Epidemiology of childhood diarrhoea in Iran

Thesis submitted in accordance with the requirement of the University of Liverpool for the degree of Doctor in Philosophy

By

Bahman Khalilidehkordi

July 2004

Liverpool School of Tropical Medicine

Abstract

The impact of diarrhoeic disease on childhood morbidity and mortality in developing countries is represented by more than one billion episodes and 3 to 6 million deaths annually in Asia, Africa, and America. The greatest morbidity and mortality is seen among children less than two years of age (Griffin et al., 1988).
This study investigated the epidemiology of childhood diarrhoea in Shahrekord, Iran. For this purpose we carried out one cross- sectional study, one case-control and one longitudinal study. Stool samples were collected from 259 children hospitalised with acute diarrhoea, 245 children with acute diarrhoea seen as outpatients in the hospital outpatients departments (OPD) and 114 children hospitalised without diarrhoea (controls). Samples were screened using ELISA, negative stain EM, and standard diagnostic techniques for parasites and bacteria. Pathogens were identified in 62%, 44% and 18% of the children hospitalised children with diarrhoea, the outpatients and the hospital controls respectively. Viruses were the pathogens most frequently detected, followed by bacteria and parasites. Rotavirus was the most frequent virus, present in 35% of the children hospitalised. The RT-PCR for rotavirus successfully typed 95% of the rotavirus G types and 85% of the P types. The most frequent G and P types were G1, G2, P[8] and P[4] which is consistent with other studies from Iran and the Middle East (Kurugol *et al.*, 2003; Youssef *et al.*, 2000; Zarnani *et al.*, 2004).

To identify the characteristics of the children that are risk factors for hospitalisation, hospitalised children with diarrhoea were compared with two groups of controls who also had diarrhoea but were not hospitalised. These control groups were selected from the OPD (245) and from health centres (245). The variables statistically significant after bivariate and multivariate analysis were the presence of blood in the stools (Adjusted Odds ratios (AOR), 124.6), a history of vomiting in the week before consultation (AOR, 6.6) and a history of a previous hospitalisation (AOR, 3.1). Some risk factors such as the use of spring water (AOR, 11), having an unhealthy (AOR, 3.2) or illiterate mother (AOR, 3) were only identified when comparing the cases with the OPD controls. The presence of watery stools (AOR, 31), lack of use of ORS (AOR, 11.8) and breast-feeding for less than 7 months (AOR, 4.7) were also identified by comparing the cases with the health centre controls.

The AOR were higher when comparing cases with health centre controls than with hospital controls. Theses finding are consistent with studies worldwide (a *et al.*, 1992; Anonymous, 1989; Arifeen *et al.*, 2001; Brussow *et al.*, 1993; Curtis *et al.*, 2000a; Moulin *et al.*, 2002).

Two hundred and fifty-six and 196 serum samples from children hospitalised with and without diarrhoea were also tested to identify their micronutrient status. The geometrical mean vitamins A and E and selenium concentrations were significantly lower in children with diarrhoea than in controls. Zinc concentrations were higher in cases than controls (p= 0.06), while copper was similar in both groups. There was no clear pattern between the micronutrient concentrations and gender. However serum vitamin E concentrations were significantly higher in females than in males (p= 0.04). The frequency of vitamins A and E and selenium deficiency was statistically higher in children with diarrhoea than in controls. The proportions of children who were zinc and copper deficient (< 9.94 and 1.5 μmol/L respectively) were similar in children with and without diarrhoea. More than 75% of the children were vitamin E and zinc deficient. A similarly high prevalence of these deficiencies has been reported from Cameroon and Pakistan (Gouado *et al.*, 1998; Paracha *et al.*, 2000; Paracha and Jamil, 2001).

Factors associated with micronutrient deficiencies were related to the characteristics of the clinical episodes. Watery diarrhoea was associated with changes in vitamin E, zinc and copper concentrations. Dehydration modified selenium, zinc and copper levels and a history of vomits in the week before consultation modified vitamin A and E and zinc concentrations. The presence of enteropathic viruses was associated with lower vitamin A and selenium concentrations and rotavirus modified selenium and copper.

The longitudinal study revealed that 153 (73%) of the 211 children recruited experienced no further diarrhoea episodes after hospital discharge and 58 (27%) had subsequent episodes. The most frequent number of days with recurrent diarrhoea per child was one-day (59%), followed by 2 and 3 days. The multivariate logistic regression showed that nutritional parameters such as weight-for-height z score <-1, vitamin A and zinc deficiencies identified during the initial diarrhoea episode were associated with children experiencing subsequent diarrhoea episodes. In addition, children who had micronutrient concentrations below selected cut off points for vitamin A and zinc deficiency had a high risk of subsequent diarrhoea (vitamin A deficiency, OR =2.9 and zinc deficiency OR=2.8). Our study however demonstrated

that these deficiencies are multifactorial and often associated with each other. These findings are in agreement with other worldwide studies which have documented that there is an association between malnutrition and diarrhoea and other infectious diseases (Brown, 2003; Rice *et al.*, 2000; Uysal *et al.*, 2000; Wekell *et al.*, 1997).

DEDICATION

To the memory of my brother who died many years ago in a car accident like many other Iranian children who die every day in car accidents, the most frequent killer in my country

To the memory of my aunt who suffered from liver cancer, she taught me to be kind and care for my people

To my dear wife Malliheh, for her patience and support by always being on my side and to my lovely children Saeed, Neda and Nastaran who make my life so pleasant for their encouragement, love and patience during this study

To my dear parents, who taught me to dream and gave me the strenght to realise my dreams

To my brothers Bahram, Karam, Ahmad, Ghasem and Mehdi and my dear sister Mohtaram

To all the people who supported me

To Iranian malnourished children

Acknowledgment

I wish to extend my sincerely gratitude to:

My supervisors:

- Dr. Luis Cuevas for his unwavering support and valuable assistance throughout the thesis and my study in general and because he was kind enough to share his experiences with me. I will always be profoundly grateful to him for his guidance.
- Professor C.A. Hart for his endless encouragement and professional support in laboratory work. Words fail me to express my gratefulness to him.
- My sincere thanks to Dr. N. Reisi for her help during fieldwork in Iran and her full co-operation during the study.
- My wholehearted gratitude also goes to The Ministry of Heath and Medical Education of Iran and Shahrekord University of Medical Sciences, which offered me the opportunity to proceed with this degree, financial support and their assistance and insightful comments during the study.
- Many thanks and appreciations also go to
- Dr. Frank McArdle in The Royal Hospital University Trust for his help for the tests to measure zinc, selenium and copper
- Mr. Gregory Harper for his help to measure vitamin A and vitamin E at the Liverpool School of Tropical Medicine
- Mr. B. Getty who examined all stool samples to detect viruses by electron microscopy
- Mrs. W. Dove in the Department of Medical Microbiology and Genitourinary Medicine of Royal Hospital of Liverpool for her co-operation to detect rotavirus and genotyping.
- Mr. Barry Moody, computer programmer for his technical support during the study.
- My brother Ghasem and his wife Dr. Chupani who supervised the study when I was away from field. Without their help the study would not have materialised
- My brother Dr. Mehdi who also helped me with sampling and data collection
- Dr. N. A. Cunliffe for his technical support for rotavirus genotyping and revising drafts for publication

- Dr. J.W. Baily for her technical support in laboratory work
- Dr. Ian Hastings for his assistance and comments during the study
- Secretaries, Mrs. Jeni Howley, Mrs. Joy Herbert, Mrs. Pauline Anderson and Mrs. Clare Kelly in Medical Microbiology and Genitourinary Medicine Department of Royal Hospital of Liverpool who helped me during the study
- My colleagues in Iran Mr. Soulati, Mr. Mansouri, Mr. Fatahian, Mr. Mobini and all the staff of Hajar Hospital laboratory for their endless help
- Dr. Shahabi for his technical support in laboratory and also Dr. Khoshdel and Dr. Kathiri for their help in the paediatric wards
- To all members of Faculty of Medicine of Shahrekord and especially to Mr. Zahedi and all his staff in the health centres for collection of the data in the health centres
- Especial and hearted thanks to the manager, head of paediatric wards of Hajar Hospital in Iran for their endless help especially the nurses, without their help the study would not have been completed
- Last, but not least, my thanks go to the various people who helped me at various of the time of study, especially my friends at Liverpool School of Tropical Medicine, Drs Sathosi Mayama, Mohamed Yassin, Jailson B.Correia and all families in Iran who were fully co-operating with the research team and certainly without their help this study would have remained no more than a dream.
- I am grateful to them all

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AD	Annu Domini
AIDS	Acquired Immuno Deficiency Syndrome
ALRI	Acute Lower Respiratory Infection
AOR	Adjust Odds Ratio
ARI	Acute Respiratory Infection
BSA	Bovine Serum Albumen
CI	Confidence Intervals
CSA	Cryptosporidium Specific Antigen
Cu	Copper
EAEC	Enteroaggregative *Escherichia coli*
EIA	Enzyme Immunoassay
EIEC	Enteroinvasive *Escherichia coli*
EHEC	Enterohemorrhagic *Escherichia coli*
ELISA	Enzyme- linked Immunosorbant Assay
EM	Electron Microscopy
ETEC	Enterotoxigenic *Escherichia coli*
DAEC	Diffusely Adherent *Escherichia coli*
DDC	Diarrhoeal Disease Control
DNA	Deoxyribonucleic Acid
HIV	Human Immunodeficiency Virus
GP	General Practitioner
IU	International Unit
LBW	Low Birth Weight
NHANES	National Health And Nutrition Examination Survey
NT	Not Tested
NS	Not Significant
OPD	Out Patients Department
ORT	Oral Rehydration Therapy
ORS	Oral Redydration Solution
OR	Odds Ratio
PAGE	Polyacrylamide Gel Electrophoresis
PEM	Protein-Energy Malnutrition
RR	Relative Risk

RNA	Ribonucleic Acid
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SD	Standard Deviation
SDB	Specimen Dilution Buffer
SE	Standard Error
Se	Selenium
TMB	Terra Methyl Benzidine
UNICEF	United Nations Children's Fund
WHO	World Health Organisation
Zn	Zinc

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Chapter 1

General introduction

1.1. Introduction

This study investigated 952 children under 5 years of age, comprising 259 hospitalised children with acute diarrhoea, 245 children with diarrhoea attending the OPD of the hospital, 245 children attending three health centres and 203 hospitalised children without diarrhoea, to investigate the aetiology of diarrhoea, risk factors for hospitalisation, the prevalence of micronutrient deficiencies, and the effect of micronutrient deficiency on the subsequent incidence diarrhoea in children in Shahrekord, Iran. On the aetiology of the diarrhoeal episodes stools were screened for parasites, bacteria and viruses using ELISA, negative stain EM, selective and specific media for culture, the stool concentration method and direct stool smears.

