CHAPTER I

1. INTRODUCTION

The term diarrhoea describes wide range of condition where the stools are loose. Therefore, the best description of diarrhoea is “an abnormal increase in the frequency and liquidity of stools. In epidemiological studies diarrhoea is defined as the passage of three or more loosely or watery stools in 24 hours periods, loose stool being one that would take the shape of container. Diarrhoeal disease causes an estimated 1.7 million death per year, 90% of these occurs in children, the vast majority in developing countries where many people lack access to safe drinking water (Colindres *et al.*, 2007).

In developing countries, diarrhoeal diseases account for 1.5 million death each year among children aged 1-4 years. The risk of children in this age group dying from diarrhoeal disease is 600 times greater in developing countries than in developed countries. In some developing countries, children suffer ten or more episodes of diarrhoea a year (WHO 2004). The main cause of death from acute diarrhoea is dehydration, which results from the loss of fluid and electrolytes in diarrhoea stools. Other important causes of deaths are dysentery and under nutrition.

Diarrhoea is an important cause of undernutrition. The disease also represents an economic burden for the developing countries. In many nations more than a third of the hospital beds for children are occupied by patients with diarrhoea. These patients are often treated with expensive intravenous fluids and ineffective drugs. The infectious agents that cause diarrhoea usually spread by the fecal-oral route, which includes the ingestion of faecally contaminated water or food, person to person transmission and direct contact with infected faeces. The factors responsible for contaminating drinking water at source points in Nepal includes the lack of protection and proper treatment of water, leakage in pipe distribution system, intermittent supply of water, poor drainage system and poor environment surrounding of water sources (NHRC/WHO 2002, DWSS/UNICEF 2000).

Several types of bacteria consumed through contaminated food or water can cause diarrhoea. Common bacterial pathogens include *Campylobacter, Salmonella, Shigella, and Escherichia coli*. Many viruses cause diarrhoea, including rotavirus, norwalk virus etc. Parasites can enter the body through food or water and settle in the digestive system. Parasites that cause diarrhoea include *Giardia lamblia, Entamoeba histolytica*, *Cyclospora cayetanensis, Cryptosporidium, Ascaris lumbricoides* and hookworm.

Distinct seasonal pattern occurs in many geographical areas. In temperate climates bacterial tends to occur more frequently during the warm season, whereas, viral diarrhoea, particularly disease caused by rotavirus, peak during the winter. In tropical areas, rotavirus diarrhoea tends to occurs throughout the year, increasing in frequency in drier, cooler month, during the warmer and rainy season. The incidence of persistent diarrhoea follows the same seasonal pattern as acute watery diarrhoea. Across the world, rotavirus is thought to be responsible for more than 125 million cases of diarrhoea each year in children and infants. Rotavirus is responsible for the deaths of as many as 600000 children each year (Hendricks *et al*., 1995).

In Nepal, every year 30,000 to 40,000 people die from diarrhoeal disease (Bista *et al.,* 1993). Nepal being the developing country, diarrhoeal disease is major problems. Diarrhoea is one of the major public health problems among children below five years of age in Nepal. The report from ministry of health showed that the total diarrhoea death from acute diarrhoea per 1000 in less than five is 194 in 2003/04, 244 in 2004/05 and 82 in 2005/06 in nation wide. The case fatality rate (CFR) from acute diarrhoea per 1000 less than five years is 0.2% in 2003/04 0.3% in 2004/05 and 0.1% in 2005/06 in nation wide. Similarly the total diarrhoeal visit is 7, 87,094 in 2003/04, 7, 85,336 in 2004/05 and 7, 39,915 in 2005/06 (DoHS, Annual report, 2006). The data of the Oral Rehydration Therapy Centre of Kanti children's Hospital showed 1,189 diarrhoeal patients were admmited in 2007 among them 1092 were cured, 2 were death, 26 with dysentery and 36 with cholera.

The study therefore is aimed to reveal the present status of children that affected with diarrhoeal disease and also to isolate and identify the possible enteric organisms from their stool samples. This study also allows tracking of current levels and trends in diarrhoeal incidence and mortality and provides the basis for future projection and evaluations of different control strategies.

CHAPTER II

2. OBJECTIVES

2.1 General objective

- To isolate and identify etiological agents of diarrhoea among children under 10 years of age who attend in Kanti Children's Hospital of Kathmandu.

2.2 Specific objectives

- To compare the etiological agents of diarrhoeal patients among oral rehydration therapy (ORT) centre and outdoor patient department (OPD) of Kanti Children's Hospital.
- To find out the co-occurrence of different enteropathogens.
- To study the distribution pattern of microorganisms involved in causing diarrhoea in relation to socio-medical aspects.
- To find out the seasonal distribution of diarrhoea.
- To study the burden of rotavirus infection among children.

CHAPTER III

3. LITERATURE REVIEW

Diarrhoea is the passage of loose or watery stools that may contain blood, pus, or mucus. Diarrhoea is a major contributor to childhood mortality and morbidity in the developing world, causing an estimated 2.5 million death each year and long-term effects on growth and cognitive function. Hippocrate defined diarrhoea as an abnormal frequency and liquidity of faecal discharge. Diarrhoea is one of the most important causes of death in childhood and is still a considerable public health problem in developing countries especially among less than five years old (Pauline *et al.*, 2004). Diarrhoea is a clinical syndrome of diverse etiology, often associated with vomiting and fever.

The term acute watery diarrhoea refers to diarrhoea that begins acutely, last less than 14 days (most episodes last less than 7 days), and involves the passage of frequent loose or watery stools without visible blood. There is possibility of occurrence of vomiting and fever during course of disease. Acute watery diarrhoea causes dehydration; when food intake is reduced, it also contributes to malnutrition. When death occurs it is usually due to acute dehydration. The most significant etiological agent of acute watery diarrhoea in young children in developing countries is rotavirus, enterotoxigenic *E. coli*, *Shigella, Campylobacter jejuni and Cryptosporidium.* In some area *Vibrio cholerae* O1, *Salmonella* and enteropathogenic *E. coli* are also important.

The term dysentery refers to diarrhoea with visible blood in feaces. Important effects of dysentery include anorexia, rapid weight loss and damage to the intestinal mucosa by the invasive bacteria. The most important causes of acute dysentery are *Shigella* other causes are *C. jejuni* and infrequently, enteroinvasive *E. coli* or *Salmonella*. *Entamoeba histolytica* can cause serious dysentery in young adults but rarely a cause of dysentery in young children (WHO, 1995).

Persistent diarrhoea begins acutely but is of unusually long duration (at least 14 days). The episode may begin either as watery diarrhoea or dysentery. Marked weight loss is frequent. Diarrhoeal stool volume may also be large with a risk of dehydration. There is no single microbial cause, although *Shigella, Salmonella,* enteroaggregative *E. coli* and *Cryptospridium* may play a greater role than other agents. Generally persistent diarrhoea is associated with extensive changes in the bowel mucosa, especially flattening of villi and reduced production of disaccharides enzymes, which causes reduced absorption of nutrients and may perpetuate the illness.

The infectious agents that cause diarrhoea are usually spread by the faeco-oral route, which includes the ingestion of feacally contaminated water or food, person to person transmission; and direct contact with infected faeces.

3.1 Behaviors that increase the risk of diarrhoea

A number of specific behaviors help enteric pathogens to spread and thus increase the risk of diarrhoea. These include:

1. Failing to breast-feed exclusively for the first 4-6 months of life. The risk of developing severe diarrhoea is many times greater in non-breast-fed infants than in infants who are exclusively breast fed; the risk of death from diarrhoea is also substantially greater.

2. Using infant feeding bottles. These easily become contaminated with faecal bacteria and are difficult to clean. When milk is added to an unclean bottle it becomes contaminated; if it is not consumed immediately, further bacterial growth occurs.

3. Storing cooked food at room temperature. When food is cooked and then saved to be used later; it may easily be contaminated, for example, by contact with contaminated surfaces or container. If food is kept for several hours at room temperature, bacteria in it can multiply many times.

4. Drinking water that contaminated with faecal bacteria. Water may be contaminated at its source or during storage in the home. Contamination in the home may occur when the storage container is not covered, or when a contaminated hand comes into contact with the water while collecting it from the container.

5. Failing to wash hands before handling food, after defecation or after handling faeces.

6. Failing to dispose of faeces (including infant's faeces) hygienically. It is often believed that infant faeces are harmless, whereas they may actually contain large numbers of infectious viruses or bacteria; animal faeces also can transmit enteric pathogens to man.

Host factor that increase susceptibility to diarrhoea. Several host factors are associated with increased incidence, severity, or duration of diarrhoea. They includes

1. Undernutrition: The frequency, severity duration, and risk of death from diarrhoea are increased in undernourished children, especially those with severe undernutrition.

2. Current or recent measles: Diarrhoea and dysentery are more frequent or severe in children with measles or who have had measles in the previous four week. This presumably results from immunological impairment caused by measles.

3. Immunodefieciency or immunosuppresion: This may be a temporary effect of certain viral infections (e.g., measles), or it may be prolonged, as in persons with the acquired immunodeficiency syndrome (AIDS).

Observations in studies suggested that active immunization through repeated exposure and prolonged breastfeeding may protect against the diarrhogenic effect of these agents (WHO, 2004).

3.2 Immunity against infection in gastrointestinal tract

The human host has numerous defenses that normally prevents or control disease produced by enteric pathogens. The presence of normal flora from mouth to anus prevents the colonization by pathogenic organisms. Thus infection is prevented in normal condition. Saliva possesses mild bactericidal action. A significant reduction in number of normal microflora, due to the antibiotic therapy or some host factor, resistant to gastrointestinal infection is greatly reduced. Similarly, the anaerobic colon bacteria produce fatty acids with antibacterial activity. The term colonization resistance refers to resistance offered by the predominant normal flora to infection. The inner lining of the stomach, the mucosa contain millions of glands that secrete mucus and various other components of gastric juice. Hydrochloric acid secreted by stomach keeps the pH of the stomach extreme low up to 1 to 2 and thus the acidic environment of stomach provides a barrier to establishing disease. The normal propulsive movement of gastrointestinal tract decreases the possibility of adherence of foreign pathogenic organisms to the mucosa. The continuous flow of liquid between intestinal wall and blood vessel is also another important factor of defense. The shedding and replacement of epithelium and the lymphoid tissues (Payer's patches) also provide defense against pathogens to attach on mucosal layer. Secretary immunoglobulin A (IgA) and phagocytic cells within the gut is particularly active against the parasites (Forbes *et al.*, 2002).

3.3 Acquisition and transmission of gastrointestinal pathogens

Infection of the gastrointestinal tract can be grouped into those, which remain localized in the gut and those, which invade beyond the gut to cause infection in other body sites. In order to spread to new host, pathogens are excreted in large amount in the faeces and must survive in the environment for a long enough time to infect another person directly or indirectly through contaminated food and fluids or via direct contact with the infected faeces.

3.4 The ability of microbial factor in pathogenic mechanism

The ability of organism to cause gastrointestinal infection depends not only on the susceptibility of the human host to the invading organism but also on the organism's virulence traits. For a microorganism to cause gastrointestinal infection, it must contains one or more factors that allow it to overcome these host defenses or it must enter the host at a time when one or more of the defense system is inactive (Forbes *et al.,* 2002). Depending on how they interact with the human host, enteric pathogens may cause disease in one or more following ways:

Viruses, such as rotavirus, replicate within the villous epithelium of the bowel, causing patchy epithelial cell destruction and villous shortening. The loss of normal absorptive villous cells and their temporary replacement by immature, secretory, crypt-like cells causes the intestine to secrete water and electrolytes. Villous damage may also be associated with the loss of disaccharidase enzymes, leading to reduced absorption of dietary disaccharides, especially lactose. Recovery occurs when the villi regenerate and the villous epithelium matures.

Mucosal adhesion: The bacteria that multiply within the small intestine must first adhere to the mucosa to avoid being swept away. Adhesion is caused by hair like antigens, termed pili or fimbriae that bind to receptors on the intestinal surface; this occurs, for example, with ETEC and *V. cholerae* O1. In some instances, mucosal adherence causes changes in the gut epithelium that may reduce its absorptive capacity or cause fluid secretion.

Toxins that cause secretion: ETEC, *V. cholerae* O1 and possibly other bacteria e.g., *Salmonella*, cause intestinal secretion by producing toxins that alter epithelial cell function; these toxins reduce the absorption of sodium by villi and may increase the secretion of chloride in the crypts, resulting in net secretion of water and electrolytes. Recovery occurs when the intoxicated cells are replaced by healthy ones after 2-4 days.

Mucosal invasion *Shigella, C. jejuni* and enteroinvasive *E. coli*: The *E. coli* may cause bloody diarrhoea by invading and destroying mucosal epithelial cells. This occurs mostly in

the colon and the distal part of the ileum. Invasion is followed by the formation of microabscesses and superficial ulcers, and hence the presence of red and white blood cells, or frank blood, in the stool. Toxins produced by these organisms cause tissues damage and possibly also mucosal secretion water and electrolytes.

Mucosal adhesion: *G. lambia* and *Cryptosporidium* adhere to the small bowel epithelium and cause shortening of the villi, which may be how they cause diarrhoea causing microabscess and ulcers. This only happens when the infecting strains of *E. histolytica* is virulent. In about 90% of human infections the stains are non virulent; in such cases there is no mucosal invasion and no symptoms occurs, although amoebic cysts are present in the faeces.

Table 1 Clue for identification of enteropathogens

Appearance	**Possible Pathogens**
Unformed containing pus and mucus mixed with blood	Shigellosis *Campylobacter enteritis*
Unformed with blood and mucus (acid pH)	Amoebic dysentery
Unformed or semi formed often with blood and mucus	Schistosomiasis
Watery stool	ETEC infection, Rotavirus enteritis
Rice watery stools with mucous flakes	Cholera
Unformed or watery and sometimes with blood, mucus and pus	Salmonellosis
Unformed, pale coloured, frothy, unpleasant smelling stools that float on water (high fat content)	Giardiasis, other conditions that cause malabsorption e.g. post infective tropical malabsorption
Fluid stools (containing lactose with pH below 6	Lactase deficiency

Unformed of semi formed black stools (positive occult blood test)	Malaena (gastrointestinal bleeding), Hookworm diseases, Iron therapy

Note: Blood can also be found in the stools of patients with haemorrhoids, ulcerative colitis, or tumors of the intestinal tract.

Source: Cheesbrough, (2000)

There are various types of pathogens responsible for causing gastrointestinal infections. Among bacteria, the common ones are *Shigella* spp., *Salmonella* spp., *V. cholerae*, *Helicobacter pylori*, *Aeromonas* spp., *Plesiomonas* spp., *Campylobacter* spp., *Yersinia enterocolitica*, *Clostridium perfringens*, *Clostridium difficile*, ETEC, EHEC, EAEC, and EIEC etc. Similarly Rotavirus, Norovirus, Astrovirus, Calcivirus and Enterovirus are the major viruses responsible for causing gastrointestinal infections. Among the intestinal parasites, the common ones are *Cryptosporidium paravum*, *C. caytanensis*, *E. histolytica*, *G. lamblia*, *A. lumbricoides*, Hookworm, *T. trichiura*, *Strongyloides stercoralis* etc. The fungi like *Candida albicans* are also found to cause the intestinal infections.

The organism *Shigella* was isolated in 1896 by Japanese microbiologist, Kiyoshi Shiga during a major epidemic of severe shigellosis, thus making it one of the oldest identified enteric pathogen. This disease differs from profuse watery diarrhoea, as is commonly seen in choleric diarrhoea or in ETEC diarrhoea, in that the dysenteric stool is scant and contains blood, mucus, and inflammatory cells. Shigellosis is an acute invasive enteric infection caused by bacteria belonging to the genus *Shigella*; it is clinically manifested by diarrhoea that is frequently bloody. Shigellosis is endemic in many developing countries and also occurs in epidemics causing considerable morbidity and mortality (WHO, 2005). Gram-negative, non-motile facultative anaerobes of the genus *Shigella* are the principal agents of bacillary dysentery. Each of the approximately 40 serotypes of *Shigella* is divided into four groups depending on serological similarities and fermentation reaction. Serotypes are characterized by their 'O' antigens. The four named groups of *Shigella* are *S. dysenteriae* (Serological group A), *S. flexneri* (Serological group B)**,** *S. boydii* (Serological group C) and *S. sonnei* (Serological group D).

Among the four species of *Shigella*, *S. dysenteriae* type I causes other epidemic and endemic shigellosis with high case fatality rate, it produces 1000 folds more toxin than others (Bartlett *et al.,* 1986). Commercial antisera are available to differentiate these four groups.

There are close relationships between the O antigens of many *Shigella* serotypes and those of serotypes in other *Shigella* species and certain serotypes of *E. coli* and its alkalescens-dispar varieties. For example, the O antigen of *S. dysenteriae* serotype 1 is related to the O antigens of *E. coli* O-group 1 and A-D group 1. *Shigella* is spread by direct contact with an infected person, or by eating contaminated food or drinking contaminated water. Flies may also transmit the organism. The low infective dose, as few as 200 viable organisms, facilitates person to person spread. Humans and a few primates are the only reservoir of *Shigella.*

Shigella species are pathogens of humans and other primates, and the pathogenesis of infection with these bacteria and EIEC is very similar. The infective dose is small: bacillary dysentery may follow the ingestion of as few as 10 viable bacteria. The site of infection is the M cells in the Payer's patches of the large intestine. Strains of *Shigella* spp. have been examined for a range of putative pathogenic mechanisms. Since the infective dose for this organism is small, *Shigellae* might have innate tolerance to the low pH and bile encountered in the human digestive tract (Lewis, 1997). Species of *Shigella* are non-motile and it is not known how the bacteria reach and adhere to the M cells.

Shigellosis has two basic clinical presentations: Watery diarrhoea associated with vomiting and mild to moderate dehydration, and dysentery characterized by a small volume of bloody, mucopurulent stools, and abdominal pain. The incubation period is usually between 2-3 days but may be as short as 12 hours. The onset of symptom is usually sudden and frequently the initial symptom is abdominal colic. This is followed by the onset of watery diarrhoea and in all but the mildest cases this is accompanied by fever and malaise. Koster *et al.,* (1978) observed acute haemolytic anaemia, thrombocytopenia, oligouria and

sometimes kidney failure due to haemolytic uraemic syndrome. Vomiting and nausea are not prominent, anorexia is common. The symptoms typically last about 4 days but may continue for 10 days or more (Lama, 2006).

Salmonella possesses one or more 'O' somatic antigen, flagellar 'H' antigen and virulence 'Vi' antigen. Up to now more than 2500 serovars of combination of somatic (O) and flagellar (H) antigens of genus *Salmonellae* have been described. The modified Kauffman-White scheme gives specific serological differentiation, which constitutes powerful epidemiological tools (Christie, 1980). Salmonellosis includes various syndromes such as enteric fevers, septicemia, focal infections and an asymptomatic carrier state. Particular serovars show a strong tendency to produce a particular syndrome (*S. typhi, S. paratyphi* A, and *S. schottmuelleri* produce enteric fever; *S. cholerae-suis* produces septicemia or focal infections; *S. typhimurium* and *S. enteritidis* (produce gastroenteritis); however, on occasion any serotype can produce any of the syndromes. In general more severe infections occur in infants, in adults over the age of 50, and in subjects with debilitating illness. The organisms almost always enter via the oral route usually with contaminated food or drink. The mean infective dose to produce clinical or sub-clinical infection in human is 10^5-10^8 *Salmonellae* (but perhaps as few as 10^3 *Salmonella typhi* organisms).

Among the host factors that contribute to resistance to *Salmonella* infection are gastric acidity, normal intestinal microbial flora and local intestinal immunity (Brooks *et al.*, 2004). After ingestion, the organisms colonize the ileum and colon, invade the intestinal epithelium, and proliferate within the epithelium and lymphoid follicles. Attachment and invasion are under genetic control and involve multiple genes in both chromosomes and plasmids. After invading the epithelium, the organisms multiply intracellularly and then spread to mesenteric lymph nodes and throughout the body via systemic circulation; they are then taken up by the reticuloendothelial cells. The reticuloendothelial system confines and controls spread of the organism. However, depending on the serotype, some organisms may infect the liver, spleen, gallbladder, bones, meninges, and other organs. Most serovars

are killed promptly in extra intestinal sites, and the most common human *Salmonella* infection, gastroenteritis remains confined to the intestine.

