CHAPTER I

1. INTRODUCTION

Gastroenteritis is an infection the gastrointestinal tract that may be acute or chronic. It is characterized by diarrhoea and symptoms of gastric irritation (e.g. Nausea, vomiting, epigasstric pain). Diarrhoea is the most common presenting symptoms of lower intestinal tract infection. When there are more than three times bowel movements daily and the stool takes a form that is softer than or more liquid than usual, it is called diarrhoea (Chakraborty, 2005). Diarrhoea may also be defined as increase in frequency, fluidity or volume of bowel movements relative to usual habits of each individual (Pohkrel, 2004). According to pathogenecity, three clinical syndromes of diarrhoea are defined as: acute watery diarrhea (lasts less than 14 days), dysentery (faeces with mucus and blood) and persistent diarrhoea (lasts at least14 days).

Diarrhoeal disease is one of the major public health problems among the children under five years in developing counties like Nepal. In 2003/04 nations wide, total diarrhoeal visits in were 787,094, total diarrhoeal deaths were 194. The incidence of diarrhoea in children under five years were 222/1000 population and case fatality rate of children under 5 years were 0.2/1000 population (HMG, 2003/04). The disease burden of diarrhea disease is estimated at 6,25,41,000 DALY'S in 2001 (WHO, 2002). In some developing countries, children have more than 12 episodes of diarrhoea per year and diarrhoeal disease account for 15-34% of all deaths. Conservative estimates place the death toll from diarrhoea disease at 4 to 6 million deaths per year with most of these occurring in young children. The diversity of bacterial and viral infections that may cause diarrhoea complicates accurate surveillance and diagnosis especially with little or no access to modern laboratory procedures (WHO, 2002). About 60% of deaths due diarrhoea occur in the first two years of life The main cause of death due to diarrhoea is dehydration, dysentery malnutrition and other infections (WHO, 1992).

Immense microflora are implicated in gastroenteritis. Some of them are parasites like *Entamoeba histolytica*, *Giardia* and bacteria like *Shigella, E. coli, Salmonella*, *Vibrio*

cholerea, etc. Some are recently recognized during last few decades such as bacteria like *Campylobacter jejuni, Enteroadnerant E.coli, Helicobacter pylori*, etc, protozoans like *Cyclospora cayetanensis, Cryptosporidium parvum* and variousdiartrhoeal viruses such as rotavirus Adeno virus, Norwalk agents, etc (Farthing and Keush, 1989).

Cyclospora cayetanensis is an obligate, intracellular, coccidian like protozoan parasite that causes prolonged diarrhoea in humans worldwide. Although *C. cayetanensis* infection has recently been reported in non-travelers in USA and CANADA (Serpetini et al., 1998). The C*yclospora* infection produces a characteristic illness that produces clinical symptoms such as gastroenteritis associated fever, vomiting and frequent watery diarrhoea, fatigue, and abdominal cramp, anorexia and weight loss.

From 1990 to 2000, there were 11 food borne outbreaks of Cyclosporiasis in North America that affected at least 3600 people (Mansfield and Gajadhar, 2004).

Rotavirus infections are responsible for approximately 3 million cases of diarrhoea and 55,000 hospitalized cases of diarrhoea and dehydration in children under 5 years old each year in the US. 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 death as many as 600,000 children each year (Hendrich et al, 1995). Globally in year, it is assumed that rotavirus causes approximately 111 million episodes of gastroenteritis requiring only home care, 25 million clinic visits, 2 million hospitalization and 352,000-592,000 deaths (median 440000 deaths) in children below 5 yearss of age (Parasar et al., 2003).

There are only few studies on Rota viral diarrhoeal disease and Cyclosporiasis in Nepal (Sherchand et al, 2004). A study conducted in Kanti children's hospital in 1992 by Sherchand et al showed that 27% of 198 children were infected with rotavirus and a similar proportion of diarrhoeic children attending general practitioner, 275 of 52 were infected (Sherchand et al, 1992). Another study conducted in Kathmandu showed that 16.2% of 714 samples from children were positive for rotavirus. Rotavirus was found in

6 months to 2 years of age and infection was predominant in winter season (Sherchand et al, 2004).

In a study carried out by Sherchand and cross, 2001, in different health care units in kathmandu, 24.6% stool samples were found to be positive for *C. cayetanensis* with 41.9% infection in children of age group 3-5 years in 1995 and 1998.

In present study, both Rota viral infection and cyclosporiasis were studied. EIA (Enzyme Immuno Assay) was used for detection of rotavirus in stool samples and routine microscopic examination has been parformed for detection of *C. cayetanensis* and other enteric parasites. Besides, culture of same stool samples was done to identify bacterial pathogens.

This study was conducted to reveal the existing condition and other related factors in the spreading of disease in the socio regional background of our country. Furthermore, it was assumed that present study on etiological agents may establish certain data in one hand and it will also be useful for further research work in this field. So this study has been carried out in order to fulfil the following objectives.

CHAPTER II

2. OBJECTIVES

2.1 General objectives

The aim of this study is to find out the possible cause of gastroenteritis in children with special attention to parasites and viruses.

2.2 Specific objectives

- To find the prevalence of enteropathogens in children.
- To evaluate the relation of the age and gender with prevalence of enteropathogens.
- To find out prevalence of *Cyclospora caytenansis* in relation to age, gender, month and habit of drinking water of the patient.
- To compare the rotavirus infection between diarrhoeal and non- diarrhoel cases.
- To Asses the parasites infestation related with rotavirus.

CHAPTER III

3. LITERATURE REVIEW

3.1 DIARRHOEA

According to oxford dictionary (4th Edition, 1998) diarrhoea is an illness in which waste matter is emptied from the bowels frequently and in a liquid form. Diarrhoea is the most common presenting symptom of lower intestinal tract infection. According to him, when there are more than three bowel movements daily and the stool takes on a form that is softer than usual, it is called as diarrhoea (Chakraborty, 2005). Though many use variety of terms to describe diarrhoea, three kinds of clinical features have been defined. These are briefly defined below:

3.1.1 Acute watery diarrhoea (AWD)

This term refers to diarrhoea that begins acutely, lasts less than 14 days, and involves the passage of frequent loose or watery stools without visible blood. There is possibility of occurrence of vomiting and fever and it causes dehydration. The most significant etiological agent of acute watery diarrhoea in young children in developing country is rotavirus, enterotoxigenic *E.coli, Shigella* spp*, Campylobacter jejuni* and *Cryptosporidium.* In some area *V. cholerae*01, *Salmonella* and enteropathogenic *E.coli* are also important.

3.1.2 Dysentry

It is characterized by the frequent passage of stools with low volume that contain pus, mucus, and blood and accompanied by fever, cramping abdominal pain, and tenesmus (Chakraborty, 2005). It is caused especially by *Shigella flexneri* and *Shigella dysenteriae*. Other cause includes *Campylobacter jejuni, E.coli, or Salmonella* species. *Entamoeba histolytica* can cause serious dysentery in young children (WHO, 1995) and is known as amoebic dysentery and former is known as bacillary dysentery. A table is

appended below to show the microscopic and macroscopic difference between the stool of amoebic and bacillary dysentery.

Table 1 Differences between Amoebic and Bacillary dysentry

	Amoebic dysentry	**Bacillary dysentery**
Macroscopic		
Number	6-8 motions per day	Over 10 motions per day
Amount	Relatively copious	Small
Odour	Offensive	Odourless
Color	Dark red	Bright red
Nature	Blood and mucus Mixed with feaces	Blood and mucus; No feaces
Reaction	Acid	Alkaline
Consistency	No adherant to the Container	Adherant to the bottom Of the container
Microscopic		
R.B.C	In clumps; reddish- Yellow in color	Descrete or in rouleaux; bright red in color
Pus cells	Scanty	Numerous
Macrophages	Very few	Large and numerous; many of them contain R.B.C; hence mistaken for *E.histolytica*
Eosinophils	Present	Scarce
Pyknotic bodies	Very common	Nil
Ghost cells	Nil	Numerous
Parasite	Trophozoite of *E.histolytica*	Nil
Bacteria	Many motile bacteria	Nil
Charcot-Layden Crystals	Present	Nil

Source: Chatterjee, 2002

3.1.3 Persistent diarrhoea

This is diarrhoea that begins acutely but is of unusually long duration (at least 14 days). The episode may begin either as watery diarrhoea or dysentery. Diarrhoeal stool volume may also be large with a risk of dehydration. There is no single microbial cause, although *Shigella* spp, *Salmonella* spp , Enteroagreesive *E.coli*, and *Cryptosporidium* may play a greater role than other agents. It is associated with extensive changes in the bowel mucosa, esp. flattening of villa reduced production of disaccharides enzymes, which causes reduced absorption of nutrients and may perpetuate the illness (WHO, 1992). Persistent diarrhoea should not be confused with chronic diarrhoea, which is recurrent or long lasting diarrhoea due to non-infectious causes such as sensitivity gluten or metabolic disorders (WHO, 1994).

3.2 ANATOMY AND GENERAL FEATURES OF GASTROINTESTINAL TRACT

The gastrointestinal tract is actually in continuation with the external environment. Ingested materials that enters the gastrointestinal tract passes through the stomach, through the duodenum, the jejunum, the ileum, and finally through the caecum and colon to anus. The duodenum, jejunum, and ileum are collectively known as the small intestine and the caecum and the colon comprises the large intestine. The small intestine is lined with the small projections; called villi that greatly increase the surface area. The function of the villi is ti absorb fluids and nutrients from the intestinal contents. Other cells of the lining secrete fluids and metabolites into the lumen. Many of the cells in the lining of the large intestine are numerous and mucus secreting and there are no villous projections into the lumen. The cells lining the large intestine reabsorb the remaining excess fluid within gastrointestinal tract before waste is finally discharged through anus. The lining of the gastrointestinal tract including the cells and their closely adjacent secretion is called the mucosa. The appendix, a long narrow tube of bowel tissue extends from the ceacum, near the ileum. The appendix may become inflamed and become gangrenous when there is a state within its lumen or when there is an adjacent

inflammatory process. Appendicitis is the most common initial event leading to abdominal abcess in children.

The gastrointestinal tract contains a vast normal flora. Although the acidity of the stomach prevents any significant colonization in a normal host, many species can survive passage through the stomach to become resident within the lower intestinal tract. Normally, the upper small intestine contains only a sparse flora 10 to 10^3 bacterium per milliliters. *Enterobacteriaceae* and *Bacteroides* are also present in this region. The normal flora of gastrointestinal tract is greatly influenced by diet (Forbes et al., 2000). Microorganisms, which may form a part of this normal flora and become opportunistic pathogens include:

Gram-positive: *Enterococci,* Anaerobic Streptococci, *Proteus* spp, *Lactobacilli* spp , and *Clostridia* spp .

Gram negative: *Escherichia coli, Enterobacter, Hafnia, Citrobacter, Providencia, Morganella, Serratia, Klebsiella, Bacteroides, Psuedomonas aeuroginosa*

Fungi: *Candida* species and yeasts.

Others*: Mycoplasma* and a variety of protozoa and viruses.

Source: Cheesebrough, 2000

Table 2 Clue for the identification of enteropathogens

Appearance	Possible Pathogens
Unformed containing pus and mucus mixed with pus	Shigellosis; Campylobacter enteritis
Unformed with blood and mucus (acid pH)	Amoebic dysentery
Unformed or semiformed often with blood and mucus	Schistosomiasis
Watery stool	ETEC infection, Rota virus enteritis
Rice watery stools with mucous flakes	Cholera
Unformed or watery and sometimes with blood, mucus and pus	Salmonellosis
Unformed, pale colored, frothy, unpleasant smelling stools that float on water (high fat	Giardiasis, other conditions that cause malabsoption e.g.

content)	Post infective tropical malabsoption
Fluid stools (containing lactose with pH below 6)	Lactase deficiency
Unformed or semiformed black stools (positive occult blood test)	Malaena (gastrointestinal bleeding), Hookworm diseases, Iron therapy

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

Source: Cheesbrough, 2000

3.3 IMMUNITY AGAINST INFECTION IN GASTROINTESTINAL TRACT

The structure of the gastrointestinal tract determines the localization of the microbial flora and to some extends, the composition of the flora as well. Microbial habitat may exist in any area from mouth to anus. Each habitat provides a different kind of environment or nutritional challenge. Some of the factors responsible for the prevention of infection are:

3.3.1 Presence of normal flora

The presence of normal flora from mouth to anus prevents the colonization by pathogenic organism. The 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. 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.

3.3.2 Acidity of stomach

The inner lining of the stomach contains millions of glands that secrete mucus and various other components of gastric juice. Hydrochloric acid secreted by stomach keeps the pH of the stomach extremely low upto 1 to 2 and thus the acidic environment provides a barrier to establishing disease.

3.3.3 Normal peristalsis

The normal propulsive movement of gastrointestinal tract decreases the possibility of adherence of foreign pathogenic organism to the mucosa.

3.3.4 Flow of liquid

The continous flow of liquid between intestinal wall and blood vessel is also an important factor of defense.

3.3.5 Shedding and Replacement of epithelium and lymphoid tissue (Peyer's Patches)

These structures also provide defense against pathogens to attach on mucosal layer.

3.3.6 Secretory IgA and Phagocytic cells within in Gut

Secretory immunoglobulin A (IgA) and phagocytic cells within the gut is particularly active against the parasites.

3.4 MICROBIAL FACTORS IN PATHOGENESIS OF GASTROINTESTINAL INFECTION

The ability of an organism to cause GI tract 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 posses one or more factors that allows it to overcome these host defenses, or it must enter the host at a time when the one or more of the defense system is inactive (Forbes et al., 2000). Depending on how they interact with the human host, enteric pathogens may cause disease in one or more following ways:

3.4.1 By proliferating within or close to intestinal mucosal cells and destroying them thus disrupting the function

Viruses such as rotavirus, replicate within the villous eoithelium of small bowel, causing patchy epithelial cells destruction and villous shortening. The normal absorptive villous cells and their temporary replacement by immature secretory, crypt like cells cause the intestine to secrete water and electrolytes.

3.4.2 Producing a toxin that effects fluid secretion, cell function, or neurological function

Enterotoxigenic *E. coli, Vibrio cholerae, Shigella* spp , *Salmonella* spp , and some other bacteria produce toxin that alters epithelial cell function. These toxins reduce the absorption of sodium by the villi and may increase the secretion of chloride in the crypts, causing secretion of water and electrolytes.

3.4.3 By invading the mucosal epithelium, causing cellular destruction and occasionally invading the blood stream and going on to systemic disease

Protozoa like *Entamoeba histolytica*; bacteria like *Shigella* spp , *Campylobacter jejuni,* Enteroinvasive *E. coli and Salmonella* spp can invade and destroy mucosal cells resulting in bloody diarrhoea. This occurs mostly in coon and distal part of the ileum. Invasion may be followed by formation of micro abscesses and superficial ulcers, hence the presence of red and white blood cells or visible blood in the stool. These organisms prduce toxin that causes tissue damage and possibly also mucosal secretion of water and electrolytes

3.4.4 By adhering to the intestinal mucosa, thus preventing the normal function of absorption and secretion

Bacteria that multiply within the small intestine must first adhere to the mucosa to avoid being swept away. Ashesion is through superficial hair like antigens termed as pilli or fimbriae that bind to receptor on the intestinal surface. Bacterial genera ETEC, *Vibrio cholerae*01, and protozoan like *G.lamblia and Cryptosporidium* cause diarrhoea by this mechanism. Mucosal adherence is associated with changes in the gut epithelium that may reduce the absorptive capacity or cause fluid secretion (Forbes et al., 2000).