Stool and serum samples were collected from children after explaining the objectives of the study to the parents and obtaining their parental consent. Serum and stool samples were collected from hospitalised children with and without acute diarrhoea, while only stool samples were collected from outpatient children with acute diarrhoea. Serum samples were collected to assess micronutrient concentration including zinc; selenium, copper, vitamin A and vitamin E. Children hospitalised with diarrhoea were then followed for 3 months to study if micronutrient deficiencies had an effect on the incidence of diarrhoea in the following months.

1.2. Literature review

1.2.1. Diarrhoeal disease

The magnitude of the impact of diarrhoeic disease on childhood morbidity and mortality in developing countries is represented by more than one billion episodes and 3 to 6 million deaths annually in Asia, Africa, and America. In the majority of the developing countries of the Americas, Asia and Africa, high infantile mortality rates have always been associated with a high frequency of diarrhoeal disease (Griffin et al., 1988). The greatest morbidity and mortality is seen among children less than two years of age. On average a child is estimated to experience 3.3 episodes of diarrhoea a year and this is higher in developing countries, where it has been estimated that each child suffers up to15 to19 episodes of diarrhoea per year. Diarrhoeal disease is thus responsible for over a quarter of the deaths of children in the world and the fourth commonest cause of death worldwide. However the majority of diarrhoea episodes are

self-limited, and only a small proportion leads to dehydration and death (Carroll and Reimer, 2000; Curtis *et al.*, 2000b; Torres *et al.*, 2001), indicating that the morbidity associated with this disease results in a high burden to the health services.

There is a substantial number of community and hospital based studies on diarrhoea. However despite the importance of diarrhoea as a cause of childhood mortality and morbidity there are still relatively few comprehensive studies that examine the risk factors for hospitalisation, and the rate of hospitalisation and death of young children due to acute gastroenteritis in developed and developing countries (Katyal *et al.*, 2000; Mitra *et al.*, 2000).

There is a general consensus that a considerable worldwide decline of childhood diarrhoea mortality and morbidity has been achieved during the past three decades mostly due to successes in the implementation of oral rehydration therapy (ORT) in developing countries (Alam et al., 2003c). Since programs of diarrhoea disease control were instituted a gradual decrease in the prevention of these diseases was registered, especially after the1980's thus helping to diminish the global infant death rate around the world, and in particular to this thesis, in Iran, which decreased to 42 per 1000 in 2001 (Lankarani and Musaiger, 1991; UNICEF, 2002). Though some diarrhoea episodes are due to metabolic errors, chemical irritation or organic disturbances, the vast majority are caused by pathogens. Agents include viruses, bacteria, protozoa and parasites. Among the aetiological agents of acute infectious diarrhoea, rotaviruses account for a large proportion of the deaths and 20%- 52% of acute episodes. In developing countries it has been estimated that more than 870, 000 children die from rotavirus infections alone every year (Esona *et al.*, 2003; Hart, 2003c).

Diarrhoea is a clinical syndrome of diverse aetiology, associated with frequent loose or watery stools, accompanied emesis, fever, abdominal bloating or pain. Because most transmission routes occur in the domestic domain, which is the child's main habitat, it can be prevented by changes in domestic hygiene and the majority of the episodes are theoretically preventable. The main factors associated with a high incidence and mortality are, unsafe water, vomiting, low socio-economic status, young age, concomitant illness, feeding practice, inappropriate rehydration therapy, inadequate sanitation, and/or physiological condition such as malnutrition and

previous hospitalisation in children with diarrhoea. Besides, other physiologic events such as weaning confer an additional risk related to increased exposure to pathogens and decreased passively acquired immunity from breast milk.

There is already strong evidence of the effect that diarrhoea has on growth in both the short-and long-term and that this effect is associated with the type of diarrhoea. Dysentery has the most deleterious consequences on both ponderal and linear growth, although other types of diarrhoea also have a similar but relatively less strong negative association with growth (Brown, 2003).

Paediatric diarrhoea is a costly disease in terms of direct or indirect monetary costs to the community, and it is a cause of emotional trauma for the child and the parents. Rotavirus alone causes approximately 111 million episodes of gastroenteritis requiring home care, 25 million clinic visits, 2 million hospitalisations, and 352,000- 870,000 deaths in children <5 years of age, and the cost of treating rotavirus infections alone have been estimated to be in excess of £ 6.3 million in the United Kingdom and $ 352 million in the United States (Parashar *et al.*, 2003a; Parashar *et al.*, 2003b). It has been estimated that more than 20 million cases of diarrhoea annually occur in the United States among children less than 5 years, resulting in approximately 2.5 million physician visits and 220,000 hospitalisations. To extrapolate these figures worldwide would require multiplying them manifold as diarrhoea is not an important cause of death in either of these countries (Glass *et al.*, 1991; Noel *et al.*, 1994; Parashar *et al.*, 2003b).

1.2.2. Global burden of diarrhoea

The first estimates of the global burden of childhood mortality and morbidity became available in the early 1980's. Diarrhoeal illnesses accounted at this time for about 4.6 million deaths originating from around 1 billion episodes of diarrhoea in children younger than five years of age (Thapar and Sanderson, 2004). As these data emerged, WHO was coordinating the worldwide implementation of ORT. A decade later, despite little change in the incidence of diarrhoea, the number of deaths attributable to the disease had fallen to 3.3 million per year. Diarrhoea, however still remains a prolific killer of children. Some data suggest that in children under 5 years of age it

accounts for 15% of case-specific proportional mortality and is exceeded only by perinatal causes (23%) and acute respiratory infections (18%). The burden of diarrhoeal illness sits firmly in the developing world, both morbidity (6-7 episodes per child per year compared with 0.5 or 2 episodes in the developed world) and mortality (Katyal et al., 2000). For example in a health and demographic survey conducted in Mali in 1987, 37% of breastfed children had had diarrhoea in the 2 weeks preceding the survey. Thirty-one percent of infants had diarrhoea in the city of Bamako, 44% in other cities and 36% in rural areas (1989).

The prevalence of malnutrition and inadequate hygiene highlights the stark inequalities that exist within our world. A quarter of the children in developing countries are still malnourished, 1.1 billion people do not have access to safe drinking water, and 2.4 billion are without adequate sanitation (Cairncross, 2003). In the developed countries deaths caused by diarrhoea are rare, and the effect of these illness are often measured in financial terms. Only in Brazil, acute diarrhoea is responsible for the death of 20, 000 children under five years of age each year, and this number is fifty times higher than estimated for children of the same age in the United States (Fagundes-Neto and de Andrade, 1999). The impact of diarrhoeic diseases is more severe in the earliest periods of life. In the Unites States, there are about 20 million episodes of diarrhoeal illness in children < 5 years of age and 220,000 hospital admissions every year, accounting for 4% of all admissions and 2% of outpatient visits at a cost of about $50 a time (Duggan et al., 1992).

1.2.3. Aspect of diarrhoea in developed countries

The major burden of infectious diarrhoea falls upon infants and young children who live in the developing world. However, despite industrialization, wealth and public health interventions to ensure water quality and sewage disposal, acute intestinal infections are also increasing in developed areas. This is particularly due to food borne infections such as *Salmonell*a, *Campylobacter jejuni* and enterohemorrhagic *E. coli* O157. Water borne infection in developed countries is also an important problem as a result of contamination of domestic water supplies with the cysts of *Giardia lamblia* and *Cryptosporidium*. Other problems faced in the industrialised world include the widespread use of broad-spectrum antibiotics, impaired host immunity due to HIV

infections, anticancer chemotherapy and the increase in foreign travel to developing countries. In developing countries estimates of the annual incidence of diarrhoea in children vary from 3.3 to 19 episodes per year, while the incidence is much lower in children in developed countries; for example in Canada and Holland it was 0.8 per year in 2003 (Hart, 2003b).

The contrast between paediatric diarrhoea in developed and developing countries is shown in table 1.1.

Table 1.1. Features of diarrhoea in developed and developing countries

Feature	Developed countries	Developing countries
Episodes per year	<1	3.3 -19
Seasonality	Winter	None
Severe dehydration	Rare	Frequent
Nutritional sequelae	Rare	Usual
Measles associated	Non-existent	15 –63%
Epidemics	Rare	Frequent
Polymicrobial	Unusual	>20
Case fatality rate	<0.01%	0.6%

1.2.4. Aetiology of diarrhoea

The prevalence of different enteropathogens varies with the age of the individuals, how the diarrhoea is acquired, between acute or chronic diarrhoea and with the state of the host's immunity (Hart, 2003b). The causes of diarrhoea include a wide array of viruses, bacteria and parasites, many of which have been recognised only in the last decade or two. While enterotoxigenic *E. coli* and rotavirus predominate in developing countries, Norwalk- like viruses, *Campylobacter jejuni*, and cytotoxigenic *Clostridium difficile* are seen with increasing frequency in developed areas. Also *Shigella* spp, *Salmonella* spp, *Cryptosporidium* spp, *and G. lamblia* are found throughout the world (Guerrant *et al.*, 1990; Schnack *et al.*, 2003).

Establishing the cause of diarrhoea often is difficult because of variations in host susceptibility and response to infection, geographical location, season, and complexity of laboratory techniques necessary to fully identify the causative agents. Diarrhoeal illness is caused by many enteropathogens, but on the basis of epidemiologic and aetiologic considerations, a number of major categories can be listed.

1. Outbreaks of diarrhoea in child care centres and hospitals
2. Food born or water born diarrhoea
3. Antibiotic associated diarrhoea
4. Diarrhoea of travellers
5. Diarrhoea in immunosuppressed hosts, including persons with AIDS
6. Other causes of diarrhoea, which include sporadic episodes in which an epidemiologic factor cannot be identified.