After invading the intestine, most *Salmonellae* induce an inflammatory response, which can cause ulceration, because of inflammatory reaction, symptoms such as fever; chills, abdominal pain, leukocytosis and diarrhoea are common. The stools may contain polymorphonuclear leucocytes, blood, and mucus (Threlfall *et al.*, 1992).

The incubation period for *Salmonella* gastroenteritis depends on the dose of bacteria. Symptoms usually begin 6 to 48 hours after ingestion of contaminated food or water and usually take the form of nausea, vomiting, diarrhoea, and abdominal pain. Myalgia and headache are common; however, the cardinal manifestation is diarrhoea. Fever and chills are also common, the duration of fever and diarrhoea vary though usually being around 2-7 days.

Enteric fevers are severe systemic forms of salmonellosis. The best studied enteric fever is typhoid fever, the form caused by *S. typhi*, but any species of *Samonella* may cause this type of disease. The symptoms begin after an incubation period of 10 to 14 days. Enteric fevers may be preceded by gastroenteritis, which usually resolves before the onset of systemic disease. The symptoms of enteric fevers are nonspecific and include fever, anorexia, headache, myalgias, and constipation. Enteric fevers are severe infections and may be fatal if antibiotics are not promptly administered (Threlfall *et al.*, 1992).

V. cholerae is responsible for causing a devastating disease, cholera due to the rapidity with which severe dysfunction occurs. The organism is classified by biochemical tests and is further subdivided into serogroups based on the somatic O antigen. The O antigen shows enormous serological diversity, with over 200 serogroups. Only the O1 and O139 serogroups cause epidemic and pandemic disease. Strains identified by biochemical tests as *V. cholerae* that do not agglutinate with O1 or O139 antisera are referred to as non-O1 and non-O139 *V. cholerae*.

The O1 serogroup is divided into two biotypes, classical and E1 Tor that can be differentiated by use of assays of haemolysis, haemagglutination, phage typing, polymixin B sensitivity, and the Voges-Proskauer reaction. The latest approach, however, is to use biotype –specific genes (e.g. *tcpA*, *rtxC*) to differentiate between the two biotypes. Each of the O1 biotypes can be further subdivided into two major serotypes, Ogawa and Inaba. Ogawa strains procuce the A and B antigens and a small amount of C, whereas Inaba strains produce only the A and C antigens. A third serotype, Hikojima, produces all the three antigens but is rare and unstable. Cholera is a potentially epidemic and life-threatening secretory diarrhoea characterized by numerous, voluminous watery stools, often accompanied by vomiting, and resulting in hypovolaemic shock and acidosis. It is caused by certain members of the species *V. cholerae* which can also cause mild or in apparent infections. Other members of the species may occasionally cause isolated outbreaks of milder diarrhoea whereas the vast majority is free living and not associated with disease.

Ingested *Vibrio* spp. from contaminated water or food must pass through the acid stomach before they are able to colonize the upper small intestine. Colonisation is aided by fimbria, filamentous protein structures called toxin coregulated pilus (TCP) extending from the cellwall, that attach to receptors on the mucosa and by the motility of bacterium, which helps to penetrate the mucus overlying the mucosa. Concentrations of *Vibrios* on the mucosal surface rapidly increase to 10^7 to 10^8 per cells. With this high concentration of *Vibrios* closely attached to the mucosa; enterotoxin can be efficiently delivered directly to the mucosal cells.

The cholera enterotoxin (choleragen) a polymeric protein (Mr 84,000) consisting of two major domains or regions, activates the adenylase cyclase enzyme in cells of the intestinal mucosa leading to increased levels of intracellular cAMP, and the secretion of H_2O, Na^+, K^+ and Cl^- into the lumen of the small intestine. The effect is dependent on the specific receptor, monosialosyl ganglioside (GM1 ganglioside) present on the surface of intestinal

mucosal cells. The net effect of the toxin is to cause cAMP to be produced at an abnormally high rate which stimulates mucosal cells to pump large amounts of Cl^- into the intestinal contents. H_2O, Na^+ and other electrolytes follow due to the osmotic and electrical gradients caused by the loss of Cl^-. The lost H_2O and electrolytes in mucosal cells are replaced from the blood. Thus, the toxin damaged cells become pumps for water and electrolytes causing diarrhoea that result in loss of electrolytes, and severe dehydration that are characteristic of cholera (Sathaporn *et al.,* 2002).

After an incubation period of between 18 hours and 5 days, symptoms are generally abrupt and include watery diarrhoea and vomiting. The most distinctive feature of cholera is the painless purging of voluminous stools resembling rice-water. The stools are sometimes described as having fishy odour. The vomits are generally a clear, watery, alkaline fluid. In adults with severe cholera, the rate of diarrhoea may quickly reach 500-1000 ml/hr, leading to severe dehydration. Signs of severe dehydration include absent or low-volume peripheral pulse, undetectable blood pressure; poor skin turgor, sunken eyes, and wrinkled hands and feet. Many patients also show respiratory signs of metabolic acidosis with gasping breathing. Most patients have no urine output until the dehydration is corrected.

Helicobacter pylori are responsible for large number of gastrointestinal disease throughout the world. It 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.

In developing country *C. jejuni* causes disease mostly in infants. It also infects animals, especially chickens and dogs and is spread by contact with their faeces or consumption of contaminated food, milk or water. *C. jejuni* can cause both watery diarrhoea and dysentery.

Clostridium perfingens produces an enterotoxins that is 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 temperature.

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 primarily in the colon, where it induces inflammation and ulceration with formation of a 'pseudomembrane'.

Human infections with *Yersinia enterocolitica* have been reported mainly from northern Europe, Japan and the United States of America. The majority of isolates have been identified from children with sporadic diarrhoea.

At least six different classes of diarrhoea producing *E. coli* have been identified: EPEC, ETEC, EHEC, EIEC and EAEC. Four of them are common causes of diarrhoeal diseases in developing countries. However, identification of these strains requires serological assays, toxicity assays in cell culture, pathogenicity studies in animals and gene-probe techniques that are beyond the capacity of intermediate-level clinical laboratories.

ETEC is an important cause of acute watery diarrhoea in adults and children in developing countries. ETEC does not invade the bowel mucosa and the diarrhoea it causes is toxin-mediated; there are two ETEC toxins-heat-labile (LT) and heat-stable (ST). Some strains produce only one type of toxin, some both. The LT toxin is closely related to cholera toxin. ETEC is spread mostly by means of contaminated food and water.

Intestinal parasitic infections are among the most common infections in the world, being responsible for considerable morbidity and mortality especially among children. It is highly prevalent in developing countries, mainly due to deficiency of sanitary facilities, unsafe human waste disposal system, inadequacy and lack of safe water supply, low socio-economic status and lack of health education. The public health importance of the intestinal parasitosis continues because of its high prevalence, virtually global distribution, and effect

on both nutritional and immune status of individual as well as affects the mental development (Rai *et al.*, 1995).

3.5 Reason for examination feacal specimens for parasites

In district laboratories the examination of faecal specimens for parasites is requested:

1. To identify the parasites that cause of blood and mucus in faeces and differentiate between amoebic dysentery from bacterial dysentery.

Table 2 Difference between amoebic and bacillary dysentery

	Amoebic dysentery	**Bacillary dysentery**
Macroscopic		
Number	6-8 motions per day	Over 10 motions per day
Amount	Relatively copious	Small
Odour	Offensive	Odourless
Colour	Dark red	Bright red
Nature	Blood and mucus mixed with faeces.	Blood and mucus; no faeces
Reaction	Acid	Alkaline
Consistency	Not adherent to the container	Adherent to the bottom of the container
Macroscopic		
R.B.C.	In clumps; reddish-yellow in colour	Discrete, bright red in colour
Pus cells	Scanty	Numerous
Macrophages	Very few	Large and numerous; many of them contain R.B.C.; hence mistaken for *E. histolytic*
Eosinophils	Present	Scarce

Pyknotic bodies	Very common	Nil
Ghost cells	Nil	Numerous
Parasite	Trophozoites of *E.histolytica*	Nil
Bacteria	Many motile bacteria	Nil
Charcot-Leyden crystals	Present	Nil

Source: Chatterjee, 1980 (Parasitology)

2. To identify intestinal parasitic infections that require treatment, i.e. those associated with serious ill health, persistent diarrhoea, weight loss, intestinal malabsorption and impairment of development and nutrition in children.

3. To identify chronic significant infections that if not treated can lead to serious complications developing later in life, e.g. intestinal schistosomiasis leading to portal hypertension, or chronic *Opisthorchis viverini* infection leading to cancer of the bile duct.

4. To detect serious hookworm infection in patients with severe iron deficiency anemia, especially in pregnant woman or child.

5. To assit in the surveillance and control of local parasitic infections caused by geohelminths (soil transmitted nematodes), and helminthes transmitted by the ingestion of infected meat, fresh water fish, crabs or shell-fish (Cheesbrough, 1999).

The intestinal parasites comprise mainly protozoa and helminthes some of them are depicted below:

Protozoal parasites consist of a single “cell like unit” which is morphologically and functionally complete. Protozoa are small ranges from 1.5 μm (microsporidia) to 80 μm

(*Balantidium coli,* a ciliate). Some are intracellular and require multiple isolation and staining methods for identification.

E. histolytica is a parasite that causes amoebic dysentery and liver abscess in man. Lambl in 1859 first discovered the parasite; Losch (1875) proved its pathogenic nature, while Schaudinn (1903) differentiated pathogenic and non-pathogenic types of amoebae. The parasite belongs to class Rhizopodia of family protozoa. It causes an infectious disease known as amoebiasis, which may be non-invasive or invasive, causing colonic ulceration (amoebic dysentery) and confined to the lumen of the colon. Invasive amoebiasis constitutes amoebic liver abscess (ALA), pulmonary amoebiasis, cerebral amoebiasis, cutaneous amoebiasis and splenic amoebiasis, etc. Amoebiasis has worldwide distribution. The prevalence rates are highest in areas of crowding and poor sanitation in tropics and sub-tropics than in temperate zone.

The amoeba occurs in three stages: trophozoites, precyst and cyst. Trophozoites are motile and are feeding and growing phase. They are pleomorphic and size ranges from 23-30µm. The cytoplasm is differentiated into clear transparent ectoplasm and granular endoplasm. Nucleus is spherical and size varies from 4 to 6 µm; numerous vacuoles and often RBC's and occasionally WBC's are present in the endoplasm. Trophozoites are actively motile with ectoplasm behaving as pseudopodia. Precystic stage is stage between trophozoites and cyst, and their size varies from 10-20µm. Cysts are the infective stage and their size is 6-15µm. They are quadrinucleated and the cytoplasm is clear, hyaline and free of all nutritional inclusions. They are surrounded by a thick wall that provides resistance to gastric juice, bactericidal concentration of chlorine in water and other adverse conditions.

The life cycle of *E. histolytica* is simple and is completed in a single host, the man. Man acquires infection by ingestion of water and food contaminated with mature quadrinucleate cysts**.** Man can also acquire the infection directly by ano-genital or oro-genital sexual contact. On ingestion, the cysts were excyst in the small intestine. The amoebae inside the host become active in the neutral or alkaline environment of the small intestine. The cyst

wall is lysed by intestinal trypsin liberating a single trophozoite with four nuclei (excystation). The trophozoite quickly undergoes a series of cytoplasmic and nuclear divisions to form eight small metacystic trophozoites. These trophozoites are carried by persistalsis through the small intestine to the ileo-caecal area of the large intestine. Here they grow and multiply by binary fission. They colonize on the mucosal surfaces and in crypts of the large intestine.

In the amoebic dysentery, trophozoites invade the colonic epithelium and lyses host cells causing necrosis areas of mucosa are excavated, leaving multiple flasks shaped ulcers. Though the exact molecular mechanism of intestinal inflammation is unknown, the enterotoxin isolated from axenically cultivated *E. histolytica* may play an important role in the production of inflammation and leading to cause diarrhoea. During growth, the parasite secretes a proteolytic ferment of histolysin which brings about destruction and necrosis of tissues and thereby helps the parasite obtain nourishment. Through absorption of these dissolved tissue juices (Chatterjee, 1980).

The incubation period may vary from few days to months, depending upon the area of endemicity. Normally, the time ranges from 1 to 4 months. *E. histolytica*, besides being an intestinal pathogen, it is able to invade tissues and the presentation of disease may range from an asymptomatic infection to a disseminated fatal disease. The four major intestinal syndromes caused by the infection are asymptomatic colonization; acute amoebic colitis, which usually presents with lower abdominal pain, frequent bloody stools over a period of several weeks, and fever; fulminant colitis, which occurs most often in children who present with diffuse abdominal pain, profuse bloody diarrhoea, and fever, which presents as a completely asymptomatic lesion or as a tender mass accompanied by symptomatic dysentery (Reed, 1992).

G. lamblia is a well-established cause of diarrhoea and is one of the most frequent intestinal pathogens in both developing and developed countries. *G. lamblia* is found worldwide, particularly in temperate and tropical location and where sanitary conditions are

suboptimal. *G. lamblia* was first of all observed by Leeuwenhoek in 1681, while examining his own stool. Lambel described the species in the year 1859 and named it as *G. lamblia*. The flagellate inhabits the jejunum and duodenum of man. It is the causative agent of a 'Giardiasis' and is the most common parasite.

The life cycle of *G. lamblia* posses two distinct forms: trophozoites (feeding and growing) and cyst (resting and infective form). Trophozoites are pear-shaped with a broad rounded anterior end and tapering posterior end. They measure 14 µm in length and 7 µm in breadth. Dorsal surface is convex while ventral surface is concave with a sucking device, the organ of attachment. Cysts are infective stage and are oval or ellipsoidal in shape with 12µm length and 7 µm in breadth. They have thick wall, four nuclei are present in pairs at two opposite ends and consists of an axostyles and margins of sucking disc.

G. lamblia may remain attached to intestinal mucosa and rarely invades the submucosa and brings abnormalities related to the function and morphology of upper small intestine. As few as 10-25 cysts cause giardiasis. Malabsorption of fat and carbohydrates in children and diarrhoea are the important clinical manifestations. The precise mechanisms for this change are as follows:

1. Mechanical blockage of the intestinal mucosa by large numbers of trophozoites.
2. Competition of essential nutrients.
3. Deficiencies of secretory IgA antibody in the intestine.
4. Inflammation of intestinal mucosa.

Bacterial (e.g. *Enterococcus*) induced decongugation of bile and altered jejunal motility with or without overgrowth of intestinal bacteria and yeast. The attachment of *Giardia* to the duodenal mucosa may also be facilitated by a lectin produced by the parasites and activated by duodenal secretions.

The incubation period varies from 1-3 week and majority of infected persons in the endemic area are asymptomatic. The acute giardiasis is characterized by the sudden unset of anorexia, nausea abdominal distension, discomfort, diarrhoea, etc. Steatorrhoea is frequently accompanied by epigastric and abdominal cramp. Stool is voluminous; foul smelling and greasy in appearance, typically no mucus or blood is present in faeces. The acute phase, which lasts 3 or 4 days, can resemble other causes of traveller's diarrhoea and is often not recognized as being due to giradiasis (Farthing and Keush, 1989).

C. cayetanensis is a coccidian protozoan parasite that causes prolonged diarrhoea in human's worldwide (Ortega *et al.*, 1993). It was first reported from Papua New Guinea (Ashford, 1979) and the infection due to a coccidian parasite *C. cayetanensis* is referred to as 'Cyclosporiasis'. Organisms that resemble *Cyclospora* protozoa have been discovered in human stool samples around the world and have been isolated from children, immunocompetent adults, and human immunodeficiency virus (HIV) seropositive individuals (Wurtz, 1994).

C. cayetanesis resembles blue-green algae or cyanobacteria like body; is non-refractile, double-shelled spheres, 8-10 µm in diameter. The oocyst has two sporocysts, each with two sporozoites. The organism can be easily identified in the wet mount preparation by a microscopic familiar with the organism. The organism floats in sheather sucrose solution and on modified acid-fast stain, the oocysts appear as round, irregularly stained bodies; they often contain red staining granules, some oocysts may appear as unstained glassy wrinkled spheres.

The reaction of the organisms with the modified acid-fast stain is highly variable: some organisms stain dark red and have a variable number of dark inclusion bodies, whereas others do not stain at all and appear as transparent spheres. In addition, the organisms show fairly strong green auto fluorescence when observed under ultraviolet epifluorescence (Ortega *et al.*, 1993). The *Cyclospora* is resistant to free chlorine.

Humans ingest sporulated oocysts (the infectious stage) of *C. cayetanensis*, which only infects humans. The oocyst excysts in the small intestine, usually in the jejunum, and invades the intestinal epithelial cells. The next process is schizogony, which begins with the formation of a trophozoite that grows into a mature schizont that contains 8-12 merozoites, which are then released, presumably by cell rupture, to invade other epithelial cells and repeat the process. These merozoites are called type I meronts, which are asexual forms. After several cycles of type I schizogony, type II meronts (sexual forms) develop, with each cell containing 4 merozoites. After invading epithelial cells, some of these form single macrogametes and others divide multiple times to form microgametes. When released, a microgamete fertilizes a macrogamete, which develops into a zygote. The zygote, in turn, develops into an oocyst with an environmentally resistant wall. The oocyst passes into the environment in the feces, as a nonsporulated noninfectious oocyst.

Consequently, human-to-human transmission does not occur. During infection, best evidence suggests that oocysts are continuously excreted. In the environment, the oocyst sporulates, becoming infectious for humans. During sporulation, the sporont divides into 2 sporocysts, each containing 2 sporozoites. Time course in the environment is days to weeks. In culture, 10-20% of sporonts have completed the process in 5 days. In other experimental studies, sporulation at ambient temperature occurs in 7-12 days. The preferred temperature is 78.8-86°F (26-30°C). Contamination of food or drinking water leads to human ingestion and infection. The infectious inoculums is small but has not been precisely quantitated.

Cyclosporiosis is the disease associated with diarrhoea and the exact mechanism of pathogenesis is not understood. However, the study carried out by Connor *et al*., (1993) in Nepal shows: the organism is adherent to the intestinal mucosa and there is marked erythema of the distal duodenum, epithelial disarray with acute or chronic inflammation, partial villous atrophy, and crypt hyperplasia.

Food and waterborne coccidia including *C. parvum*, *C. cayetanensis, Sarcocystis hominis* and *Sarcocystis suihominis,* and *Isospora belli* are cyst-forming apicomplexan protozoa

that cause intracellular infections, predominantly in the epithelial cells of the intestine. They are transmitted by oocysts from person-to-person by the faeco-oral route or via contaminated water or food. The most common symptom of infection is diarrhoea, however, asymptomatic infections occur. Infections are associated with intestinal inflammation, with pathological lesions such as villus blunting, and abnormal function such as malabsorption. Mild to moderate, self-limiting diarrhoea is common in healthy individuals ingesting infective stages of these organisms. However, patients with immune dysfunction can have severe intestinal injury and prolonged diarrhoea (Mansfield *et al.*, 2004).

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The *Cyclospora* infection produces a characteristic illness that produces clinical symptoms such as gastroenteritis associated with fever, vomiting, and frequent watery diarrhoea. This acute phase is followed by severe fatigue, anorexia, and intermittent diarrhoea and nausea ensues; and symptoms can be consistent from day to day. Other symptoms like abdominal discomfort, tenesmus, constipation flatulence weight loss, myalgia etc are also common.

Cryptosporidium paravum is a coccidian parasite that causes disease in infants, immunodeficient patients, and a variety of domestic animals. In developing countries infection is frequent and most episodes of illness occur in the first year of life. Thereafter, infections are usually asymptotic. Diarrhoea is usually neither severe nor prolonged, except in immunodeficient patients such as those with severe malnutrition or AIDS. In such individuals, *C. paravum* is an important cause of persistent diarrhoea with wasting.

The Helminths are worm like parasites. These are multicellular and bilaterally symmetrical elongated, flat or round bodies. Nematodes are unsegmented worms belonging to phylum Aschelminthes. They have separated sex; males are slightly smaller than females. The parasitic nematodes are generally a light cream white colour but the female may appear darker with coloured eggs.

