasing the rates of parasitic infection in these countries (Sayyari *et al*, 2005). Since these parasitic infections are the major public health problems of the country, it is obligatory to conduct surveys in different areas to find out their prevalence (Sherchand *et al*, 1998).

Improvement in sanitation has played important role in reducing the prevalence of parasitic infection but large-scale improvement cannot be achieved instantly in developing countries (Waikagul *et al*, 2002). A decline in the incidence rate can be expected through behavioral change in people like wearing shoes/slipper, avoiding raw meat, appropriate hand washing, avoiding green salads etc. Similarly, the sanitary measures like personnel and community hygiene, planned urbanization, development of proper sewerage system can be contributory in disease prevention. The use of paper and electronic media for publicity and health education programmes can be effective means of public awareness. Likewise, anti-parasitic treatment through mass chemotherapy can bring sharp decline in symptomatic patients as well as the carriers (Cheesebrough, 1998; Oda and Sherchand, 2002; Rai, 2004).

6.2 Conclusion

Hence, the parasitic infections are closely related with the conditions of living and environmental sanitation in a community. They are more prevalent among socio-economically depressed community where standard of living and environmental hygiene, health education and other amenities are inadequate or lacking. So it can be effectively controlled when the socioeconomic status of a community is improved, when better living conditions, environmental sanitation, and other interventions such as regular deworming, health education and improved nutritional status are made possible. Appropriate water management, safe disposal of excreta, prevention of food contamination by faecal material and avoidance of faecal material as agricultural fertilizer can contribute a lot in parasite control. Similarly particular attention should be paid to diet, ways of taking meal, child education and care and social structures and religious patterns.

CHAPTER VII

7 SUMMARY AND RECOMMENDATION

7.1 Summary

1. Stool samples were collected from 235 elderly people in Kathmandu; 122 from government elderly home, 66 from private elderly home and 47 from a rural community. Among them 41.7% had intestinal parasitic infection and 30.6% of the total infected had multiparasitism.

2. The government elderly home had higher parasitic prevalence (50.8%) followed by the rural community (46.8%) and private elderly home (21.2%). Males were marginally more infected (43.8%) than female (40.4%) and nearly equal protozoal and helminthic infection was found in both genders.

3. *Trichuris trichiura* (39.4%) was the commonest helminth and *Entamoeba histolytica* (19.7%) was the commonest protozoa found. Other parasites recovered were *A. lumbricoides*, Hookworm, *S. stercoralis*, *E. coli*, *B. hominis*, *G. lamblia*, *E. hartmanni*, *C. cayetanensis* and *C. parvum*.

4. *Indo-Aryans* had higher parasitic infection rate than the *Tibeto-Burmans* (46.9 Vs. 37.1%). Literate people were less infected (26.2%) than the illiterate people (45.1%). Vegetarians were less infected (30.1%) than non-vegetarian (48.0%).

5. The people of age group 70-79 years were most affected with the parasites followed by 80+ and 60-69 years.

6. 44.1% of people with abdominal complain were infected with parasites and 34.3% people without such complains were infected with parasites. 31.2% of people with clinical disease other than gastrointestinal were infected with the parasites and 50.8% of the people without such disease were infected with the parasites.

7. The parasitic infection rates were 41.0% and 40.0% for those from Kathmandu Valley and those from out of the Valley respectively for those residing in elderly homes. Those people having the habit of eating outside the mess were found to be more infected with the parasites than those with out the habit.

8. *Salmonella* spp. in three samples and *Vibrio* spp. in two samples were found where as *campylobacter* spp and *Shigella* spp. were found in none in the study.

7.2 Recommendations

- It is recommended to conduct study on elderly people at large scale, possibly at national level based on geography, socioeconomic level and seasons.

- Bacterial and parasitic study of food, water and utensils and other sanitary parameters of the elderly homes should be done and correlated.

- The study of prevalence of *H. pylori* and other infections like lower respiratory tract infections, genitourinary tract infection etc. is recommended, as they are quite common among elderly people.

- It is recommended to conduct study of enteropathogens on other institutionalized groups like prisoners, people at rehabilitation centers, hostel students etc..