The aetiological profile of infantile diarrhoea has changed in recent years as more microorganisms have been identified and the reassessment of the diarrhoeagenic role of many *E. coli* strains that were frequently considered non pathogenic. In addition at least five new categories have also been recognised, including enteroinvasive *E.coli* (EIEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enterohemorrhagic *E. coli* (EHEC) and diffusely adherent *E. coli* (DAEC) ((Niyogi *et al.*, 1994; Regua-Mangia *et al.*, 2004a; Regua-Mangia *et al.*, 2004b). A five-year study from the USA reporting the epidemiological and microbiological pattern of childhood diarrhoea were found to be very similar to those seen in developing countries. The most frequent aetiological agents were rotavirus, ETEC and *Shigella* (Sack et al., 1995). However it seems that the frequency and gravity of diarrhoea due to *E. coli* strains in developed countries are undoubtedly less important than in developing countries (Batikhi, 2002; Forestier *et al.*, 1996; Khan *et al.*, 1988; Vesikari *et al.*, 1999). Many studies have also reported that rotavirus is more often isolated from children with acute diarrhoea. The average proportion of rotavirus isolated from 21 studies worldwide shows a predominant role of rotavirus (30%), with bacteria being the second most frequent pathogens (table 1.2). However a few other studies (e.g. in Brazil) have reported that bacterial infections predominate over viral infections. There is also a difference in the peak age of diarrhoea infection between developed and developing countries. The median age of children hospitalised with rotavirus

gastroenteritis in Africa is 6 months and 81% of them are under 1 year of age, whereas in developed countries the median age is 11 months (Hart, 2003c; Souza *et al.*, 2002).

The annual incidence and the aetiological profile of diarrhoea may also vary with the presentation of predisposing factors in the community. The following factors predispose to high incidence rates and a bacterial aetiology; young age, nutritional deficiencies, inadequate physical and food hygiene, early weaning, densely populated homes and work places, lack of basic sanitation, contaminated water suppliers and hot season (Larry K Pikering and Thomas G. Cleary, 1998).
The highest frequency of enteropathogens identified worldwide was (86%) reported from Kuwait and in contrast, the lowest frequency of identified pathogens was from Greece (23%). The distribution of enteropathogens responsible for diarrhoea in children varies across the world and a summary of selected studies is shown in tables 1.2 & 1.3.

Table 1.2. Prevalence of viruses, bacteria and parasites in hospital-based studies

Country	N	HRV	*E. coli*	*Salmonella*	*Shigella*	*Campylobacter*	Parasites	*V. cholera*	Other bacteria	% pathogen isolated	Reference
Asia											
Kuwait	274	25	21	20	6	2	-	-	-	75	(Khuffash et al., 1982)
	621	45	16	24	4	7	-	-	1	86	(Sethi et al., 1989)
Indonesia	3875	38	19	-	-	-	11	-	21		(Oyofo *et al.*, 2002b)2)
Jordan	1400	27	4	11	1	1	-	0		52	(Batikhi, 2002)
Bahrain	805	14	-	10	-	-	-	-	-	-	(Ismaeel et al., 2002)
Pakistan	3500	10	57	3	4	-	1	-		-	(Khan et al., 1988)
Korea	4668	46	-	-	-	-	-	-	-	-	(Seo and Sim, 2000)
Africa											
Zambia	639	-	15	0	10	-	-	3	-	28	(Nakano et al., 1998)
Europe											
Sweden	144	45	-	-	-	5	1		9	77	(Uhnoo et al., 1986)
Italy	-	20-40	-	-	-	-	-	-	-		(Ruggeri and Declich, 1999)
Turkey	920	40	-	-	-	-	-	-	-	-	(Kurugol et al., 2003)
UK	-	23	-	-	-	-	-	-	-	-	(Akhter et al., 1994)
Greece	132	15	5	10	1	7	3	-	5	48	(Maltezou et al., 2001)
France	752	51	-	9	-	-	-	-	-	-	(Moulin et al., 2002)
Finland	-	54	-	-	-	-	-	-	-	-	(Vesikari et al., 1999)
Americas											
Brazil	154	19	7	7	8	-	-	-	1	42	(Orlandi et al., 2001)

Numbers are percentages due the pathogen, HRV= human rotavirus

Table 1.3. Prevalence of viruses, bacteria and parasites in community and OPD studies

Country	Setting	N	HRV	*E.coli*	*Salmonella*	*Shigella*	*Campylobacter*	Para site	V. cholera	% pathogen isolated	Refrence
Africa											
Kenya	C	1362	11	17	6	6	-	10	-	56	(Aihara, 1997)
Ghana	C	1717	39	-	-	-	-	-	-	-	(Binka et al., 2003)
Europe											
Poland	C	757	41	-	-	-	-	-	-	-	(Mrukowicz et al., 1999)
America											
Brazil	OPD	94	1	4	0	0	-	85	-	-	(Schnack et al., 2003)
Asia											
Saudi-Arabia	C	853	12	-	34	15	-	24	-	49	(Al-Freihi *et al.*, 1993)
Korea	OPD	231	47	43	-	-	-	-	-	76	(Kim et al., 1989)
Pakistan	C	-	16	16	-	11	-	-	-	68	(Huilan et al., 1991)

Numbers are percentages due the pathogen, HRV = human rotavirus

1.2.4.1. Bacterial diarrhoea

The epidemiology of diarrhoea due to bacteria varies by region, climate, age and season. Some studies reported that bacteria causes up to 50% of childhood diarrhoea in developing countries. Among bacteria, Enteropathogenic *E. coli* (EPEC), *Shigella, Salmonella, V. cholera, Campylobacter* and *Clostrium difficile* are the most important isolated bacteria in children with diarrhoea, however since many laboratories in developing countries have no capacity to investigate the presence of *Campylobacter* and *Clostrium difficile,* then the prevalence of these two and of other newly recognised bacteria is not well established.

In contrast to rotavirus which is most often seen during the winter, bacterial diarrhoea most often occurs during the summer and the hot seasons. The pathogenic bacteria most often associated with acute infectious diarrhoea are shown in table 1.4 (table was adapted from Pikering and Cleary (Larry K Pikering and Thomas G. Cleary, 1998).

Table 1.4. Pathogenic bacteria associated with acute infectious diarrhoea

pathogen	Epidemiologic considerations
Aeromonas hydrophila	Water, food, or animal exposure
Bacillus cerous	Food exposure
Campylobacter jejuni	Animal or food exposure
Clostridium difficile	Exposure to antimicrobial agents
Clostridium perfingens	Food exposure
Listeria monocytogenous	Food exposure
Plesiomonas shigelloides	Water, fish or animal exposure
Salmonella species	Exposure to carrier or food
Shigella species	Exposure to an infected person or to contaminated food
Staphylococcus aureus	Food exposure
Vibrio cholerae O1	Food or water exposure
Vibrio cholerae O139	Food or water exposure
Vibrio parahaemolyticus	Seafood exposure
Yersina enterocolitica	Food or animal exposure

EPEC is a major aetiological agent of diarrhoeal illness, responsible for as much as 25% of all diarrhoeal disease in developing countries. However a few studies have reported that up to 50% of diarrhoea is due to *E. coli* strains (Larry K Pikering and Thomas G. Cleary, 1998; Thapar and Sanderson, 2004). The pathogenic *E. coli*

associated with acute infectious diarrhoea is shown in table 1.5 (table adapted from Pikering and Cleary (Hart, 2003b; Larry K Pikering and Thomas G. Cleary, 1998)).

Table 1.5. Epidemiology of *E.coli* strains

Class of E. coli	Abbreviation	Usual presentation of disease	Site of action
Enterotoxigenic	STEC/ETEC	Bloody diarrhoea, watery-and food borne outbreak	Small bowel (secretory)
Enteroaggregative	EAEC	Acute and chronic watery diarrhoea in children in developing countries	Large bowel (inflammatory)
Enterohaemorrhagic	EHEC	Associated with haemolytic- uremic syndrome	Large bowel (inflammatory)
Enteropathogenic	EPEC	Acute & chronic diarrhoea in infants in nurseries and in children <1 year of age in developing countries	Small bowel (osmotic)
Enteroinvasive	EIEC	Dysentery in adults and watery diarrhoea, occasionally food borne out breaks	Large bowel (inflammatory)
Diffusely adherent	DAEC	Diarrhoea in children 2-5 years*	?

* (Paciorek, 2002)

1.2.4.2. Viral diarrhoea

Much of the gastroenteritis in children is caused by viruses belonging to 5 distinct families; rotaviruses, caliciviruses, astroviruses, coronavirus and adenoviruses. Other viruses, such as the Toro viruses, picobirnaviruses, breadavirus, picornavirus (the Aichi virus) and enterovirus 22 may play a role as well (Glass *et al.*, 2001; Hart., 2003).

A review by Cook et al (Cook et al., 1990) reported that rotavirus was found in between 11- 71% of children with diarrhoea in 34 studies with a median of 33%.

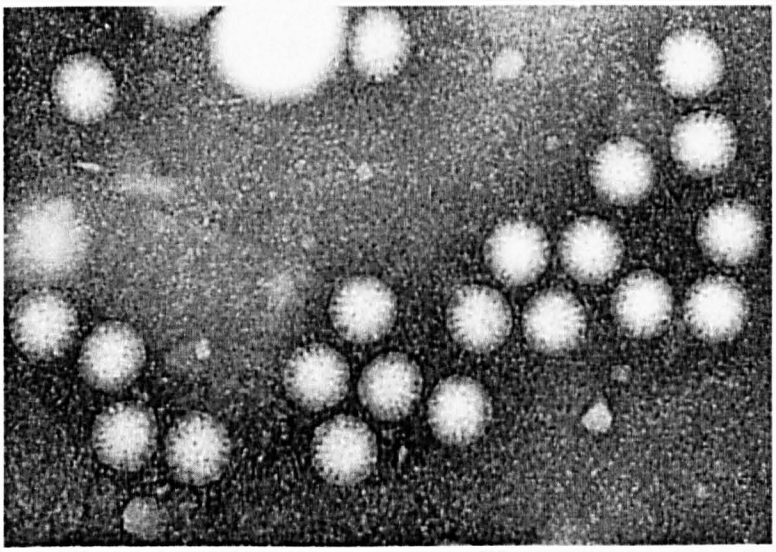
The relative contributions of viral enteropathogens to childhood gastroenteritis are shown in table 1.6 (table adapted from Hart's review (Hart, 1999)).