3.5 AETIOLOGICAL AGENTS OF GASTROENTERITIS

Immense microflora including bacteria, parasites, fungi, and viruses are implicated in GI infection. The most common enteric pathogens encountered in the stool of diarrhoeal patients are listed below but only few organisms will be taken into in detail.

Bacteria

Gram Positives: *Clostridium perfringens* type A and C, *Clostridium difficle, Bacillus cereus* and *Staphylococcus aureus.*

Gram Negatives: *Shigella* species, *Shigella* species, *Campylobacter* species, *Escherichia coli (***ETEC, EPEC, EHEC, EIEC***), Vibrio cholerae, Yersinia enterocolitica and Aeromonas hydrophila.*

Viruses: Rotaviruses, Norwalk agent, Coxsackie viruses, Echoviruses and Polioviruses.

Fungi*: Candida albicans*

Parasites:

Amoeba: *Entamoeba histolytica*

Flagellates: *G. lamblia, Trichomonas hominis*

Ciliates: *Balantidium coli*

Larvae: *Strogiloides stercoralis*

Cysts: *Entamoeba histolytica, Giardia intestinalis, Isopora belli (Oocysts), Cryptosporidium (Oocysts), Cyclospora caytanensis (Oocysts)* and *Balantidium coli*

Ova of: *Ascaris lumbricoides, Hookworm, Trichuris trichuria, Schistosoma* species, Fasiolopsis buski, *Fasciola hepatica, Paragonimus* species, *Taenia* species, *Hymenolepsis* species, *Diphylobothrium* species, *Opisthorchis* species, *Heterophyles heterophyes, Dipylidium caninum, and Enterobbius vermicularis*.

Source: Cheesbrough, 2000

3.5.1 BACTERIA

There are large numbers of bacteria involved in gastroenteritis. Some of the important ones are as follows:

3.5.1.1 Salmonella

Salmonella spp. is well grown in Salmonella-Shigella agar with black centered transparent colony. *Salmonella* spp. possesses one or more 'O' somatic antigen, flageller 'H' antigen, and Virulence Vi antigen. Upto now more than 2500 serovars of combination of somatic 'O' and flageller (H) antigens of genius *Salmonellae* have been described. The modified Kauffman-White scheme gives specific serological differentiation, which constitutes a powerful epidemiological tool (Christie, 1980).

Salmonellosis is an important enteric disease and it includes several clinical syndromes like enteric fever, septicaemia, focal infections, and an asymptomatic carrier state. Particular serovars show a strong tendency to produce a particular type of syndrome (*S. typhi S. paratyphi*A, and *S. schottmuelleri* produceenteric fever; *S. cholerae-suis* produces septicaemia or focal infections; *S. typhimurium* and *S. enteitidis* 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 organism enters via the oral route usually with contaminated food or drink. The 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 factor that contributes to resistance to *Salmonella* infection are gastric acidity, normal intestinal microbial flora and local intestinal immunity (Brooks et al., 2004).

After ingestion, the organism colonizes the ileum and colon, invade the intestinal epithelium, and proliferate within the epithelium and lymphoid follicles. After invading the epithelium, the organisms multiply intracellularly and then spread to mesentery lymph nodes and throughout the body via systemic circulation; the reticuloendothelial cell then take them up. The e reticuloendothelia system controls the spread of the organism. However, depending on serotype, some may infect the liver, spleen, gallbladder, bones meninges, and other organs, most serovars are e killed promptly in

extra-intestinal sites, and the most common human *Salmonella* infection, gastroenteritis remains confined to the intestine.

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

The incubation period for *Salmonella* depends on the number of bacteria. Symptoms usually begin 6 to 48 hours after ingestion of contaminated food or water and take the form of nausea, vomiting diarrhoea and abdominal pain. Mayalgia 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 systemic forms of Salmonellosis. The best-studied enteric fever is typhoid fever, the form caused by *S. typhi*, but any species of *Salmonella* may cause this type of disease. The symptoms begin after an incubation period of 10 to 14 days. Enteric fever may be preceded by gastroenteritis, which usually resolves before the onset of systemic disease. The symptoms of enteric fevers are non-specific and include fever, anorexia, headache, myalgia, and constipation. Enteric fevers are severe infections and may be fatal if antibiotic are not promptly administered (Threlfall et al., 1992).

Salmonellosis is caused by animal pathogens through food and drink, particularly poultry products including eggs and is acquired via faecal-oral route. Foodstuffs get contaminated by polluted water or via the hands of the carriers. Typhoid mary a cook in USA was a famous carrier and responsible for outbreaks in USA.

Contaminated food is a major mode of transmission for non-typhoidal *Salmonellae* because salmonellosis is a zoonosis and has an enormous animal reservoir (Chakraborty, 2005).

The epidemiology of the typhoid fever and other enteric fevers primarily involves person- to -person spread because these organisms lack a significant animal reservoir. Contamination with the human faeces is the major mode of spread, and the usal vehicle is contaminated water. Approximately 3% of person infected with *S. typhi* and 0.1% of those infected with non - typhoidal salmonellae become chronic carriers (Chakraborty, 2005).

General salmonellosis treatment measures include replacing fluid loss by oral and intravenous routes and controlling pain, nausea, and vomiting. Typhoid fever and enteric fevers should not be treated with antibiotics. *Salmonella* are difficult to eradicate from the environment. However, because the reservoir is poultry and livestock, reducing the number of *Salmonella* harbored in these animal reservoirs would significantly reduce human exposure.

Vaccines are available for the typhoid and are partially effective, especially in children. No vaccines are available for non- typhoidal salmonellosis.

3.5.1.2 Shigella

Shigellosis is an acute infection of intestine with frequent passage of blood stained mucopurulent stool and caused by the genus *Shigella*. Bacillary dysentery constitutes a significant proportion of acute untestinal disease in the children of developing countries. It is one of the major cause of mortality in the developing countries and causes most of the estimated 370,000 deaths from dysentery occur worldwide each year in children under five years (WHO, 1996).

Each of the approximately 40 serotypes of shigella is divided into four groups depending on serological similarities and fermentation reaction. Their 'O' antigens characterize serotypes. The four named groups of *Shigella* are as follows:

S. dysenteriae (Serological group A)

S. flexneri (Serological group B)

S. boydii (Serological group C)

S. sonnei (Serological group D)

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

Shigella species are pathogens of humans and other primates, and the pathogenesis of infection with these bacteria and enteroinvasice S. coli (EIEC) is very similar. The infective dose is small; bacllary dysentery may follow the ingestion of as few as 10 viable bacteria. The first site of infection is the M cells in the Peyer's patches of the large intestine. Since the infective dose for these organisms is small, *Shigella* might have innate tolerance to the low pH and bile encountered in the human digestive tract (Greenwood et al., 2003).

Association with the intestinal mucosa initiates mucosal inflammation leading to apoptosis, which is thought to facilitate the invason of the M cells, after which the bacteria are phagocytosed. The *Shigella* multiplies within the epithelial cells and spread into the adjacent cells and deep into the lamina propia. The infected epithelial cells are killed and the lamina propia and submucosa develop an inflammatory reaction with capillary thrombosis. Patches of necrotic epithelium are sloughed off and ulcers forms. The cellular response is mainly by polymorphonuclear leucocytes, which can be seen readily on microscopic examination of the stool, together with red cells and sloughed epithelium. Dysentery bacilli rarely invade other tissues. Transient bacteraemia can occur but septicaemia with metastatic infection is rare.

S. dysentriae serotype 1 expresses Shiga toxin, an extremely potent, ricin like, cytotoxin that inhibits protein synthesis in mammalian cells. This toxin also has enterotoxic activity in rabbit ileal loops, but in human diarrhoea is unclear, since *Shigella* apparently expresses a number of enterotoxins. More importantly, shiga toxin is associated with the haemmolytic uraemic syndrome, a complication of infection with *S. dyseteriae* 1. Closely related toxins are expressed by enterohaemorragic E.coli (EHEC) including the potentially lethal, food borne serotypeO157-H7 (Rammamurty et al., 1992).

Shigellosis has two basic clinical presentations:(1) Watery diarrhoea associated with vomiting and mild to moderate dehydration, and (2) Dysentry characterized by a small amount of bloody, mucopurulent stools and abdominal pain. The incubaton period is usually between 2 and 3 days but may be as short as 12 hours. The onset of symptomis usually sudden and frequently the initial symptom is abdominal colic. This is followed by the onset of watery diarrhea, and in all but the mildest cases this is sccompanied by fever and malaise. Keush, 1979 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.

Infection with *S. dysentriea* 1 causes more severe, prolonged, and more frequently fatal illness than that infection by other *Shigella.* Antimicrobial resistance develops more quikly and occurs more frequently in JS. Dysentriea 1 than in other groups (WHO, 2003). Complications of Shigellosis, including rectal prolapses, toxic mega colon, bacteraemia, hyponatraemia, hypoglcaemia, and hypoproteinaemia occur mostly in children whose illness is clinically severe (Bennish et al., 1993).

Man is the only source of infection and there is no animal reservoir. The disease is transmitted by "finger, food, faeces, and flies"; from person to person by direct cintact or the faecal oral route. In general, *S. sonnei* is more common in developed countries where sporadic common source outbreaks ae transmitted by uncooked food or contaminated water and *S. flexneri* and *S. dysentriae* serotype 1 occur more frequently in developing countries. Shigellosis is endemic in developing countries and most cases occur in young children under 10 years of age. In tropical areas, about 5 million cases of shigellosis require hospitalization and some 600,000 patients die every year (Chakraborty, 2005).

3.5.1.3 Vibrio cholerae

Vibrio cholerae is classified by biochemical tests and further subdivided into serotypes 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' and 'O139' antisera are reffered to as non-O1 and non-O139 *V. cholerae.*

The 'O1' serogroup is divided into two biotypes, classical and EI Tor that can be differentiated by use of assays of haemolysis, haemagglutination, phage typing, polymixin sensitivity, and the Voges –Prokauer reaction. Each of the O1 biotypes can further be subdivided into two major serotypes, Ogawa and Inaba. Ogawa strains produce 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 three types of antigens but is rare and unstable.

Vibrios are ingested with contaminated weter or food and passes through the acid stomach and colonise upper small intestine. Colonization is aided by fimbria, filamentous protein structures called toxin coregulated pilus (TCP) extending from the cellwall, that attach to recepter on te mucosa and by the motility of the bacterium, whch 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. This high number of vibrios efficiently delivers enterotoxin to the mucosal cells.

The cholera enterotoxin also called as choleragen is a polymeric protein, that activates the adenylcyclase enzyme in the cells of the intestinal mucosa leading to increased levels of intracellular camp, and the secretion of H_2O, Na^+, K^+, Cl^- into the lumen of the small intest ine. 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 andelectrical gradients caused by the loss of Cl^-. Thus, the toxin damaged cells become pumps for water and electrolytes causing diarrhoea, which results in the loss of electrolytes, and severe dehydration that are characteristic of the cholera (Sathoporn et al., 2002).

After an incubation period of 18hrs to 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. In adults with severe cholera, the rate of diarrhoea may quikly reach 500-1000 ml/hr, leading to severe dehydration. Cholera is most commcnly transmitted through faecal-oral route via contaminated water and food.

In endemic areas, the incidence of cholera is highest in children, and decreases with the age due to aquired immunity. In non-endemic areas, choleraprevalence is not age – dependent, as the majority of the population has no immunity to the bacterium (Sack et al., 2004).

The control measure include a safe drinking water supply, cooking of high risk foods, improving sanitaion, and health education. Treatment of the cases decreases the case fatality rate from about 50% to some lower value, whuch includes replacement of fluids, and administration of proper antibiotic to shorted the illness. However, use of antibiotic greatly increases the risk of development of resistance.

3.5.2 PARASITES

A parasite is an organism that is entirely dependent on another organism, reffered to as its host, for all, or part of its life cycle and metabolic requirements.

Intestinal parasitic infections are among the most common infections in the world, being responsible forconsiderable 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)

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 most major public health problems of the country (Sherchand et al., 1997).

Some of the important intestinal parasites are depicted below:

3.5.2.1 *Cyclospora cayetanensis*

Cyclospora cayetanensis is a coccidian protozoan parasite that causes prolonged diarrhoea in humans worldwide (Ortega et al., 1993).

The first published report of *Cyclospora cayetanensis* in humans appears to be by Ashford (1979), who found unidentified *Isospora*-like coccidia in the feces of three individuals in Papua, New Guinea. At least the photomicrographs in the paper reveal an organism morphologically identical to that we see now. Later, Narango et al. (1989) reported what may be the same organism from several Peruvians with chronic diarrhea and termed the organism *Cryptosporidium muris*-like. Other investigators thought the unsporulated oocysts appeared more similar to cyanobacteria, and the name "cyanobacterium-like body" or CLB became prevalent in the literature (occasionally, authors also used the term "coccidian-like body" for CLB). Eventually, Ortega et al. (1992) published an abstract reporting that they had sporulated and excysted the oocysts, resulting in placement of the parasite in the genus *Cyclospora*. They also created the name *Cyclospora cayetanensis* at this time. However, since no morphologic information was presented in the abstract, *C. cayetanensis* technically became a *nomen nudum* (a named species without a description). Although Ortega et al. (1993) later published additional details about this coccidian, it wasn't until 1994 that a complete morphologic description was published to validate the name (Ortega et al., 1994). Thus, the correct name for this parasite is *Cyclospora cayetanensis* Ortega, Gilman, & Sterling, 1994, and the etymology of the *nomen triviale* is derived from Cayetano Heredia University in Lima, Peru.

It was first reported from Papua New Guinea (Ashford, 1979) and the infection due to coccidian parasite *Cyclospora cayetanensis* is reffered to as 'Cyclosporiasis'. *Cyclospora cayetanensis* is an emerging pathogen. *Cyclospora cayetanensis, a* coccidian protozoan parasite that causes protracted, relapsing gastroenteritis, has a short-recorded history. Infection with *C. cayetanensis* has been repoted from several countries including the USA, Peru, Nepal, and the UK (Arora and Arora, 2005). However, *Cyclospora* infection has recently been reported in non-travellers in the United States and Canada (Serpentini et al., 1999).

Susceptible humans are supposed to be infected by ingesting sporulated oocysts. Water is probably an important vehicle, either drinking parasite contaminated water directly or indirectly when water is used to grow plant foods. Water has been implicated in outbreaks in the United States, (Huang et al., 1995) and in Nepal (Raboldet al., 1994; Sherchand and Cross, 1999,2001).

Oocysts of *C. cayetanensis* are non-refractile, spherical to oval, slightly wrinkled bodies (mulberry appearance) measuring 8-10um in diameter. They contain two ovoid sporocysts, 4x6 um in size. Each contains two sporozoites. Unsporulated oocysts are exreted in the faeces. Diagnosis can be made by faecal examination. Oocyst of *C. cayetanensis* are acid fast and stain light pink to deep red in color. Older cells may fail to stain (Arora and Arora, 2005).

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

C. cayetanensis are transmitted by oocysts from person to person by faecal oral route or via contaminated water or food. The most common symotom of infection is diarrhoea, however, asymptomatic infections occur. Infections are associated with intestinal inflammation, with pathological lesions such as villous blunting, and abnormal function

such as malabsoption. Mild- to – moderate, selflimiting 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 (Mansfeild and Gajadhar, 2004).

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.

The results observed by Sherchand and Cross, 2004 suggest that sewage water and green leafy vegetables are pssible sources of infestion in Nepal although more studies are needed to clarify the direct link between *Cyclospora* infection and these sources.