- Assessment of the correlation of bacterial and parasitic infections with the individual clinical parameters should be done by taking the hospitalized and diseased elderly people as the study groups.

CHAPTER VIII

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APPENDIX-1

Materials and chemicals used

1 Chemicals and reagents

Sodium Chloride	:	Qualigens, India
Basic fuschin	:	Qualigens, India
Ethanol	:	Bengal, India
Diethyl ether	:	Qualigens, India
Formaldehyde	:	Qualigens, India
Crystal Violet	:	Qualigens, India
Iodine crystals	:	Loba chemie, India
Sulphuric acid	:	Qualigens, India
Hydrochloric acid	:	Qualigens, India
KI crystals	:	Qualigens, India
Methanol	:	Qualigens, India
Malachite Green	:	Loba chemie, India
Phenol	:	Qualigens, India
Sucrose crystals	:	Qualigens, India
Safranine	:	Qualigens, India
Hydrogen Peroxide (3%)	:	Qualigens, India
Kovac's Reagent	:	Qualigens, India
MR Reagent	:	Qualigens, India
Barrit's Reagent (VP Reagent)	:	Qualigens, India
2.5 % Potassium Dichromate	:	Qualigens, India

2 Materials

Test-tube
Conical flask
Beaker
Petri dishes
Screw cap test-tube
Measuring cylinder
Glass Slide and Cover slips
Droppers
Pipettes
Glass rod
Test tube Stand
Inoculating loop
Forceps
Sterile Swab

3 Equipments

Microscope	:	Olympus (Japan)
Incubator	:	Memmert, Germany
Refrigerator	:	LG, Korea
Centrifuge	:	Remi, India
Autoclave	:	ALP, Japan
Laminar air flow	:	Olympic Gold, India

4 Media

Salmonella Shigella Agar
MacConkey Agar
Nutrient Agar
Nutrient Broth
Thiosulphate Citrate Bile salt Sucrose Agar
Campylobacter Blood Free Selective Agar Base (Modified CCDA-Preston)
Mueller Hinton Agar
MR-VP Broth
Triple Sugar Iron Agar (TSI)
Sulphide Indole Motility Agar (SIM)
Urease Broth
Simmon Citrate Agar

Antibiotic discs

Antibiotic discs used were from Hi-Media Laboratories Pvt. Ltd., Bombay, India 23, Vadhani Industrial Estate, L.B.S. Marg, Bombay-4000 086

Name of antibiotics	**Concentration**
Ampicillin	10 ⇑g
Ciprofloxacin	5 ⇑g
Erythromycin	15 ⇑g
Nalidixic acid	30 ⇑g
Tetracycline	30 ⇑g
Chloramphenicol	30 ⇑g
Gentamicin	10 ⇑g
Cephalexin	30 ⇑g
Amikacin	30 ⇑g
Amoxicillin	10 ⇑g

APPENDIX-2

Type and frequency of multiple infections detected

S.N.	Pattern of multiple infection	Frequency				% of total multiple infection
		Govt. elderly home	Private elderly home	Community	Total	
1	TT+AL	2	-	1	3	10.0%
2	TT+HK	1	1	2	4	13.3%
3	HK+AL	-	-	1	1	6.7%
4	AL+TT+HK	1	-	-	1	3.3%
5	EH+TT	3	-	3	6	20.0%
6	EC+TT	2	1	1	4	13.3%
7	EC+HK	1	-	-	1	3.3%
8	AL+TT+EC	1	-	-	1	3.3%
9	TT+HK+EC	1	-	-	1	3.3%
10	BH+TT+EH	1	-	-	1	3.3%
11	BH+GL	1	-	-	1	3.3%
12	BH+EH	1	1	1	3	10.0%
13	AL+TT+CP	1	-	-	1	3.3%
14	CC+CP	1	-	-	1	3.3%
15	EC+SS	1	-	-	1	3.3%
	TOTAL	18	3	9	30	100%

Note:

TT = *Trichuris trichiura*
AL = *Ascaris lumbricoides*
HK = Hookworm
SS = *Strongyloides stercoralis*
EH = *Entamoeba histolytica*
EC = *Entamoeba coli*
BH = *Blastocystis hominis*
GL = *Giardia lamblia*
CP = *Cryptosporidium parvum*
CC = *Cyclospora cyatenensis*

APPENDIX-3

Questionnaire and report form

CODE NO.
Date:

Subject's name :
Age :
Gender :

Belonging district :
Date of admission :
Complaints (Symptoms, if any) :

Clinical History
Any disease being suffered (current disease) :
Transplantation :
Surgery undergone :
Medication (current) :

- Type of diet : veg. / nonveg.
- Drinking water : boiled / filtered / tapwater
- Alcoholism : yes / no
- Smoking : yes / no
- Educational status : literate / illiterate
- Do you wash your hands before meal? : yes / no
- Do you wash your hands after toilet? : yes / no
- Do you have the habit of eating raw meat? : yes / no
- Do you wash fruits and vegetables before raw consumption? : yes / no
- Do you eat outside mess? : yes / no
- How often are you provided health check up?
- When was the last time you had health check up?

Report of stool examination

Macroscopic Examination Colour: Consistency: Blood and mucus: Adult worms and segments:	**Microscopic Examination** Gram's staining: Saline wet mount: Iodine wet mount: Kinyoun modified Z-N staining
Culture Report Organism isolated: Drug of choice: Suggestion based on the study:	

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APPENDIX-4

Biochemical tests of *Salmonella* spp., *Shigella* spp. and *V. cholerae.*

Tests	**Typical reactions of**		
	***Salmonella* Spp.**	***Shigella* spp.**	***V. cholerae***
Urease	-	-	-
Motility	+	-	+ (Darting type)
Indole	-	-	+

Citrate	+1	-/+	-/+
Triple Sugar Iron	Alk/A	Alk/A	Alk/A
Gas from Glucose	+2	- (rarely+)	-
Acid from Glucose	+	+	+
Acid from Lactose	-	-	- (+ on prolonged incubation)
Hydrogen Sulphide	+3	-	-
Catalase	+	+	+
Oxidase	-	-	+
M-R Test	+	+	+
V-P Test	-	-	-/+

Note: +1 = All strain positive except *S. typhi* and *S. paratyphi*

+2 = All strain positive except *S. typhi*

+3 = All strain positive except *S. paratyphi*

Alk/A = Alkali/Acid

- = Negative

+ = Positive

-/+ = Negative / Positive (Variable)

(Source: District Laboratory manual in tropical countries Part 2)

APPENDIX-5

Tests used to identify presumptively *Shigella* spp. and *Salmonella* spp.

				KIA Medium Reactions			
	Motility	**Indole**	**LDC**	**Slope**	**Butt**	**Black (H2S)**	**Cracks (Gas)**
Shigella dysenteriae	-	d	-	R	Y	-	-
Shigella flexneri	-	d	-	R	Y	-	-[1]
Shigella boydii	-	d	-	R	Y	-	-[2]
Shigella sonnei	-	-	-	R	Y	-	-
***Salmonella paratyphi* A**	+	-	-	R	Y	-[3]	+
***Salmonella paratyphi* B**	+	-	+	R	Y	+	+

***Salmonella paratyphi* C**	+	-	+	R	Y	+[4]	+
Salmonella typhi	+	-	+	R	Y	+ Weak	-
Other *Salmonella* serovars	+[5]	-	+	R	Y	+[6]	d

Key: KIA = Kligler iron agar, LDC =Lysine decarboxylase, d =different strains give different results, R = Red-Pink (alkaline reaction), Y =Yellow (acid reaction).

Notes:
1 Some strains of serotype 6 produce gas. **2** Serotype 13 and 14 produce gas. **3** About 12% of strains produce H_2S weakly. **4** A minority of strains do not produce H_2S. **5** *Salmonella pullorum* and *Salmonella gallinarum* are non-motile. **6** A minority of strains do not produce H_2S.

(Source: District Laboratory manual in tropical countries Part 2)