A. lumbricoides, the common round worm, is the most common intestinal nematode of the human (Parija, 2004). It is worldwide in distribution. Approximately 20% of world's population is infected by this parasite with a very high prevalence (90%) in certain tropical and subtropical areas.

It is the largest intestinal nematode parasite of the human. Freshly passed adults worms in the stool are pink in color and look like earth worm with large and cylindrical tapering ends. Adult males are relatively smaller than (15 cm to 30cm in length and 3mm to 4mm in diameter) than the females of 20 cm to 40 cm in length and 2mm to 6 mm in diameter. Adult worms live in the lumen of the small intestine. A female may produce approximately 200000 eggs/day, which are passed with the faeces. Embryonated eggs are infective to humans. Both fertilized and unfertilized eggs are present in the faeces.

The life cycle of *A. lumbricoides* is completed in a single host man. Man is the only host; no intermediate host is required in the life cycle. Man acquired infection by ingestion of food, water or raw vegetables contaminated with embryonated eggs of the round worm. The ingested eggs hatch in the small intestine (duodenum) to liberate the larvae. These larvae then penetrates the intestinal wall, enter lymphatics and venoules. The larvae are carried by the portal circulation to the liver, where they live for 3 to 4 days. They are then carried to right heart, then to the lung. Where they moults twice and becomes fourth stage larva. The larvae then break out of the pulmonary capillaries and reach alveoli. From the alveoli, they ascend up the respiratory tract and are swallowed back to the small intestine.

An initial pathological lesion in ascariasis is associated with migrating larvae. The severity of lesions depend upon the

1. Sensitivity of the host.
2. Nutritional status of the host, and
3. Number of the migrating larvae.

The migrating larvae, in the persons repeatedly infected with *Ascaris* and sensitized to the parasite antigens; produce inflammatory and hypersensitive reactions in the lung and liver. The reaction is associated with the formation of granuloma and eosinophilic infiltrates. This leads to pneumonis and a condition known as loefflers syndrome.

It is the most important clinical manifestations of ascariasis. The severity of intestinal disease depends upon the worm load of the intestine and nutritional status of the host. *A. lumbricoides* infection caused by few worms is usually asymptomatic. Increased numbers of worms produces symptoms of abdominal pain, nausea, vomiting, fever, weight loss, and diarrhoea. This manifestation is seen during initial stages of the infection and is caused by the migrating larvae in the lung tissue. During the lung phase of larval migration, pulmonary symptoms can occur (cough, dyspnea, hemoptysis, eosinophilic pneumonitis-Loeffler's syndrome) (Jaime *et al.*, 1999).

Laboratory diagnosis is mainly achieved by parasite detection (mainly adult worm and eggs) by a direct microscopical examination of a saline emulsion of the stool. Concentration by floatation method may be employed for the detection of eggs in the stool. It is also to be noted that unfertilized eggs do not float in salt solutions. The drugs of choice for treatment of ascariasis are albendazole, mebendazole, and pyrantel pamoate (Cheesebrough, 2000).

T. trichiura, also called the whipworm that causes trichinosis in humans, an intestinal infection, caused by invasion of mucosa of the colon by the adult worm. Linnaeus (1771) first of all observed the parasite and Stiles in 1901 described the pathogenesis. It is commonly called whipworm and has worldwide distribution with approximately 10% of infection, especially in tropical areas where sanitation is poor and children with poor hand washing practices (Lama, 2006).

Males are relatively smaller with coiled posterior end. The anterior end is thin and long (two third of body length), whereas the posterior end is thick and stout. Mouth is simply

opening and does not contain lips. One fertilized female worm lays 1000-7000 eggs per day. Eggs are barrel shaped, golden brown in color and measures 5×25 µm in size. Embryonation takes place in the environment. Life cycle begins with the ingestion of the embryonated eggs containing first stage larvae present in contaminated food or water. The larvae hatch out in the small intestine, penetrate the villi and start to develop. Young worms develop after 7 days and migrate from the small intestine to the caecum; they develop into sexually mature male and female adults. After fertilization, the female worms start to lay eggs after about 3 months of infection. These unembryonated eggs are passed in the faeces. In damp and warm soil, eggs are embryonated with rhabditiform larvae.

Most infections are asymptomatic, but heavy infections causes abdominal discomfort, anemia, bloody diarrhoea, rectum prolapse, and appendicitis. Diagnosis is established by laboratory investigation of the stool specimen. Characteristic barrel-shaped eggs are found in the stool and are very easily recognized. In heavy infection, the stool is often mucoid and contains Charcot-Leyden crystals. The adult worms may be demonstrated in the rectal mucosa in heavy infection.

Infected men are the reservoir and contaminated soil, food and water are the chief sources of infection. Children are more susceptible than adults due to their habit of playing with soil contaminated with embryonated eggs. Mebendazole is the drug of choice. It is effective in reducing the egg load in the host by 90% and offers a cure rate of 60% after treatment.

Hookworm infection is major disease in many countries of the developing world and is an important cause of anaemia in endemic areas. It is also known as ‘Old World Hookworm’ which is common hookworm of man. The hookworm causes ancyclostomiasis in humans, which is characterized by iron-defidiency anemia and hypoalbuminaemia. Two important species of hookworm that infect human are *A. duodenale* (the old world hookworm) and *Necator americanus* (the new world hookworm). An Italian physician Angelo Dubini *discovered A. duodenale in* 1838 while Stiles discovered *N. americanus* in 1903. The hookworm infection is common in tropical farming areas of Africa, Asia, South America,

and Caribbean countries in association with poor sewage disposal, which allows the eggs to remain and hatch in the soil.

The worm is about 9 mm long and female *Ancyclostoma* produces 30000 eggs per day and *Necator* produces 9000 eggs per day. Both the organism's posses similar life cycle and their eggs are almost identical and these are not differentiated. The eggs are the diagnostic form and are passed in the stools. Eggs are oval or elliptical in shape having 60×40 µm in size, colourless and surrounded by a transparent hyaline shell membrane.

Hookworm transmission occurs by skin contact with infective third-stage larvae (L3) that have the ability to penetrate through the skin, frequently entering the body through the hands, feet, arms, or legs. *A. duodenale* L3 also can be ingested. L3 migrate through the body and enter the lungs from which they are expelled by cough and swallowed into the intestine where they first moult twice to become adults. Adult hookworms are approximately 1 cm long parasites that cause host injury by attaching to the mucosa and sub mucosa of the small intestine and producing intestinal blood loss. The presence of between 40 and 160 adult hookworms in the human intestine results in blood loss sufficient to cause anaemia and malnutrition.

The hookworm pathogenesis may be considered either produced by larvae while entering the skin of the host and during migration through the lungs or produced by adult worm in the intestine. The pathogenic effects due to larvae are *Ancyclostoma dermatitis*, creeping eruptions and lesions in the lungs.

The clinical symptoms are associated with the area of infection and includes cutaneous phase known as ‘‘ground itch" a local dermatitis at the site of infection. The pulmonary phase may be characterized by pharyngitis, cough, and production of bloody sputum. The intestinal stage involves causing diarrhoea, nausea and vomiting.

Hookworm infection is common in rural areas where there is poor sewage disposal system and human faeces are used as fertilizers. Infection occurs through skin and the prerequisite of wide spread hookworm infection in a population of significant parts is: direct defecation into the soil and those persons who not necessarily wear shoes at least during part of year. Primarily to tropics and subtropics, the ambient temperature, sufficient rainfall and sandy loose soil are major factors in disseminating the parasite.

H. nana, a cestode is widely distributed in countries with warm climates including those of South America, Mediterranean region, Africa, and South East Asia. Children are more commonly affected than adults. It is commonly called dwarf tapeworm. *H. nana* is one of the smallest intestinal cestode infecting man. The worm is small and thread like, measuring 1 to 4 cm in length with a maximum diameter of 1 mm. The worm may be present in large numbers (1000-8000). Life span is very short (about 2 weeks). The scolex is globular, has 4 suckers and is provided with a short retractile rostellum armed with single row of hooklets numbering 20 to30. The neck is long.

Eggs are liberated in the faeces and the eggs are spherical in shape, measuring 30 to 45 µm in diameter. There are two distinct membranes and the egg contains the oncosphere or embryo. No intermediate host is required and the entire development from larval to the adult stage takes place in one host. The first infection is acquired through the ingestion of food or water contaminated with eggs of *H. nana* liberated along with the faeces of an infected man or rodents. Afterwards, autoinfection increases the number of parasites. The infection is diagnosed by finding the characteristic eggs in a microscopical examination of a stool sample.

The genus rotavirus is recognised as the most important cause of infantile diarrhoea thorought the world. Rotaviruses are major cause of diarrhoeal disease in human and also cause diarrhoea in young of a wide variety of mammals and birds including calves, mice, piglets, foals, rabbits, dogs, deers, lambs, monkey, and sheep. Rotavirus infections are

usually mild to moderately severe in developed countries but can become very severe and cause high mortality in developing countries**.**

Rotavirus infection can cause gastroenteritis. It most often infects infants and young children, and in children ages 3 months to 2 years, rotavirus is one of the most common causes of diarrhoea. In the United States, it leads to outbreaks of diarrhoea during the winter months and is particularly a problem in child-care centers and children's hospitals. Almost all children have had a rotavirus infection by the time they are 3 years old**.**

Morphologically, rotaviruses are polyhedrons of 75 nm diameter displaying characteristic sharp-edged double-shelled capsid, which in electron micrographs look like spokes grouped around the hub of wheel (Latin, rota). The name rotavirus was derived from this appearance, which is pathognomonic. More detailed structural studies have shown that the double-shelled capsid is penetrated by a large number of channels and that it carries on the surface 60 protrusions that consist of dimmers of VP4 (viral protein 4) molecules. Rotaviruses have a genome of 11 segments of double stranded RNA which can be easily separated by polyacrylamide gel electrophoresis. The RNA segments code for 6 structural (VP1, VP2, VP3, VP4, VP6 and VP7) and 5 non structural protein (NSP1- NSP5). The structural proteins are located in the core (VP1- VP3), inner shell (VP6) and outer shell (VP4, VP7) (Desselberger, 1998).

Rotaviruses infect cells in the villi of the small intestine (gastric and colonic mucosa are spared). They multiply in the cytoplasm of enterocytes and damage their transport mechanisms. One of the rotavirus-encoded proteins, NSP4, is a viral enterotoxin and induces secretion by triggering a signal transduction pathway. Damaged cells may slough into the lumen of the intestine and release large numbers of virus, which appear in the stool (up to 10^{10} particles per gram of feaces). Viral excretions usually lasts 2-12 days in otherwise healthy patients but may be prolonged in those with poor nutrition. Diarrhoea caused by rotavirus may be due to impaired sodium and glucose absorption as damaged

cells on villi are replaced by nonabsorbing immature crypt cells. It may take 3-8 weeks for normal function to be stored (Jawetz *et al.*, 1980).

The viral factors determining pathogenicity of rotaviruses have investigated as the product of RNA segment 4, VP4, but products of other structural gene (RNA 3 coding for VP3 and RNAs 8 and 9 coding for VP7) and non structural genes (RNA 5 coding for NSP4, RNA 8 coding for NSP2 and RNA 10 coding for NSP4) have also been associated with pathogenicity.

Rotaviruses cause acute gastroenteritis. Infantile diarrhoea, winter diarrhoea, acute nonbacterial infectious gastroenteritis, and acute viral gastroenteritis are names applied to the infection caused by the most common and widespread group A rotavirus. The onset of symptoms is abrupt after a short incubation period of 1-2 days. Diarrhoea and vomiting are seen in the majority of infected children and lasts for 2-6 days. Although symptoms of respiratory tract infection are frequently observed at the time of rotavirus infection, there is no evidence that rotaviruses replicate in the respiratory tract. Clinical symptoms can range from mild to very severe, in part depending upon the rotavirus strain. It has been estimated that about half of all gastro-enteritis cases in children are caused by rotaviruses. Infection has been detected in older children and adults, but is usually asymptomatic. Rotavirus infection can be life-threatening if children are already malnourished.

The primary mode of transmission is fecal-oral, although some have reported low titers of virus in respiratory tract secretions and other body fluids. Because the virus is stable in the environment, transmission can occur through ingestion of contaminated water or food and contact with contaminated surfaces. In the United States and other countries with a temperate climate, the disease has a winter seasonal pattern, with annual epidemics occurring from November to April. The highest rates of illness occur among infants and young children, and most children in the United States are infected by 2 years of age. Adults can also be infected, though disease tends to be mild.

Outbreaks of group A rotavirus diarrhoea are common among hospitalized infants, young children attending day care centers, and elder persons in nursing homes. Among adults, multiple foods served in banquets were implicated in 2 outbreaks. An outbreak due to contaminated municipal water occurred.

Several large outbreaks of group B rotavirus involving millions of persons as a result of sewage contamination of drinking water supplies have occurred in China since 1982. Although to date outbreaks caused by group B rotavirus have been confined to mainland China, seroepidemiological surveys have indicated lack of immunity to this group of virus in the U.S. The newly recognized group C rotavirus has been implicated in rare and isolated cases of gastroenteritis. However, it was associated with three outbreaks among school children: one in Japan, 1989, and two in England, 1990.

At the peak of the disease as many as 10^{11} virus particles per millilitre of faeces are present. Therefore, the diagnosis is not difficult. Agglutination test using latex particles coated with rotavirus-specific antibody and different forms of Enzyme-Linked Immunosorbent Assay (ELISA) are most commonly used. Electron microscopy, when routinely applied the diagnosis of viral diarrhoea in infants and young children, will easily detect the characteristic virus particles. Recently, reverse transcription polymerase chain reaction (RTPCR) with gene and type-specific primers has been widely applied as a very reliable typing procedure.

In the majority of cases there are sufficient numbers of virions in faeces to allow identification of RNA profiles by PAGE. Although they are fastidious, rotaviruses can be propagated in secondary or continuous cultures of monkey kidney cells. To ensure success it is necessary to incorporate trypsin in the culture medium. Cell culture, however, is not used for routine diagnosis. Specific diagnosis of the disease is made by identification of the virus in the patient's stool. Enzyme immunoassay (EIA) is the test most widely used to screen clinical specimens, and several commercial kits are available for group A rotavirus. Electron microscopy and polyacrylamide gel electrophoresis are used in some laboratories

in addition or as an alternative to EIA. A reverse transcription-polymerase chain reaction has been developed to detect and identify all three groups of human rotaviruses.

Oral rehydration therapy (drinking enough fluids to replace those lost through bowel movements and vomiting) is the primary aim of the treatment. For persons with healthy immune systems, rotavirus gastroenteritis is a self-limited illness, lasting for only a few days. If the diarrhoea becomes severe, it may be necessary to hospitalize the patient so that fluids can be administered intravenously. Some important preventive measures are as follows:

1. An oral, live, tetravalent, rhesus-based rotavirus vaccine (RRT-TV) for infants is available commercially. This vaccine should be administered to infants between the age of 6 weeks and 1 year. The recommended schedule is a 3-dose series, with doses to be administered at ages 2, 4 and 6 months. The first dose may be a minimum interval of 3 weeks to 6 months; subsequent doses should be administered with a minimum interval of 3 weeks between any two doses.

2. The effectiveness of other preventive measures is undetermined. Hygienic measures applicable to disease transmitted via the feaco-oral route may not be effective in preventing transmission.

3. In day care, dressing infants with overall to cover diapers has been demonstrated to decrease transmission of the infection.

4. Preventive exposure of infants and young children to individuals with acute gastroenteritis in family and institutional (day care or hospital) settings by a high level of sanitary practices.

5. Passive immunization by oral administration of IG has been shown to protect low-birth weight neonates and immunocompromised children (Control of communicable diseases manual, 2003).

3.6 Global scenario of intestinal infections

An analysis carried out by WHO in 1998 of data from surveys and other sources indicates that over 1.3 thousand million episodes of diarrhoea occurs each year in children under 5 years of age in Asia (excluding China), Africa and Latin America. Four million children in above age group die annually from diarrhoea, 80 % of these deaths occur in the age of first two years of life (WHO, 1990). The incidence of EHEC infections varies by age group, with the highest incidence of reported cases occurring in children aged under 15 years (0.7 cases per 100 000 in the United States). The 63 to 85% of cases are a result of exposure to the pathogen through food. The percentage of EHEC infections which progress to HUS varies between sporadic cases (3%-7%) and those associated with outbreaks (20% or more). In epidemiological terms, there is generally a background of sporadic cases, with occasional outbreaks. Some of these outbreaks have involved a high number of cases, such as in Japan in 1996, where an outbreak linked to contaminated radish sprouts in school lunches caused 9, 451 cases. Data on the situation in developing countries are limited, as surveillance for this pathogen is not done routinely (WHO, 2005). *Escherichia coli* O157:H7 is a leading cause of food borne illness. Based on a 1999 estimate, 73,000 cases of infection and 61 deaths occur in the United States each year. There are an estimated 79,420 cases of ETEC in the United States each year. A total of 24 diarrhoeagenic *E. coli* strains were isolated from 79 stool samples from children with diarrhoea and from 60 samples from children without diarrhoea. The prevalence of diarrhoeagenic *E. coli* in both groups was significantly different (P=0.003) (Kordidarian *et al.*, 2007).

Shigellosis is endemic in most developing countries and is the most important cause of bloody diarrhoea worldwide. It is estimated to cause at least 80 million cases of bloody diarrhoea and 700,000 deaths each year. Ninety-nine percent of infections caused by *Shigella* occur in developing countries, and the majority of cases (~70%), and of deaths (~60%), occur among children less than five years of age. Probably less than 1% of cases are treated in hospital (WHO, 2005). In 1994, an explosive outbreak among Rwandan refugees in Zaire caused approximately 20,000 deaths during the first month alone. Between 1999 and 2003, outbreaks were reported in Sierra Leone, Liberia, Guinea,

Senegal, Angola, the Central African Republic and the Democratic Republic of Congo. In Central America, the most recent large epidemic lasted from 1969 to 1973 and was responsible for more than 500,000 cases and 20,000 deaths.

V. cholerae is the infectious agent responsible for cholera. Only *V. cholerae* serogroup O1 and serogroup O139 are known to cause epidemics of cholera. During 2001, 58 countries officially notified WHO of a total of 184 311 cases (one third more than in 2000) and 2 728 deaths. The reported overall case-fatality rate (CFR) has dropped to 1.48% with regards to the 3.6% reported in 2000. This absolute decline in CFR reflects contrasting realities, as CFR for South Africa is very low (0.22%) whereas rates of up to 30% have been observed in other parts of Africa. With a total of 173 359 cases, Africa accounted for 94% of the global total. Asia reported a total of 10 340 cases, which represents a small decrease compared to 2000. However, globally, the actual figures are likely to be higher, owing to the under-reporting and other limitations of surveillance systems. The year 2001 was marked by major outbreaks of cholera in several African sub regions.

The enteric protozoan *E. histolytica* causes an estimated 50 million cases of amoebic colitis and liver abscess in human; resulting in 100,000 deaths annually (Tachibana *et al.,* 2007). Ameoebiasis has a worldwide in distribution and is a major health problem. More than 10% of the world's population estimated to be infected every year and 80-90 % of these patients have symptoms related to the intestinal mucosa and in the remaining 2 -20 %, the amoebae invade beyond the intestinal mucosa (WHO, 2003).

The prevalence of *G. lamblia* in stool specimens ranges from 2–5% in industrialized countries to 20–60% in developing countries (Bilenk *et al.,* 2004). The infection occur world wide an incidence varying from 1.5-2.5 %. Giardiasis shows two distinct epidemiology patterns i.e. endemic and epidemic with a high prevalence rate 15-20 % occurring in children less than 10 years of age in developing countries while all age are equally susceptible in developed world.