Outbreaks seem to occur most frequently in late spring and summer, and these warmer temperatures are clearly needed to get oocysts to sporulate with any rapidity. In addition, this time of year correlates with increased import of fruits and vegetables into the US from our more southern neighbors. Individuals become infected when they ingest contaminated food or water containing viable, sporulated oocysts. Because so many of the foods we consume are shipped over long distances and involve contact by many individuals, transportation of pathogens such as *Cyclospora* between states and countries has become unavoidable. However, the odds of becoming infected with *Cyclospora*, and many other foodborne pathogens, can be greatly diminished by simply washing fruits and vegetables well prior to consumption. However, it should be noted that simply washing foods does not removed 100% of the oocysts (Ortega et al., 1997).

Whereas study conducted in Guatemala and Peru suggested that having certain animals (e.g. dogs, chicken, and ducks) increased the risk for human infection with *Cyclospora*. In a survey conducted in and around Leogane, Haiti, from 1997 to 1998, *Cyclospora* was not identified in the faeces of any of the 327 stool specimens collected from 11 types of

domestic animals (e.g. dogs, chicken, pigs, goats, and ducks). However, *Cyclospora* like oocysts were identified in at least one sample from each group of animal feaces by DIC (Differential inference contrast) and flouresence microscopy in a study carried out by chu et al., (2004) while detecting *C. cayetanensis* in animal fecal isolates from Nepal using an FTA filter base polymerase chain reaction method.

The laboratory diagnosis of newly recognized infectious agents, such as *Cyclospora cayetanensis*, is frequently problematic because appropriate diagnostic techniques and algorithms are not available. The methods currently available for diagnosis of *Cyclospora* are concentration procedures, examination of wet preparations, various staining techniques, and the use of molecular based assays. Because of the auto fluorescent properties of the oocysts, particular attention is drawn to the role of fluorescent microscopy in providing a rapid inexpensive, and sensitive technique for the diagnosis of *Cyclospora* infection in stool samples (Eberhard et al., 1997).

In a study carried out by Kimura et al., (2004), the direct smear technique was found to be an effective and rapid technique for diagnosis of *C. cayetanensis* when they compared three microscopic techniques such as formalin ether sedimentation, sucrose centrifugal floatation, and direct smear; maling it a technique of choice for routine epidemiological investigation of the prevalence of this infection in human populations. However, they obtained highest number of oocyst by the sucrose centrifugal floatation technique and in contrast; the formalin ether sedimentation technique was found to be least reliable concentration technique for the detection of *Cyclospora* in human faeces.

Modified acid-fast technique is mostly used to detect oocysts in faeces. Oocyst of *C. cayetanensis* are acid fast and stain light pink to deep red in color. Wet mount can also be done. For easy detection of oocysts, sporulation may be enhanced by keeping the stool in 2.5% $K_2Cr_2O_7$ for 15 days at room temperature. Phase contrast microscopy can also be done to detect the oocysts in faecal smears (Ortega, 1998).

Genome amplification by PCR and subsequent analysis is a very sensitive tool (Ortega, 1998) Trimethoprim-Sulphamethoxazole was first used to treat Cyclosporiasis in

1993(Madico et al., 1993) and since 1995 it has been the drug combination of choice (Hoge et al., 1995). Immunocompetent patients are given 7-days course of Trimethoprim-Sulphamethoxazole (160 and 800 mg respectively). For immunocompromised patients; the same dosage is recommended but is given for 10 days and afterwards three times a week indefinitely (Guerrant et al., 2001).

For those who are allergic to sulpha drugs, two stidies have been published using alternative medication: one in Nepal (Shlim et al., 1997) using trimethprim alone and other in Haiti using ciprofloxacin (Verdier et al., 2000).

3.5.2.2 *Entamoeba histolytica*

Entamoeba histolytica is world wide but most common intropical and subtropical countries. Lambl in 1859 first discovered the parasite, Losch (1875) proved its pathogenic nature, while Schaudin (1903) differentiated pathogenic and non-pathogenic types of amoebae. 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 colcn. Amoebiasis is world wide in distribution. The prevalence rates are highest in areas of crowding and poor sanitation in tropics and sub tropics than in temperate zone.

Entamoeba histolytica causes intestinal and extra intestinal amoebiasis. WHO estimates that every year more than 70,000 people die because of amoebiasis (Arora and Arora, 2005). During growth, the parasites secrete a proteolytic ferment of the nature of histolysin, which brings about destruction and necrosis of the tissues and thereby helps the parasite obtain nourishment through absorption of these dissolved tissue juices (Chaterjee, 2002).

The incubation period may vary from few days to months, depending upon the areas of endemicity. Normally, the time ranges from 1 to 4 months. *Entamoeba 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 disseminate 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 occur most often in children who present with diffuse abdominal pain, profuse bloody diarrhea, and fever; ameboma, which presents as a completely asymptomatic lesion or as a tender mass accompanied by symptomatic dysentery (Reed, 1992).

Trophozoites are detected in diarrhoel stool, liver aspirated, rectal exudates, or rectal ulcer tissue. Cysts are detected in formed stool via microscopic observation of wet mount preparation either iodine wet mount or simply saline mount.

Molecular techniques such as countercurrent immunoelectrophoresis and DNA probes can be used to identify *Entamoeba histolytica* in stools or biopsy and aspiration specimens. Serological methods such as indirect haemaglutination, fluorescent antibody test, and ELISA are used to detect the serum anti-amoebic antibody in invasive intestinal and extra- intestinal amoebiasis. Recently an ELISA is developed to detect circulating antigen to diagnose ALA.

Infections with *Entamoeba histolytica* occur worldwide, and it has been suggested that 12% of the world's population is infected with this organism. About 10% of those infected every year have clinical symptoms, 80 to 90% of these patients have symptoms related to the intestinal mucosa and in the remaining 2 to 20%, the amoebae invade beyond the intestinal mucosa (WHO, 2003). Food and water contaminated with human faeces thet contain cysts are the main sources of reservoirs of the infections. The transmission is either via faecal oral route or person-to-person spread, by vectors like flies, cockroaches and by sexual, contact esp. in homosexuals. Infections is common in poor personal hygiene, unsafe drinking water, poor sanitation, and where human faeces are as fertilizers. Moreover, it is important cause of diarrhea in AIDS patients.

3.5.2.3 *Giardia lamblia*

Giardia was first discovered by Leeuwenhoek in 1681 in his own stool but was not described until 1859 by Lambl. The organism was named after professor A. Giard of

Paris and Professor F. Lambl of Prague. It inhabits duodenim and upper part of jejunum of man.

Giardia exists in two forms: Trophozoite and Cyst form. Trophozoite is pear-shaped with rounded anterior and pointed posterior end. It measures 10-20µm in length and 5-15µm in width. Dorsal surface is concave and 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 7µm in breadth. They have thick wall, four nuclei are present in two pairs at two opposite ends and consists of an axostyles and margins of sucking disc.

Giardia lamblia may remain attached to intestinal mucosa and rarely invades the submucosa. As few as- 10-25 cysts cause giardiasis. With the help of sucking disc the parasite attaches itself to the surface of the epithelium cells in the duodenum and jejunum and in appreciable number of cases it may cause duodenal and jejunal irritation leading to duodonitis and jejunitis. The stool is voluminous, foul smelling and contains large number of mucus and fat but no blood. When the parasite localize in the billiary tract, it may lead to chronc cholecystis and jaundice (Arora and Arora, 2005).

The incubation period varies from 1-3 weeks and majority of infected persons in endemic area are asymptomatic. The acute giardiasis is characterized by the sudden unset of anorexia, naudea, abdominal distension, discomfort, diarrhoea, etc. Steatorrhoea is frequently accompanied by epigastic 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 three or four days, can resemble other causes of travelers diarrhoea and is often not recognized as being due to giardiasis (Farthing and Keush, 1989)

Giardiasis can be diagnosed by identification of cysts of *Giardia Lamblia* in the formed stools and the trophozoits of the parasite in diarrhoeal stools by normal saline and iodine preparation as in case of *E. histolytica*.

For the detection of *G. lamblia* in faecal specimens, a fluorescent method using monoclonal anti bodies is extremely sensitive and specific. ELISA test has been developed for the detection of *Giardia* antigen in faeces. After multiple stool examinations, examination of bile aspirated from duodenum and enterotest are negative, biopsy from multiple duodenal and jejunal sites may confirm the dignosis of Giardiasis.

Giardia lamblia infection occurs worldwide with an incidence varying from 1.5-2.5%. Giardiasis shows two distinct epidemiological patterns: 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 group are susceptible in developed world. The transmission is mainly by faeco-oral route, usually by contaminated water, food, direct person-to-person spread in person with poor sanitation and poor faeco-oral hygiene and sexually transmitted in homosexuals.

Giardiasis can be prevented and controlled by improving clean water supply, proper human excreta disposal, proper maintenance of food and conducting health education. Treatment of giardiasis is carried out with metronidazole, tinidazole, and furazolidone (Garcia and Brukner, 1993).

3.5.2.4 *Ascaris lumbricoides*

Ascaris lumbricoides has worldwide distribution, being especially prevalent in the tropics, such as India, China, and Southeast Asia. It is estimated that it effects about 25% of the world's population. The highest prevalence is in malnourished people residing in developing countries. Areas with modern water and waste treatment have low incidence of the infection with this parasite. Linnaeus first of all observed *A. lumbricoides* in 1758 and detail of the life cycle of the parasite was after 1976. The organism is commonly known as roundworm. *A. Lumbricoides* is the largest nematode parasitizing the human intestine (Adult female: 20 to 35 cm; adult male: 15 to 30 cm). The posterior end of the male is slightly curved. The tail is bluntly pointed. The spicules in male genital organ are simple and measure 2-3µm in length. In female, vulva is present at one third of body length from the anterior end.

Adult worm reside in small intestine, particularly the jejunum of man. Fertilized eggs containing unsegmented ovum are passed in the faeces. Man acquires infection by ingestion of food, water, or raw vegetables contaminated with embryonated eggs. In the small intestine, the ingested eggs hatch to liberate the larvae. These larvae then burrow their way through the mucus membrane of the small intestine and are carried by the portal circulation to the liver, where they reside for 3-4 days. Then they are passed via systemic circulation to lungs. The mature further in the lungs, penetrate the alveolar walls, ascend the bronchial tree to throat, and are swallowed. Upon reaching the small intestine, they develop into adult worm. Between 2-3 months are required from ingestion of infective eggs to oviposition by the adult female. Adult worms can live 1-2 years.
Disease produced by *Ascaris* is known as ascariasis and is caused by both adult worms and migrating larvae. During the lung phase of larval migration, pulmonary symptoms can occur (cough, dyspnea, hemoptysis, eosinophilic pneumonitis-Leoffle's syndrome) (Jaime et al., 1999).

Laboratory diagnosis of *A. lumbricoides* infection can be made by finding adult worms in stool or vomit. It can be done by X-ray diagnosis in which *A. lumbricoides* can be demonstrated with a barium emulsion, which being ingested by worm within 4-6 hrs, casts an opaque shadow. The drugs of choice for treatment of ascariasis are albendazole, and pyrantel pamoate (Chatterjee, 2002).

3.5.2.5 *Trichuris trichuria*

Linnaeus first described this worm in 1771 and the pathogenesis was first described by Stiles in 1901. It is commonly called whipworm and has worldwide distribution with approximately 10% infection. It is cosmopolitan in distribution but is more common in the warm, moist regions of the world. The disease is called trichuriaisis and the parasite invades large intestine especially the caecum and appendix, but can also be found in the rectum.

The worms are white in color. The males are relatively smaller and coiled posteriorly. The anterior end is thin, long (two third of body length), whereas the posterior end is

thick and stout. Mouth is simply opening and doesn't contain lips. One fertilized female lays 1000-7000 eggs per day. Eggs are barrel shaped, golden brown in color and measures 5x25 µm in size. Embryonation takes place in the environment.

Man acquires infection by ingesting embryonated eggs with contaminated food or water. Most infection are asymptomatic, but heavy infections cause abdominal discomfort, anemia, bloody diarrhoea, rectum prolapsed, and appendicitis. Diagnosis is established by laboratory investigation of the stool specimen. Characteristic barrel shaped eggs are found in the stool 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.

Trichuriasis can be treated by oral administration of mebendazole in a dosage of 100mg twice daily for 3 days.

3.5.2.6 *Hymenolepsis nana*

H. nana is the smallest tapeworm infecting humans. The name hymenolepsis refers to thin membrane covering the egg (hymen- membrane, lepsis- covering) and nana to small size (nanus- dwarf or small). It was first discovered by Bilhharz in 1857.It not only infects human but also rodents such as mice and rats.

It is cosmopolitan in distribution but more is common in warm than in cold climates. It is estimated that approximately 20 million people are infected with *H. nana.*

It is widely distributed in countries with warm climates including those of South America, Mediterranean region, Africa, and South East Asia. Children are more commonly infected than adults. It is commonly called dwarf tapeworm (Chatterjee, 2002).

In humans the adult tapeworms are found in the upper two thirds of the ileum, whereas in mice and rats they are found in posterior part of the ileum. The worm is thread like,

measuring 1 to 4 cm in length with a maximum diameter of 1 mm. The worm may present in large numbers (1000-8000). Life span is very short (about 2 weeks).

H. nana even in large numbers, is well tolerated. The mechanism by which symptoms are produced is an allergic reaction and in heavy infections enteritis may be produced. The infection is more common in children. Patients develops headache, dizziness, anorexia, pruritus of nose and anus, abdominal pain, diarrhoea, restlessness, epileptiform convulsions and eosinophilia in excess of 5% (Arora and Arora, 2005).

The diagnosis is made by demonstration of characteristic eggs in faeces by direct microscopy, salt floatation, and formalin-ether concentration methods. The drug of choice is praziquantel, of which a single dose is highly effective. The drug of second choice is niclosamide (Arora and Arora, 2005).

3.5.2.7 Hookworm

Two major hookworm species that infect humans are *Ancyclostoma duodenale* and *Nectar americanus*. An estimated 700-900 million people worldwide are infested with these parasites (mostly *A. duodenale*), 0.2% of whom suffer from severe anaemia. *N. americanus* prevails in tropical and sub tropical regions whereas *A. duodenale* tends to occur in the cooler and somewhat drier regions. Mixed infections also occur especially in northern India and the middle of china (Arora and Arora, 2005). An Italian physician Angelo Dubini discovered A. duodenale in 1838 while Stiles discovered *Nectar americanus* in 1903.

The worm is about 9mm long, female *Ancyclostoma duodenale* produces 30000 eggs per day, and *N. americanus* produces 9000 eggs per day. Both the organism possesses similar life cycle after 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 on shape having 60x40µm in size; colorless and surrounded by a transparent hyaline shell membrane.

The migrating larvae or adult worm may cause clinical disease in hookworm infection. The pathogenic effects due to larvae are Ancyclostome dermatitis, creeping eruptions and lesions in the lungs. The clinical symptoms are associated with the area of the 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 diarrhea, nausea, and vomiting.

Hookworms flourish under primitive conditions where people go barefoot, modern sanitary conditions do not exist, and human faeces are deposited on the ground. Therefore, for the prevention of hookworm infection, living conditions and sanitation should be improved and there should be sanitary disposal of human faeces. Wearing of shoes and gloves provides personal protection.

Mebendazole, 100mg twice daily for 3 days, or pyrantel pamoate in a single dose of 11 mg per kilogram of body weight, or albendazole, 400mg in a single doze may be used for the treatment of *Ancyclostoma duodenale* and *Nectar americanus* infection (Arora and Arora, 2005).