Table 1.6. Contribution of viral enteropathogens to childhood gastroenteritis

Virus	% of community - based	% of cases hospital-based
Rotavirus	5 -40	25- 65
Calicivirus	10-25	20-30
Adenovirus 40/41	10-25	5 -12
Astrovirus	10-25	5-10
Coronavirus	1-3	1-2
Breadavirus	?	1-2
Torovirus	?	0- 0.35
Picobirnavirus	?	<1

Rotavirus infection is found as frequently in developed as in developing countries. Rotavirus was first discovered under microscopy in duodenal epithelium cells of patients with diarrhoea by Bishop et al (1973), since then, the virus has been recognised as the most important agent of infantile diarrhoea. At first the virus was called duovirus and subsequently named rotavirus because of its characteristic wheel shape morphology (figure 1.1). Rotavirus can be classified into groups A- G according to antigenic group on VP6, the major capsid antigen. Only group A, B, and C rotaviruses have been shown to infect humans, and most human rotavirus disease is caused by group A. Group A rotavirus often causes severe diarrhoea, vomiting and fever.

Figure 1.1. Negative –stain electrograph of rotavirus



Rotavirus mortality rates are low in developed countries but approximately 900 000-1,000,000 infants die of rotavirus infection in developing countries each year (Katyal *et al.*, 2000; Nishio *et al.*, 2000). Most rotavirus severe infections in young children are caused by serotype G1-4, and during the last two decades, G1 infections appear to have predominated worldwide. In general the more densely populated countries show the most complex pattern of occurrence of serotypes. Tong et al reported that rotavirus G1 caused between 11% and 40% of the deaths due to rotavirus in children <5 of age in China (Tong et al., 2003). The hospitalisation rate due to rotavirus in children is shown in table1.7.

Table 1.7. Hospitalisation rate in children due to rotavirus diarrhoea

Country	Hospital rate <2 years	Hospital rate < 5 years	Hospital visits	Case fatality	Reference
Denmark	3%	5.4%	-	-	(Fischer, 2001)
Argentina	-	8%	50%	<1 /1000	(Gomez et al., 2002)
Hawaii	-	3 %	-	-	(Effler et al., 2000)
China	-	16/1000	-	-	(Fang et al., 2000)
USA	-	136-236 /10 000	-	-	(Holman et al., 1999)
	-	13%	-	2 /1000	(Chang et al., 2003)
France	-	9% -11%	-	-	(Grimprel et al., 2001)
Australia	11.6/1000	7.5/1000	10,000/year	-	(Carlin et al., 1998)

Numerous studies with different study design and laboratory techniques have investigated the prevalence of rotavirus in different populations, however, the highest frequency of viral enteropathogens reported so far is from France, which could detect viruses in 83% of faecal specimens of hospitalised children. In contrast in the hospital based –study from Brazil the lowest prevalence of rotavirus reported was 1% (Desenclos *et al.*, 1999; Schnack *et al.*, 2003). In developed countries, while deaths from diarrhoea are less common, much illness leads to hospitalisation or doctor visits. The frequency of isolated viruses in children with diarrhoea is shown in table 1.8. (table adapted from Hart's review (Hart, 1999)).

Table 1.8. Frequency of pathogens (emphasis on viruses) in childhood diarrhoea

Country	Mexico	China	Philippines	Malawi	Canada	Chile	Australia
Number	271	186	236	168	206	90	4637
Duration	4.5 m	22 m	25 m	2 m	24 m	12	13 years
Setting	Community	Inpatient	Inpatient	OPD	Inpatient	OPD	Inpatient
% Positive							
Rotavirus	3.7	56	65	42	3.9	1.1	39.6
Astrovirus	61	8.5	0.4	1.2		5.6	NT
Adenovirus	12.9	2.5	0.4	4.2	3.9	1.1	6
Calicivirus	NT	7.6	0.4	1.2		NT	NT
Toroviruses	NT	NT	0	0	3.5	NT	NT
Other virus	77.6	74.6	66.2	48.6	42.8	7.8	45.6
Bacteria	20.3	NT	23.4	NT	NT	32.2	9.2
Protozoa	NT	NT	2.1	4.2	NT	3.3	NT
>1 pathogen	7.4**	NA	7 **	-	NT	30**	0
No pathogen	-	25.4	1.2	-	-	42	43.5

Numbers are percentage due to pathogen

*Include classical calicivirus and Norwalk- like viruses

** Predominantly viruses, NT=not tested, m = duration of the study in month

1.2.4.3. Parasitic diarrhoea

Diarrhoea is only one of the many manifestations of intestinal parasites and is neither a usual nor a frequent feature of parasitic infection. In tropical regions virtually all under privileged children carry intestinal parasites, but these parasites are only responsible for 5 –10% of acute diarrhoea in infancy and slightly more in cases of chronic diarrhoea. In developed countries, in temperate zones, parasitic infections are much less frequent, however parasitic diarrhoea is not uncommon (Gendrel, 2003b).

Diarrhoea is quite common in children who travel and in nurseries, where the cases primarily seem to be due to *G. lamblia* or *Cryptosporidium spp* (Dupont and Sullivan, 1986). The prevalence of such cases is probably underestimated in Europe and North America, where many doctors do not routinely look for them.

Among the numerous pathogenic parasites only some parasites can cause diarrhoea: the protozoa *E. histolytica, G. lamblia, Cryptosporidium, Isospora belli, Sarcocystis hominis, Balantidium coli, Blastocystis hominis* and *Cyclospora spp* do so more often than the helminths (*Strongyloide*s and *Trichuris trichiura*) while occasionally *Ascaris* and *Ancylostoma* are involved. Schistosoma is not uncommon (Gendrel, 2003b). Also Anguillula is a significant cause of diarrhoea in undernourished children in endemic areas. The host-parasite relationship is very poorly understood.

Natural immunity to intestinal parasites is quite weak and does not eliminate them, thus serology is of little use for diagnosis, and the parasite must always be demonstrated directly. Antibodies, whether locally produced or present in the circulation, prevent neither continuing infection nor later re-infection. The parasite tends to neutralize host immune defences, mainly by antigenic variations during the different phases of its life cycle. The WHO estimates that although 480 million people are carriers of *E. histolytica*, it causes only 40, 000 deaths per year (probably most are *E. dispar* carriers). *E. histolytica* is the only amoeba that is a pathogenic for humans. Its hematophagous form is found in dysenteric stools, in colonic abscesses and in visceral metastases. *E. histolytica* cysts are identical in appearance to the non-pathogenic parasite *E. dispar* that is a new species name for what was previously called non-invasive or non-pathogenic *E. histolytica.* This explains the very large

differences in the reported numbers of carriers or infected persons in various countries: from 3 – 47% of inhabitants in some regions of India, 12-20% in Nigeria, and 50% in Colombia (Gendrel, 2003b).

G. lamblia infections are very common throughout the world and are considered one of the main non -viral causes of diarrhoea in industrialized countries. The prevalence of infection varies considerably with the level of hygiene, ranging from 1-5% in western populations and from 20% to 50% in developing countries. Giardiasis leads to severe atrophic villosity requiring appropriate specific treatment.

In children some protozoa such as *Cryptosporidium* spp may be symptomatic or lead to diarrhoea especially in cases associated with malnutrition or immunodeficiency related in particular to AIDS. *Cryptosporidium* is a small protozoa of the sub-class coccidia, which multiplies on the surface of intestinal and respiratory epithelia. Since 1907, it has been known to exist in animals, but the first description in humans, in a case of transient gastroenteritis in a three- year old toddler, dates from only 1976 (Dillingham et al., 2002). New cases became apparent only upon investigating patients with AIDS, in whom *Cryptosporidium* causes severe, chronic diarrhoea. Despite the difficulties in demonstrating the oocyst, numerous reports appeared with epidemiological data from the general population: 4.2% cases of childhood diarrhoea in Costa Rica and Australia and 10.8% in Liberia and Rwanda were found to be due to *Cryptosporidium.* The degree of pathogenicity of *Cryptosporidium* is still poorly understood. There are many healthy carriers, but the parasite is often found in childhood diarrhoea. Some people believe that its pathogenicity, although weak, is greater than that of other protozoa such as *G. lamblia* or amoeba, especially in malnourished patients. Infection by this parasite should be sought in those with diarrhoea, particularly those who developed it soon after weaning (as breast feeding seems to be protective). Several epidemiologic studies have demonstrated that *Cryptosporidium* is more prevalent in developing countries (5% to >10%) than in developed countries (1% to < 3%) (Iqbal et al., 2001).

The role of *B. hominis* as a human pathogen is controversial; although there is some evidence suggesting that it is implicated as a cause of diarrhoea in immunocompromised patients (Gassama et al., 2001).

Isospora belli and *Sarcocystis hominis* are two coccidia of worldwide distribution, but they are uncommon in children. Isospora was established as a cause of diarrhoea in humans in the early 1900s' when sporadic cases of isosporiasis and a few clinical series were reported in the medical literature. *I. belli* has gained importance with the advent of AIDS, where it has been shown to be an important cause of severe and prolonged gastroenteritis (Larry K Pikering and Thomas G. Cleary, 1998).

Balantidium coli is the only ciliated parasites found in humans. It is not as common as other protozoa, several mammals; particularly pigs can be infected and can infect children (Gendrel, 2003a, b).

Cyclospora cayetanensis (formerly cyanobacteria or blue-green algae – like bodies) is a coccidian protozoa that was first diagnosed as causing infection in human in 1977. It is only in the past few years that this organism has come into prominence as a result of food borne outbreaks in the United States and Canada. A strong association of this parasite with diarrhoea in patients with AIDS, travellers, and paediatric groups has been found in health centre populations. In developing countries, cyclosporiasis and associated symptoms occur more often in children. Infection rates as high as 20% have been reported (Bern *et al.*, 1999; Bern *et al.*, 2000; Soave, 1996). Environmental, socio-economic, demographic and health- related behaviour is known to influence the transmission and distribution of these infections. Environmental influences are inescapable, regardless of an individual's state of health; in a highly endemic region, intestinal parasitic colonization is almost the rule. The expression of the parasitosis however is largely determined by host defence, and when they are weakened, parasite diarrhoea is frequent and severe. Protein-energy malnutrition is by far the most important cause of immunodeficiency in developing countries. Diarrhoea caused by *Strongyloides spp* or *G. lamblia* is common and severe in malnourished children, while well-nourished children remain healthy carriers. These parasites need specific treatment in the malnourished children (Gendrel, 2003a; Norhayati *et al.*, 2003). The distribution of most parasites causing diarrhoea in selected countries is shown in table1.9.