C. cayetanensis has emerged as an important cause of acute chronic gastroenteritis worldwide (Sherchand *et al.,* 1996). From 1990-2000, there were 11 food borne outbreaks of cyclosporosis in North America that affected at least 3600 people (Mansfield and Gajadhar, 2004). Where as studies conducted in Guatemala and Peru suggested that having certain animals (e.g. Guatemala, Peru, Indonesia and Nepal). However, the seasonality is not uniform among these countries and defies easy explanation (Lopez *et al.,* 2003). In 1993, in Lima, Peru, Ortega *et al.,* characterized and clarified remaining taxonomic issues for *C. cayetanensis*. Also in 1993, a prospective study of 1042 stool specimens in patients with diarrhoea at the Lahey Clinic in Massachusetts yielded 3 patients with *Cyclospora* infection. In the late spring and early summer of 1996, an outbreak affecting approximately 1450 individuals (70% laboratory confirmed) was described in Canada and the United States. Since then, numerous reports have documented its endemicity in 27 countries around the world.

C. cayetanensis has been reported as endemic in at least 27 countries, mostly tropical.

1. It has been reported as a cause of traveler's diarrhoea after international travel by at least 11 countries.

2. It has been reported as a cause of food borne diarrhoea in outbreaks secondary to food imported to Canada.

3. It has been reported as a cause of an outbreak in Mexico secondary to watercress (within the country).

The distribution of worm burdens among different human hosts is highly over dispersed so that often only 10% of the infected population carries 70% of the worms. Because most helminthes do not replicate in humans, the rate of morbidity from infections with helminthes is typically highest among patients with the heaviest worm burdens. Unlike other soil-transmitted helminthes infections, such as ascariasis and trichuriasis, in which the highest intensity infections occur primarily in school-aged children, high intensity hookworm infections also frequently occur in adult populations. This is an important health

threat to adolescent girls, women of reproductive age, and to outcomes in pregnancy. Up to 44 million pregnant women are estimated to be infected with hookworm. In pregnant women, anemia resulting from hookworm disease results in several adverse outcomes for both the mother and her infant, including low birth weight, impaired milk production, and increased risk of death for both to the mother and child. The greatest number of hookworm cases occurs in Asia, followed by sub-Saharan Africa. In China alone, approximately 190 million people are infected, an estimate based on a nation wide study involving the examination of fecal specimens obtained from almost 1.5 million people between 1988 and 1992.(Peter, 1989)

The prevalence of viral, bacterial and parasitic pathogens among children of Jeddah, Saudi Arabia, was investigated. During December 1995-October 1996, 576 faecal samples were collected from children (0-5 year(s) old) suffering from acute diarrhoea and attending hospitals and outpatient clinics in Jeddah. One or more enteropathogen(s) were identified in 45.6% of the stool specimens. Mixed infections were detected in 12.2% of the diarrhoeal cases. Rotavirus was detected in 34.6% of the specimens of the hospitalized patients and in 5.9% of the specimens of the outpatients. Fifty-one percent of the rotavirus-positive specimens were long electropherotype, 26% were short electropherotype, and 23% could not be electropherotyped specifically. Among those of the long electropherotype, there were six patterns; and of the short electropherotypes, there were four patterns. Serotyping of these specimens revealed a distribution of 39.6%, 4.2%, 6.3%, and 15.6% for rotavirus serotype 1, 2, 3, and 4 respectively. Mixed serotypes were found in 3.1%, and 31.3% of the specimens were untypeable. Other aetiologic agents recognized included *E. coli* (13%), of which 3.8% were EPEC and 1.9% EHEC. Among the *E. coli* (EPEC) serotypes, O111:K58:B4, O55:K59:B11, and 0127:K63:B8 were found in 31.8%, 18.2%, and 13.6% of the cases respectively. Serotype 026:K60:B6, 0124:K72:B17 and 0112:K66:B11 each was found in 9.1% of the EPEC cases. 0128:K67:B12 and 0125:K70:B13 each was found in one case only. Other detected pathogens were: *Klebsiella pneumoniae* (4%), *G. lamblia* (3.1%), *Salmonella* spp. (3%), *S. flexneri* (2.6%), *E. histolytica* (2.2%), *T. trichiura, H. nana*, and *A. lumbricoides* (0.7% each), and *Candida albicans* (0.5%). Based on the results

of this study, it is concluded that the high prevalence of the various enteropathogens among young children is a significant public health problem (Sheikh and Assouli, 2001).

Rotavirus infections occur worldwide. Globally, each year, 2 million children are hospitalized and 700,000 children die due to rotavirus diarrhoea (Uchida *et al.,* 2006). Most symptomatic infections are seen in children under 2 years of age; by the age of 3 years, more than 90% of children have been infected by most of the major serotypes. Five million children under the age of 2 years die from diarrhoeal disease in developing countries each year; rotavirus infections account for about 20% of these deaths (Desselberger, 2003). About 120 million rotavirus infections occur every year, causing the death of 600 000 to 650 000 children (Wikipedia). Each year, rotavirus cause approximately 111 million episodes of gastroenteritis only home care, 25 million clinic visits, 2 million hospitalizations, and 352,000-592,000 deaths (median 440,000 deaths) in children <5 yrs of age. By age 5, nearly every child will have episodes of rotavirus gastroenteritis, 1 in 5 will visit a clinic, 1 in 65 will be hospitalized, and approximately 1 in 293 will die. Children in the poorest countries account for 82% of rotavirus deaths where resources are scarce and health systems are inadequate. The tremendous incidence of rotavirus disease underscores the urgent need for intravenous such as vaccines, particularly to prevalent childhood deaths in developing countries (Parashir *et al.,* 2003 and PAHO *et al.,* 2004).

A total of 271 stool specimens were collected from children (diarrheagenic, n = 115 and control, n = 54) and adults (diarrheagenic, n = 73 and control, n = 29) from Tunis, Tunisia, and processed to detect bacterial enteropathogens, parasites, and viruses. Diarrheagenic *E. coli* (DEC) were identified by their virulence genes (polymerase chain reaction) and adherence patterns (tissue culture assays). The most frequently isolated enteric pathogens from diarrheagenic children were ETEC (32.3%), EAEC (11.3%), EIEC (11.3%), adenovirus (10.4%), EHEC (10.4%), and *Salmonella* spp. (9.5%). For children in the control group, ETEC (37%), EAEC (15%), EHEC (11.1%), and typical EPEC (11.1%) were the most common enteric pathogens. In adults in the diarrheagenic group, *Salmonella* spp. (34.2%), ETEC (12.3%), adenovirus (7%), and *Shigella* spp. (4%) were the most

common enteric pathogens. In adults in the control group, ETEC (31%) was the most common enteric pathogen. Multiple pathogens were recovered from 22% of the diarrheagenic children and 7% of the diarrheagenic adults. Escherichia coli strains showed high resistance rates to tetracycline, streptomycin, and ß-lactams. The most frequent combinations were ETEC-rotavirus and ETEC-adenovirus. Pulsed-field gel electrophoresis for DEC indicated a large number of DEC clones (five major clones) persistent in the community reservoir for a considerable period of time that caused diarrhea in the population. This suggests the confluence of small epidemics by clonally related DEC strains circulating in this region (Nazek *et al*., 2007).

Enteropathogens and clinical features associated with diarrhoea were investigated in 1526 children admitted over a 5-year period to the paediatric ward of a hospital in the highlands of Papua New Guinea. Overall, a recognized pathogen was isolated from 39 per cent of the children admitted with diarrhoea. The most commonly isolated agents were rotavirus (23 per cent), *Shigella* spp. (13 per cent), *Campylobacter* spp. (12 per cent), *C. parvum* (10 per cent) and EPEC (8 per cent). The clearest clinical associations were rotavirus with vomiting and *Shigella* with blood and pus in the stool. A control series of children admitted with other complaints was also included, and the odds ratios for diarrhoea for the above five pathogens were 18.2, 9.6, 3.7, 2.2, and 1.6, respectively (Howard *et al*., 2000).

The incidence of nosocomial infection due to rotavirus was studied in 80 children aged 3-24 months from November 2003 to April 2004 in the Alzahra Hospital, Isfahan, Iran. Rotavirus antigen was detected by latex aggulutation in stool samples obtained during hospitalization and up to 72 hours after discharge from the hospital. The prevalence of nosocomial infection due to rotavirus was 26.25%, which is a considerable prevalence compared to similar studies which reported a prevalence of 27.7%, 19.4%, and 14.6%. Overall, 15% of the 21 children with positive rotavirus antigen in their stools had acute diarrhoea during hospitalization and up to 72 hours after discharge (symptomatic nosocomial infection), and 11.25% of all children (n=80) studied had asymptomatic nosocomial infection. Regarding the low frequency of nosocomial infection due to rotavirus in other studies which have only studied symptomatic cases during hospitalization

and reported a prevalence of 3.3 and 9%, it is suggested that the real estimation of nosocomial infection due to rotavirus in asymptomatic cases that might become symptomatic after discharge from hospital should also be considered. Due to the relatively high frequency of nosocomial infection in the Alzahra Hospital, it is necessary to follow stricter health issues, e.g. isolation of patients with diarrhoea and hand-washing before and after the examination of every patient (Kordidarian *et al*., 2007).

This study was carried out on 1600 rectal swabs from children under 5 years of age admitted at the health centre in Islamshahr, Tehran province, Islamic Republic of Iran, during 1998-99. The specimens were examined for various bacterial pathogens. Isolation rates were: EPEC 6.8%, *Shigella* spp. 3.4%, *Salmonella* spp. 2.9%, *Campylobacter* spp. 0.9%, *Yersinia* spp. 0.7%. The isolation rate was highest in the summer, except for *Yersinia* spp., which was predominantly isolated in spring. The results of this study demonstrate the significance of *Yersinia* spp. and *Campylobacter* spp. in patients with diarrhoea (Dallal *et al*., 2006).

The objective was to determine the prevalence of parasitic agents among under-five children with diarrhea in Ilesa, Nigeria and the clinical correlates of diarrhea associated with parasitic infestation. All under-five children presenting with diarrhoea in the hospital had stool microscopic examination. Children with parasites in diarrhoeic stools (cases) were compared with those without (controls) for clinical features. Out of 300 under-five children with diarrhoea, 70 (23.3%) had parasites. There were 18 (6%) helminthes and 52 (17.3%) protozoas. These included the' ova of *A. lumbricoides* (13; 18.6%), cysts and trophozoites of *E. histolytica* (46; 65.7%), cysts of *Entamoeba coli* (1; 1.4%), *G. Lamblia* (5; 7.1%), *Necator amer*ican*us* (1; 1.4%) and *T. trichiuria* (4; 5.7%) (Tinuade *et al*., 2006).

To determine the relative frequencies and prevalence rates of pathogenic *E. coli* and intestinal parasites in hospitalised infants (0-5 years) in Kumasi. A prospective descriptive study of screening 162 (83 males and 79 females) infants with diarrhoea and 122(64 males and 58 females) non-diarrhoeal infants controls for pathogens (*E. coli* and intestinal parasites) by standard microbiological methods was done. The sample was collected from

Komfo Anokye Teaching Hospital and Maternal and Child Hospital, Kumasi, Ghana. From the 162 in the diarrhoeal group 96(59.6%) pathogens, and from the 122 in the control group, eight (6.6%) pathogens were isolated. Enteropathogenic E. coli (EPEC) was the most frequently detected pathogen, accounting for 24(14.8%) of the findings in the diarrhoeal group, five (4.1%) in the non-diarrhoeal control group. Of the 26 EPEC isolates, there were nine serotypes with the three dominant ones being 0125 (6), 0119 (5), and 026 (15). Other agents isolated included *A. lumbricoides* 18(11.1%) and two (1.6%), *Cryptosporidium* 13(8.0%) and one (0.8%) for diarrhoeal and non-diarrhoeal infants respectively. The following were detected only in diarrhoeal stools. *G. lamblia*, six (3.7%); *Trichomonas hominis*, three (1.9%); *T. trichiura* one (0.6%) and Hookworm, one (0.6%) (Addy *et al*., 2000).

This study investigated the frequency of *E. coli*, *Shigella* and *Salmonella* spp. in stool specimens from patients with diarrhoea presenting to health centers in Hamedan province, Islamic Republic of Iran. From 144 samples, *Shigella* strains were isolated in 17 cases (11.8%): 10 *S. flexneri*, 3 *S. sonnei*, 2 *S. boydii* and 2 untyped strains. No *Salmonella* strains were isolated. Using molecular diagnostic methods, diarrheogenic *E. coli* were detected in 37 cases (25.7%), the majority were ETEC (22 cases) and Shiga toxin-producing (STEC) strains (15 cases). In 14 cases (9.7%) there was co-infection (Alizadeh *et al*., 2007).

3.7 Asian scenario of intestinal infections

The International Centre for Diarrhoeal Disease Research (ICDDR), Bangladesh is a major center for research into diarrhoeal diseases. The center treats more than 100,000 patients a year. To obtain useful information representative of all patients, a surveillance system in which a 4% systematic sample of all patients is studied in detail, including etiological agents of diarrhoea, was installed in October 1979. The first paper on etiology for the surveillance patients was published in 1982, which identified a potential enteric pathogen in 66% of patients. The study was conducted from February 1993 to June 1994 under 5years old children. A potential enteric pathogen was isolated from 74.8% of diarrhoeal

children and 43.9% of control children (P = 0.0001). It identified rotavirus, *C. jejuni*, ETEC, *Shigella* spp., and *V. cholerae* O1 as major pathogens (Albert *et al.*, 1999).

In Asia 3 to 23 % of all diarrhoea persisted for longer than 2 weeks. Information from 2 countries (India and Bangladesh) indicates that 45% diarrhoea associated deaths with persistent no dysenteric diarrhoea (45%) and 20 % with dysentery of any duration (Black, 1993). As far as South-East Asia Region is concerned, the household surveys carried out during 1994-1995 show that in under 5 years children diarrhoea episodes ranged from 0.7-3.9 episodes per child per year (WHO, 2004).

Samples (1,318) of ETEC isolated in 1994-1995 from children with diarrhoea from Nepal, Indonesia, Peru, and Thailand were examined for colonization factor antigen (CFA) and coli surface (CS) antigens. Fifty-five percent of 361 heat-labile and heat-stable (LT-ST), 14% of 620 LT-only, and 48% of 337 ST-only ETEC had CFA/CS antigens. LT-ST ETEC strains were predominantly in the CFA II group, and ST only strains were in the CFA IV group. Additional studies are needed to identify ETEC strains that do not have CFA/CS antigens (Nirdnoy *et al.*, 1997). *Shigella* species are responsible for 10-15% of acute diarrhoea in children less than 5 years old and the most common etiologic agents of childhood dysentery. An outbreak of *S. dysentery* in West Bengal, India, had a high attack rate in children less than 5 years old and was resistant to many drugs (Niyogi *et al.*, 2004). In 2000, outbreaks of bloody diarrhoea due to Sd1 that were resistant to fluoroquinolones occurred in India and Bangladesh (WHO, 2005). *V. cholerae* O1 is endemic in parts of Africa and Asia (e.g., 5-10% of hospitalized diarrhoea patients). The El Tor cholera biotype is responsible for the 7th pandemic (Niyogi *et al.*, 1994).

The contribution of amebiasis to the burden of diarrhoeal disease in children and the degree to which immunity is acquired from natural infection were assessed in a 4 year prospective observational study of 289 preschool children in an urban slum in Dhaka, Bangladesh. *E. histolytica* infection was detected at least once in 80%, and repeat infection in 53%, of the children who completed 4 years of observation. Annually there were 0.09 episodes per

child of *E. histolytica* associated diarrhoea and 0.03 episodes/child of *E. histolytica* associated dysentery (Jain *et al.*, 1976).

The etiology of rotavirus in acute diarrhoeal illness in children 0-5 years of age, admitted to the pediatric wards of Kasturba Medical College Hospital, Manipal was studied over a period of 5 years. Rotavirus in the faeces detected by Latex agglutination test accounted for 19.56% of the diarrhoea with maximum incidence (65%) in the 7-12 months of age group. A total of 283 (95.6%) cases of rotavirus infection occurred in children under 5 years of age. Serotype G1 (62%) was most prevalent followed by G2 (28.5%), G3 (5%) and G4 (2%) (Lama, 2006).

Children between the ages of 2-5 years attending the primary health centers (PHCs) at Sursand and Parihar (in Sitamarhi District of Bihar) for the treatment of diarrhoea, from the border villages of neighboring Nepal as well as from India, were the subjects of this study designed to explore the different aspects of the problem of diarrhoea in children and their practical management in villages at the Indo-Nepal border. Attendants were interviewed to elicit the etiology, progress of the disease, and treatment administered prior to making a detailed clinical examination and subsequent relevant laboratory investigation in selected cases. Criteria for diagnosis of diarrhoea, i.e., passing of 3 or more soft or liquid stools within 12 hours and/or even a large single soft or liquid stool containing mucus and/or blood were strictly adhered to in all the cases. Of 650 children attending the 2 PHCs for various ailments, 225 (34.6%) suffered from diarrhoea. The boys outnumbered the girls (130:95). Dug wells in an unhygienic state were the main source of the water supply to the well-to-do families. 78% of the families used ponds and shallow rivers for both cooking and cleaning. The majority of children (55.1%) were ill-nourished, and of the remaining 44.9%, the larger percentage consisted of thin children (33.3%) and only 11.6% were normal. Household treatments of diarrhoea included "heeng," opium powder, "ajawain," and "ishapgol" husk and were freely used in 50% of the children on the advice of elderly persons in the family. The lack of education, superstition, and poverty were the main reasons for delay in consulting the proper person for the treatment of the diarrhoea in

children. Dehydration of different degrees was present in all the children and was gross in 25.7% of the cases. 44.8% and 29.3% of the cases had mild and a moderate degree of dehydration, respectively. Oral rehydration therapy (ORT) heralded the commencement of treatment in cases of dehydration having scores of less than 4. The quantity of freshly prepared ORT was in direct proportion of 2 ounces for every liquid stool passed. Different available commercial preparations of ORT were used. In children with dehydration of more than a 4 score and accompanied by intermittent vomiting, ORT was combined with simple parenteral transfusion with fluids of variable composition according to the different commercial preparations available. In regard to chemotherapy, mostly co-trimoxazole and furazolidone combination in suitable dosage for 3-5 days yielded satisfactory results. In addition to ORT fluid in small but frequent doses, breastfeeding, milk, rice-gruel, and parboiled and overcooked rice were given from the outset. 48 of the children died, but in 50% of the cases it was due to delayed treatment (Gupta, 1985).

Cholera caused by either *V. cholerae* O1 or O139 is endemic in Delhi and its peripheral areas. The study was carried out to understand the changing epidemiology of *V. cholerae* in terms of prevalence of serotypes, antibiogram pattern and phage types. A total of 9858 stool samples from the admitted diarrhoea patients were used for the isolation of *V. cholerae* O1 and O139. Subsets of isolates were tested against thirteen antimicrobials and phage typed. Among 4251 (43.1%) confirmed cases, 41.6 per cent were *V. cholerae* O1 and rest (1.5 %) *V. cholerae* O139. Detection of *V. cholerae* O1 serotype Inaba was 87.7 per cent during 2005 and rest were serotype Ogawa. Majority of cases (93.1%) were from Delhi. male: female ratio remained 1.5:1.0. Children below 5 yr age group constituted 32.7 per cent cases. Shift in the age groups and seasonal incidence were recorded. All 226 strains of V. cholerae O1 and O139 were resistant to nalidixic acid; 96 per cent V. cholerae O1 isolates were multidrug resistant (FX NA SXT). Phage type 27 (98.7%) was the most prevalent and the new phage types were 4, 16 and 25 in this area (Sharma *et al.*, 2007).

3.8 National scenario of intestinal infections

A diarrhoeal disease is one of the major public health problems among children less than five years of age in Nepal. The National Control of Diarrhoeal Diseases Programme (NCDDP) has been in priority status by the government of Nepal. The main objective of the NCDDP is to reduce mortality due to diarrhoea and dehydration (from the estimated 30,000 deaths per year in the past) to a minimum, and to reduce morbidity from 3.3 episodes per child per year to a minimum (Nepal population report, 2007).