3.5.2.8 *Cryptosporidium parvum*

Cryptosporidium parvum is obligate, intracellular, coccidian protozoan pathogens that cause prolonged diarrhoea during childhood (Griffiths, 1998 and looney, 1998). It produces mild and self-limited (1—2 weeks) diarrhoea in immunocompetent persons. It is spread through faecal-oral route, and nosocomial outbreaks have been reported. The diarrhoea is profuse and watery; it may contain mucus but rarely blood and leucocytes, and it is often associated with weight loss. Fluid loss in AIDS patients with Cryptosporidiosis is often excessive; 3-6 liters of watery stool per day has been reported. In 75% cases, diarrhoea is accompanied by crampy abdominal pain; approximately 255 of patients have nausea and/or vomiting.

C. parvum is not always confined to gastrointestinal tract; additional symptoms (respiratory cryptosporidiosis, cholecystitis, hepatitis and pancreatitis) have been associated with extra intestinal lesions. (Arora and Arora, 2005).

A study conducted by Sherchand et al., in Nepal, from 2002 to 2004, showed that water and food are important vehicles for this coccidian transmission. This study also revealed that, the lowest age of *Cryptosporidium* infected infant was of 2 months, while the highest age of *Cryptosporidium* infected person was 64 of age (Sherchand et al., 2005).

C. parvum can be diagnosed by using a standard formalin-ethyl acetate concentration method and examined by two methods: direct light microscopy and stool smear stained with modified acid fast stain. Patient can minimize their risk of developing cryptosporidiosis by avoiding contact with human and animal faeces and by not drinking water from lakes or rivers.

3.5.3 ROTA VIRUSES

Rotavirus is a double stranded RNA virus measuring about 70nm, is considered as common cause of diarrhoea in children. It has been detected from all over the countries, though the prevalence differs from place to place. Rotavirus is frequently be demonstrated in the stools of neonates, but in them the infection is generally asymptomatic, probably due to maternal immunity. The disease peaks from the age of six month to two years. The mode of spread is believed to be faecal-oral route. The incubation period is 2-4 days. Typically, vomiting is a prominent early symptom, often preceding diarrhoea. The stools are watery, sometimes with flakes of mucus. Mild fever and respiratory symptoms may occur (Sherchand et al., 1992).

Rotavirus is the most important cause of early childhood nonbacterial gastroenteritis in both developed and developing countries. The infection is also observed in older children and adults. In developed countries 50% of pediatric hospitalization is due to

acute diarrhoea, while in developing countries it is responsible for an estimated one million deaths annually (Shariff M, Deb M, Singh R, 2003).

Rotaviruses infect cells in the villi of the small intestine. They multiply in the cytoplasm of enterocytes and damage their transport mechanisms. Damaged cells may slough into the lumen of the intestine and release large quantities of virus, which appear in the stool (up to 10^{10} particles per gram of faeces). 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 glues absorption as damaged cells villi are replaced by nonabsorbing immature crypts cells. It may take 3-8 weeks for normal function to be stored (Brooks et al., 2004).

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. Asymptomatic infections of neonates with 'nursery strains' are not uncommon. It has been estimated that about half of all gastroenteritis 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 (Greenwood et al., 2003).

Rotavirus is an etiological agent of diarrhoea in infants and young children. The study showed that out of 714 stool samples from children, 116 (16.2%) was positive for rotavirus. Hospital samples showed higher percentage of rotavirus positive (30.6%) compared to village samples (4.1%). The highest rate of rotavirus positive was also found in diarrhoeic stools (30.4%) compared to normal stools (0.87%). The high incidence of diarrhoea and rotavirus was observed in winter. Rotavirus was found in 6 months to 24 months children (Sherchand et al., 2004).

Study conducted in children under 6 years attending OPD of Kanti Children's Hospital and General practitioner in Kathmandu showed as follows: Total 198 diarrhoeic samples from OPD of Kanti Children's Hospital collected and tested for rotavirus, 53 (27%) were found positive but no one in 5-6 age group. Similarly 52 samples from General practitioner in Kathmandu were collected and 14 (275) were found positive, which were all below 3 years of age. At the same time 92 samples from children without diarrhoea was collected and 7 (7.6%) were found positive (Sherchand et al., 1992).

A few studies on occurrence of gastro-intestinal infections in Nepal are available. According to the Nepal Human Development Report published in 1998, 16 to 25% of childhood deaths occur due to diarrhoea (Shariff M et al., 2003). This meta- analysis of approximately 800000 children in Japan under the age of 6 years visited in pediatric department showed that rotavirus gastroenteritis rate was 11 cases /100 person/ year, and one in two children will visited paediatrician before they go to primary school. Such visits most frequently occur below the age of 1 year. This emphasizes that magnitude of the burden of rotavirus disease among Japanese children is substantial (Michiyo et al., 2004).

In a study of "diarrhoea among children in eastern Nepal with special reference to rotavirus" conducted by Shariff M et al., 2003, showed that One hundred and sixty children with acute diarrhoea were included in the study, of which 108 (67.5%) were male and 52 (32.5%) were female. Rota 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.

Rotavirus is the leading recognized cause of diarrhoea-related illness and death among infants and young children. Each year, rotavirus is associated with 25 million clinic visits, 2 million hospitalizations, and 600,000 deaths worldwide among children <5 years of age. Development of a safe and effective rotavirus vaccine is therefore a high priority, particularly but not exclusively in developing countries where the burden of disease is highest (Ruiz Palacious G. et al., 2006).

During the 1980 autumn diarrhoea season, 19 patient and 12 control subjects were selected from among outpatients under two years of age and were interviewed and studied for bacterial and viral enteric pathogens. 11 (58%) of 19 patients and 2 (17%) of 12 controls were positive for faecal rotavirus. 10 (91%) of rotavirus positive patient were under one year of age. The most significance risk factor for illness was the presence of household contact under the age of two years. Dog handlers were found to be associated with infection (Engle berg et al., 1982).

Electron microscopy, when routinely applied for 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.

The rotavirus vaccine licensed in the US, Rotataq has shown to be quite effective against rotavirus disease. The vaccine will prevent 74% of all rotavirus cases, about 98% of severe cases, and about 90% of hospitalization due to rotavirus (CDC, 2007).

A live attenuated human rotavirus (HRV) vaccine containing the RIX4414 strain of G1P[8] specificity has been developed from the parent vaccine strain 89-12. clinical trails with the HRV vaccine in Finnish and Latin American infants showed that two doses were well tolerated and immunogenic. In phase 2 clinical trails, the efficacy of the vaccine against severe rotavirus gastroenteritis reached 90-100 percent. Protection started as early as the first dose, lasted until the subjects were up to two years of age, and was demonstrated against both G1P[8] and G9P[8] rotaviruses (Ruiz Palacious G. et al., 2006).

CHAPTER IV

4. MATERIALS AND METHODS

4.1 Subject

This study was carried out in T.U. IOM, Health Research Laboratory, Kathmandu between May 2006 to September 2006. Stool samples were collected from children below 5 years old with gastroenteritis from Kanti Children's Hospital.

4.2 Sample collection

Stool samples from children below 5 years of age attending oral rehydration treatment (ORT) ward and out patient department (OPD) ward of Kanti Children's Hospital were collected in a grease free wide mouthed vessel with air tight cap and side by side interview was taken with parents of patient. During collection of sample Doctors prescription was strictly observed to determine whether the case was diarrhoel or non-diarrhoeal. Then the collected stool samples were brought immediately to the laboratory. The collected stool samples were processed according to the standard laboratory methods (Collee et al 1998). Descriptive statistics like Chi square test was used to analyze the data to show association between enteropathogen infection and predisposing factors.

4.3 Laboratoy processing of samples

Each fresh stool sample was processed in the following steps as:

4.3.1 Macroscopic examination

The stool samples were examined for the presence of blood mucus adult or larvae of parasites. The color and consistency of stool samples were also observed (Cheesebrough 1999)

4.3.2 Microscopic examination

Direct microscopy of slide preparation was done to examine the cyst, ova, or oocyst of the parasites. It was also helpful to observe the RBC, Puscells, and WBC in faeces. The detection of oocyst, cysts and trophozoites of protozoa, ova or egg of helminthes was carried out at low power 10x magnification by wet mount saline preparation and followed by iodine preparation method. Ziehl Neelson staining of each sample was also done for examination of *Cyclospora* and *Crptosporidium*.

Wet mount preparation

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 covering with the cover slip. In case of watery stool, a single drop of the sample was placed on a slide, covered with cover slip, and then examined under the microscope (Cheesebrough, 1999).

Iodine/ KOH preparation

KOH preparation was performed exactly the same as saline wet mount; the only difference was use of KOH instead of normal saline (Cheesebrough, 1999).

Hanging drop

For the cholera suspected sample they were subjected to hanging drop preparation for special darting motility exhibited by *Vibrio cholerae* (Collee et al, 1996)

Ziehl Neelson staining

Modified Ziehl Neelson stain is used for detection and identification of *C.caytanensis* and *C. parvum*. A thin smear of faeces was made on clean slide. It was fixed on methanol. It was then flooded with carbon fuschin for 5 minutes and then washed with water. It was then decolorized with 5% acid alcohol for 30 seconds, washed with water. It was then counter stained with Malachite green/ methylene blue for 1 minute. Finally washed and dried.

4.3.3 Culturing on enrichment differential and selective media

Alkaline peptone water was used for the enrichment of *Vibrio* spp. and selenite F broth was used for the enrichment of *Salmonella* spp. and *Shigella* spp. SS agar, MacConkey agar was used for isolation of *Salmonella* and *Shigella* spp., and TCBS agar was used for isolation of *Vibrio* spp.

For *Salmonella* and *Shigella* spp.

Several loopfuls of faeces was inoculated in selenite F broth and 2-3 loopfuls of faeces was inoculated on SS agar and MacConkey agar and were incubated overnight at 37^{o} C.

For *Vibrio* spp.

Stool samples of about 2 ml were inoculated on 20 ml of alkaline peptone water (pH 6.6) and several loopfuls were also inoculated in TCBS agar. All the tubes and plates were incubated at 37^{o}C for overnight.

4.3.3.1 Examination of culture plates

The overnight-incubated culture plates were observed for their characteristic colony morphology. MacConkey plate was observed for lactose fermenting pink colonies 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 center of S*almonella* spp. Similarly on TCBS agar plate was observed for characteristic sucrose fermenting yellow colonies of V*ibrio* spp.

4.3.3.2 Identification

The colonies from SS plates were subjected to Gram staining and macroscopic morphology of isolated bacteria was observed. The isolated colonies from SS agar, MacConkey agar, and TCBS agar were sub cultured on Nutrient agar followed by incubation at 37^{o}C for overnight. The well-isolated colony was subjected to biochemical identification. The principles and methodology are described in Appendix.

4.4 Purity plate

Purity plate shows whether or not the experiments were proceeded aseptically. It was done in each biochemical lots. 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 on pre and post inoculation is an indication of free of contamination or of an aseptic condition.

4.5 Quality control

For the maintenance of quality control, each batch of test medium was incubated without inoculating the test organism. For selective media like MA, SS agar, and stock culture of *E coli and Salmonella* spp. were incubated along with the test organism and after incubation the results were compared.

4.6 Micro plate Enzyme-Immunoassay for the quantitative detection of Rotavirus Antigen in human stool (Maridian *Bioscience,* Inc.)

Assay protocol

1. Faecal sample was collected by use of transfer pipette (first mark0 and it was emulsified into 1 ml 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, sealed, and mixed.
4. It was then incubated at room temperature for one hour.
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) of stop solution was added.
10. The result was read visually or spectrophotometrically at 450nm

CHAPTER-V

5. RESULTS

5.1 Laboratory analysis

Within the study period (May to September 2006), a total of 500 stool specimens were collected from children under 5 years of age suffering from gastroenteritis attending Kanti Children's Hospital and processed in Health research Laboratory for microscopic examination of parasites and identification of bacterial agents and also the detection of rotaviral antigen.

Table 4 Frequency distribution according to hospital registration

Registration	Diarrhoeal cases		Non-Diarrhoeal cases	
	Frequency	%	Frequency	%
In patient	250	64.8	000	0.0
Out-patient	136	35.2	114	100.0
Total	386	100.0	114	100.0

The table shows that out of total diarrhoeal children (n= 500) enrolled for the study 250 (64.8%) were inpatient, admitted in ORT ward and 136 (35.2%) were attended in OPD. Total 114 (100%) non-diarrhoel cases were included in the study from OPD patients.

Table 5 Age wise distribution of diarrhoeal and non-diarrhoeal cases

Age group in months	Diarrhoeal cases		Non-diarrhoeal cases		Total
	Frequency	%	Frequency	%	
0 – 6	40	10.4	12	10.5	52
7 – 24	190	49.2	52	45.6	242
25 -60	156	40.4	50	43.9	206
Total	386	100.0	114	100.0	500

The table shows that out of total (n=386) diarrhoeic children 40(10.4%) were from age group 0-6 months, in age group 7-24 months show 190 (49.2%) number of cases and in age group 25-60 months show 156 (40.4%) number of cases. Similarly in non-diarrhoeic children (n=114), 12(10.5%) cases were from age group 0-6 months, 52 (45.6%) cases were from age group 7-24 and 50 (43.9%) cases were from age group 25-60.

Table 6 Sex-wise distribution of diarrhoeal and non-diarrhoeal cases

Sex	Diarrhoeal cases		Non –diarrhoeal cases	
	Frequency	%	Frequency	%
Male	245	63.5	66	57.9
Female	141	36.5	48	42.1
Total	386	100	114	100

The table shows that out of total diarrhoeic patient (n=386) enrolled for the study, the incidence rate of male patient were 245 (63.5%), and female patient were 141 (36.5%) and out of total non-diarrhoeic patient 114, the male patient were 66 (57.9%) and female patient were 48 (42.1%).

Table 7 Frequency distribution according to source of drinking water used by patients

Source of drinking water	Diarrhoeal cases		Non-diarrhoeal cases	
	Frequency	%	Frequency	%
Tap	290	75.1	62	54.4
Well	9	2.4	3	2.6
Boiled	52	13.5	36	31.6
Filtered	35	9.0	13	11.4
Total	386	100.0	114	100.0

The table shows drinking water source based on response given by respondents in the hospital, out of total diarrhoeal cases (n=386); the majority 290 (75.1%) patients were using tap water for drinking purpose. Like wise, well water 9 (2.4%), boiled water 52 (13.5%) and filtered water 35 (9.0%). Similarly in non-diarrhoel cases (n=144), the majority 62 (54.4%) was using tap water for drinking purpose. Like wise well water 3 (2.6%), boiled water 36 (31.6%) and filtered water 13 (11.4%).

Table 8 Frequency distribution according to clinical symptoms in diarrhoel cases

Symptoms	Frequency				
	Yes	%	No	%	Total
Fever	196	50.8	190	49.2	386
Abdominal pain	302	78.2	84	21.7	386
Vomiting	201	52.0	185	47.9	386
Nausea	195	50.5	191	49.4	386

The table shows clinical symptoms observed in diarrhoeal cases as follows; out of total (n=386) cases, 196 (50.8%) had fever and 190 (49.2%) had no fever, similarly, 302

(78.2%) had abdominal pain and 84 (21.7%) had no abdominal pain, 201 (52.0%) had vomiting and 185 (47.9%) had no fever, and 195 (50.5%) had nausea and 191 (49.4%) had no nausea.