Table 1.9. Distribution of the most frequent parasites causing diarrhoea in selected countries

Country	N	*E.histolytica/ dispar*	*G. lamblia*	*Cryptosporidium*	Cyclospora	Other protozoa	Reference
Asia							
Kuwait	3549	-	-	10	-	15-38	(Iqbal et al., 2001)
India	127	11*	*	19	-	6	(Kaur et al., 2002)
Indonesia	3875	-	1	-	-	10	(Oyofo et al., 2002b)
Bangladesh	289	8	11	8	-	-	(Haque et al., 2003)
America							
Venezuela	212	-	-	-	6	-	(Chacin-Bonilla et al., 2003)
Mexico	132	-	-	8	-	-	(Solorzano-Santos et al., 2000)
Venezuela	301	1	21	89	-	38	(Miller et al., 2003)
Brazil	94	56	4	85	-	-	(Schnack et al., 2003)
Africa							
Zambia	222	-	-	18	-	-	(Nchito et al., 1998)
Kenya	200	-	26	0	-	31**	(Chunge et al., 1992)
Egypt	65	5	11	14	1	-	(Osman et al., 1999)
Nigeria	215	1	1	0	-	2	

* prevalence of *G. lamblia* included with *E. histolytica/dispar*

** 31% related to *A. lumbricoides*

N = number

1.2.5. Risk factors for diarrhoea

Infective diarrhoea is predominantly a disease of poverty, overcrowding and environmental contamination. Case-control and longitudinal epidemiological investigations have revealed that young age, male gender, insufficient use of health care services, non-compliance with treatment, unhygienic behaviour (disposing of children's and household members faeces in open places rather than in a latrine, bathing children in a river rather than using wells, children eating with their hands rather than with spoons, houses without sewage systems), poor feeding practices for infants and young children, living in a house with few rooms, socio-economic, environmental, maternal reproductive, dietary and nutritional variables and absence of electrical appliances are important risk factors for diarrhoeal diseases (Aulia *et al.*, 1994; Pelto, 1991a, b; Teklemariam *et al.*, 2000). In addition poor washing and purifying of fruit and vegetables, the presence of wastewater, refuse storage, lack of collection of household refuse, poor parental education, poor domestic water reservoirs and the presence of vectors in the house and short birth interval have been highlighted as important factors (Heller *et al.*, 2003; Manun'ebo *et al.*, 1994). A case-control study in Santo Domingo to assess the relation between the presence of protozoa and risk factors has found that inadequate garbage disposal facilities, crowded conditions (more than 3 persons per room), drinking non-potable water, lack of piped water, and not having facilities were more frequent in children with parasites (Tavarez et al., 1991).

1.2.6. Diarrhoea and risk of hospitalisation

The risk of an individual acquiring a gastrointestinal tract infection varies with age, environment, season, exposure, and immune status. Diarrhoea causes an estimated one-third of all hospitalisations. Few comprehensive studies have been done to identify risk factors for hospitalisation due to diarrhoea in children under 5 years. These studies have reported that, age under 6 months, malnutrition, the presence of dehydration, recent illness, lower socio-economic level, inadequate sanitation, unhygienic behaviour and lack of the sanitation are risk factors for hospitalisation. (Do

Carmo-Leal *et al.*, 1996; D'Souza R and Bryant, 1999; Gupta *et al.*, 1998; Lindtjorn, 1991; Sachdev *et al.*, 1991).

Recent estimates of rotavirus disease burden indicate that in developed countries hospitalisation rates are between 2.5 and 5 per l000 in children under 5 whereas in developing countries it may be as high as 30 per 1000. A study from USA reported a significant association between birth weight and the risk for hospitalisation due to rotavirus. Very low birth weight (< 1500 grams) children were at the highest risk, low birth weight (1500 –2499 gram) at intermediate and large infant (> 4000 grams) at reduced risk. Other characteristics that increased the risk of gastrointestinal hospitalisation were male gender, maternal smoking, having an unmarried mother, lack of medical insurance and maternal age <20 (Khuffash *et al.*, 1982; Lindtjorn, 1991; Molbak *et al.*, 1997b; Newman *et al.*, 1999; Sempertegui *et al.*, 1995; Yoon *et al.*, 1997). A selection of the risk factors for hospitalisation due to diarrhoea is shown in table 1.10.

Table 1.10. Risk factors for hospitalisation due to diarrhoea in a representative sample of studies

Country	Setting	N	Age <1 year	Dehydration	Recent major illness	Short breast feeding	Lack of hygiene	Malnutrition	Reference
Ecuador	C	230	-	-	-	-	+	-	(Sempertegui *et al.*, 1995)5)
Philippines	C	9942	+	-	-	-	-	+	(Yoon et al., 1997)
Ginua Bissau	C	531	+	+	+	+	-	-	(Molbak et al., 1997c)
Kuwait	H	274	+	+	+	-	-	-	(Khuffash et al., 1982)
Brazil	H	406	+	-	+	-	-	+	(Do Carmo-Leal *et al.*, 1996)
USA	H	1606	+	-	+	-	-	+	(Newman et al., 1999)

H = hospital, C = community, N = number

1.2.7. Risk factors associated with diarrhoeal death

In the early 1980s, diarrhoeal diseases were the main cause of death, while nowadays it is second to acute respiratory infection. Despite this reduction, diarrhoea is responsible for more than 3 million children deaths each year. Diarrhoea deaths worldwide vary from 1% to 40% of all deaths in the first five years of life in developing countries. About 80% of these deaths occur in the first two years of life (Hart, 2003b).

Few case- control studies have identified the risk factors associated with diarrhoeal death in children < 5 years age in developing countries. Studies in hospitalised children due to diarrhoea have found that age <6 months, moderate to severe dehydration, severe malnutrition, having an illness in two weeks preceding a recent episode, low weight for height and low height for age, co-existent sepsis, lack of breastfeeding, shigellosis, hypoalbuminemia and metabolic acidosis were significant risk factors for death due to diarrhoea (Griffin *et al.*, 1988; Uysal *et al.*, 2000). A three- year longitudinal study of 9942 children from Philippines documented that in the first 6 months of life, failing to initiate breastfeeding or ceasing to breastfeed resulted in an 8-10 fold increase in the rate of diarrhoeal mortality. The rate of mortality associated with both acute lower respiratory infection (ALRI) and diarrhoea was increased almost 6 times by not breastfeeding, but the rate of ALRI mortality alone was not increased (Yoon et al., 1997).

A study in Mexico with emphasis on seasonality of death in children < 5 years of age showed that the distribution of deaths in 1989-1990 was bimodal, with one peak during the winter and a more pronounced one during the summer (Villa et al., 1999). In this study, diarrhoea mortality was associated with poor hygienic facilities as the mortality rate for districts with the most slums had the highest mortality rates (1.9% to 4.3%) and the districts with most services and fewest slums had the lowest mortality rates (0.79 – 0.38). Similarly a study from Bangladesh reported a strong association between the condition of the latrine at home and other environmental factors and death due to diarrhoeal disease (Hoque et al., 1999). In Pakistan the main risk factors for under 5 years mortality were the use of traditional healers, poor nutrition, incomplete or no immunisation, changing the antibiotics or using the traditional healer after

uncompleted antibiotic dosage, inappropriate child care arrangements, mother's literacy, who decides to ask for outside treatment, short birth interval, bottle feeding, and an unclear family structure. Risk factors for death due to diarrhoea are shown in table 1.11.

Table 1.11. Risk factors for diarrhoea death

Country	N	Malnutrition	Age < 1 year	Low family income	Dehydration	Recent major illness	Not breast fed	Illiterate Parents	HRV	Lack of hygiene	Mortality Rate	Reference
Asia												
Bangladesh	928	+	+	+	+	+	+	+	-**	-	-	(Teka et al., 1996)
India	387	+	NT	-	+	+	+	-	NT	+	-	(Zodpey et al., 1998)
	400	+	+	-	+	+	-	-	-	-	7%	(Uysal et al., 2000)
Pakistan	347	+	-	+	-	NT	+	+	NT	+	NT	(D'Souza R and Bryant, 1999)
America												
Brazil	406	+	+	-	-	+	-	-	-	-	-	(Do Carmo-Leal *et al.*, 1996)
	500	+	-	-	-	-	-	-	-	+	-	(Blake et al., 1993)
Africa												
Ethiopia	105	+	+	-	+	+	-	-	NT	+	-	(Lindtjorn, 1991)
Ghana	1717	+	NT	NT	NT	+	NT	NT	+	NT	-	(Binka et al., 2003)
South-Africa	-	+	+	NT	+	-	NT	NT	NT	NT	19%	(Chopra et al., 1997)

NT =not tested or risk factor not investigated, ** This study revealed that HRV was a protective factor

1.2.8. Diarrhoea classification

Definitions of diarrhoea include increase in volume or fluidity of stools, changes in consistency and increased frequency of defecation. The measurement of stool fluid content is impractical and assessment of stool frequency is preferred for diagnostic purposes. WHO defines diarrhoea as the "passage of loose or watery stools at least three times in 24 hours period" but emphasises the importance of changes in stool consistency rather than frequency, and the usefulness of parental insight in deciding whether children have diarrhoea or not. However, parents may use a variety of terms to describe diarrhoea, depending on an increase in the number, volume and water content of the stools.

The definition does not apply to babies in whom breast-feed is a major component of the diet. These babies often pass more than three soft stools each day even when healthy but the child is said to have diarrhoea when the mother notices that the stools are more frequent or watery (Fruhwirth *et al.*, 2001; Thapar and Sanderson, 2004).

1.2.8.1 Acute watery diarrhoea

This term refers to diarrhoea lasting less than 14 days involving the passage of frequent loose or watery stools without visible blood. Vomiting may occur and fever may be present. Watery diarrhoea in children often leads to moderate or severe dehydration. The major categories of acute infectious diarrhoea are shown in table 1.12, which was adapted from Pikering and Cleary (Larry K Pikering and Thomas G. Cleary, 1998).