One hundred and sixty children under five years of age suffering from acute gastroenteritis who attended the out patient department and those who were admitted at the BP Koirala Institute of Health Sciences (BPKIHS), a tertiary care hospital situated in Eastern Nepal were included. The stool samples were collected over a period of one year from July 1999 to June 2000. Faeces of children showing the presence of blood and mucus (dysentery) were excluded from the study. Of which 108 (67.5%) were male and 52 (32.5%) were female. Rotavirus antigen was detected in 62(38.7%) samples. In 9 (14.5%) samples antigen was detected in combination with parasite and bacterial agents. The majority (70.9%) of infections were observed in patients between 6 months and 2 years of age. In that study, patients are categorized as rotavirus positive (Group I) and negative (Group II). Vomiting (56%) and dehydration (76%) were predominantly seen in Group I, due to which more children in this group needed hospitalization (66%) as compared to their Rotavirus negative counterparts. Fever was seen in both groups. Presence of pus cells and RBCs were detected more often in Group II. Bacterial pathogens were isolated in 11% in Group I. These included six strains of *E. coli* and one strain of *Shigella flexneri*. In Group II bacterial pathogens were isolated in 17 (19%) samples which included 10 strains of *E. coli*, six strains of *S. flexneri* and one strain of *Shigella boydii*. One each of *Cryptosporidium* and *E. histolytica* cysts was seen in Group I. *E. histolytica* in three samples and *Trichomonas* in one sample were detected in Group II (Shariff *et al.*, 2003).

Stools from 124 Nepalese children aged 6 to 60 months with diarrhoea were examined for organisms of the coccidian genus *Cyclospora* and for other enteric pathogens. ETEC, *G. Lamblia, Campylobacter* spp., *Cyclospora* spp. and *Cryptosporidium* spp. were the most common pathogens identified. *Cyclospora* spp. were detected in none of 74 children < 18

months of age compared with 6 (12%) of 50 children > or = 18 months of age (P = 0.004) (Hoge *et al.,* 1994).

A cross sectional descriptive study was conducted among school children at public school in the urban settings of Kathmandu valley, Nepal from June to November 2006. Among 309 study subjects aged 5-14 years, the prevalence of helminthic infections was found nearly 345. Such infection was found equally among male and female population. *T. trichuris* was the most common parasites among the study subjects (nearly 55%), followed by *A. lumbricoides* (26%), Hookworm (12%), *H. nana* (%) and *S. stercoralis* (2%) (Adhikari *et al*., 2007).

Four hundred and forty stool samples were collected from children under 11 years of age who developed diarrhoea and were admitted to Kanti Children's Hospital between May to October 2005. Of them 285 (64.8%) enteropathogens were identified. The highest infection was due to intestinal parasites 104/285 (36.5%) followed by rotavirus 92/285 (32.3%), pathogenic bacteria 57/285 (20%) and *Cyclospora* 32/285 (11.2%). Among the pathogenic bacteria, the predominant were *Shigella* spp. (36.8%), *Vibrio spp*., (26.3%), *E. coli* (22.8%) and *Salmonella* spp. (14.03%) respectively (Lama and Sherchand, 2007).

Rotavirus, an etiologic agent of causing diarrhoea, was identified in 25-40 percent of children with diarrhoea in urban Kathmandu valley of Nepal, but the data in remote rural areas was inadequate (Sherchand *et al.,* 2004). In Nepal, due to lower socio-economic status and poor hygienic condition of the people, intestinal parasitosis is very much prevalent and intestinal pathogens are important causative agents of diarrhoea and are one of the major public health problems of the country (Sherchand *et al.*, 2004).

One hundred and eighty-one patients attended the gastroenteritis ward of Sukra Raj Tropical and Infectious Disease Hospital (STIDH) with acute diarrhoea and were investigation for etiology of diarrhoea. Bacterial pathogens were isolated among 33% of the patients. Among them EPEC was isolated in 8.28%, *Shigella* species in 13.25% and *V.*

cholerae in 1.1% of the patients. Mixed infections with bacterial pathogens, helminthes and protozoan parasites were commonly observed in the study. *T. trichiuria* was detected in 27.6%, Hookworm in 12.7% and *A. lumbricoides* in 11.04%. *E. histolytica* and *G. lamblia* each in 11.7 and 7.73 of the patients, respectively. A large number of *Cryptosporidium* (7.73%) and *Cyclospora* species (3.86%) commonly present in immunocompromised patient were also detected in acute diarrhoeal cases. The results showed that widest ranges of bacterial pathogens were isolated from the inhabitants of Kathmandu prior to monsoon. These findings indicate that the bacterial pathogens especially different species of *E. coli*, *Shigella* and protozoan parasites need to be given additional attention in the diagnosis and treatment of acute diarrhoea (Pandey, 2000).

The study carried out in Kanti Children's Hospital from November 2005 to January 2006 in which a total of 374 stool samples were tested, out of these 262 samples were diarrhoeal and 112 were non-diarrhoeal samples. Out of 262 diarrhoeal samples 125(47.7%) were positive for rotavirus. Out of 112 non-diarrhoeal samples 15(13.4%) were positive for rotavirus. Among the diarrhoeal cases highest rate of infection 87/150 (58.0%) were seen in age group 7-24 months. Whereas, among the non-diarrhoeal cases highest rate of infection 3/16(18.7%) were seen in age group 0-6 months. The highest rate of infection 86/16(50.8%) was seen in male children. In comparison, rotavirus infection was significantly higher in diarrhoeal cases 125/140(89.2%) than non-diarrhoeal cases 15/140 (10.7%) [P-value 0.000, odds ratio (OR) 5.9, at 95% confidence interval (CI)]. The highest rate of infection were seen in the month of January 61/107 (57.0%) (Maharjan *et al.*, 2007).

The annual report of children diarrhoea under 5 years of age in fiscal year (FY) 2060/61, 2061/62 and 2062/63 which shows that the decreasing trend in the number of diarrhoeal visits in central developmental region (CDR) and also shows fewer diarrhoea epidemics in the country than there were a few years ago. In fiscal year 2062/63 the total number of diarrhoeal visits decreased slightly in comparison to fiscal year 2061/62 and 2060/61. Similarly, diarrhoeal death is also decreasing in fiscal year 2062/63 in comparison to the

fiscal year 2061/62 but in fiscal year 2061/62 it is slightly higher in fiscal year 2060/61 and 2062/63 in central developmental region.

CHAPTER IV

4. MATERIALS AND METHOD

A list of materials, reagents, media, equipments and chemicals are presented in Appendix 2, 3 and 4.

4.1 Subject

The study was carried out in collaboration with Central Department of Microbiology and Institute of Medicine, Health Research Laboratory, Maharajgunj, Kathmandu, Nepal during October 2006 to September 2007. Stool samples were collected from Kanti Children's Hospital of children's aged under 10 years with acute diarrhoea attending ORT centre and OPD who passed watery or loose stools with or without mucus or blood, with or without vomiting; dehydration and abdominal pain were taken in this study.

4.2 Sample collection

Stool samples from children attending both ORT centre and OPD of Kanti Children's Hospital below 10 years of age were collected in a clean and sterile screw capped container with spatula and simultaneously data on predisposing factors associated with gastroenteritis were collected according to the questionnaire designated. Then the collected stool samples were brought immediately to the laboratory. Upon arrival the stool samples were processed according to the standard laboratory methods.

Descriptive statistics was used to analyze the data to show association between enteropathogens infection and predisposing factors by using chi-square test and other relevant statistical tools. Clinical data from each patient were collected by using a questionnaire and statistical analysis was performed with MS Excel and SPSS 11.5.

4.3 Laboratory processing of samples

Each fresh specimen was processed in the following four steps as:

i. Macroscopic examination

ii. Microscopic examination

iii. Culturing on differential, selective and enrichment medium for isolation of, *Salmonella* spp., *Shigella* spp. and *V. cholera*.

4.3.1 Macroscopic examination

The stool samples were examined macroscopically for the presence of blood, mucus and adult or larvae of helminthic parasites. The color and consistency of the stool samples were also observed at the same time.

4.3.2 Microscopic examination

Microscopic examination was the part of the study and carried out for the detection of oocyst, cyst, trophozoites, of protozoa and the detection of larva or eggs of helminthes. The detection was carried out at low power (10x) followed by high power (40x) of the microscopes, the suspected and possible parasite was observed under microscope by wet mount and iodine staining with special preference for *C. cayetanensis*. This preparation was done to examine the ova and cyst of the parasite. On the other hand, it was also helpful for the examination of red blood cells (RBC) and white blood cells (WBC) in faeces. The *C. cayetanensis* thus observed was confirmed by modified Zeihl Neelson staining.

a. Wet mount preparation

Wet mount gives detailed original structural morphology in the natural form. A small amount of the stool was taken by touching the various parts and then mixed with a drop of normal saline on a clean microscopic slide followed by the covering with cover slip. In case of watery stool sample, a single drop or the sample was placed on a slide and covered with cover slip and then examined under microscope.

b. Iodine preparation

Iodine preparation was performed exactly the same as saline wet mount the only difference was use of iodine instead of normal saline. Iodine preparation helps in identification of the parasite by making the nuclear and other inner parts of trophozoites and cysts more clear.

c. Hanging drop

For the cholera suspected stool samples, they were subjected to hanging drop preparation for special darting motility exhibited by *Vibrio* spp.

Procedure

1. One drop of the broth culture was placed in the center of a 22×22 mm coverslip.

2. A small drop of wax on each corner of the cover slip was placed.

3. Then the cover slip was inverted over the depression slide.

4. After that microscopic observation was done in 40x

4.3.3 Culturing on differential, selective and enrichment medium

Salmonella-Shigella agar and MacConkey agar were used for isolation of *Salmonella* spp. and *Shigella* spp. and TCBS agar was used for isolation of *Vibrio* spp. Alkaline peptone water was used for enrichment of *V. cholera* and selenite-F broth was used for enrichment of *Salmonella* and *Shigella* spp.

a. For *Salmonella* and *Shigella spp.*

A large loopful of faeces was inoculated separately on SS-agar plate and MacConkey agar plate and several loopfuls were inoculated in selenite-F broth and were incubated at 37^{o}C for overnight. From the overnight enrichment broth, a loopful of specimen was again inoculated on SS agar plate and incubated at 37^{0}C for 24 hours.

b. For *Vibrio* spp.

Stool sample of about 2 ml was inoculated in 20 ml of alkaline peptone water (pH 8.6) and several loopfuls were inoculated on Thiosulphate citrate bile sucrose agar plate. All the tubes and plates were incubated at 37^0 C for overnight.

4.3.3.1 Observation of culture plates

The overnight-incubated culture plates were observed for their characteristic colony morphology. MacConkey agar was observed for Lactose fermenting pink and non-lactose fermenting pale colonies. SS agar plates were observed for non-lactose fermenting pale colonies of *Shigella* spp. and pale colonies with black centers for *Salmonella* spp.

4.3.3.2 Identification

The colonies from SS agar plates, MacConkey agar plates and TCBS agar plates were observed by both macroscopic and microscopic. The macroscopic observation by observing colony characteristics and microscopic observation by performing gram staining test. The isolated colonies from SS and MacConkey agar after performing gram staining were sub-cultured on Nutrient agar. After incubation, the well isolated colony was subjected to biochemical identification.

4.3.4 Enzyme-immunoassay detection of rotavirus antigen in human stool

Procedure

1. Feacal sample was collected by use of transfer pipette (first mark**)** and it was emulsified into 1ml of sample diluents in a test tube.

2. Two drops (100µl) of diluted stool, positive control and negative control (sample diluent) was added in the appropriate wells

3. Two drops (100µl) of enzyme conjugate was added to each well and sealed and mixed.

4. It was then incubated at room temperature for 60 minutes.

5. The wells were then washed 5 times with deionised water.

6. Two drops (100µl) of substrate was added to each well.
7. Two drops (100µl) of chromogen solution was added to each well.

8. It was incubated at room temperature for 10 minutes.

9. Two drops (100µl) stop solution was added into each well.

10. The result was read visually.

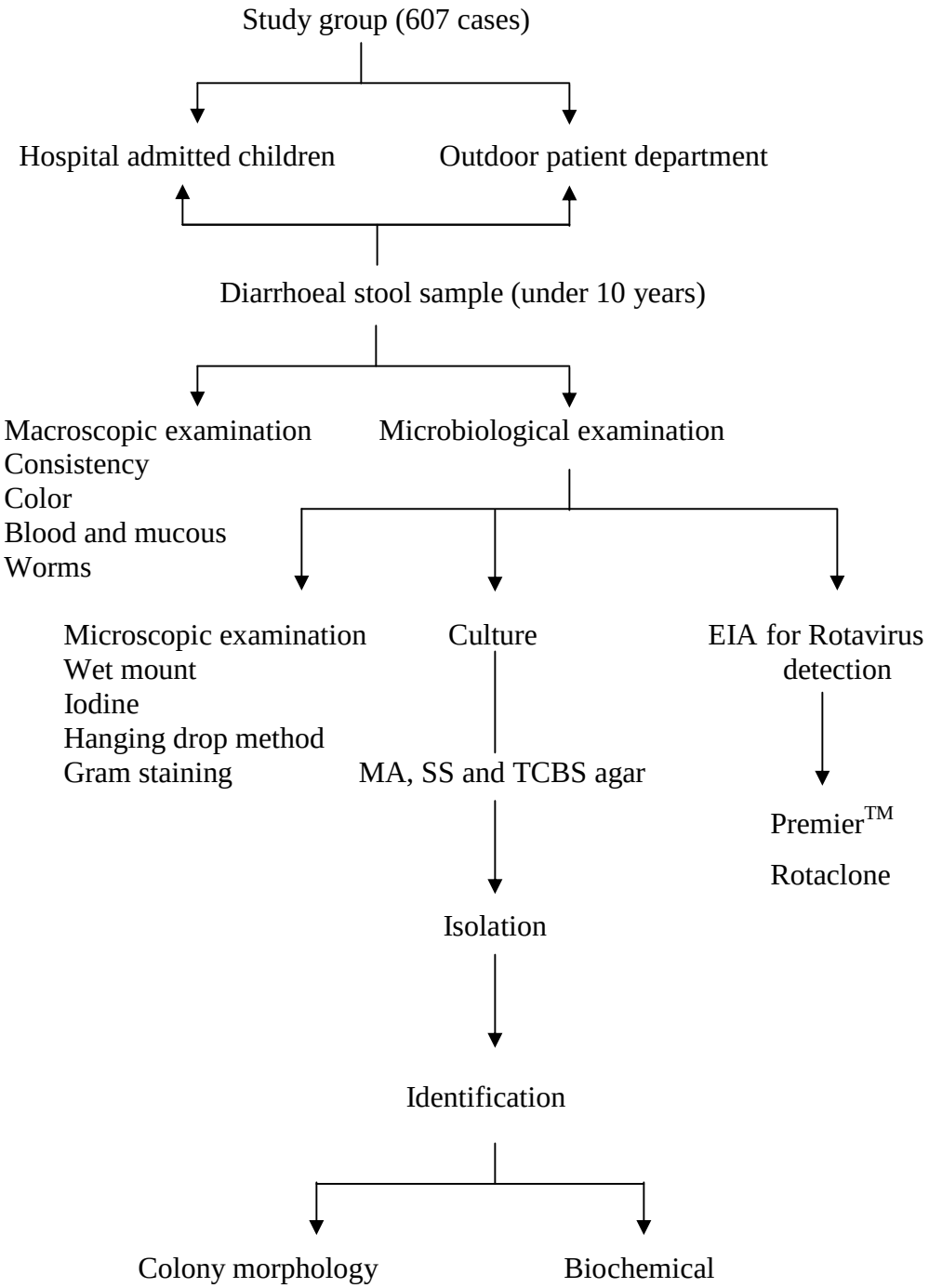
4.3.5 Purity plate

Purity plate shows whether or not the experiments were preceded aseptically. It was done in each biochemical tests. Each half of NA plates was inoculated before the test and another half after the test performed. The growth of the same organism in pure form both in pre and post inoculation is an indication of free of contamination or of an aseptic condition.

4.3.6 Quality control

The quality control was done by incubating each batch of test medium without inoculating the test organisms. In study, the selective media like MacConkey agar and SS agar were inoculated with stock culture of *Salmonella* spp. and *E. coli* along with test cultures and were incubated at 37^0C for 24 hours. After that results were compared.

FLOW CHART OF METHOD



CHAPTER V

5. RESULTS

Within the study period (October 2006 to September 2007), a total of 607 diarrhoeal stool specimens of gastroenteritis suspected patients were collected from inpatient and out patient department of children under 10 years of age attending Kanti Children's Hospital and processed in Health Research Laboratory for microscopic examination of parasites as well as isolation and identification of bacterial etiological agents and EIA for detection of Rotavirus antigen.

5.1 Number of cases of enteropathogens according to hospital admission

Out of total diarrhoeal cases 607, enrolled for the study, 320 (52.7%) were of oral rehydration therapy (ORT) ward and 287 (47.3%) were of out patient department (OPD). This showed that the patient visited in ORT ward was higher than OPD. The prevalence of enteropathogens as shown in table 3 was found to be higher in ORT 169 (63.3%) than OPD 98 (36.7%).

Table 3 Number of cases of enteropathogens according to hospital admission

Enteropathogens / **Hospital admission**	**Enteropathogens**		**Total**	**Percentage (%)**
	Positive	**Negative**		
Inpatient	169	151	320	63.3
Outpatient	98	189	287	36.7
Total	**267**	**340**	**607**	**100**

5.2 Prevalence of enteropathogens in total processed sample

Among the 607 cases, potential enteropathogens were identified in 267 (44.0%) cases and the prevalence of bacteria was 6.6%, rotavirus was 27.0% and parasites were 15.2% (Table 4).

Table 4 Prevalence of enteropathogens in total processed sample

Enteropathogens **Total sample processed**	**Either of enteropathogens**	**Bacteria**	**Rotavirus**	**Parasites**
607	267 (44%)	40 (6.6%)	164 (27%)	92 (15.2%)

5.3 Distribution of enteropathogens

The distribution of enteropathogens (Figure 1) showed that rotavirus was found highest constituting 27% of the total cases. Among parasites, protozoan (12.2%) dominated over helminthes (3.0%). Among the protozoa, *E. histolytica* (6.9%) was major causative agent of dysentery followed by *G. lambia* (3.8%), *C. cayetanensis* (1.2%), and *Entamoeba coli* (0.3%). Among the helminthes, *A. lumbricoices* was the major pathogen constituting 1.3% followed by *T. trichiura* (0.8%), Hookworm (0.5%) and *H. nana* (0.3%) of the total cases. Similarly, among bacteria *Shigella* spp. was highest of the total cases. *S. dysenteriae* in 2.0% (12 out of total 607); followed by *S. flexneri* in 1.0% and *S. boydii* in 0.3%. In case of *V. cholera* it constitutes about 2.0% and S*almonella* spp. in 1.3% of total cases. The study shows that the prevalence of *S. paratyphi* A in 0.3% (2 out of 607) followed by *S. paratyphi* B in 0.3% and *S. typhi* in 0.6%.

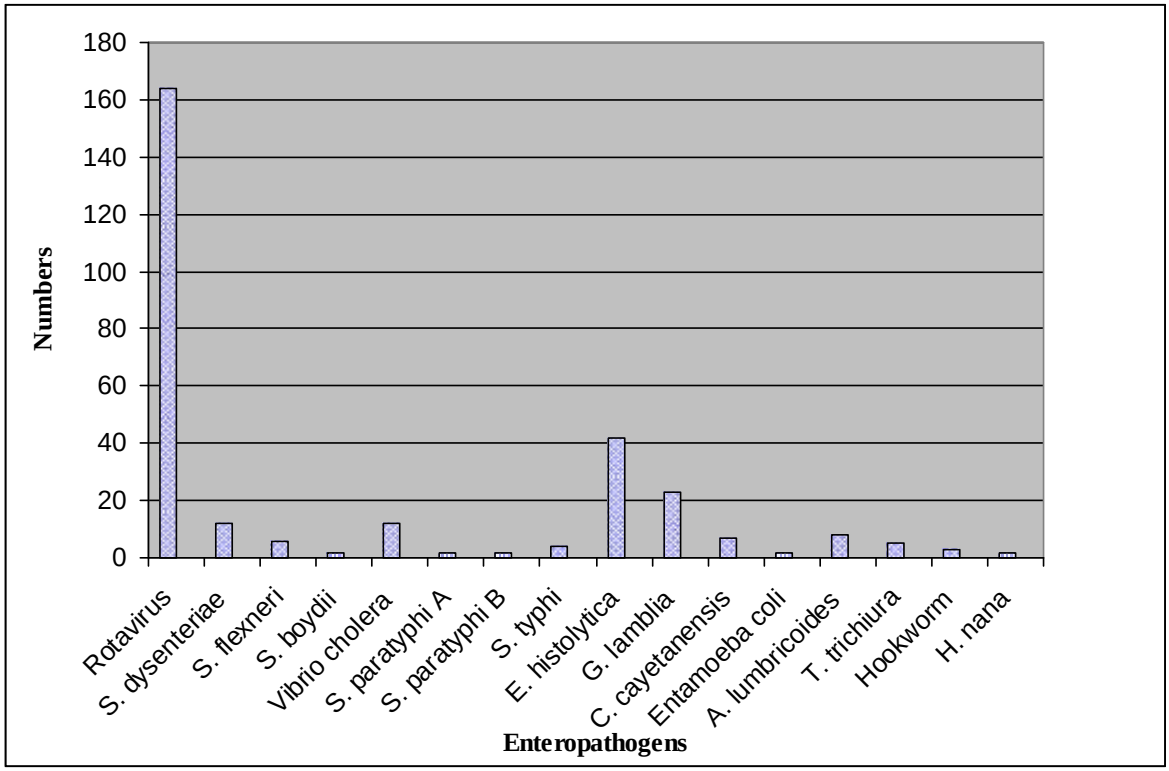


Figure 1 Distribution of enteropathogens

5.4 Proportion of co-infections among total cases

Out of 607, 30 (11.2%) cases showed positive only for bacteria (*Salmonella* spp. or *Shigella* spp. or *V. cholera*), 75 (28.1%) cases showed positive for only parasites and 133 (49.8%) cases were positive for only rotavirus.