Table 9 Frequency distribution based on number of stool/day in diarrhoel cases

Frequency of stool/day	Frequency	%
3-5	167	43.3
5-10	129	33.4
>10	90	23.3
Total	386	100.0

The table shows that out of total patient (n=386) enrolled for study 167 (43.3%) had frequency of stool between 3-5 times per 24 hour, 129 (33.4%) had frequency of stool between 5-10 times per 24 hour, and 90 (23.3%) had stool more than 10 times per 24 hours.

Table 10 Frequency distribution based on consistency of stool in diarrhoel cases

Stool type	**Frequency**				**Total**
	Yes	**%**	**No**	**%**	
Watery	266	68.9	120	31.0	386
Mucus	211	54.6	175	45.3	386
Blood	5	1.3	381	98.7	386

The table shows that out of total diarrhoeal cases (n=386), 266 (68.9%) had watery stool and 211 (54.6%) had mucus in stool and 5 (1.3%) had blood in stool. Whereas 120 (31.0%), 175 (45.3%) and 381 (98.7%) had no watery stool, mucus, and blood in stool respectively.

Table 11 Frequency distribution based on status of dehydration in diarrhoel cases

Degree of dehydration	Frequency	%
Mild	88	22.7
Moderate	94	24.4
Severe	204	52.9
Total	386	10000.0

The table shows that out of total patients (n=386), 88 (22.7%) had mild dehydration, 94 (24.4%) had moderate dehydration and 204 (52.9%) had severe dehydration.

Table12 Frequency distribution based on treatment in diarrhoel cases

Treatment	Yes	%	No	%	Total
ORS	95	24.6	291	75.3	386
Antibiotics	74	19.2	312	80.8	386
IV Fluids	13	3.4	373	96.6	386
Others	169	43.7	217	56.2	386

The table shows that out of total diarrhoeal patients (n=386), 95 (24.6%) were treated with ORS whereas 291 (75.3%) were not. Similarly, 74 (19.2%) were treated with antimicrobials, 312 (80.8%) were not 13 (3.4%) were using IV fluid and 373 (96.6%) were not using IV fluids.

Table 13 Month wise distributions of diarrhoeal and non-diarrhoeal cases

Month	Diarrhoeal cases		Non-diarrhoeal cases	
	Frequency	%	Frequency	%
May	83	21.50	15	13.16

June	94	24.35	19	16.67
July	79	20.46	26	22.80
August	80	20.74	30	26.32
September	50	12.95	24	21.05
Total	3866	100.00	114	100.00

The table shows month wise distribution of patients.out of total (n=386) diarrhoeal samples, month June shows the highest number, 94 (24.35%), followed by May 83 (21.50%), August 80 (20.74%), July 79 (20.46%) and September 50 (12.95%). Similarly in non-diarrhoeal cases, out of total (n=114), month shows August the highest number 30 (26.32%), followed by July 26 (22.80%), September 24 (21.05%), June 19 (16.67%), May 15 (13.16%).

Table 14 Distribution of rotavirus in diarrhoel and non-diarrhoel cases

Rotavirus	Diarrhoel cases		Non-diarrhoel cases	
	Frequency	%	Frequency	%
Positive	65	16.84	5	4.39
Negative	321	83.16	109	95.61
Total	386	100.00	114	100.00

The table shows that out of total diarrhoeal stool samples (n=386), 65 (16.84%) were positive for rotavirus and 321 (83.16%) were negative for rotavirus. Similarly in non-diarrhoeal stool samples (n=114), 5 (4.39%) were positive rotavirus and 109 (95.61%) were negative which was found statistically significant at 5% level of significance, $p<0.05$.

Table 15 Distribution of intestinal parasites in diarrhoel and non-diarrhoel cases

Intestinal parasites	Diarrhoel cases		Non-diarrhoel cases	
	Frequency	%	Frequency	%
Positive	119	30.8	30	26.3
Negative	267	69.2	84	73.7
Total	386	100.0	114	100.0

The table shows that out of total diarrhoeic stool samples (n=386), 119 (30.8%) were positive for intestinal parasites and 267 (69.2%) were negative for intestinal parasites. Similarly in non- diarrhoeic stool samples (n=114), 30 (26.3%) were positive for intestinal parasites and 84 (73.7%) were negative which was found statistically insignificant at 5% level of significance, $p>0.05$.

Table 16 Distribution of bacterial pathogen in diarrhoel and non-diarrhoel cases

Bacterial pathogen	Diarrhoel cases		Non-diarrhoel cases	
	Frequency	%	Frequency	%
Positive	13	3.4	1	0.9
Negative	373	96.6	113	99.1
Total	386	100.0	114	100.0

The table shows that that out of total diarrhoeic stool samples (n=386), 13(3.4%) were positive for bacterial pathogens and 373 (96.6%) were negative for bacterial pathogens. similarly in non- diarrhoeic stool samples (n=114), 1 (0.9%) were positive for bacterial pathogens and 113 (99.1%) were negative which was found statistically insignificant at 5% level of significance, $p>0.05$.

Table 16 Distribution of enteropathogens among total cases

Enteropathogens	Diarrhoel cases		Non- diarrhoel cases	
	Frequency	% (n=386)	Frequency	% (n=114)
Bacteria	13	3.36	1	0.87
Shigella spp.	0	0.00	0	0.00
Salmonella spp.	1	0.25	0	0.00
Vibrio spp.	12	3.1	1	0.87
Protozoans	264	29.01	33	28.94
Entamoeba histolytica	36	9.32	8	7.01
Giardia lamblia	32	8.29	12	10.52
Cyclospora cayetanensis	30	7.77	7	6.14
Cryptosporidium parvum	3	0.77	1	0.87
Entamoeba coli	4	1.04	1	0.87
Blastocystis hominis	7	1.81	4	3.50
Helminthes	76	2.84	6	5.26
Ascaris lubricoides	4	1.04	2	1.75
Trichuris trichuria	2	0.51	2	1.75
Hymenolepsis nana	4	1.04	1	0.87
Teania spp.	1	0.03	1	0.87
Rotavirus	65	1.68	5	4.38

The table shows that out of total diarrhoeal stool samples (n=386), among bacteria *Vibrio* spp. was highest in number 12 (3.1%), followed by *Salmonella* spp. 1 (0.25%) and *Shigella* spp was not found. Among parasites, protozoans 112 (29.01%) dominated over helminthes 11 (2.84%). Among protozoans, *Entamoeaba histolytica* was highest in number 36 (9.32%), followed by *Giardia lamblia* 32 (8.29%), *Cyclospora cayetanensis* 30 (7.77%), *Blastocystis hominis* 7 (1.81%), *Entamoeba coli* 4 (1.04%) and *Cryptosporidium parvum* 3 (0.77%).
Among the helminthes, *Ascaris lumbricoides4* (1.04%), *Hymenolepsis nana* 4 (1.04%), *Trichuris trichuria* 2 (0.51%) and *Taenia* spp. 1 (0.03%).

Out of total non-diarrhoeal samples (n=114), *Vibrio* spp. 1 (0.87%) was the only bacterial pathogen. Among parasites, protozoans 33 (28.94%) dominated over helminthes 6 (5.26%). Among protozoans, *Giardia lamblia* was highest in number 12 (10.52%), followed by *Entamoeba histolytica* 8 (7.01%), *Cyclospora cayetanensis* 7 (6.14%), *Blastocystis hominis* 4 (3.50%), *Entamoeba coli* 1 (0.87%) and *Cryptosporidium parvum* 1 (0.87%).
Among helminthes, *Ascaris lumbricoides* 2 (1.75%), *Trichuris trichuria* 2 (1.75%), *Hymenolepsis nana* 1 (0.87%) and *Taenia* spp. 1 (0.87%).

Table 18 Age wise distribution of rotavirus

Age group in month	Diarrhoeal cases			Non- diarrhoeal cases		
	Frequency	Rotavirus positive	%	Frequency	Rotavirus positive	%
0-6	40	5	12.5	12	0	0.00
7-24	190	41	21.57	52	3	5.76
25-60	156	19	12.17	50	2	4.00
Total	386	65	46.24	114	5	9.76

The table shows age wise comparison of rotavirus infection between diarrhoeal and non-diarrhoeal cases. Among the diarrhoeal cases in age group 0-6 months out of 40 cases 5 (12.5%) were positive for rotavirus. In age group 7-24 months out of 190 cases 41 (21.57%) were rotavirus positive. In age group 25-60 months out of 156 cases 19 (12.17%) were rotavirus positive. Among the non-diarrhoeal cases in age group 0-6 months out of 12 no rotavirus positive cases found. In age group 7-24 months out of 52 cases 3 (5.76%) were rotavirus positive, in age group 25-60 months out of 50 cases 2 (4%) were rotavirus positive which was found statistically insignificant, p>0.05.

Table 19 Sex wise distribution of rotavirus

Sex	**Diarrhoeal cases**			**Non- diarrhoeal cases**		
	Frequency	**Rotavirus positive**	**%**	**Frequency**	**Rotavirus positive**	**%**
Male	245	44	17.95	66	3	4.54
Female	141	21	14.89	48	2	4.16
Total	386	65	32.84	114	5	8.7

The table shows sex wise distribution of rotavirus. Among diarrhoeal cases, in males out of 245 cases 44 (17.95%) were rotavirus positive; in females out of 141 cases 21 (14.89%) were rotavirus positive.whereas in non-diarrhoeal cases, in males out of 66 cases 3 (4.54%) were rotavirus positive; in females out of 48 cases 2 (4.16%) were rotavirus positive which was found statistically insignificant, p>0.05.

Table 20 Month wise distribution of rotavirus

Month	Diarrhoeal cases			Non- diarrhoeal cases		
	Frequency	Rotavirus positive	%	Frequency	Rotavirus positive	%
May	83	17	20.48	15	2	13.33

June	94	15	15.95	19	1	5.26
July	79	10	12.65	26	0	0
August	80	9	11.25	30	0	0
September	50	14	28.00	24	2	8.33
Total	3866	65	88.33	114	5	26.92

The table shows month wise distribution of rotavirus. Among diarrhoeal cases, in month May out of 83 cases 17 (20.48%) were rotavirus positive; in June out of 94 cases 15 (15.95%) were rotavirus positive; in July out of 79 cases 10 (12.65%) were rotavirus positive; in August out of 80 cases 9 (11.25%) were rotavirus positive and in September out of 50 cases 14 (28.00%) were rotavirus positive. Whereas in non-diarrhoeal cases in month May out of 15 cases 2 (13.33%) were rotavirus positive, in June out of 191 (5.26%) rotavirus positive, in July and August no rotavirus positive cases found; in September out of 24 cases 2 (8.33%) were rotavirus positive. Therefore in diarrhoeal cases highest proportionate percentage was seen in month September (28.00%), whereas in non-diarrhoeal cases highest percentage was seen in May month (13.33%) which was found statistically insignificant, $p>0.05$.

Table 21 Distribution of rotavirus according to source of drinking water

Source of drinking water	Diarrhoeal cases			Non- diarrhoeal cases		
	Frequency	Rotavirus positive	%	Frequency	Rotavirus positive	%
Tap	290	55	18.96	62	3	4.83
Well	9	1	11.11	3	0	0.00
Boiled	52	3	5.76	36	2	5.55
Filtered	35	3	8.57	13	0	0.00
Total	386	62	44.4	114	5	10.38

The table shows comparison of rotavirus infection according to source of drinking water between diarrhoeal and non-diarrhoeal cases. Among patients using tap water, out of 290 cases 55 (18.96%) were positive for rotavirus; whereas in non-diarrhoeal cases, among tap water users, out of 62 cases 3 (4.83%) were positive. Among patients using well water, out of 9 diarrhoeal cases, 1 (11.11%) was positive; whereas in 3 non-diarrhoeal cases, none was positive. Among patients using boiled water, out of 52 diarrhoeal cases, 3 (5.76%) were positive; whereas in 36 non-diarrhoeal cases, 2 (5.55%) were positive. Similarly, among patients using filtered water, out of 35 diarrhoeal cases, 3 (8.575) were positive; whereas in 13 non-diarrhoeal cases, none was positive for rotavirus.

Table 22 Distribution of rotavirus according to clinical symptoms of patient

Symptoms	Yes			No		
	Frequency	Rotavirus positive	%	Frequency	Rotavirus positive	%
Fever	196	31	15.81	190	39	20.52
Abdominal pain	302	47	15.56	84	23	27.38
Vomiting	201	43	21.39	185	26	14.05
Nausea	195	46	23.58	191	24	12.56
Total	894	167	76.34	650	112	74.51

The table shows Distribution of rotavirus according to clinical symptoms. Out of 196 fever present cases, 31 (15.81%) were present for rotavirus; whereas in those without fever (190), 39 (20.52%) were positive. In those with abdominal pain, out of 302 cases, 47 (15.56%) were present for rotavirus; whereas in those without abdominal pain, out of 84 cases, 23 (27.38%) were positive for rotavirus. In those with vomiting, out of 201 cases, 43 (21.39%) were positive for rotavirus; whereas in those without vomiting, out of 185 cases, 26 (14.05%) were positive. In those with nausea, out of 195 cases, 46 (23.58%) were positive for rotavirus; whereas in those without nausea, out of 191 cases, 24 (12.56%) were positive for rotavirus.

Table 23 Age wise distribution of *Cyclospora cayetanensis*

Age in month	Diarrhoeal cases			Non-diarrhoeal cases		
	Frequency	*Cyclospora* positive	%	Frequency	*Cyclospora* positive	%
0-6	40	5	12.5	12	0	0.00
7-24	190	12	6.31	52	1	1.92
25-60	156	13	8.33	50	6	12.00
Total	386	30	7.77	114	7	6.14

The table shows age wise comparison of *Cyclospora cayetanensis* infection between diarrhoeal and non-diarrhoeal cases. In diarrhoeal cases in age group 0-6 months out of 40 cases 5 (12.5%) were positive for *C. cayetanensis*; whereas in non-diarrhoeal cases, out of 12 cases none were positive. Among the age group 7-24 months, in diarrhoeal cases, out of 190 cases 12 (6.31%) were positive for *C. cayetanensis*, whereas in non-diarrhoeal cases, out of 52 cases 1 (1.92%) was positive. Among the age group 25-60 months, in diarrhoeal cases, out of 156 cases 13 (8.33%) were positive for *C. cayetanensis*; whereas in non-diarrhoeal cases, out of50 cases 6 (12.00%) were positive which was found statistically insignificant, $p>0.05$.

Table 24 Sex wise distribution of *Cyclospora cayetanensis*

Sex	Diarrhoeal cases			Non-diarrhoeal cases		
	Frequency	*Cyclospora* positive	%	Frequency	*Cyclospora* positive	%
Male	245	22	8.97	66	4	6.06
Female	141	8	5.67	48	3	6.25
Total	386	30	7.77	114	7	6.14

The table shows sex wise comparison of *Cyclospora cayetanensis* infection between diarrhoeal and non-diarrhoeal cases. In diarrhoeal cases among the males out of 245

cases 22 (8.97%) were positive for *C. cayetanensis*; whereas in non-diarrhoeal cases out of 66 cases 4 (6.06%) were positive. Among the females in diarrhoeal cases out of 141 cases 8 (5.67%) were positive for *C. cayetanensis*; whereas in non-diarrhoeal cases out of 48 cases 3 (6.25%) were positive which was found statistically insignificant, p>0.05.