Table 1.12. Major categorises of acute infectious diarrhoea

Category of diarrhoea	Epidemiologic consideration	Most commonly involved enteropathogens
Outbreak	Childcare centres, hospital	Enteric viruses, *G. lamblia*
Food borne or water borne	Other people involved after common food or water exposure	*E. coli* O157, ETEC, *S. areus*, *Y. entercolitica*, *V. cholerae*
Antimicrobial associated	Recent administration of antimicrobial agents	*Clostridium difficile*
Traveller's	Recent travel to a developing country	ETEC, *Campylobacter*, *Shigella* and *Salmonella*
Immunosuppressed host	Underlying disease, including HIV infection, recent administration of an immunosuppressive drug	*Salmonella*, *Shigella*, *Campylobacte*, *E. histolytica*, *C. difficile*

1.2.8.2. Persistent diarrhoea

Persistent diarrhoea is defined as any watery or bloody episode lasting more than 14 days. Episodes of persistent diarrhoea usually arise secondary to infection in the presence of complications. Persistent diarrhoea often has an origin in malnutrition.

The following factors are the main causes of persistent diarrhoea; enteric infections, malnutrition, micronutrient deficiencies, impaired immunity, history of measles and lactose intolerance. Persistent diarrhoea can be due to infections with many well-known organisms, although the intracellular protozoa, *C. parvum*, *Cyclospora cayetanensis*, *microspora,* EPEC, and other *E. coli* have been implicated (Fruhwirth *et al.*, 2001; Thapar and Sanderson, 2004).

1.2.8.3. Dysentery

Dysentery may be simply defined as diarrhoea containing blood. It has many effects such as weight loss, anorexia and damage of intestinal mucosa by the invasive bacteria. A number of other complications may occur. The main causes of acute dysentery are invasive enteropathogens such as *Shigella, non-typhoid Salmonella, Campylobacter jejuni,* diarrhoeagenic *E. coli* and *E. histolytica.* The latter is uncommon in children under 3 years of age. Among *Shigella, S. flexneri* affects many children aged 6 months to 3 years and peaks in the warm seasons.

1.2.9. Seasonality of diarrhoea

Epidemiological investigations of childhood diarrhoea have revealed that diarrhoea outbreaks vary in terms of aetiology and climate. Rotavirus infections are responsible for a large proportion of diarrhoeal disease and have been called a winter disease in temperate zone. Its incidence shows a striking seasonal pattern during the colder months. Some studies however have reported a peak incidence in the hot, dry and low relative humidity season. For example the peak of rotavirus infection in Ghana was in February and September. Rotavirus incidence peak in winter primarily in the Americas and peaks in the autumn or spring are common in other parts of the world (Kurugol *et al.*, 2003; Seo and Sim, 2000). In the tropics, the seasonality of such infections is less distinct and within 10 degrees latitude (North or South) of the equator, eight of ten locations exhibited no seasonal trend (Cook et al., 1990). Throughout most of the world, rotavirus is present all the year round, which suggests that low-level transmission could maintain the chain of infection. The epidemiology of rotavirus, its seasonality in the cooler months, its universal spread in temperate and tropical zones in developed and less developed settings more closely resemble that of childhood viruses that are spread by the respiratory route than that of common enteric pathogens that are spread predominantly by the faecal-oral route (Armah *et al.*, 1994; Cook *et al.*, 1990; Mutanda *et al.*, 1984; Rytlewska *et al.*, 2000).

In contrast bacterial and protozoan diarrhoea tend to occur in the wetter seasons in the tropics and during the summer in temperate countries. In temperate countries cryptosporidiosis peaks are reported in the spring with a lower peak in the autumn.

However some outbreaks of bacterial origin such as water borne or food borne diarrhoea occur in the spring and other seasons. For example In Bangladesh it was shown that the incidence of ETEC diarrhoea in infants was positively correlated with the frequency of consumption of weaning food contaminated with fecal coliforms and the seasonal peak of ETEC diarrhoea coincided with the time when food was most contaminated due to higher bacterial growth caused by high temperatures (Maltezou *et al.*, 2001; Robins-Browne, 1984; Rowland, 1986).

1.2.10. Diarrhoea and malnutrition

The nutrients that humans require for their health, productivity longevity and well being come from foods of varying complexity. Global food production needs to be doubled over the next half century in order to support a population that is expected to increase to more than 10 billion. Nearly half of the world's people are malnourished. Some 840 million have insufficient intake of protein/energy and more than two billion peoples consume diets that are less diverse than 30 years ago, leading to deficiencies in micronutrients, especially vitamin A, iodine, zinc and selenium. The populations greatest risk are the underprivileged, especially resource-poor women, infants and children (Kossmann *et al.*, 2000a; Kossmann *et al.*, 2000b; Stephenson *et al.*, 2000).

The interactions between diarrhoeal disease and nutritional status are complex and synergistic. This is a serious issue globally because it affects hundreds of millions of young children and annually cause >3 millions deaths in children under 5. Despite intensive field-based and laboratory studies over three decades, many questions remain unanswered about the causes, pathophysiology and best approaches to the management and prevention of this "diarrhoea- malnutrition"syndrome (Gracey, 1999).

Malnutrition, particularly wasting, is a strong predictor of diarrhoeal disease duration and prolonged illness can exacerbate nutritional faltering, thereby increasing the subsequent risk of death. Poor appetite, vomiting, deliberate withholding of food resulting in poor intake; malabsorption of macro and micro-nutrients; hastening of intestinal transit time; disturbance of metabolic and endocrine functions; and direct loss of protein and other nutrients into the gastrointestinal tract are some of the known

mechanisms which have an impact on nutrition during an episode of diarrhoea (Patwari, 1999; Stephenson *et al.*, 2000). Over half the children in South Asia and a third of those in Africa south of the Sahara and millions more around the world are malnourished, and some six million young children die every year when they would be unlikely to die if they were well nourished (Stephenson et al., 2000). Malnutrition can be seen in several forms, but the four most important forms of malnutrition globally are protein-energy malnutrition (PEM), iron, iodine, zinc and vitamin A deficiency (Stephenson et al., 2000).

PEM affects an unacceptably large proportion of the children under 5 in developing countries. The prevalence of stunting and underweight are high especially in South Asia where one in every two preschool children is stunted. Besides protein-energy malnutrition, Asian children also suffer from micronutrient deficiencies (Khor, 2003). Although prevalence varies greatly between areas and regions, stunting and underweight move in parallel, and both are most prevalent among South Asian children, followed by sub-Saharan children, south Central Asia and Eastern and Western Africa (Stephenson et al., 2000). Whereas studies with different study designs have used different assessment scales such as conjunctival impression cytology, serum retinal, clinical symptoms, dietary vitamin A intake or using anthropometrical classifications to identify malnutrition, they have all reported a high prevalence of malnutrition and micronutrient deficiencies.

Malnutrition contributes by an estimated 54% to two-thirds of the deaths around the world which are directly or indirectly associated with diarrhoea (Caballero, 2002; Stephenson *et al.*, 2000). Moreover their nutrient requirements are increased as a result of the infection. Each episode of diarrhoea contributes to malnutrition; when episodes are prolonged, their impact on growth is increased. Recurrent episodes of diarrhoea lead to malnutrition as the result of anorexia, catabolism from infection, and lack of adequate caloric and protein intake because of the widespread custom of "starving " diarrhoea. Pre-existing malnutrition increases the duration, severity and case fatality rate of diarrhoea (Stephenson et al., 2000).

1.2.10.1. Nutrition and economic factors

Studies have shown that in developing countries the nutritional status of children has a significant inverse relationship with household income. Socio-economic factors such as household income, the education level of parents, distribution of food in the family, demographic factors, immunisation status and childhood illness, intestinal parasitosis, and childhood nutrition, also have significant association with the nutritional status of children. A significant decrease in weight –for age with increasing number of parasite infections per child has been observed among children in 3- 8 years of age (Norhayati et al., 2003).When there is serious poverty, as in many native populations in developing countries, failure to breast feed is likely to cause serious malnutrition, because the mothers' can not afford to buy the artificial feeds, or for financial reasons they are able to give only excessively diluted feeds. It is estimated that 200 million children suffer from malnutrition as a result of the decline of breast-feeding. The protection afforded by breast-feeding against infection, and therefore against the need of medical and hospital treatment, is of considerable financial importance.

Breast-feeding seems to influence growth through two separate pathways, firstly growth is influenced through the provision of energy and essential nutrients in breast milk, and secondly breast-feeding reduces diarrhoea morbidity, which in turn affects the growth of infants (Oddy, 2002).

1.2.10.2. Diarrhoea in Iran

Despite a sharp decrease in the mortality rate of children <5 years old in the last three decades in Iran, diarrhoeal disease still remains one of the most important health problems. In recent years very few comprehensive studies have investigated the aetiology of diarrhoea, although some studies have reported that rotavirus is the main pathogen responsible for 15 –20% of childhood diarrhoea in Tehran and two studies have reported that *E. coli* is responsible for 20 –26 % of childhood diarrhoea. Recently a case-control study with emphasis on *Aeromonas* spp role in childhood diarrhoea has reported that *Aeromonas* spp was responsible for 5% of diarrhoeal episodes and the other most frequent pathogens were *Shigella* (18%), followed by EPEC, *Salmonella*

and *G. lamblia* with 3 % each (Katouli *et al.*, 1988; Katouli *et al.*, 1990; Moddares, 1995; Zarnani *et al.*, 2004).

The primary health care programs in Iran have played an important role in reducing the morbidity associated with this disease; however, the amount of diarrhoeal illness in the population, particularly in young children, remains a concern. Although the local knowledge of its causes is limited, methods currently used in public health laboratories allow for the identification of *Salmonella, Shigella, E. histolytica/dispar, and G. lamblia,* but they do not have the capacity to routinely detect, rotavirus, *Cryptosporidium, Campylobacter* and diarrhoeagenic strains of *E. coli.* Because an aetiologic agent is not detected for a large proportion of the patients with diarrhoea, it is likely that a high proportion of the undiagnosed illness is attributable to one or more of these latter enteropathogens. Several studies have examined the role of specific enteropathogens in childhood diarrhoea in Iran; however, no comprehensive studies describing the prevalence of viral, bacterial and parasitic enteropathogens, especially newly recognised ones, has been published.

1.2.10.3. Malnutrition in Iran

The current nutritional problems of Iran are malnutrition in general, the lack of energy and proteins, anaemia and health disturbances arising from the shortage of iodine in the diet (goitre). At present, on average 30% of children under 5 suffer from varying degrees of malnutrition as well as from protein and energy deficiency. Anaemia is suffered by 13- 53% of women and girls, 12 – 30% of children and 8% of men (Jazayeri, 2003). A study in Tehran also reported a high prevalence of zinc deficiency in junior-high school students with 65%, 49% and 1.3% of the samples of plasma, erythrocyte and hair being deficient respectively. Overall zinc deficiency was seen in 31% of the subjects (Mahmoodi and Kimiagar, 2001).