The co-occurrence of enteropathogens was also found in this study as depicted in table 5. There were 29 cases (10.9%) of co-infections either with bacteria and rotavirus; parasites and rotavirus; or bacteria and parasites and that of bacteria and parasites. Co-infection of rotavirus and bacteria was 0.8% co-infection of rotavirus and parasite was 7.1% and that of bacteria and parasites was 3%. There were 8 cases (3%) of multiple parasitic infections with two or more than two parasites, the frequency being highest (1.5%) in combination protozoa and protozoa. But there was not a single case of multiple bacterial infections found in this study.

Table 5 Proportion of co-infections among total cases

Total infection	Positive number	Percentage (267)
Total single type of pathogen	**238**	**89.1**
Only bacteria	30	11.2
Only parasites	75	28.1
Only rotavirus	133	49.8
Total co-infections	**29**	**10.9**
Rota-virus and bacteria	2	0.8
Rotavirus and parasite	19	7.1
Bacteria and parasite	8	3.0
Total pathogens positive	**267**	**100**
Total multiple parasites	**8**	**3.0**
Protozoa and helminthes	1	0.4
Protozoa and protozoa	4	1.5
Helminthes and helminthes	2	0.7
Protozoa and protozoa and protozoa	1	0.4
Total multiple bacteria	-	-

5.5 Age and gender wise distribution of enteropathogens

The distribution of different enteric pathogens among various age groups indicated that the highest number of enteropathogens was found in age group 0-2 yrs (50.2%) i.e.134 out of 267 followed by age group 2-4, 4-6, 6-8 and 8-10 yrs with 20.2, 15.0, 9.5 and 5.2% respectively (Table 6).

Table 6 Age and gender wise distribution of enteropathogens

Sex / Age	Positive		Total	% (+ve) (267)	Negative		Total	Grand Total
	Male	Female			Male	Female		
0-2 yrs	90	44	134	50.2	131	105	236	370
2-4 yrs	35	19	54	20.2	32	11	43	97
4-6 yrs	29	11	40	15.0	20	9	29	69
6-8 yrs	19	6	25	9.4	6	5	11	36
8-10 yrs	9	5	14	5.2	15	6	21	35
Total	**182**	**85**	**267**	**100**	**204**	**136**	**340**	**607**

5.6 Ethnic group wise distribution of enteropathogens

Ethnic group wise distribution of enteropathogens revealed that the group Magar/Rai/Gurung/Limbu/Tamang were highest 40.1% among total bacterial positive followed by Chhettri 22.1%, Brahmin 18%, Newar 12%, Yadav 5.6% and Minority 2.2% of total positive cases (Table 7).

Table 7 Ethnic group wise distribution of enteropathogens

Enteropathogens Ethnic group	Total number	Enteropathogen positive	% (267)
Magar/Rai/Gurung/ Limbu/Tamang	196	107	40.1
Chhettri	133	59	22.1
Brahmin	126	48	18
Newar	109	32	12
Yadav	33	15	5.6
Minority	10	6	2.2
Total	**607**	**267**	**100.0**

5.7 Month wise distribution of total enteropathogens

The highest numbers of samples (74) were processed in July and least (33) were processed in September. The prevalence of enteropathogens was found highest in the month of July 11.2% of total enteropathogens positive cases followed by January (10.8%), February (9.4%), and August (9.0%) was shown in table 8.

Table 8 Month wise distribution of total enteropathogens

Enteropathogens Seasons	Month	Total number	Pathogens positive	% (267)
	March	55	23	8.6
	April	43	20	7.5
	May	60	19	7.1
Spring		**158**	**62**	**23.2**
	June	61	21	7.9
	July	74	30	11.2
	August	42	24	9.0
Summer		**177**	**75**	**28**
	September	33	20	7.5
	October	34	18	6.7
	November	41	19	7.1
Autumn		**108**	**57**	**21.3**
	December	43	19	7.1
	January	61	29	10.8
	February	60	25	9.4
Winter		**164**	**73**	**27.3**
	Total	**607**	**267**	**100.0**

5.8 Seasonal distributions of enteropathogens

The prevalence of enteropathogens was highest in summer season i.e. 28% (75 out of 267), followed by winter season 27.3% (73 out of 267), spring season 23.2% (62 out of 267), and autumn season 21.3% (57 out of 267).

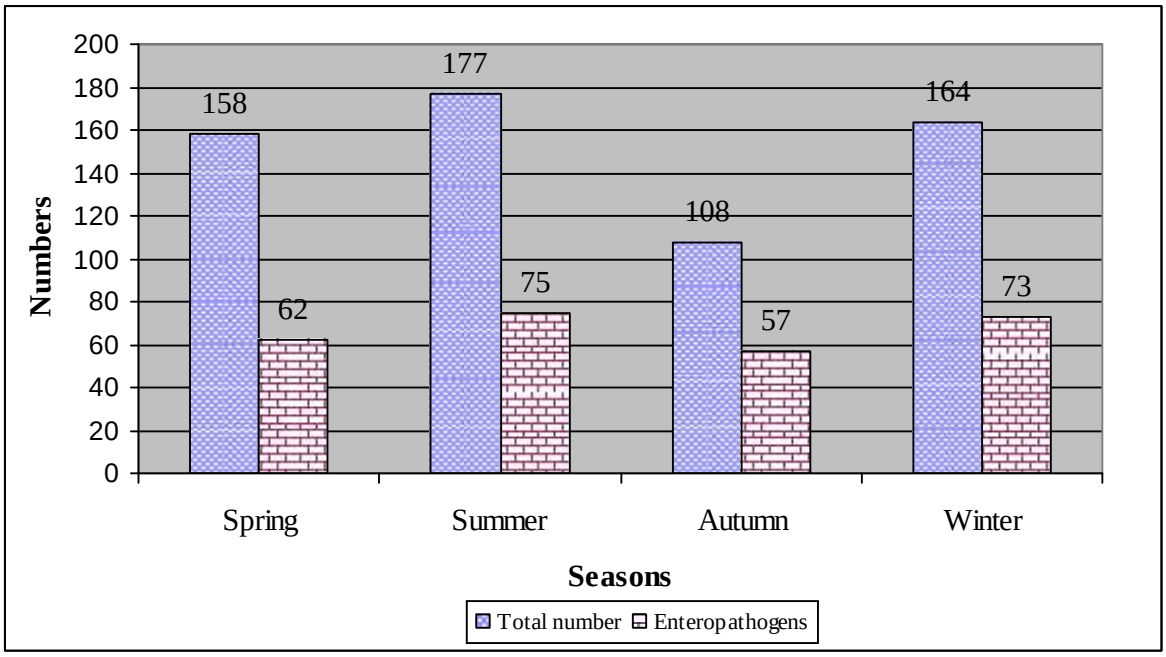


Figure 2 Seasonal wise distribution of enteropathogens

5. 9 Distribution of enteropathogens on the basis of drinking water

The study showed that most of the patients used raw water (331) for drinking purpose followed by filtered (188) and boiled (88). Among them prevalence of enteropathogens was highest in raw water user 53.6% (143 out of 267) followed by filtered water 34.8% (93 out of 267) and boiled water 11.6% (31 out of 267) users (Figure 3).

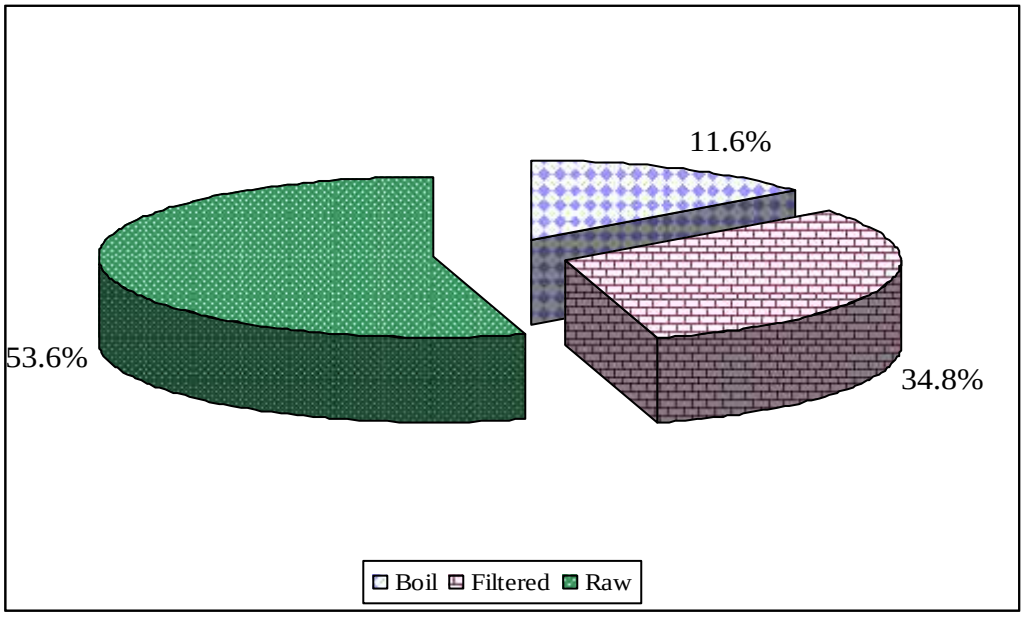


Figure 3 Distribution of enteropathogens on the basis of drinking water

5.10 Distribution of enteropathogens according habit of hand washing

The prevalence of enteropathogens was found higher in children who didn't wash hand before meal 71.9% (192 out of 267) than wash hand before meal 28.1% (75 out of 267) as shown in table 9.

Table 9 Distribution of enteropathogens according habit of hand washing

Enteropathogens / Washing hand before meal	Total	Enteropathogens		% Positive (267)
		Positive	Negative	
No	412	192	220	71.9
Yes	195	75	120	28.1
Total	**607**	**267**	**340**	**100.0**

5.11 Distribution of enteropathogens with father's occupation

The enteropathogens were found to be high in children with father's having occupation service (67.8%) followed by driver (8.6%), agriculture (7.5%) labor (4.9%), business (4.1%) and teacher (3.7%) of the total enteropathogens positive cases.

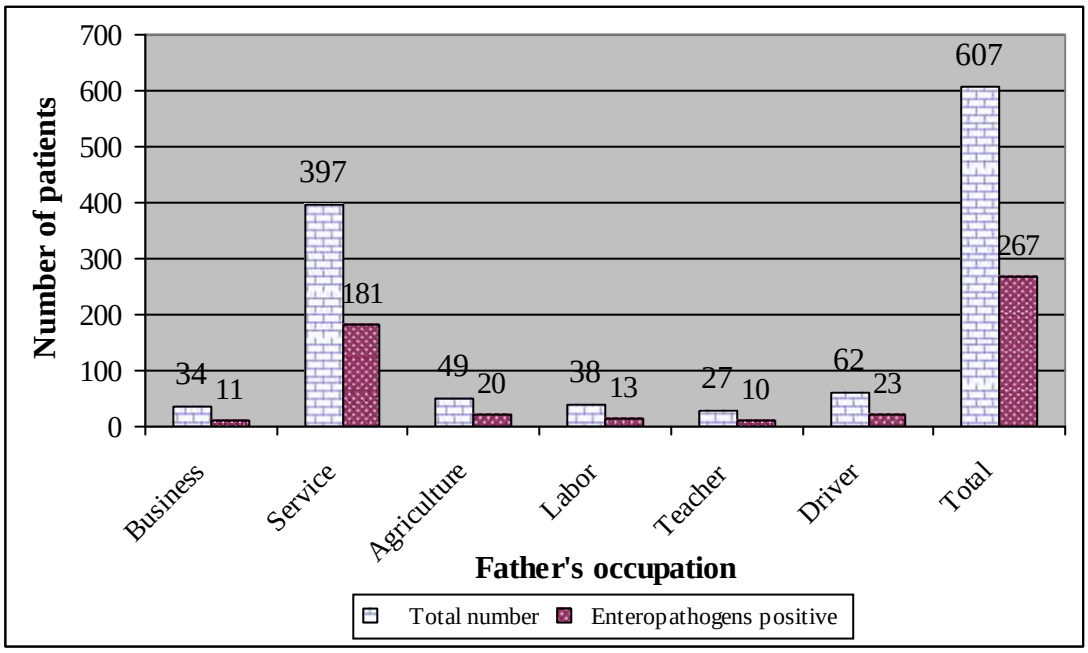


Figure 4 Distribution of enteropathogens with father's occupation

5.12 Distribution of enteropathogens with mother's education

The prevalence of enteropathogens was found to be higher in children with illiterate mother constituting 61% than literate mother constituting 39% which was statistically significant at 5% level of significance.

Table 10 Distribution of enteropathogens with mother's education

Enteropathogens / Mother's education	Total number	Pathogen positive	Percentage (%)
Illiterate	398	163	61.0
Literate	209	104	39.0
Total	**607**	**267**	**100**

5.13 Number of cases according to clinical symptoms

Out of 607 case, 323 (53.2%) had nausea and vomiting followed by 241(39.7%) had abdominal pain and vomiting 43 (7.1%) had fever, abdominal pain and vomiting among the total cases.

Table 11 Number of cases according to clinical symptoms

Enteropathogens / Clinical symptoms	Number of cases	Percentage (%)
Nausea / vomiting	323	53.2
Abdominal pain / vomiting	241	39.7
Fever / abdominal pain / vomiting	43	7.1
Total	**607**	**100.0**

5.14 Number of cases according to consistency of stool

Of the total cases, 370 (60.9%) had watery stool that was highest followed by 213 (35.1%) watery stool with mucous, 12 (1.9%) stool with mucous and blood and 12 (1.9%) rice watery stool.

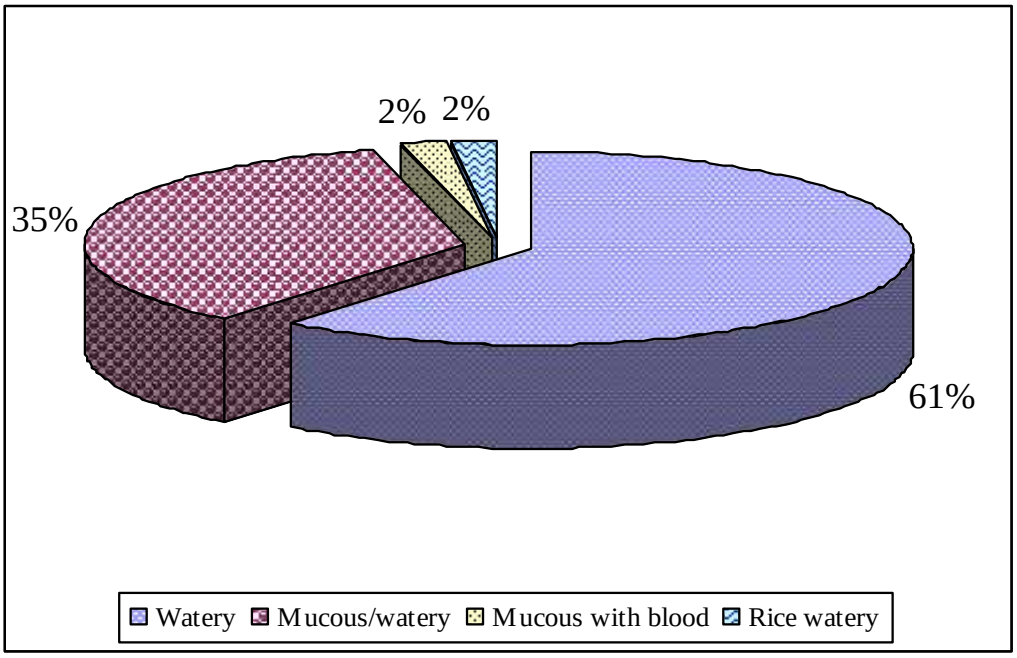


Figure 5 Number of cases according to consistency of stool

5.15 Number of cases according to degree of dehydration

Out of 607 cases, 323(53.2%) were severe, 246 (40.5%) were moderate and less were mild i.e. 38 (6.3%).

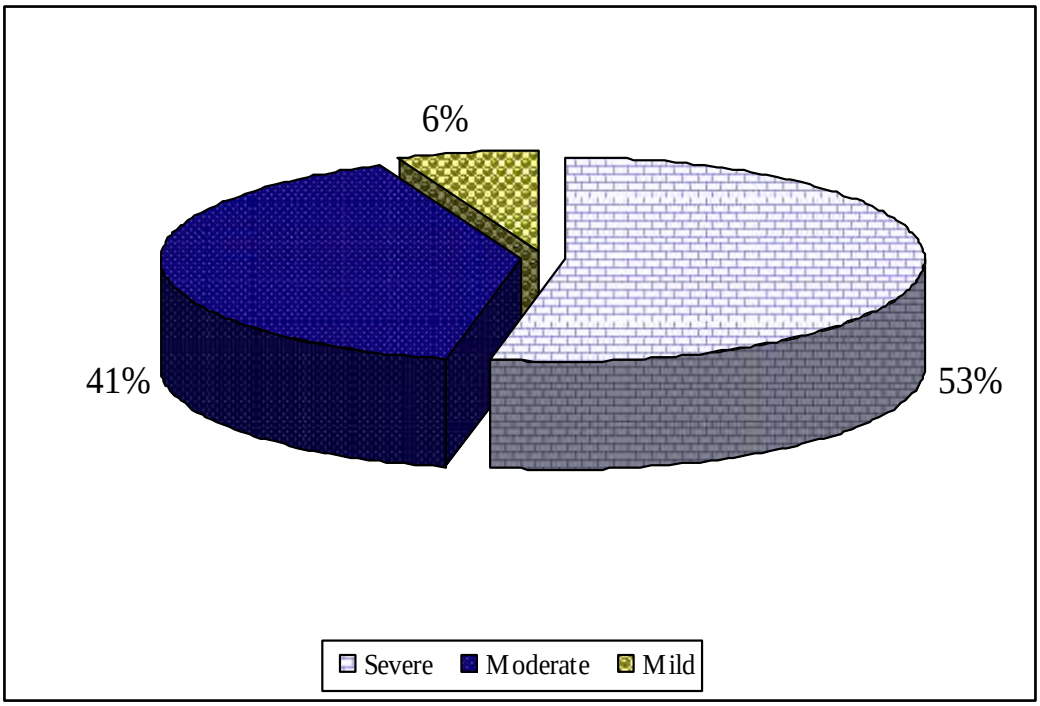


Figure 6 Number of cases according to degree of dehydration

5.16 Number of cases based on hospital treatment

Out of total cases, highest number were treated with oral rehydration therapy 236 (39%) followed by intravenous fluid 165 (27%), ORS and IV 93 (15%) others 58 (10%) and antibiotics 55 (9 %).