Table 25 Month wise distributions of *Cyclospora cayetanensis*

Month	Diarrhoeal cases			Non-dairrhoeal cases		
	Frequency	*Cyclospora* positive	%	Frequency	*Cyclospora* positive	%
May	83	0	0.00	15	0	0.00
June	94	8	8.51	19	3	15.78
July	79	13	16.45	26	1	3.84
August	80	5	6.25	30	3	10.00
September	50	4	8.00	24	0	0.00
Total	3866	30	7.77	114	7	6.14

The table shows month-wise comparison of *Cyclospora cayetanensis* infection between diarrhoeal and non-diarrhoeal cases. Among diarrhoeal cases, in May month out of 83 cases none was positive for *C. cayetanensis*, whereas in non-diarrhoeal cases out of 15 cases none was positive for C. *cayetanensis*. In June, among diarrhoeal case out of 94 cases 8 (8.51%) were positive; whereas in non-diarrhoeal cases out of 19 cases 3 (15.78%) were positive. In July, among 79 diarrhoeal cases, 13 (16.45%) were positive; whereas in 26 non-diarrhoeal cases, 1 (3.84%) was positive. In August, among 80 diarrhoeal cases 5 (6.25%) were positive; whereas in 30 non-diarrhoeal cases 3 (10%) were positive. In September, among 50 diarrhoeal cases 4 (8%) were positive; where as in 24 non-diarrhoeal cases none were positive for *C. cayetanensis* which was found statistically insignificant, p>0.05.

Table 26 Distribution of *Cyclospora caytanensis* according to source of drinking water

Source of drinking water	Diarrhoeal cases			Non- diarrhoeal cases		
	Frequency	*Cyclospora* positive	%	Frequency	*Cyclospora* positive	%
Tap	290	25	8.62	62	4	6.45
Well	9	0	0.00	3	0	0.0
Boiled	52	4	7.69	36	2	5.55
Filtered	35	1	2.85	13	1	7.69
Total	386	30	7.77	114	7	6.14

The table shows distribution of *Cyclospora caytanensis* according to source of drinking water in diarrhoeal and non-diarrhoeal cases. Among patients using tap water, out of 290 cases 25 (8.62%) were positive for *C. cayetanensis*; whereas in non-diarrhoeal cases, among tap water users, out of 62 cases 4 (6.45%) were positive. Among patients using well water, out of nine diarrhoeal cases, none was positive; whereas in three non-diarrhoeal cases, none was positive. Among patients using boiled water, out of 52 diarrhoeal cases, 4 (7.695) were positive; whereas in 36 non-diarrhoeal cases, 2 (5.55%) were positive. Similarly, among patients using filtered water, out of 35 diarrhoeal cases, 1(2.85%) was positive; whereas in 13 non-diarrhoeal cases, 1 (7.695) was positive for *C. cayetanensis.*

Table 27 Distribution of *Cyclospora cayetanensis* according to clinical symptoms

Symptoms	**Yes**			**No**		
	Frequency	***Cyclospora* positive**	**%**	**Frequency**	***Cyclospora* positive**	**%**
Fever	196	16	8.16	190	15	7.89
Abdominal pain	302	27	8.94	84	5	5.95

Vomiting	201	19	9.45	185	13	7.02
Nausea	195	19	9.74	191	13	6.80

The table shows Distribution of *Cyclospora cayetanensis* according to clinical symptoms. Out of 196 fever present cases, 16 (8.16%) were present for *C. cayetanensis*; whereas in those without fever (190), 15 (7.89%) were positive. In those with abdominal pain, out of 302 cases, 27 (8.94%) were present for *C. cayetanensis*; whereas in those without abdominal pain, out of 84 cases, 5 (5.95%) were positive for *C. cayetanensis*. In those with vomiting, out of 201 cases, 19 (9.45%) were positive for *C. cayetanensis*; whereas in those without vomiting, out of 185 cases, 13 (7.02%) were positive. In those with nausea, out of 195 cases, 19 (9.74%) were positive for *C. cayetanensis*; whereas in those without nausea, out of 191 cases, 13 (6.80%) were positive for *C. cayetanensis*, which was found statistically significant, $p<0.05$.

Table 28 Age wise distribution of bacterial pathogen

Age	Diarrhoeal cases				Non-diarrhoeal cases			
	Frequency	Shigella spp.	Salmonella Spp.	Vibrio spp.	Frequency	Shigella spp.	Salmonella Spp.	Vibrio spp.
0-6	40	0(0%)	1(2.5%)	1(2.5%)	12	0(0%)	0(0%)	0(0%)
7-24	190	0(0%)	0(0%)	2(1.05%)	52	0(0%)	0(0%)	0(0%)
25-60	156	0(0%)	0(0%)	9(5.76%)	50	0(0%)	0(0%)	1(2.0%)
Total	386	0(0%)	1(0.25%)	12(3.10%)	114	0(0%)	0(0%)	1(3.33%)

The table shows age wise distribution of bacterial pathogen. Among age group 0-6,in diarrhoeal cases out of 40 cases, 1 (2.5%) was positive for S*almonella spp*. and *Vibrio spp*. each. In 7-24 age group, out of 190 cases, 2 (1.05%) were positive for *Vibrio spp*. and *Salmonella* and *Shigella* spp. was absent. In 25-60 age group, out of 156 cases, 9 (5.75%) were positive for *Vibrio* spp. and *Salmonella* and *Shigella* spp. was absent.

Similarly in non-diarrhoeal cases, in 0-6 age group, out of 12 cases none of the bacterial pathogen was present. Among 7-24 age group also no bacterial pathogen was present. Among 25-60 age group, 1 (2.0%) was positive for *Vibrio spp.*, and *Salmonella* and *Shigella* spp. were absent which was found statistically insignificant, p>0.05.

Table 29 Sex wise distribution of bacterial pathogens

Sex	Diarrhoeal cases				Non-diarrhoeal cases			
	Frequency	Shigella spp.	Salmonella Spp.	Vibrio spp.	Frequency	Shigella spp.	Salmonella Spp.	Vibrio spp.
Male	245	0(0%)	1(0.40%)	5(2.04%)	66	0(0%)	0(0%)	0(0%)
Female	141	0(0%)	0(0%)	7(4.96%)	48	0(0%)	0(0%)	1(2.08%)
Total	3866	0(0%)	1(0.25%)	12(3.10%)	114	0(0%)	0(0%)	1(3.33%)

The table shows gender wise distribution of bacterial pathogen. In diarrhoeal cases, out of 245 males, 1 (0.4%) was positive for *Salmonella* spp., 5 (2.04%) were positive for *Vibrio* spp. out of 141 female cases, 7 (4.96%) were positive for *Vibrio* spp. and *Salmonella* and *Shigella* spp. were absent. In non-diarrhoeal cases, out of 66 male cases, no bacterial pathogen was found. Out of 48 female cases, 1 (2.08%) was positive for *Vibrio* spp. and *Salmonella* and *Shigella* spp. were absent which was found statistically insignificant, p>0.05.

Table 30 Month wise distributions of bacterial pathogens

Age in months	Diarrhoeal cases				Non-diarrhoeal cases			
	Frequency	Shigella spp.	Salmonella Spp.	Vibrio spp.	Frequency	Shigella spp.	Salmonella Spp.	Vibrio spp.

May	83	0(0%)	0(0%)	0(0%)	15	0(0%)	0(0%)	0(0%)
June	94	0(0%)	0(0%)	0(0%)	19	0(0%)	0(0%)	0(0%)
July	79	0(0%)	0(0%)	2(2.53%)	26	0(0%)	0(0%)	0(0%)
August	80	0(0%)	1(1.25%)	2(2.53%)	30	0(0%)	0(0%)	1(3.33 %)
September	50	0(0%)	0(0%)	8(16%)	24	0(0%)	0(0%)	0(0%)
Total	386 6	0(0%)	1(0.25%)	12(3.10%)	114	0(0%)	0(0%)	1(0.87 %)

The table shows month wise distribution of bacterial pathogens. *Shigella* spp. was absent in whole study. Bacterial pathogens were absent in May and June. In July, out of 79 diarrhoeal cases, 2 (2.53%) were positive for *Vibrio* spp. whereas in non-diarrhoeal cases bacterial pathogen was absent. In august, out of 80 diarrhoeal cases, 1 (1.25%) and 2 (2.53%) were positive for *Salmonella* spp. and *Vibrio* spp. respectively. out of 30 non-diarrhoeal cases 1 (3.33%) was positive for *Vibrio* spp. In September, out of 50 diarrhoeal cases, 8 (16%) were positive for *Vibrio* spp.out of 24 non-diarrhoeal cases, bacterial pathogen was nil.

Table 31 Age wise distribution of parasites

Age in month	Diarrhoeal cases			Non-diarrhoeal cases		
	Frequency	Parasite Positive	%	Frequency	Parasite Positive	%
0-6	40	9	22.5	12	4	33.33
7-24	190	56	29.47	52	10	19.23
25-60	156	34	27.79	50	17	34.00
Total	386	99	79.76	114	31	86.56

The table shows the age wise distribution of parasites. In diarrhoeal cases highest numbers of parasites were fond in the age group 7-24 (29.47% i.e. 56/190) followed by age group 25-60 (27.79% i.e. 34/156) and 0-6 (22.5% i.e. 9/40). Whereas in non-diarrhoeal cases, the highest number of parasite were found in the age group 25-60

(34.0% i.e.17/50) followed by 0-6 month (33.33% i.e. 4/12) and 7-24 (19.23% i.e. 10/52) which was found statistically insignificant, p>0.05.

Table 32 Sex wise distribution of parasites

Sex	Diarrhoeal cases			Non-diarrhoeal cases		
	Frequency	Parasite Positive	%	Frequency	Parasite Positive	%
Male	245	57	23.26	66	21	31.81
Female	141	34	24.11	48	8	16.66
Total	386	91	47.37	114	29	48.47

The table shows sex wise distribution of parasites. Among diarrhoeal cases, highest numbers of parasite were positive in females (24.11%) than males (23.26%). Similarly in non-diarrhoeal cases, highest numbers of parasites were found in males (31.81%) than females (16.66%), which were found statistically significant (p<0.05).

Table 33 Month wise distributions of parasites

Month	Diarrhoeal cases			Non- diarrhoeal cases		
	Frequency	Parasite positive	%	Frequency	Parasite Positive	%
May	83	15	18.07	15	1	6.66
June	94	26	27.65	19	7	36.84
July	79	24	30.37	26	10	38.46
August	80	16	20.00	30	3	10.00
September	50	13	26.00	24	4	16.66
Total	3866	94	122.09	114	25	108.62

The table shows month wise distribution of parasite. Among diarrhoeal cases, in month May out of 83 cases 15 (18.07%) were parasite positive; in June out of 94 cases 12 (27.65%) were parasite positive; in July out of 79 cases 24 (30.37%) were positive; in August out of 80 cases 16(20.0%) were positive and in September out of 50 cases 13 (26.00%) were positive. Whereas in non-diarrhoeal cases in month May out of 15 cases, 1 (6.66%) were positive, in June out of 19 cases7 (36.84%) were positive, in July, out of

26 cases 10 (38.46%) were positive, in August out of 30 cases 3(10.00%) were positive and in September out of 24 cases 4 (16.66%) were positive which was statistically not significant (p>0.05).

Table 34 Association between pathogenic parasites and bacteria

Parasites (N=162)	Bacteria (N=14)			
	Salmonella (n=1)	*Shigella* (n=0)	Vibrio (n=13)	Total (n=14)
Entamoeaba histolytica	0	0	3	3
Giardia lamblia	0	0	1	1
Cyclospora cayetanensis	1	0	3	4
Trichuris trichuria	0	0	1	1
Total	1(100%)	0(0%)	8(60.53%)	9(64.28%)

There was some association between bacteria and pathogenic parasites. It was found that 9 (64.28%) cases out of 162 parasite-infected cases were associated with diarrhoea causing bacteria (i.e.9/162). Similarly, 64.28% of bacterial isolates were in association with the parasites among the total bacteria infected cases (i.e.9/14). The higher number of association was shown by *Vibrio* spp. (8/13) followed by *Salmonella* spp. (1/140).

Table 35 Association between Cyclospora cayetanensis and bacterial infection

Bacteria (N=14)	*Cyclospora cayetanensis* (N=37)	%(N=14)
Salmonella spp	1	7.14
Shigella spp.	0	0.00
Vibrio spp.	3	21.42
Total	4	42.56
%(N=37)	10.81	

The table shows that some of the *Cyclospora* infection (10.81%i.e. 4 out of 37) was associated with bacterial infection and similarly, 42.56% i.e. 4 out of 14 bacterial pathogens were in association with *Cyclospora* infection.

Table 36 Association between rotavirus and bacterial infection

Bacteria	Rotavirus	Total
Salmonella spp	-	-
Shigella spp.	-	-
Vibrio spp.	5	5
Total	5	5

Among the total cases processed, there was only one case in which there was co-infection of *Vibrio spp.* and rotavirus.

Table 37 Association between rotavirus and parasitic infection

Parasites (N=162)	**Rotavirus positive (N=70)**	**%**
Ascaris lumbricoides	1	1.42
Blastocystis hominis	2	2.85
Cyclospora cayetanensis	3	4.28
Entamoeaba histolytica	7	10.0
Giardia lamblia	4	5.71
Hymenolepsis nana	1	1.42
Entamoeaba coli	1	1.42
Total (N=162)	89(27.1?%)	11.72

Among the total rotavirus infections (70), 19 cases (27.1% i.e. 19 out of 70) were found in association with the enteric parasites. Similarly, 19 cases of parasitic infections (11.72% i.e. 19 out of 162) were in association with rotavirus infection. The highest association was shown by *Entamoeaba histolytica* (10.0%).

CHAPTER-VI

6.1 DISCUSSION

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).

Diarrhoeal disease is one of the major public health problems among children below five year in Nepal. In Nepal, every year, 30-40 thousand people die from this disease (Bista et al., 1993). There are high rates of morbidity and mortality due to this disease in the country. In the year 2000/01, 8836 cases and 247 deaths from acute diarrhoeal diseases were reported to epidemiology Division (DoHS, Annual report, 2001).

The identification of etiological agents is very important which helps in precise diagnosis and proper treatment of patient in time. So, to determine common etiological agents of diarrhoeal diseases, 500 stool samples from children below five years were studied in Kanti Children's Hospital. The study was conducted from May 2006 to September 2006. Out of 500 samples, 386 samples were diarrhoeic and 114 were non-diarrhoeic samples.

Out of 500 samples, 311 were males and 189 were females, which shows normal practice to seek medical treatment for male children preferably in our society. Chand, 2000 and Agrawal et al., 1989, observed similar results in a study carried out in India. However, in the study carried out in Lahore, Pakistan by Mahmud et al., 1993 and in China, there were no gender differences (Huang et al., 1995).

In this study, the prevalence of enteropathogens observed was 201(52.07%) in diarrhoeal cases and 45(39.47%) in non-diarrhoeal cases including bacteria, *Cyclospora cayetanensis*, other parasites, and rotavirus. These findings were in accordance with the study carried out by Sheikh and Assouli in 2001 in Saudi Arabia (47.6%) and 66.4% in

Jordan by Youssuf et al., 2000, 28.7% in India by Neerja et al., 1991and 39% by Howard et al., 2000.

The study done in Dhaka, Bangladesh by Albert et al., 1999 showed that enteropathogens were isolated from 74.8% in 1993/94 and 66% in 1982 from diarrhoeal patients. Similarly enteropathogens were identified in 58.4% cases in a study carried out in republic of the Philippines by Adkins et al., 1987, in Somalia 61% by Casalino et al., 1984,in Bangladesh 59% by Moyenddin et al., 1982/83, in Denmark 54% by Olesen et al., 2005 and in Srilanka 53.7% by Mertens et al., 1990. This showed marked variations in the prevalence of enteropathogens in various places. These variation in prevalence of enteropathogens May be due to different factors like geographic set up, living conditions, economic status, traditions and practices, hygienic conditions, water and disposal system, sanitary measures, time period of study carried out, etc.