1.2.11. Prevention and control of diarrhoea

A major advance during the second half of this century was the development of the glucose-electrolyte solution for the oral rehydration of infants and young children with

acute watery diarrhoea. Because of its simplicity, this therapeutic approach has been implemented worldwide under the auspices of the WHO and is estimated to have saved millions of lives. However, despite its efficacy in rehydrating and maintaining hydration in individuals with watery diarrhoea, stool volumes do not decrease and `sometimes paradoxically increase, thereby raising doubts in the mind of a child's carer as to whether the treatment is actually working. Reducing the osmolality of standard glucose-electrolyte solutions either by using a polymer such as rice starch has been shown to reduce stool volume in some circumstance, however, the diarrhoea will still continue until the infection resolves (Farthing, 2000). Even though the lives of an estimated three quarters of a million children are being saved each year, over three million children are still dying from readily preventable diarrhoeal disease (Urio et al., 2001). To manage and reduce the effect of diarrhoeal disease there are many recommendations mainly in 3 aspects:

1) ORT programmes should move strongly toward promoting home treatment, building on local traditions of giving food-based preparation, with ORS available from health workers and health facilities for those who need it. Recent developments in the science of gastroenteritis management have substantially altered case management. Physicians now recognize that zinc supplementation can reduce the incidence and severity of diarrhoeal disease (Sazawal et al., 1996b) and an ORS of reduced osmolarity (i.e., proportionally reduced concentration of sodium and glucose) has been developed for global use.

2) Nutritional support is just as important as rehydration. Diarrhoeal precipitates and accelerates the progression of malnutrition, which lowers resistance. Improved weaning practices using high density, easily digestible, local foods are especially important during and after an episode of diarrhoea.

3) For long-term prevention, breaking the transmission cycle of the many common pathogens that cause diarrhoea will be necessary.
Because diarrhoeal disease mortality can be effectively reduced at a reasonable cost by ORT and possibly other measures, it is a priority target for primary health care programs in many countries. Other interventions are needed in addition to oral rehydration programs, to reduce mortality from chronic or dysenteric diarrhoeas in

which ORT is of limited use, and to reduce mortality rates. The Diarrhoeal Disease Control (DDC) programme of the WHO advocates a four- part strategy for diarrhoea control consisting of improved case management, improved maternal and child health care, improved use and maintenance of drinking water and sanitation facilities, and improved food hygiene, and detection and control of epidemics (Feachem *et al.*, 1983; Taylor and Greenough, 1989). Continuation of breast-feeding and nutrient rich, food-based ORT is vital. ORT benefits illnesses caused by *Shigella, Salmonella,* or *Campylobacter*. Promotion of breastfeeding reduced 8-20% of diarrhoea- related morbidity and 24 –27% of mortality up to 6 months of age. Improved weaning practise by education cut mortality by 2 –12% for under 5 years children (Arifeen et al., 2001).

An expansion of the immunisation program could reduce the morbidity and mortality of diarrhoeal disease due to some pathogens, for example a rotavirus immunisation program could reduce the incidence of diarrhoea by 6% and 20% of deaths in children < 5 years. *Cholera* immunization with an efficacy of 70 % could eliminate 4% of diarrhoea episodes and 8% of diarrhoea deaths. Measles immunization coverage of 60% reduces diarrhoea morbidity by 2% and mortality by 13% in the target group.

Hygienic practices play a fundamental role in the prevention of infectious disease; they also serve other needs such as diarrhoeal disease control. Nowadays hygiene promotion is increasingly favoured by policymakers because of its potential to deliver reductions in diarrhoeal disease at low cost. In the past, improved water supply and sanitation alone lowered diarrhoeal morbidity by 22%. It is now widely accepted that water supplies and sanitation, though necessary for the prevention of diarrhoeal diseases in young children, are not sufficient, unless they are accompanied by changes in domestic hygiene behaviour (Curtis *et al.*, 2000b; Strina *et al.*, 2003; Taylor and Greenough, 1989). Hand washing with soap and water reduces the incidence of dysentery and watery diarrhoea by 14-48% (Curtis and Cairncross, 2003).
Whereas diarrhoea may occur for varied reasons; most episodes of diarrhoea in developing countries are infectious in origin. Antimicrobial agents play a vital role in the management of some acute invasive diarrhoeas particularly shigellosis and amoebiasis. In persistent diarrhoea, nutritional therapy, including dietary manipulations is a very important aspect in its management, in addition to rehydration

therapy. Rehydration may be carried out either by the oral or intravenous route depending upon the degree of dehydration (Alam and Ashraf, 2003; Alam *et al.*, 2003b; Alam *et al.*, 2003c).

1.3. Description of study location

1.3.1. History of Iran

The name of Iran comes from an ancient term "a-eer-ya-nem va-ee-jo" in Avesta, the holy book of Zoroastrianism, meaning the land of the Aeers'. This term refers to a certain plateau that Indo-Iranians, a branch of the Aryans, selected for their settlement. With the passage of time, the term "Air" changed to "Er" and later to "Ir". "Er" or "Ir", in ancient languages of the time, meant "nobel". The official name of the country in the Sassanid period (400-600 A.D.) was Iranshatr or Iranshahr. "Shatr" or "Shahr" means country. Thus, Iranshahr means The Country of the noble. Iran became an Islamic republic in 1979 after the ruling shah was forced into exile.

Iran is one of the world's most mountainous countries. Its mountains have helped to shape both the political and the economic history of the country for several centuries. The mountains enclose several broad basins, or plateaus, on which major agricultural and urban settlements are located. Until the Twentieth Century, when major highways and railroads were constructed through the mountains to connect the population centres, these basins tended to be relatively isolated from each another. Typically, one major town dominated each basin, and there were complex economic relationships between the town and the hundreds of villages that surrounded it. In the higher elevations of the mountains rimming the basins, tribally organized groups practiced transhumance, moving with their herds of sheep and goats between traditionally established summer and winter pastures. Historically transportation was by means of caravans that followed routes traversing gaps and passes in the mountains.

Almost all Iranians are Muslim including 89% Shi'a and 10% Sonny Muslim. The 1% remaining population are Jewish, Zoroastrian, Christian and Bahaei. The formal language in Iran is Farsi (Persian) but Arabic and English are taught in school.

1.3.2. Geography of Iran

With an area of 1,648,000 square kilometres, Iran ranks sixteenth in size among the countries of the world. Iran is located in South-western Asia and shares its entire northern border with the former Soviet Union. This border extends for more than 2,000 kilometres, including nearly 650 kilometres of water along the Southern shore of the Caspian Sea. The western borders are with Turkey in the north and Iraq in the south. The Persian Gulf and the Gulf of Oman litorals form the entire 1,770-kilometer southern border. To the East lie Afghanistan and Pakistan on the South. Iran's diagonal distance from Azerbaijan in the Northwest to Baluchestan va Sistan in the Southeast is approximately 2,333 kilometres. The rainfall varies from 40- 1370 mm per year with a minimum in the Southeast and a maximum in the North. The mountains also impede easy access to the Persian Gulf and the Caspian Sea. Most of the country is above 1,500 feet, one-sixth of it over 6,500 feet high. In sharp contrast are the coastal regions outside the mountain ring. In the north, the 400-mile strip along the Caspian Sea, never more than 70 miles wide and frequently narrowing to 10, falls sharply from the 10,000-foot summit to 90 feet below sea level. In the South, the land drops away from a 2,000-foot plateau, backed by a rugged escarpment three times as high, to meet the Persian Gulf and the Gulf of Oman. The lowest and highest points in Iran are the Caspian Sea and the Kuh-e Damavand with -28 m and 5,671 m from the sea level respectively. Iraq has the longest border with Iran (1609 km) followed by Turkmenistan (1206 km), Pakistan (978 km), Afghanistan (945 km), Azerbaijan (767 km), Turkey (489 km) and Armenia (40 km).

1.3.3. Iran's economy

Iran is very rich in natural resources with almost one-tenth of the world's oil and one-fifth of the world's natural gas reserves. Its total oil reserves stands at 92.9 billion barrels. At the present extraction rate, it will last 70 years. Iran is ranked second in the world in view of its natural gas reserve, totalling 20.7 trillion cubic meters. At the current daily consumption rate, it will take Iran 360 years to exhaust it. Besides natural resources and unlike many other Middle Eastern countries, Iran possesses strong agricultural and service sectors. These together with a population of more than 66 million make Iran a substantial domestic power from the political and economical point of view. The Iranian government spends 55%, 26% and 19% of its annual budget for services, industry and agriculture respectively. However still 53% of its

population live below the poverty line. The economy of Iran is mainly based on exports; including petroleum (85%), carpets, fruits, nuts, iron, steel and chemicals and its Gross Domestic Product is $1640 per capita.

1.3.4. Iran's climate

Iran is a vast country made up of very different climatic regions. The climate can change from one time of the year to another as well as between different regions at the same time of the year. There are generally four seasons: spring, summer, autumn and winter. Spring starts from mid March and the weather is pleasant with warm and sunny days and occasional spring showers. The average temperature during spring is around 20-25°C. In the summer, the heat is often overwhelming with the temperature frequently rising above 35°C in Tehran. Autumn starts from mid September with rather cool and sunny days. In winter, the cold can be intense and the temperature frequently drops below freezing. Shemshak and Dizin in Tehran and Kouhrangh in Shahrekord are popular ski slopes during winter. In the west, and especially in the North-West, winters are cold with heavy snowfall and subfreezing temperatures during December, January and February. Spring and autumn are relatively mild, while summers are dry and hot. In the south, winters are mild and the summers are very hot, having average daily temperatures in July exceeding 38°C. On the Khuzestan plain, summer heat is accompanied by high humidity. In general, Iran has an arid climate in which most of the relatively scant annual precipitation falls from October through April. In most of the country, annual precipitation averages 25 centimetres or less. The major exceptions are the higher mountain valleys of the Zagros and the Caspian coastal plain, where precipitation averages at least 50 centimetres annually. In the western part of the Caspian, rainfall exceeds 100 centimetres annually and is distributed relatively evenly throughout the year. This contrasts with some basins of the Central Plateau that receive ten centimetres or less of precipitation annually. Periodic droughts, floods, dust storms, earthquakes along the western border and North and East are the main natural hazards in Iran.