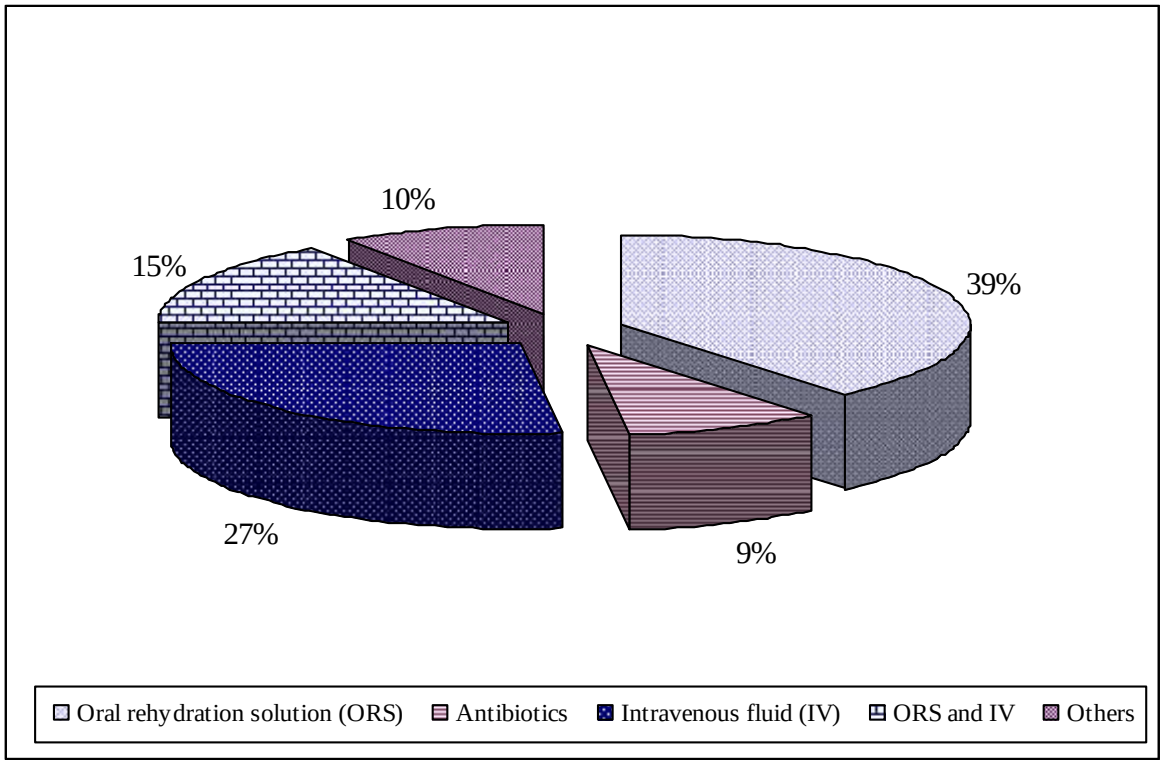


Figure 7 Number of cases based on hospital treatment

5.17 Number of cases of rotavirus depending on hospital admission

The rotavirus was higher 105 (64%) in hospital inpatient than 59 (36.0%) in hospital outpatients of total rotavirus positive cases.

Table 12 Number of cases of rotavirus depending on hospital admission

Rotavirus / **Hospital admission**	**Rotavirus**		**Total**	**Positive %**
	Positive	**Negative**		
Inpatient	105	215	320	64.0
Outpatient	59	228	287	36.0
Total	**164**	**443**	**607**	**100.0**

5.18 Age and gender wise distribution of rotavirus infected patients

A total of 164 cases were found infected with rotavirus accounting for 27.0% of the total cases (164/607). Age wise distribution showed that the infection rate was found to be highest in age group 0-2 yrs holding 30.3% i.e.112 out of 370 followed by the age group 2-4 yrs (26.8%), 4-6 yrs (21.7%), 6-8 yrs (19.4%) and 8-10 yrs (11.4%).

Table 13 Age and gender wise distribution of rotavirus infected patients

Sex / Age	Total No of Samples			Male			Female		
	Total	**+ve cases**	**+ve %**	**Male**	**+ve cases**	**+ve %**	**Female**	**+ve cases**	**+ve %**
0-2 yrs	370	112	30.3	225	72	32	145	40	27.6
2-4 yrs	97	26	26.8	71	18	25.3	26	8	30.8
4-6 yrs	69	15	21.7	49	10	20.4	20	5	19.2
6-8 yrs	36	7	19.4	25	4	16	11	3	27.3
8-10 yrs	35	4	11.4	24	2	8.3	11	2	27.3
Total	**607**	**164**	**27.0**	**394**	**106**	**26.9**	**213**	**58**	**27.2**

5.19 Ethnic group wise distribution of rotavirus

The prevalence of ethnic wise distribution showed Magar/Rai/Gurung/ Limbu/Tamang was highest containing 30.5% followed by Chhettri 21.9%, Brahmin 18.3%, Newar 17.7%, Yadav 8.5% and Minority 3% of total rotavirus positive cases.

Table 14 Ethnic group wise distribution of rotavirus

Rotavirus Ethnic group	Total number	Rotavirus positive	% (164)
Magar/Rai/Gurung/ Limbu/Tamang	196	50	30.5
Chhettri	133	36	21.9
Brahmin	126	30	18.3
Newar	109	29	17.7
Yadav	33	14	8.5
Minority	10	5	3
Total	**607**	**164**	**100**

5.20 Month wise distribution of rotavirus

The highest positive cases were observed in winter season 74 out of 164 constitute 45.1% followed by spring season 24.7%, autumn season 24.1% and summer season 14.1 % (Table 15).

Table 15 Month wise distribution of rotavirus

Rotavirus / Seasons	Month	Rotavirus		Total	%
		Positive	Negative		
	March	12	43	55	21.8
	April	9	34	43	20.9
	May	18	42	60	30
Spring		**39**	**119**	**158**	**24.7**
	June	13	48	61	21.3
	July	6	68	74	8.1
	August	6	36	42	14.3
Summer		**25**	**152**	**177**	**14.1**
	September	6	27	33	18.1
	October	10	24	34	29.4
	November	10	31	41	24.4
Autumn		**26**	**82**	**108**	**24.1**
	December	18	25	43	41.9
	January	32	29	61	52.5
	February	24	36	60	40
Winter		**74**	**90**	**164**	**45.1**
	Total	**164**	**443**	**607**	**27.0**

5.21 Number of cases of parasites depending on hospital admission

The table 16 showed that the parasitic infestation was highest 53.3 % (49/92) in hospital outpatient than inpatient department i.e. of 46.7 % (43/92).

Table 16 Number of cases of parasites depending on hospital admission

Parasites / Hospital admission	Parasites		Total	Positive %
	Positive	Negative		
Inpatient	43	277	320	46.7
Outpatient	49	238	287	53.3
Total	**92**	**515**	**607**	**100.0**

5.22 Age and gender wise distribution of parasites infected patients

The highest prevalence 33.3% (out of 12/36 cases) of parasites were seen in age group 6-8 yrs followed by age group of 8-10 yrs 25.7% (9 out of 36), 4-6 yrs 21.7% (15 out of 69), 2-4 yrs 18.6% (18 out of 97) and 0-2 yrs 10.3% (38 out of 370) respectively.

Both males (15.0% i.e.59/394) and females (15.5 % i.e.33/213) were equally infected among total cases. However, in age group 0-2, 2-4 and 6-8 yrs, females were infected higher than males (11.0 % females and 9.8% in males in age group 0-2 yrs, 23.1 % females and 16.9% males in age group 2-4 yrs, and 36.4 % females and 32.0% males in age group 6-8 yrs of age). Males were dominated in age group 4-6 yrs that constitute 22.4% in male and 20.0% in female, in age groups 8-10 yrs 25.0% male and 27.3% females that showed females dominated in this age group.

Table 17 Prevalence of parasites in relation to age and gender

Sex / Age	Total no of samples			Male			Female		
	Total No	**+ve cases**	**+ve %**	**Total no**	**+ve cases**	**+ve %**	**Total no**	**+ve cases**	**+ve %**
0-2 yrs	370	38	10.3	225	22	9.8	145	16	11.0
2-4 yrs	97	18	18.6	71	12	16.9	26	6	23.1
4-6 yrs	69	15	21.7	49	11	22.4	20	4	20.0
6-8 yrs	36	12	33.3	25	8	32.0	11	4	36.4
8-10 yrs	35	9	25.7	24	6	25.0	11	3	27.3
Total	**607**	**92**	**15.2**	**394**	**59**	**15.0**	**213**	**33**	**15.5**

5.23 Ethnic group wise distribution of parasites

The prevalence of ethnic wise distribution showed Magar/Rai/Gurung/ Limbu/Tamang was highest containing 44.6% followed by Brahmin 16.3%, Chhettri 14.1%, Yadav 10.8%, Newar 9.8% and Minority 4.4%.

Table 18 Ethnic group wise distribution of parasites

Parasites **Ethnic group**	**Total number**	**Parasite positive**	**%** **(92)**
Magar/Rai/Gurung/ Limbu/Tamang	196	41	44.6
Chhettri	133	13	14.1
Brahmin	126	15	16.3
Newar	109	9	9.8
Yadav	33	10	10.8
Minority	10	4	4.4
Total	**607**	**92**	**100**

5.24 Month wise distribution of parasites

From the study, it was found that *E. histolytica* infection was highest in the month of July (7 cases) followed by June (6 cases) and May and September (5 cases), while *G. lamblia* infection seemed to be most prevalent in the month of April, May, June, July, September and November (3 cases for each month). The prevalence of *Cyclospora* was highest in month of June (4 cases) followed by July (3 cases). In this study, there were only two cases of *Entamoeba coli*, which were found in the month of July and August one cases in each month.

Same number of *A. lumbricoides* was found in month of June and August (1 case in each month) and in month July, January and November (2 case in each month). *T. trichiura* was found in month of June, July, and January (1 case in each month) and in month October 2 cases. *H nana* was found in the month of June and January (1 case in each month). Hookworm was found in month of July (2 cases) and August (1 case). The prevalence of parasites were high in the season summer 43 cases (43/92) followed by autumn season 21 cases (21/92) spring season 16 cases (16/92) and winter season 12 cases (12/92).

Table 19 Month wise distribution of parasites

Parasites **Seasons**	**Month**	*E. histolytica*	*G. lamblia*	*C. cyetanensis*	*E. coli*	*A. lumbricoides*	*T. trichiura*	*H. nana*	Hookworm	Total
	Mar	2	0	0	0	0	0	0	0	2
	Apr	3	3	0	0	0	0	0	0	6
	May	5	3	0	0	0	0	0	0	8
Spring		**10**	**6**	**0**	**0**	**0**	**0**	**0**	**0**	**16**
	Jun	6	3	4	0	1	1	1	0	16
	Jul	7	3	3	1	2	1	0	2	19
	Aug	4	1	0	1	1	0	0	1	8
Summer		**17**	**7**	**7**	**2**	**4**	**2**	**1**	**3**	**43**
	Sep	5	3	0	0	0	0	0	0	8
	Oct	1	1	0	0	0	2	0	0	4
	Nov	4	3	0	0	2	0	0	0	9
Autumn		**10**	**7**	**0**	**0**	**2**	**2**	**0**	**0**	**21**
	Dec	1	1	0	0	0	0	0	0	2
	Jan	3	2	0	0	2	1	1	0	9
	Feb	1	0	0	0	0	0	0	0	1
Winter		**5**	**3**	**0**	**0**	**2**	**1**	**1**	**0**	**12**
	Total	**42**	**23**	**7**	**2**	**8**	**5**	**2**	**3**	**92**

5.25 Number of cases of bacteria depending on hospital admission

The table showed that bacterial infection was highest in hospital inpatient 29 (72.5% i.e. 29/40) than in hospital outpatient 11 (27.5% i.e.11/40).

Table 20 Number of cases of bacteria depending on hospital admission

Bacteria / Hospital admission	Bacteria		Total	Positive %
	Positive	Negative		
Inpatient	29	291	320	72.5
Outpatient	11	276	287	27.5
Total	**40**	**567**	**607**	**100**

5.26 Age and gender wise distribution of bacterial pathogens

Of the total bacterial infection, the prevalence rate of bacterial enteropathogens was highest in age group 0-2 yrs (32.5%) followed by 8-10 yrs (25%), 2-4 yrs (20%), 4-6 yrs (12.5%), and 6-8 yrs (10%) among the total bacteria positive.

Among the bacterial isolates prevalence of *Shigella* spp. was highest in age group 0-2 yrs (50% i.e.10/20) followed by age group 4-6 (20% i.e. 4/20), 8-10 (15% i.e. 3/20), 2-4 (10% 2/20) and 6-8 yrs (5% i.e. 1/20). On the other side *Salmonella* spp. was high in age group 2-4 yrs (50% i.e. 4/8) with only distribution in age group 0-2 and 8-10 yrs. However, prevalence of *V. cholera* was found higher in age group 8-10 yrs (50% i.e.6/12); followed by age group 6-8 (24% i.e. 3/12), 2-4 (16.7% 2/12) and 4-6 yrs (8.3% i.e. 1/12).

With regard to gender wise distribution, the prevalence of bacterial pathogens among males were highest in *V. cholera* (66.7% i.e.8/12) followed by *Shigella* spp. (60% i.e. 12/20), and *Salmonella* spp. (50% i.e. 4/8). Likewise, the distribution of bacterial pathogens among females were, *Salmonella* spp (50% i.e. 4/8), *Shigella* spp. (40% i.e. 8/20), *and Vibrio* spp. (33.3% 4/12) respectively.

Table 21 Age and gender wise distribution of bacterial pathogens

Sex / **Age Group (yrs)**	***Shigella* spp. (20)**		***Salmonella* spp. (8)**		***Vibrio* spp. (12)**		**Total**	**%**
	Male	Female	Male	Female	Male	Female		
0-2 (n=370)	5	5	1	2	0	0	**13**	**32.5**
2-4 (n=97)	2	0	3	1	1	1	**8**	**20**
4-6 (n=69)	2	2	0	0	1	0	**5**	**12.5**
6-8 (n=36)	1	0	0	0	2	1	**4**	**10**
8-10 (n=35)	2	1	0	1	4	2	**10**	**25**
Total (607)	**12**	**8**	**4**	**4**	**8**	**4**	**40**	**100**

5.27 Ethnic group wise distribution of bacteria

Ethnic group wise distribution (table 22) showed that the prevalence of bacteria was highest in Magar/Rai/Gurung/ Limbu/Tamang i.e. 30% among total positive bacterial isolates followed by Brahmin 35%, Chhettri 15%, Minority 10%, Yadav 7.5%, and Newar 2.5 %.

Table 22 Ethnic group wise distribution of bacteria

Bacteria / Ethnic group	**Total number**	**Bacteria positive**			**Total positive**	**%**
		***Shigella* spp.**	***Vibrio* spp.**	***Salmonella* spp.**		
Magar/Rai/Gurung/ Limbu/Tamang	196	7	2	3	12	30
Chhettri	133	2	2	2	6	15
Brahmin	126	7	5	2	14	35
Newar	109	1	0	0	1	2.5
Yadav	33	1	2	0	3	7.5
Minority	10	2	1	1	4	10
Total	**607**	**20**	**12**	**8**	**40**	**100**

5.28 Month wise distribution of bacterial pathogens

Bacterial pathogens were found to be highest in the month June, July and August constituting 25, 22.5 and 12.5% respectively (table 23). Similarly, *Shigella* infection was found highest in June (6 cases) followed by April and August (3 cases of each month) there was equal number of isolates, in May, October and November (2 cases in each month) and February and July there was equal number of isolates (1 in each month). There were no cases in month September, December, January and March.

And *V. cholera* was found highest in July (7 cases) followed by June (3 cases) and August (2 cases) there was no *V. cholera* isolated in May in this study.

More specifically *Salmonella* infection was found highest in October and November (2 cases) followed by March, May, June and July (1 cases) and no *Salmonellae* were detected in January, February, April, August, and September.

Table 23 Month wise distribution of bacterial pathogens

Seasons / **Bacterial pathogens**	**Spring**			**Summer**			**Autumn**			**Winter**			**Total**
	Mar	**Api**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	
***Shigella* spp.**	0	3	2	6	1	3	0	2	2	0	0	1	20
Vibrio cholera	0	0	0	3	7	2	0	0	0	0	0	0	12
***Salmonella* spp.**	1	0	1	1	1	0	0	2	2	0	0	0	8
Total	1	3	3	10	9	5	0	4	4	0	0	1	40
Percentage (40)	**2.5**	**7.5**	**7.5**	**25**	**22.5**	**12.5**	**0**	**10**	**10**	**0**	**0**	**2.5**	**100**

CHAPTER VI

6. DISCUSSION AND CONCLUSION

6.1 DISCUSSION

Diarrhoeal disease remains one of the largest health problems in many parts of the world. The disease is often mild and self-limiting but, particularly in the elderly and young children, the symptoms may be very severe. Studies in developing countries have shown that children in the first 2 years of the life may have up to 10 episodes of diarrhoeal disease, often with significant mortality (Black, 1982).

Diarrhoeal disease occupied the second place among the top ten diseases in Nepal (Nepal population report, 2007). This disease was directly related to the quality of water. However it varied according to seasons. The factors responsible for contaminating drinking water at source points in Nepal included the lack of protection and proper treatment of water, leakage in pipe distribution system, intermittent supply of water, poor drainage system and poor environment surroundings of water sources (Pradhan, 2004). Therefore, the identification of etiological agents is extremely important which helps in precise diagnosis and proper management of treatment procedures.

Diarrhoeal disease caused by bacteria, parasites and viruses continues to be an important cause of morbidity and mortality among young children in developing countries. In this study, 607 cases with diarrhoea were enrolled who visited Kanti children's hospital, the study period started from October 2006 to September 2007. Out of 607 cases, the prevalence of either of enteropathogens were observed 267 (44.0%) including bacteria, parasites and viruses. These findings were in accordance with study done in Bangladesh (59 %) by Moyenddin *et al.,* 1982/82 and in Denmark (54 %) by Olsen *et al.*, 2005 (Lama, 2006).

The prevalence of enteropathogens showed highest in males 68.2% i.e.182 out of 267 than females 31.8% i.e. 85 out of 267, which was found statistically not significant (P>0.05).

The result was consistent with the finding of Lama, 2006 at TUTH, in which the male were infected more (61.6%) than the female (38.2%). The result found was statistically insignificant at 5 % level of significance.

The diarrhoeal disease also related with socio economic status of family. So, in this study occupation of father was also mentioned. The enteropathogens were found to be high in children with fathers having occupation service (67.8%) followed by driver (8.6%), agriculture (7.5%), labor (4.5), business (4.1%) and teacher (37%) of the total positive cases. The prevalence of enteropathogens was significantly higher in children with illiterate mother constituting 61% than literate mother constituting 39% at 5% level of significance. This may suggests that it was the better hygiene knowledge and practice of literate mothers that reduces the risk of childhood diarrhoea than illiterate mother. Literate mothers were more likely to give correct care to their children when they have diarrhoea and also more likely to seek medical care for a child with diarrhoea. The prevalence of enteropathogens was found significantly higher in children who didn't wash hand before meal 71.9% (192 out of 267) than wash hand before meal 28.1% (75 out of 267) at 5% level of significance.

In this study, the frequency of distribution of enteropathogens was found to be high in hospital admitted patients. Out of total cases, 320 (52.7%) were found to be of ORT ward and 287 (47.3%) were found to be of OPD. Similarly, the enteropathogens were found significantly higher in ORT centre constituting 169 (63.3%) than OPD 98 (36.7%).

National ethnic group's development committee has listed 103 caste/ethnic groups including "unidentified group" in 2001 census (Nepal population report, 2007). Ethnic group wise distribution of enteropathogens revealed that the group Magar/Rai/Gurung/ Limbu/Tamang were highest 40.1% among total bacterial positive followed by Chhettri 22.1%, Brahmin 18%, Newar 12%, Yadav 5.6% and Minority 2.2%. The prevalence of ethnic wise in rotavirus, parasites and bacteria showed highest in Magar/Rai/Gurung/ Limbu/Tamang containing 30.5%, 44.6% and 30% respectively.