In 386 diarrhoeal samples, potential enteric pathogens were identified in 201(52.07%) cases. Thirteen cases (3.36%)were positive for bacterial pathogen, 123cases (31.85%) were positive for parasites with 30 cases (7.77%) of *Cyclospora cayetanensis* and 65cases (1.68%) were positive for rotavirus. Out of 114 non-diarrhoeal samples, potential enteric pathogens were identified in 45(39.47%); one case (0.87%) was positive for bacterial pathogen, 39cases (34.20%) were positive for parasites with 7 cases (6.14%) of *Cyclospora cayetanensis* and5 cases (4.38%) were positive for rotavirus.

The distribution of different enteric pathogens among various age group indicated that the highest number of bacterial and parasitic pathogen was found in age group 25-60 months, whereas the highest number of rotavirus was found in age group 7-24 months. The gender wise distribution shows that males are more infected by parasitic and rotaviral infection, whereas females are more infected by bacterial pathogen.

Highest number of samples (113) was processed in June and the least (74) were processed in September. The prevalence of rotavirus was found highest in September (21.62% i.e. 16 out of 74), followed by May (19.38%), June (14.15%), July (9.52%),

and August (8.1%). The prevalence of bacterial pathogen was found highest in September (10.8%), followed by august (3.6%), July (1.9%).

Rotavirus is known as a causative agent of winter diarrhoea. In this study, overall rotavirus infection was seen in70 cases. In diarrhoeal cases rotavirus infection was in 65 cases (16.8%) and in non-diarrhoeal cases rotavirus infection was in 5 cases (4.8%) which was statistically significant. In hospital based studies the varying percentages of rotavirus infection have been found. A hospital based study conducted in US showed up to 54% in winter months. The exact cause of increase infection by rotavirus in winter season is not known but May be due to poor hygienic condition such as hand washing and improper food handling.

The incidence of rotavirus infection is similar in both industrialized and developing countries, suggesting that a decrease in infection cannot be affected by improvements in hygiene or sanitation. Nearly every child around the world will have a rotavirus infection in their early childhood – many more than once.

The highest rates of disease are typically in children under two years of age, who are also at the greatest risk for severe rotavirus disease. There are several different serotypes of rotavirus and the prevalence of these serotypes varies by geographic region.

Altogether, 70 cases were found infected with rotavirus accounting for 14% of the total processed samples (70/500). Similar types of findings have been found in the study carried out by Albert et al., 1999 in which the overall infection rate was 20.3% rotavirus positive during a 17-month study period from February 1993 to June 1994.Similarly the study by Saravanan et al., 2004 in Chennai found 22.5% among the children with acute diarrhoea during the period of March 1995 to August 1999.

In Nepal study carried out by Sherchand et al., 1992 found 27% rotavirus positive cases in a hospital-based study in Kanti children's hospital. Similarly another study carried out by Shariff et al., in BP Koirala Institute of Health sciences, Dharan, Nepal found 35.6%

rotavirus positive cases. Another study by Sherchand et al., 2004 found 30.6% rotavirus positive cases.

The possible reason for the differences in the rotavirus positivity may be due to the differences in geographic set up, seasonal variation, and the endemicity of the disease. In this study the prevalence of rotavirus is (14%) which is lower than the results found by other researchers in winter season. This is due to the reason that the rotavirus infection peaks during the winter season and our study was carried out during hotter months, from May to September 2006.The low prevalence of rotavirus, most likely relates to the limited seasons of the study. Ideally, etiology studies should be continued over one year (Hoge et al., 1995).

This study showed the highest rate of rotavirus infection is seen in hospitalized patients than the patients who visited OPD. In hospitalized cases rotavirus infection was seen 57 (22.8%), which was significantly higher than in non-hospitalized cases it is 12 (4.8%).

Rotavirus infection is prevalent in between age group 0-5 years (Ananthanarayan, 1994). This study showed that in age group 7-24 months rotavirus infection was highest (21.57%) comparing to age group0-6 months (12.55%) and age group 25-60 months (12.17%). But in non-diarrhoeal cases highest percentage of infection was seen in age group 7-24 months (5.7%); in age group 25-60 months (4.0%) and in age group 0-6months (0%) which was statistically insignificant. Rotavirus is the major paediatric 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., 2004). Sherchand et al., 2004 found highest frequency i.e. 42.9% and 31.4% rotavirus positives respectively in age group 6-11 months and 1-2 years among total cases. Likewise, major proportion of the rotavirus positive cases fell under the age group between 7-18 months in a study by Saravanan et al., 2004.

The gender wise distribution of rotavirus infection was found to be higher in males (17.95% i.e.44/245) than in females (14.89%) that were found statistically insignificant. Whereas in non-diarrhoeal cases the rotavirus infection was found to be highest in males

(4.54%) than in females (4.16%) that was found statistically insignificant. However no association of rotavirus infection could be discerned between male (23.9%) and females (21.1%) children in a study carried out by Saravanan et al., 2004.

Among the total diarrhoeal samples, the highest rotavirus infection was seen in September (28.0%) followed by May (20.48%), June (15.95%), July (12.65%), and August (11.25%) respectively. Whereas in non-diarrhoea cases, the highest rotavirus infection was seen in May (13.33%) followed by September (8.33%), June (5.26%), and in July and August (0%), respectively which was found statistically insignificant.

The results obtained in this study carried out in hotter months are relatively lower than that found in colder months. Sherchand et al., 2004 found that rotavirus was predominant in winter, particularly in December to February accounting for more than 60% of pediatrics diarrhoea. Saravanan et al. obtained similar results, 2004 while analyzing the seasonal variation pertaining to rotavirus that the hotter months (March-August) in three years (1996, 1997and 1998) had decreased rate of rotavirus associated diarrhoea (17.6%, 17.05 and 14.3% respectively) than the cooler months (September to February) i.e. 21.8%, 31.8% and 22.55% respectively.

This study showed that the source of drinking water was associated with rotavirus infection. The study showed that rotavirus infection was found to be highest in those using tap water (18.96%), followed by well water (11.11%), filtered water (8.57%) and boiled water (8.57%) in diarrhoeal case. But in non-diarrhoeal cases, rotavirus infection was found to be highest in boiled water (5.55%) followed by tap water (4.83%) and 0% in well and filtered water drinker.

Symptoms of rotavirus infection constitute of mild fever, abdominal pain, nausea, vomiting, diarrhoea and dehydration. The study showed that in diarrhoeal cases, children having nausea and vomiting have highest number of rotavirus infection (23.58% and 21.39% respectively) than those with fever and abdominal pain (15.81% and 15.56% respectively). But in non-diarrhoeal cases, children having fever and abdominal pain had more rotavirus infection (20.52% and 27.38% respectively) than those with nausea and

vomiting (14.05% and 12.56% respectively). The questionnaire regarding abdominal pain is taken from parents, which may not be exactly representative with the cases since infants can't answer.

In this study, the prevalence of *Cyclospora cayetanensis* was found to be 7.77% in diarrhoeal cases and 6.14% in non-diarrhoeal case, which is third most prevalent protozoan. These findings are similar with the findings of Lama, 2005 (7.1%), Rai, 2003 (7.6%), Chaudhary, 2003 (7.6%), Nimri, 2003 (6%) and Sherchand and Cross-, 2004 (found 6.4% positive cases in a study carried out in different healthcare institutions in different parts of Nepal). However, the result is different from the findings of Sherchand et al., 2001 (24.6%), Sherchand et al., 1996 (3.9%), Sherchand et al., 1999 (29.8%). These differences in the rate of infection with *Cyclospora cayetanensis* May be due to different geographical areas, seasons, time period, climatic condition, socioeconomic factors of the population, etc. In the study carried out by (Ghimire et al., 2002-2004), *Cyclospora* was identified in 8.2%.

The *Cyclospora* infection was found highest in age group 0-6 months (12.55% i.e. 5 out of 386) followed by 25-60 (8.33%), 7-24 (6.31%). But in non-diarrhoeal cases, the *Cyclospora* infection was found highest in age group 25-60 months (12%) followed by 7-2 (1.92%), 0-6 months (0%) that was found statistically insignificant. The result is somehow consistent with the findings of Sherchand et al., 2001,2004. Males were more infected (8.97%) than females (5.67%). But in non-diarrhoeal cases, females were more infected (6.25%) than males (6.06%) out of total cases, which was statistically insignificant.

In particular month, *C. cayetanensis* infection was found highest in the month of July (16.45%) followed by June (8.51%), September (8%), August (6.25%) and May (0%). But in non-diarrhoeal cases, *C. cayetanensis* infection was found highest in the month of June (15.78%) followed by August (10%), July (3.84%), May, and September (0%) that was found statistically insignificant. These findings are similar with the findings of Sherchand et al., 2001 (found highest incidence in June and July) and Sherchand and Cross, 2004. Water has been implicated in outbreaks in United States (Huang et al.,

1995). In this study, *C. cayetanensis* infection was found highest in the children consuming tap water (8.62%) followed by boiled (7.69%), filtered (2.85%) and well (0%). In non-diarrhoeal cases, *C. cayetanensis* infection was found highest in the children consuming tap water

In diarrhoeal cases, *Cyclospora* infection was highest in the children having Nausea (9.75%) followed by vomiting (9.45%), abdominal pain (8.94%) and fever (8.16%). Similarly in non-diarrhoeal cases, *Cyclospora* infection was highest in the children having fever (7.69%), followed by vomiting (7.02%), nausea (6.80%) and abdominal pain (5.95%) that was found statistically significant.

Out of total diarrhoeal samples processed (386), 13 cases (3.36%) showed positive for pathogenic bacterial growth for *Salmonella* spp. and *Vibrio* spp. and *Shigella* spp. was not found in a single sample. Whereas in non-diarrhoeal cases (114), only one case was positive for *Vibrio* spp.

Among the bacterial pathogens *Vibrio* spp. was highest in numbers (13), 12 in diarrhoeal cases (3.1%) and in non-diarrhoeal cases1 (0.8%) followed by *Salmonella* spp.1 (.25%in diarrhoeal case). This result is lower (2.8% i.e.14 / 500 cases) than the findings of Rai, 2003, in which bacterial infection accounted for 5.7% of total population. But, Chand, 2000 found 34.6% positive bacterial infection; Mubashir et al., 1990 in Islamabad found 52.2% possible bacterial pathogens, Taylor et al., 1998 found 47% bacterial pathogens in expatriate patients of Nepal and Ballal and Shivananda, 2002 found 69.6% bacterial pathogens. The relatively low number of bacterial isolates in this study May be due to use of antibiotics by patients before visit to the hospital; geographic distribution, the type of bacteria being sought; for example pathogenic *E.coli was* isolated in the most of the samples but not included in the result and *Campylobacter* spp. were not found in this study. Also this study is mainly focused on *Cyclospora* and Rotavirus.

Among the total diarrhoeal cases (386), the prevalence rate of bacterial pathogen was higher in the age group months25-60 (2.33%) followed by0-6 and 7-24 (0.51%), respectively with respect to particular age group. Among total non-diarrhoeal cases

(114), the prevalence rate of bacterial pathogen was higher in the age group 25-60 months (0.87%) followed by0-6 and 7-24 (0%) that was found statistically insignificant. The high prevalence of bacterial infection in age group 2-4 years is probably due to the combined effects of declining levels of maternal immunity and lack of active immunity in the child of this age. Also the introduction of food that May be contaminated with faecal pathogens and direct contact with human and animal faeces when child starts to crawl May attribute to high infection among the children of this age group. Most enteric pathogen stimulates at least partial immunity against repeated infection or illness, which helps to explain the declining incidence of disease in older children and adults (WHO, 1994).

Among total diarrhoeal cases females were more infected (1.81%) than males (1.55%). In non-diarrhoeal cases also, females were more infected (0.87%) than males (0%), which was statistically insignificant.

In particular month, bacterial infection was found highest in the month of September (16%) followed by August (3.75%) July (2.53%), June, and May (0%). But in non-diarrhoeal cases, bacterial infection was found only in the month of August (3.33%) and in other months no bacterial infection was found.

In our study, *Vibrio* spp was isolated at the rate of 2.6%, *Salmonella* was found in 1 case (0.2%) and *Shigella* was not found. This rate is very low in comparison to the findings of other researchers like Chand, 2000 found Vibrio (17.6%), Pant, 2005 (17%), KC, 2004 (68.5%), and *Salmonella* was found by KC.2004 (3.4%), Albert et al., 1999 (2%) and *Shigella* was found by Rai, 2000(4%), Youseef et al., 2000 (4.9%)and Urbina et al., 2003 (8.0%). These differences in prevalence of the organism May be due to different time period of study, different geographical set up, the endemicity of the organism and other environmental factors such as weather, seasonal variation, etc.

Shigellosis is primarily a pediatric disease, around 80% of infection occurs in the age below 10 years old, the majority of cases occurring in children less than 5 years old (Blaser et al., 19830 and in the study *Shigella* was not in a single case.

Salmonellosis is a systemic prolonged febrile illness caused by *Salmonella* enterica serovar and continues to be a worldwide health problem, especially in developing countries with their poor sanitation, poor standards of personal hygiene and contaminated food and water. Gastroenteritis is the most common presentation and is considered as an emergent food-borne and water-borne pathogen-caused disease (Mercides Rodriguez et al., 1998).

Vibrio cholerae 01 is endemic in parts of Africa and Asia (e.g. 5-10% of hospitalized diarrhoea patients). The EI tor cholera biotype is responsible for the 7^{th} pandemic (Niyogi et al., 1994).

While studying the prevalence of *Cyclospora* and rotavirus infections among the children, the other parasites encountered during the study period were also noted. These parasites accounted for 25.0% of total enteropathogens isolated. This finding was in harmony with the findings of Chand, 2000 (27.94%), Rai et al., 1991 (30.9%), Shrestha, 2000 (36.5%)In total diarrhoeal samples (386), the parasites accounted for 24.09% and in total non-diarrhoeal samples (114), the parasites accounted for 28.07%.

However, this finding is inconsistent with the findings of Sherchand et al., 1995 (63.1%), Ishimyama et al., 2001 (72.4%), Rai et al., 2001 (76.4%), Sherchand et al., 1996 (60.1%) in Dhanusha district.

These differences in the rate of parasitic infections May be due to various factors like seasonal variation, geographic distribution, climatic condition, socio-economic status, different time of study period, literacy rate, degree of sanitatory condition, etc of the subjects being studied.

Among the total parasitic infestation, protozoan dominated over helminthes in both diarrhoeal and non-diarrhoeal cases. In diarrhoeal cases, protozoans were positive for 112 cases (29.01%) and helminthes were positive in 11 cases (2.64%). Similarly in non-diarrhoeal cases, protozoans were positive in 33 cases (28.94%)and helminthes in 6 cases (5.26%). During the study 112 types of protozoans were recorded including

Cyclospora. Among the protozoan, *Entamoeba histolytica* (9.32%) was major causative agent of diarrhoea followed by *Giardia lamblia* (8.29%), *Cyclospora* (7.77%), *Entamoeba coli* (1.04%), *Cryptosporidium parvum* (0.77%), *Blastocystis hominis* (1.81%). Whereas in non-diarrhoeal cases, 33 types of protozoans were recorded. *Giardia lamblia* (10.52%) was the major cause of gastroenteritis followed by *Entamoeba histolytica* (7.01%), *Cyclospora* (6.14%), *Entamoeba coli* (0.67%), *Cryptosporidium parvum* (0.67%) and *Blastocystis hominis* (3.50%).