The highest numbers of samples (74) were processed in July and least (33) were processed in September. The prevalence of enteropathogens was found highest in the month of July 11.2% of total enteropathogens positive cases followed by January (10.8%), February (9.4%), and August (9.0%). The study showed that more samples were processed in summer season i.e.177 followed by winter season 164, spring season 158 and autumn season 108 of total cases. The prevalence of enteropathogens was highest in summer season i.e. 28% (75 out of 267), followed by winter season 27.3% (73 out of 267), spring season 23.2% (62 out of 267), and autumn season 21.3% (57 out of 267). This may be due to favorable condition for proliferation of pathogenic organisms especially parasites and bacteria during summer season.

Among the different sources of water, enteropathogens were found to be highest in children using tap water (79% i.e. 211 out of 267) followed by others (9.0% i.e.24 out of 267), tube well (5.6% i.e.15 out of 267), well (3.7% i.e.10 out of 267) and tanker (2.6% i.e.7 out of 269) respectively. In our country, the effectiveness of water supply system has been measured in terms of the concentration of free residual chlorine (FRC) present in the disinfectant system. So, the presence of free FRC should be checked in all parts of the system. The amount of FRC in water should normally be greater than 0.2 mg/l and less than 1 mg/l (Cheesebroug, 2000).

The prevalence of enteropathogens was highest in raw water user 53.6% (143 out of 267) followed by filtered water 34.8% (93 out of 267) and boiled water 11.6% (31 out of 267) users. This study showed that the boiled water was more appropriate for drinking purpose than raw and filtered water. Since, diarrhoeal disease represents the paradigm for water-borne disease, so selecting the best available water, and then providing the barriers of storage, filtration and disinfection, are demonstrably highly effective in prevention.

In this study, the prevalence of viral enteropathogens i.e. rotavirus was highest i.e.164 (27.02%) followed by parasite of 92 (15.16%) and bacteria 40 (6.6%). Similar study was conducted in Jordanian, in that study, stool samples were examined for 265 children under

5 years of age admitted to the pediatric ward, especially for parasites, rotavirus and enteric bacteria. A single enteric pathogen was detected in 50.9% of the children, and multiple pathogens were detected in 15.5%. The prevalence of enteropathogens identified was as follows: rotavirus (32.5%), EPEC (12.8%), EAEC (10.2), ETEC (5.7%), *Shigella* spp. (4.9%), *E. histolytica* (4.9%), *Salmonella* spp. (4.5%), *C. jejuni/coli* (1.5%), *Cryptosporidium* spp. (1.5%), EIEC (1.5%), *E. coli* (0.8%), *G. lamblia* (0.8%) and *Yersinia enterocolitica* (0.4%). No *V. cholerae,* Shiga toxin-producing *E. coli*, microsporidia, adenovirus or small round virus was detected (Youssef *et al*., 2000). This shows marked variation in the prevalence of enteropathogens in various places. The different prevalent rate of enteropathogens could be due to major factors like different geographical set up, economic status, living standards, customs and practices, hygienic condition, water and sanitary measures, time period of study carried out so on (Chand, 2000).

Among the different age groups the prevalence of enteropathogens were found to be highest in age group 0-2 years (50.2%) i.e.134 out of 267 followed by age group 2-4, 4-6, 6-8 and 8-10 years with 20.2, 15.0, 9.5 and 5.2% respectively.

In this study, the clinical features of patients in relation to the microorganisms were isolated. They were diagnosed by a physician as having acute diarrhoea, on the basis of frequent watery stools (usually more than three daily), lasting for less than 2 weeks. Diarrhoea may be accompanied by cramping, abdominal pain, bloating, nausea, or an urgent need to use the bathroom. In this study, the highest clinical symptoms shown by children were nausea and vomiting 323 (53.2%) followed by abdominal pain and vomiting 241(39.7%) and fever, abdominal pain and vomiting 43 (7.1%). The study also showed consistency of stool sample, out of 607 cases 370 (60.9%) had watery stool that was highest followed by 213 (35.1%) stool with mucous, 12 (1.9%) stool with mucous and blood and 12 (1.9%) rice watery stool.

Of 607 cases, highest number were treated with ORT 236 (39%) followed by intravenous fluid 165 (27%), ORS and IV 93 (15%) others 58 (10%) and antibiotics 55 (9 %). The

management of acute diarrhoea with ORT has led to a significant decline in acute diarrhea-related mortality in the developing world. However, improvement in the rates of use of ORT was unlikely to reduce mortality due to persistent diarrhoea (Osman, 1997). On basis of patient's condition and physician reports, patients were categorized into three groups i.e. severe, moderate and mild. Of them most of the cases were severe 323 (53.2%) followed by moderate 246 (41%) and mild 38 (6.3%) condition.

Rotavirus is known as a causative agent of winter diarrhoea. In this study, the prevalence of rotavirus infection was found to be 27.0%. The study showed that the highest rate of rotavirus infection was seen in hospitalized patients than the patients who visited OPD. The prevalence of hospitalized patients was 64 % (105 out of 164) and OPD was 36 % (59 out of 164). It was also known that rotavirus usually dominate in hospital based survey in children (Sherchand and Haruki, 2004). The study conducted by Sherchand *et al.,* during October 2001 to November 2002 showed a highest rate of infection among hospital children (38.6%) than village children (22.8%). Maharjan *et al.,* 2007 found the prevalence of rotavirus infection was highest in hospitalized patients 59.9%, which was significantly higher than in non-hospitalized patients 30.9%. The study conducted in Sentinel hospital in China during 1998 June 1999 found a total of 283 (95.6%) to be rotavirus infection in children under 5 years of age. The result was found to be higher than our findings.

Rotavirus infection is commonly prevalent in between age group 0-5 yrs. (Anathnarayan and Paniker, 1994). Similarly, this study showed that the infection rate was found to be higher in age group 0-2 yrs holding 30.3 % (112 out of 370) followed by the age group 2-4 yrs (26.8%), 4-6 yrs (21.7%), 6-8 yrs (19.4%) and 8-10 yrs (11.4%). Rotavirus was a major cause of pediatric gastroenteritis and responsible for causing half of the cases to be suffered with acute diarrhoeal illness among hospitalized patients of 6-24 months of age (Sherchand *et al.,* 1992).

Rotavirus was predominant in winter, it accounted for 45.1% in winter season followed by spring season 24.7%, autumn season 24.1% and summer season 14.1% among total

rotavirus positive cases. The result was consistent with the finding of Sherchand *et al.*, 2004 in which rotavirus was predominant in winter particularly in December to February accounting for more than 60% of pediatrics diarrhoea. The prevalence of rotavirus was highest in the patients, who used municipal tap water for drinking purpose 75.6% (124 out of 164). The symptoms of rotavirus infection range from mild fever, nausea, vomiting, abdominal pain, diarrhoea and dehydration. In this study, these symptoms were noted to high nausea and vomiting percentage than abdominal pain and fever. A potent vaccine is urgently needed to reduce the incidence and severity of the disease.

In this study, the prevalence of parasitic infestation was second most etiological agent that causes diarrhoea in children. It was found 15.2% positive cases among the total sample processed. As study conducted by Chand 2000 and Lama 2006 at TUTH had found highest parasitic prevalence rate i.e 27.9% and 44.4% respectively. This was very much higher than our findings. This might be due to the reason that many of the children received antibiotics prior to attending hospital and also during hospital stay. Therefore, there was a strong possibility that certain parasitic infection were missed during the study period. On the other hand, the government policy for antihelminthic program launched at community level, which may be another reason for lowest parasitic prevalence rate in this study. Apart from this, primary school children in selected districts are being provided with deworming tablets twice yearly by World Food Program, Plan Nepal and Save the Children (US).

The parasitic infestation showed higher in outpatient department (53.3%) than the hospitalized patient (46.7%) among total positive cases of parasite. The prevalence of parasites were found to be equal in both males (15.0% i.e.59/394) and females (15.5 % i.e.33/213) among total cases. The prevalence of parasites were high in the season summer 43 cases (43/92) followed by autumn season 21 cases (21/92) spring season 16 cases (16/92) and winter season 12 cases (12/92). This may be due to favorable condition for proliferation of parasites during summer season. Parasitic infection was found highest in

children using tap water (69.6%i.e. 64/92), followed by other (9.8%i.e.9/92); tube well (8.7% i.e.8/92); well (7.6% i.e. 7/18) and tanker (4.3% i.e. 4/92).

The *Shigella* spp. was highest in number constituting 20 (50%) followed by. of *Vibrio* spp. of 12 (30%) and *Salmonella* spp. of 8 (20%) of the total bacterial pathogens. *Shigella* and EIEC are non-zoonotic bacteria that are transmitted primarily by person-to-person spread, but part of the reason for the high prevalence could also be due to exposure to wastewater contaminated with human faeces (Desselberger, 1998). Clinically, the infection was characterized by fever and abdominal. The similar study was conducted in health centers in Hamedan province, Islamic Republic of Iran, investigated the frequency of *E. coli*, *Shigella* and *Salmonella* species in stool specimens from patients with diarrhoea of 144 samples, *Shigella* strains were isolated in 17 cases (11.8%): 10 *S. flexneri*, 3 *S. sonnei*, 2 *S. boydii* and 2 untyped strains. No *Salmonella* strains were isolated. Using molecular diagnostic methods, diarrheogenic *E. coli* were detected in 37 cases (25.7%), the majority were ETEC (22) and Shiga toxin-producing (STEC) strains (15) (Alizadeh *et al.*, 2007).

In this study, among the total bacterial infection, the prevalence rate of bacterial enteropathogens was highest in age group 0-2 years (32.5%) followed by 8-10 years (25%), 2-4 years (20%), 4-6 years (12.5%), and 6-8 years (10%) among the total bacteria positive. The highest prevalence of bacterial infection in age group 0-2 years and 8-10 years might be due the introduction of food that may be contaminated with faecal pathogens and also children of age group 8-10 years were more likely to eat street foods.

Globally, *Salmonella* and *Shigella* remain the major contributors to acute enteric infections and diarrhoea (Abu *et al.*, 2007). Bacillary dysentery or shigellosis is being a public health problem mainly in developing countries especially below 10 years. Shigellosis mainly a pediatric disease is about 80% of infections occurred under 10 years old (Blaser *et al.*, 1983). In this study, *Shigella* spp. showed highest bacterial etiological agent that causes diarrhoea in children. *Shigellae* are highly communicable enteric pathogens, as illustrated by the experimentally determined infectious dose of 10-100 organisms for North American

adult volunteers (DuPont *et al*., 1989). Among total stool sample processed, 20 (3.3%) found to be *Shigella* spp. Shigellosis is a highly contagious disease of poor and crowded communities, with faeco-oral (hand-to-mouth) transmission, and an extremely low minimum infectious dose (Nicolas *et al*., 2007). Among the bacterial isolates prevalence of *Shigella* spp. was highest in age group 0-2 yrs (50% i.e.10/20) followed by age group 4-6 (20% i.e. 4/20), 8-10 (15% i.e. 3/20), 2-4 (10% 2/20) and 6-8 yrs (5% i.e. 1/20). These age-specific trends suggested that significant risk factors for acquisition of *Shigella* infections include, weaning from breast milk and introduction of children into day care centers that have an inherent potential for faeco-oral transmission of intestinal bacteria. Secondary transmission of *Shigellae* can also occur at a rate exceeding 50% in households with young children (Hale, 1998). The prevalence of *Salmonella* spp. was high in age group 2-4 yrs (50% i.e. 4/8). The global burden of typhoid is estimated as some sixteen million cases and six million deaths each year (Pang *et al.,* 1995). Important cause of bacterial food poisoning in Nepal is reported as *S. enteritidis* and *S. typhimurium* belonging to S*almonella* group (Shrestha *et al.,* 1994). The frequency of isolation was 1.8% (65/3570) for *Salmonella* spp. (Abu *et al.,* 2007). In this study, among total cases, 20 (3.29%) found to be *Shigella* spp. and the prevalence of *S. dysenteriae* is 12 (60%), *S. flexneri* is 6 (30%) and *S. boydii* is 2 (10%). Similarly, the prevalence of *S. typhi* in 0.6% followed by *S. paratyphi* A in 0.3% (2 out of 607) and *S. paratyphi* B in 0.3%.

The prevalence of *V. cholerae* was found higher in age group 8-10 yrs (50% i.e.6/12); followed by age group 6-8 yrs (24% i.e. 3/12), 2-4 yrs (16.7% 2/12), and 4-6 yrs (8.3% i.e. 1/12). With regard to gender wise distribution, the prevalence *V. cholera* was found to be highest in male constituting 66.7% (8/12). The study done by Pokharel *et al.,* 1997, in which it was reported that the outbreak of cholera generally occurred in Nepal at the end of June to September each year with peak period being June to August suggesting favorable condition for its proliferation. *V. cholera* has fully adapted itself in Kathmandu valley environment and shows periodic endemicity. The spread pattern suggested water borne infection related to contamination river water in Nepal was confirmed by field survey study

(Chand, 2000). In this study, *V. cholera* was found highest in July (7 cases) followed by June (3 cases) and August (2 cases) but there was no single cases found in September.

Out of 607, 30 (11.2%) cases showed positive only for bacteria (either *E. coli* or *Salmonella* spp. or *Shigella* spp. or *V. cholera*), 75 (28.1%) cases showed positive for only parasites and 133 (49.8%) cases were positive for only rotavirus.

The co-occurrence of enteropathogens was also found in this study. There were 29 cases (10.9%) of co-infections either with bacteria and rotavirus; parasites and rotavirus; or bacteria and parasites and that of bacteria and parasites. Co-infection of rotavirus and bacteria was 0.8% co-infection of rotavirus and parasite was 7.1% and that of bacteria and parasites was 3%. There were 8 cases (3%) of multiple parasitic infections with two or more than two parasites, the frequency being highest (1.5%) in combination protozoa and protozoa. But there was not a single case of multiple bacterial infections found.

The diarrhoeal disease mainly concerned with impure water, low socio-economic state, poor sanitation coupled with low literacy rates of parents particularly the mothers were the main causes of this prevalent malady. So, proper health education system should be developed.

6.2 CONCLUSION

After conducting this study, it can be concluded that lots of enteropathogens were involved in children diarrhoea. Among them virus were predominant followed by parasites and bacteria. Children under 2 years were more affected age groups which were directly related with mother's hygiene. So, the disease burden may be the cause of lack of health education, contaminated drinking water supply and poor hygienic condition. The information about the prevalence of a wide range of enteropathogens should facilitate the control and management of diarrhoeal diseases among infants and children in the country. In this study,

the large number of rotavirus infections suggests that these organisms are important causes of children diarrhoea.

CHAPTER VII

7. SUMMARY AND RECOMMENDATION

7.1 SUMMARY

In this study altogether 607 diarrhoeal stool specimens of the children under 10 years were collected both from ORT center and OPD of Kanti Children's Hospital, Maharajgunj, Kathmandu from October 2006 to September 2007. The samples were processed at Health Research Laboratory, IOM, Maharajgunj.

A total of 607 cases were examined for enteric pathogens with clinical sign, symptoms and other features of children in this study. Questionnaire was taken from the patients having acute diarrhoea both from ORT centre and OPD. Stool samples were processed for bacteria, parasites and virus. Bacteria were isolated and identified by microscopy, culture and biochemical tests. Parasites were identifying microscopically and EIA for rotavirus detection.

Out of 607 cases, the enteropathogens found higher in ORT center 169 (63.3%) than OPD 98 (36.7%). Similar cases were observed in bacterial and viral infection i.e. bacterial infection inpatient 29 (72.5% i.e. 29/40) than in hospital outpatient 11 (27.5% i.e.11/40) and rotavirus was highest 105 (64%) in ORT center among total rotavirus positive cases than OPD i.e. of 59 (36.0%). The parasite infestation was highest 53.3 % (49/92) in OPD than ORT center i.e. of 36.0 % (43/92).

In this study, the enteropathogens were found highest in age group 0-2 years (50.2%) i.e.134 out of 267 and least in age group 8-10 years with 5.2%. Among the total bacterial isolates, the prevalence rate was highest in age group 0-2 years (32.5%), the rotavirus infection rate was found to be higher in age group 0-2 years holding 30.3% i.e.112 out of 370 and the highest prevalence 33.3% (12/36 cases) of parasites was seen in age group 6-8 years.

The potential either of enteropathogens were identified in 267 (44%) cases. The prevalence of rotavirus was 27%, parasites were 15.2% and bacteria were 6.6% among total cases.

Among the total bacterial pathogens, *Shigella* spp. 3.3% followed by *V. cholera* 2.0% and S*almonella* spp. 1.3% of total processed samples. Among parasites, protozoan (12.2%) dominated over helminthes (2.8%). Among the protozoa, *E. histolytica* (6.9%) was major causative agent of diarrhoea followed by *G. lambia* (3.8%), *C. cayetanensis* (1.2 %) and *Entamoeba coli* (0.3%). Among the helminthes, *A. lumbricoices* was the major pathogen constituting 1.3% followed by *T. trichiura* (0.8%), Hookworm (0.5%) and *H. nana* (0.3%) of the total cases.

Out of 607, 30 (11.2%) cases showed positive only for bacteria, 75 (28.1%) cases showed positive only for parasites and 133 (49.8%) cases were positive for only rotavirus.

The prevalence of enteropathogens showed highest in males 68.2% i.e.182 out of 267 than females 31.8% i.e. 85 out of 267, which was found statistically not significant (P>0.05).

Ethnic group wise distribution of enteropathogens revealed that the groups Magar/Rai/Gurung/Limbu/Tamang were highest 40.1% among total positive cases.

The highest numbers of samples (74) were processed in July and least (33) were processed in September. The prevalence of enteropathogens was found highest in the month of July 11.2% of total enteropathogens positive cases followed by January (10.8%), February (9.4%), and August (9.0%).

Among the different sources of water, enteropathogens were found to be highest in children using tap water (79% i.e. 211 out of 267) and in raw water user 53.6% (143 out of 267). The prevalence of enteropathogens was found higher in children who didn't wash hand before meal 71.9% (192 out of 267) than wash hand before meal 28.1% (75 out of 267). The prevalence of enteropathogens was found to be higher in children with illiterate mother

constituting 61% than literate mother constituting 39% which was statistically significant at 5% level of significance. The enteropathogens were found to be highest in children with father's having occupation service.

Out of total cases, 370 (60.9%) showed watery stool. In hospital, highest number were treated with oral rehydration therapy 236 (39%) followed by intravenous fluid 165 (27%), ORS and IV 93 (15%) others 58 (10%) and antibiotics 55 (9 %). On the basis of degree of dehydration, most of the cases were severe 323 (53.2%) followed by moderate 246 (41%) and mild 38 (6.3%). In this study, the highest clinical symptoms shown by children were nausea and vomiting 323 (53.2%) followed by abdominal pain and vomiting 241 (39.7%) and fever, abdominal pain and vomiting 43 (7.1%).

The *Shigella* spp. infection was found highest in June (6 cases) followed by April and August (3 cases of each month), May, October and November (2 cases in each month) and February and July (1 cases in each month). The *V. cholera* was found highest in July (7 cases) followed by June (3 cases) and August (2 cases) there was no *Vibrio* isolated in May in this study. More specifically *Salmonella* infection was found highest in October and November (2 cases) followed by March, May, June and July (1 cases) and no *Salmonellae* were detected in January, February, April, August, and September.

7.2 RECOMMENDATION

- The study was conducted only in Kanti Children's Hospital of Kathmandu. So, it does not give total picture of whole country. Therefore this kind of study should be carried out throughout the country to get exact picture.

- In this study, only *Shigella* spp. *Vibrio* spp. and *Salmonella* spp. were investigated. Further research on *Campylobacter, Yersinia* and pathogenic *E. coli* should be done.

- Rotavirus infection and diarrhoea have shown strong association and there is no specific treatment for rotavirus infection. Thus conventional symptom based treatment practice seems the most appropriate for the management of rotavirus diarrhoea, therefore existing practice of case management should be continued.

- Bacterial, rotavirus and intestinal parasitic infestation was found to be transmitting through poor personal hygienic. Therefore health education regarding methods of prevention of feacal contamination and increasing personal hygienic need to be given especially to mother and those who take care of child.

- Serological studies should be done to ascertain the specific identification of etiological agent.

8. REFERENCES

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