Among the helminthes, in diarrhoeal cases, 11 types of helminthes were recorded. *Ascaris lumbricoides* (1.04%) and *Hymenolepsis nana* (1.04%) were the major helminthes to cause diarrhoea, followed by Trichuris trichuria (0.51%), *Taenia* spp. (0.03%). whereas in non-diarrhoeal cases, 6 types of helminthes were recorded during the study. *Ascaris lumbricoides* (1.25%) *and Trichuris trichuria* (1.25%) were found to be the major helminthes to cause gastroenteritis, followed by *Hymenolepsis nana* (0.87%), *Taenia* spp. (0.87%).

In the study carried out by Sherchand et al., 1995 reported *G. lamblia* on top followed by *E. histolytica*, *C. parvum* and *C. cayetanensis* with the prevalence rate 9.9%, 7.2%, 1.4% and 3.9% respectively and with regards to helminthic infestation hookworm topped the list which is not consistent with our findings. Similarly, Rai et al., (1985-94) reported that *G. lamblia* (5.2-26.25%) topped the list followed by *E. histolytica* (1.9-14.6%). However, Chand, 2000 found *E. histolytica* (6.62%) as the major causative agent followed by *G. lamblia* (5.88%), *C. parvum* (2.94%) and *C. cayetanensis* (1.47%) which is similar to our results and helminthic infestation distribution shows the pattern of *Ascaris lumbricoides* (6.62%) followed by hookworm (3.31%) and *Trichuris trichura* (1.10%). This shows the various pattern of infestation with these parasites, which reveals the fact that there is marked variation of infestation with the species and type of parasites. It may be due to variation of infesting sites, mode of infestation and resistant pattern among the population, etc.

The highest prevalence of parasites was found in the age group7-24 (56 cases, 29.47%) followed by age group25-60 (34 cases, 27.79%), 0-6 (9cases, 22.5%). Whereas in non-

diarrhoeal cases, the highest prevalence of parasites was found in the age group 25-60 (17cases, 34.0%) followed by age group 7-24 (10 cases, 19.23%), 0-6 (4 cases 33.33%). From this study it was found that the highest prevalence of parasites was in female (24.11%) than in males (23.26%). Whereas in non-diarrhoeal cases, the highest prevalence of parasite was in male (31.81%) than in females (16.66%). The highest prevalence of parasitic infection was found in the month of July (30.37%) followed by June (27.65%), September (26.0%), August (20.00%) and May (18.07%). Similarly in non-diarrrhoeal cases, the highest prevalence of parasitic infection was found in the month of July (38.46%), followed by June (36.84%), September (16.66%), August (10.0%) and May (6.66%).

From our study it was found that *E. histolytica* infection was highest in the month of July (13 cases) followed by August (9 cases), June (6 cases), September (5 cases), May (4 cases) whereas in non-diarrhoeal cases, *E. histolytica* infection was highest in the month of June (3 cases) followed by July and September (2 cases), May and August no *E. histolytica.* While *G. lamblia* infection seemed to be highest in the month of June (9 cases) followed by May (8 cases, August (6 cases), July and September (5 cases). Whereas in non-diarrhoeal cases, *G. lamblia* infection was highest in the month of July (4 cases) followed by August and September (2 cases) and in May no cases of. *G. lamblia. C. parvum* seemed to be present in the month of June only (2 cases) whereas in non-diarrhoeal cases, *C. parvum infection* was found in the month of July (1 case). *Blastocystis hominis* infection was highest in the month of June (3 cases) followed by May and July (2 cases) and no case in August and September. Whereas in non-diarrhoeal cases, *Blastocystis hominis* infection was found in the month of May, June, July (1 case). *Entamoeba coli* infection was highest in the month of June (3 case) followed by May (1 case), no case of *E. coli* in the month of July August and September. Whereas in non-diarrhoeal cases, *E. coli* infection was found in the month of June only (1 case).

Single case of *Ascaris lumbricoides* was found in the month of July, August and September; whereas in non-diarrhoeal cases, *Ascaris lumbricoides* infection was found in the month of August only (1 case). *Trichuris trichuria* was highest in the month of

September (2 cases) followed by July (1 case), *Ascaris lumbricoides* was not found in the month of May, June and August. Whereas in non-diarrhoeal cases, *Ascaris lumbricoides* infection was found in the month of August (1 case) and not in the month of May, June, July and September. *Hymenolepsis nana* was found in the month of June and July (2 cases) and not found in the month of May, August and September. Whereas in non-diarrhoeal cases, *Hymenolepsis nana* was found in the month of July (1 case). Similarly single case of *Taenia* spp was found in the month of June similarly in non-diarrhoeal cases also *Taenia* spp was found in the month of June.

From the study it was seen that there was some degree of association between bacteria and pathogenic parasites. It was found that 9 (5.55%) cases out of 162 parasitic infected cases were associated with bacteria (9/162). Similarly, 64.28% of bacterial isolates were in association with the parasites among the total bacteria infected cases (9/14). The higher frequency of association was shown by *Vibrio* spp. (57.14%) followed by Salmonella spp. (7.14%) and there was no *Shigella* spp. in a single sample. However with respect to particular type of organism, *Salmonella* spp. shows the higher degree of association with the parasite among the total *Salmonella* infection accounting for 100%(1/1) followed by *Vibrio* spp. (61.83%) among the total individual bacterial infection.

Some of the *Cyclospora* infection (10.81%) was associated with bacterial infection and similarly, 42.56% bacterial pathogens was in association with *Cyclospora* infection. Among the total case processed, there was 7.14 % association of *Cyclospora* infection with salmonella spp. and 21.42 % association with *Vibrio* spp. Some of the *Cyclospora* was found to be associated with other parasites.

Among the total rotaviral infections 70 cases (27.1%) were found in association with the enteric parasites other than *Cyclospora cayetanensis.* Similarly, 11.72 % of parasitic infections were in association with rotavirus infection. The highest association was shown by *Entamoeba histolytica* (10%). Among the total case processed there was co-infection between rotavirus and *Vibrio* spp.

In this study, in the diarrhoeal children, type of dehydration was categorized as mild, moderate and severe. The children having severe type of dehydration were highest in number (52.9%) followed by moderate type (24.4%) and mild type (22.7%). Among the diarrhoeic patients who visited hospital for treatment, 24.6% of them were treated with ORS. Some of the patients were also treated with Antibiotic (19.2%), IV Fluid (3.4%) and other medicine (anti-protozoal) 43.7%.

In this study the patients were also categorized into three groups on the basis of frequency of stool per day and it was found that highest number of patients were found to have 3-5 times a stool per day (43.3%) followed by 5-10 times (33.4%) and >10 times (23.3%). Similarly, patients were also grouped on the basis of types of stool (watery, mucus and blood). Highest number of patients was found having watery type of stool (68.9%.) followed by mucoid stool (54.6%) and blood in stool (1.3%).

6.2 CONCLUSION

From the results obtained, it is clear that *Cyclospora*, rotavirus and many enteropathogenic microorganisms and parasites are prevalent in Kathmandu valley and its vicinity. These pathogens are found to be water and food borne. These gastrointestinal diseases are transmitted through faeco-oral route. The level of gastrointestinal disease can be decreased significantly by implementing simple strategies, such as proper waste and water management, and education on maintenance of hygienic conditions. Studies on rotavirus infection will help in proper management of patients in health care centers and hospitals and future implementation of rotavirus vaccine.

CHAPTER VII

7. SUMMARY AND RECOMMENDATIONS

7.1 Summary

During the study period from May and September 2006, 500 samples were collected from children of age below 5 years suffering from gastroenteritis visiting Kanti Children's Hospital, Kathmandu were studied.

1. Among the total sample collected, 250 were taken from OPD and 250 were taken from ORT ward. Out of total sample collected 386 were of diarrhoeal children and 114 were of non-diarrhoeal children

2. Age and gender wise distribution of these patients showed that male children dominated in all age group.

3. Out of total diarrhoeal cases (386), prevalence of enteric pathogens was found in 201(52.07%) cases.among the pathogen positive samples thirteen cases (3.36%)were positive for bacterial pathogen, 123cases (31.85%) were positive for parasites with 30 cases (7.77%) of *Cyclospora cayetanensis* and 65cases (1.68%) were positive for rotavirus. Out of total non-diarrhoeic samples (114), potential enteric pathogens were identified in 45(39.47%); one case (0.87%) was positive for bacterial pathogen, 39cases (34.20%) were positive for parasites with 7 cases (6.14%) of *Cyclospora cayetanensis* and5 cases (4.38%) were positive for rotavirus.

4. In this study, *Cyclospora* infection was found highest in the age group 0-6 months (12.55% i.e. 5 / 386) followed by 25-60 (8.33%), 7-24(6.31%). But in non-diarrhoeal cases, it was found highest in age group 25-60 months (12%) followed by 7-24(1.92%) while there was no single case of *Cyclospora* infection in age group 0-6 months. The *Cyclospora* infection was found higher in males than females in both cases.

5. The highest *Cyclospora* infection was seen in July (16.45% i.e. 12 / 79) followed by June (8.51%), September (8%), August (6.25%) and no case was seen in May. But in non-diarrhoeal cases, it was found highest in the month of June (15.78%) followed by August (10% i.e.), July (3.84% i.e.) while there was not a single case in May and September.

6. The *Cyclospora* infection was found higher in those children consuming tap water in both cases. The *Cyclospora* infection was found higher in those children having Nausea (9.75%) followed by vomiting (9.45%), abdominal pain (8.94%) and fever (8.16% i.e. 16/196). Similarly in non-diarrhoeal cases, it was highest in the children having fever (7.69% i.e. 15/190), followed by vomiting (7.02%), nausea (6.80%) and abdominal pain (5.95%).

7. In the study rotavirus infection was found highest in the age group 7-24 months (21.57%) comparing to age group0-6 months (12.55%) and age group 25-60 months (12.17%). But in non-diarrhoeal cases highest percentage of infection was seen in age group 7-24 months (5.7%); in age group 25-60 months (4.0%) and there was not a single cases in age group 0-6 months. The rotavirus infection was found higher in males than in females in both cases.

8. The highest rotavirus infection was seen in September (28.0%) followed by May (20.48%), June (15.95%), July (12.65%), and August (11.25%) respectively. Whereas in non-diarrhoea cases, the highest rotavirus infection was seen in May (13.33%) followed by September (8.33%), June (5.26%), and in July and August there was no single case.

9. The rotavirus infection was found higher in children consuming tap water in both diarrhoeal and non-diarrhoeal cases. The rotavirus infection was found higher in children having nausea and vomiting (23.58% and 21.39% respectively). But in non-diarrhoeal cases, children having fever and abdominal pain had more rotavirus infection (20.52% and 27.38% respectively).

10. Among total bacterial isolates, *Vibrio* spp. (2.6%) was found as major causative agent followed by *Salmonella* spp. (0.12%) and *Shigella* spp. was not seen in a single case.

11. The prevalence rate of bacterial pathogens was higher in age group 25-60 months (2.33%) followed by0-6 and 7-24(.0.51%). Among total non-diarrhoeal cases, the prevalence rate of bacterial pathogen was higher in the age group 25-60 months (.0.87%) while there was not a single case seen in the age group0-6 and 7-24 months.

12. Bacterial pathogens were isolated highest in the month of September (16%) followed by August (3.75%) July (2.53%), June and May (0%). But in non-diarrhoeal cases, bacterial infection was found only in the month of August (3.33%) and in other months no bacterial infection was found. Bacterial pathogens were isolated highest in females than males in both cases.

13. Among the parasitic infestations, protozoa dominated over helminthes in both diarrhoeal and non-diarrhoeal cases. In diarrhoeal cases, protozoans were positive for 112 (29.01%) and helminthes were positive in 11 (2.64%). Similarly in non-diarrhoeal cases, protozoans were positive in 33 (28.94%) and helminthes in 6 (5.26%).

14. Among the protozoan, E. histolytica (9.32%) was major causative agent of diarrhoea followed by *G. lamblia* (8.29%), *Cyclospora* (7.77%), *E.coli* (1.04%), *C. parvum* (0.77%), *B. hominis* (1.81%). Whereas in non-diarrhoeal cases, *G. lamblia* (10.52%) was the major cause of gastroenteritis followed by *E. histolytica* (7.01%), *Cyclospora* (6.14%), *E. coli* (0.67%), *C. parvum* (0.67%) and *B.hominis* (3.50%).

15. The highest prevalence of parasite was seen in age group 7-24 (29.47%) followed by age group 25-60 (27.79%), 0-6 (22.5%). Whereas in non-diarrhoeal cases, the highest prevalence of parasites was found in the age group 25-60 (34.0%) followed by age group 7-24 (19.23%), 0-6 (33.33%).

16. The highest prevalence of parasites was in female (24.11%) than in males (23.26%). Whereas in non-diarrhoeal cases, the highest prevalence of parasite was in male (31.81%) than in females (16.66%).

17. The highest prevalence of parasitic infection was found in the month of July (30.37%) followed by June (27.65%), September (26.0%), August (20.00%) and May (18.07%). Similarly in non-diarrrhoeal cases, the highest prevalence of parasitic infection was found in the month of July (38.46%), followed by June (36.84%), September (16.66%), August (10.0%) and May (6.66%).

18. From the study it was seen that there was some degree of association between bacteria and pathogenic parasites. It was found that 5.55% parasites were associated with bacteria (9/162). Similarly, 64.28% of bacterial isolates were in association with the parasites.

19. Some of the *Cyclospora* infection (10.81%) was associated with bacterial infection and similarly, 42.56% bacterial pathogens was in association with *Cyclospora* infection. Among the total rotaviral infections (27.1%) were found in association with the enteric parasites other than *C. cayetanensis.* Similarly, 11.72 % of parasitic infections were in association with rotavirus infection.

7.2 Recommendations

i. There is no specific treatment for rotavirus infection. Therefore awareness should be created regarding the diarrhoea, its management so that some type of home based management can be started as soon as vomiting, and diarrhoea is started.

ii. In rotavirus infection, the isolated strain should be subjected to molecular level analysis for identification of prevailing serotype and strain in our country so that it May help in future for vaccine preparation.

iii. The study showed the highest incidence of Cyclosporiasis in July and June, which reveals the possibility of infection of *Cyclospora cayetanensis* in a rainy season. Therefore further research throughout the year in different geographical area to see seasonal variation and awareness on this aspect should be carried out.

iv. Wider range of enteropathogens, bacteria such as pathogenic *E.coli, Clostridium difficle, Yersinia enterocolitica, Campylobacter jejuni, Aeromonas hydrophila, Plesiomonas shigelloides*; viruses such as Norwalk agent, Ehovirus, Adenovirus and fungal cause should be studied.

v. Serological studies should be suggested to ascertain the specific identification of etiological agents that cause infantile diarrhoea.

vi. This study showed some degree of association between different entities of enteropathogen such as parasites and rotavirus, parasites and bacterial infection; rotavirus and bacterial infection; bacterial infection and *Cyclospora* infection. Therefore further research is needed in this aspect to find the exact association.

vii. A community based study and the detailed epidemiological study is need to be conducted to find out the exact epidemiology of transmission of rotavirus so that precise preventive measures can be taken to minimize burden of rota-viral diarrhoea.

viii. As this study was conducted in the Health Research Laboratory, Academic building, TUTH, in children below 5 years of age of Kanti Children Hospital, it does not actually represent the total picture of the disease burden of the whole community and the country. So, the community-based study should be carried throughout the country so that it reveals the real picture of disease burden.

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