1.3.5. Iran and health

The health characteristics of Iran are shown in table 1.13. The Iranian health system's performance "on health" is ranked 58th in the world according to WHO. Adult mortality rate is 209 and 137 per 1000 for men and women respectively. There are 85, 259, 16 and 11 physicians, nurses, dentists and pharmacists per 100, 000 population respectively. While the literacy rate for men between 7 to 19 years old is 98%, it decreases to 92% for women. Drug abuse is a serious problem, due to imports of extremely cheap heroin and opium from Afghanistan. Estimates run as high as 2 million addicts in the country. Fertility rate is now 2.6, which has decreased in recent decades after a significant increase during the revolution and the war with Iraq. Registration for marriage in Iran requires engaged couples to undergo medical tests for contagious diseases particularly sexually transmitted diseases and drug abuse. There are currently more than 6000 known HIV positive individuals and according to WHO criteria, it is estimated that the population of HIV positive individuals is between 20000 and 50000 persons. The majority of HIV positive patients are drug related and injections are the main transmission route followed by unsafe sex (UNICEF, 2002).

Figure 1.2. Population density by region in Iran

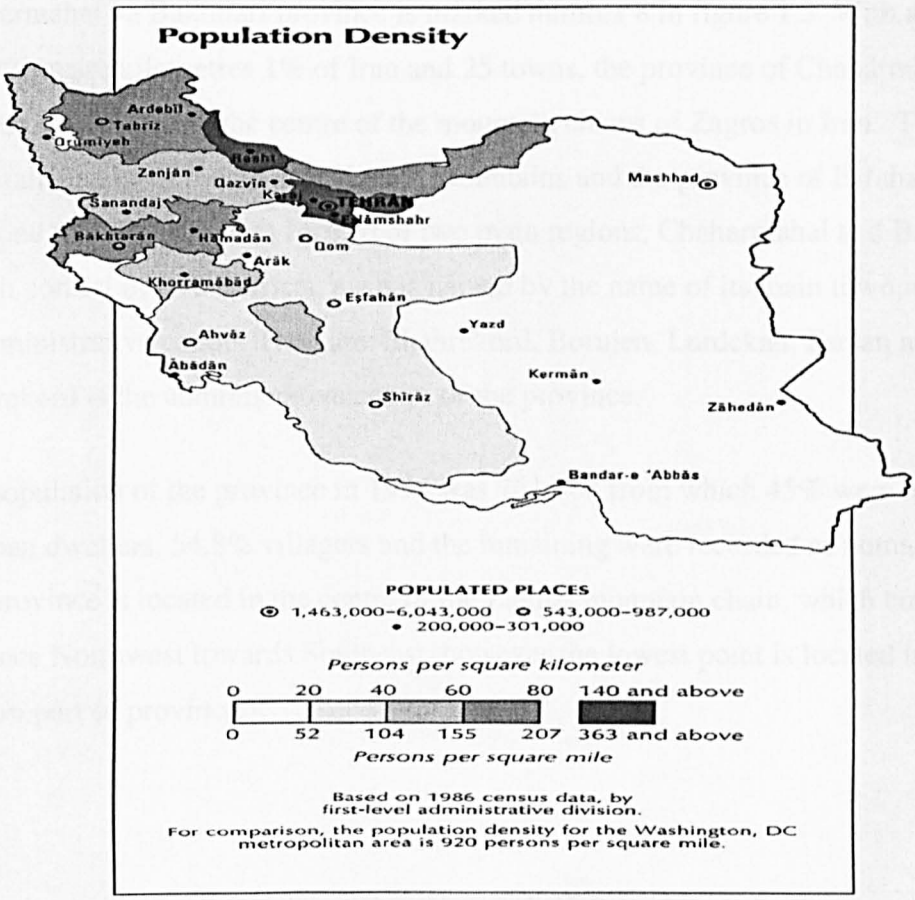


Table 1.13. Vital health statistics of Iran

Population		**Health**	
Total	66,500,000	Population growth rate	0.77%
Under one year	1.7%	Birth rate	17.54/1000
Under 5 years	11%	Death rate	5.39/1000
0-14 years	32%	Infant mortality rate (per 1000)	33.2
15-64 years	64%	< 5 years mortality rate	41.2
Over 64 years	7%	Maternal mortality rate*	37
Urban residency	61%	Vaccination coverage (urban)	99.7%

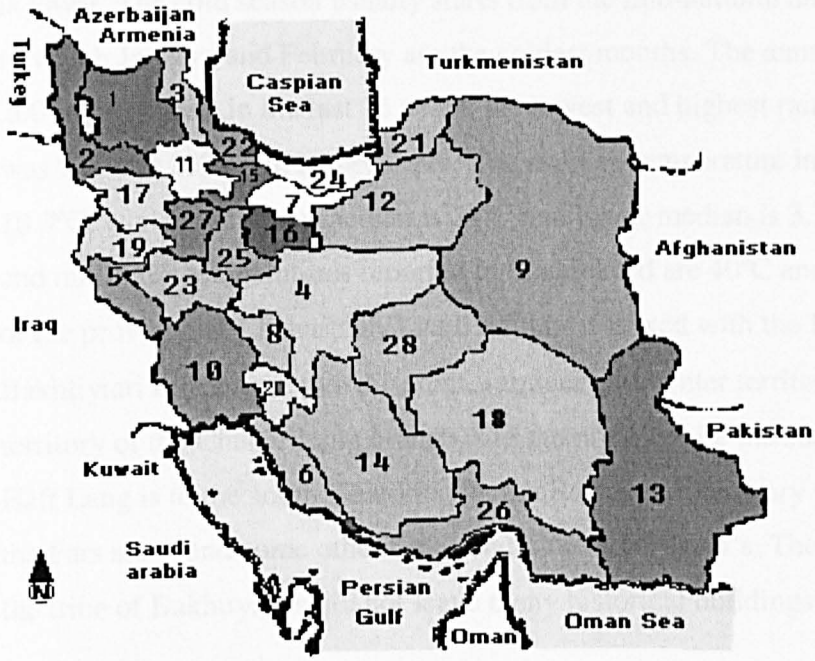
* Per 100,000 deliveries

1.3.6. Chaharmahal va Bakhtiari Province

Chaharmahal va Bakhtiari province is marked number 8 in figure 1.3. With an area of 16,532 square kilometres 1% of Iran and 25 towns, the province of Chaharmahal and Bakhtiari is located in the centre of the mountain chains of Zagros in Iran. Two mountain chains of the interior Zagros Mountains and the province of Esfahan surround the province. It is formed of two main regions, Chaharmahal and Bakhtiyiari, Which consist of five districts; each is named by the name of its main town, which is its administrative centre. They are: Shahrekord, Borujen, Lurdekan, Farsan and Ardal. Shahrekord is the administrative centre of the province.

The population of the province in 1996 was 761,168 from which 45% were registered as urban dwellers, 54.8% villagers and the remaining were recorded as nomad tribes. The province is located in the centre of the Zagros mountain chain, which crosses the province Northwest towards Southeast, however the lowest point is located in the Eastern part of province.

Figure 1.3. Iran's provinces



1.East Azerbaijan	2- West Azerbaijan	3. Ardabil
4. Esfahan	5. Ilam	6. Bushehr
7. Tehran	8. Chaharmahal & Bakhtiari	9. Khorasan
10. Khuzestan	11.Zanjan	12. Semnan
13. Sistan & Baluchestan	14. Fars	15. Qazvin
16. Qom	17. Kordestan	18. Kerman
19. Kermanshah	20. Kohgiluyeh & BoyerAhmad	21. Golestan
22. Gilan	23. Lorestan	24. Mazandaran
25. Markazi	26. Hormozgan	27. Hamedan
28. Yazd		

The province is the head springs of the largest rivers of Iran such as Karun and Zayandeh Rood. The province mountains are one of the permanent water sources of the country and many mountains are covered with snow during all seasons. The province consists mainly of highlands, and most of its area has an altitude of more than 2,000 metres. This turns the province into a very cold region, especially in the

winter. Snowfall in this province starts usually from the middle of the autumn, and it continues until the first month of the spring (October to March). There are some lowlands in which the temperature rises in the summer to more than 40°C and rainfall is lower. The cold season usually starts from the mid-autumn and it lasts 4-5 months, in which January and February are the coldest months. The annual rainfall varies from 200 to 2174 mm. In the last 15 years, the lowest and highest rainfall in the highlands was 932 and 2174 mm respectively. The average temperature in this province is 10.3°C, while the higher median is 24°C and lower median is 3.3°C. The maximum and minimum temperatures reported in Shahrekord are 40°C and – 30°C. The history of the province of Chaharmahal va Bakhtiari is mixed with the Bakhtiari tribe. The Bakhtiyiari tribes have two different, summer and winter territories. The summer territory of the Chahar Lang branch is to the north, while the summer territory of the Haft Lang is to the south. Historically, the Bakhtiyiari territory was sometimes under the Fars state, and some other times under the Khuzestan's. The nomad living style of the tribe of Bakhtiyiaris did not leave many historical buildings or physical elements.

The province has 7 hospitals with 1234 beds, 68 urban health centres and 34 rural health centres. Four universities and two colleges are responsible for 17,000 undergraduate and postgraduate students.

The vital statistics of the province are shown in table 14.1. The infant mortality rate is 21 and 29 per 1000 live birth for urban and rural areas respectively. Adult mortality rate is 4 but maternal mortality rates are 0 and 23 for the urban and rural areas respectively and 83% of the children under 6 years of age are covered by the health centres.

Table 1.14. Vital health criteria in Chaharmahal va Bakhtiari province

Criteria	Urban	Rural	Criteria	Urban	Rural
Birth mortality rate (per 1000)	14	18	Fertility rate	1.94	2.64
< 1 year mortality rate	21	29	Birth rate	16.3	19.2
< 5 years mortality rate	25	35	Access to safe water	99.7%	94%
Maternal mortality rate	0	23	Vaccination	95%	99%
Life Expectancy (years)		69	Adult mortality rate	?	3.8
Total literacy		77%			

1.3.7. Shahrekord

The town of Shahrekord is located 543 kilometres South of Tehran. It is located in a plain with 2061 altitude with 20°-32° latitude and 50°-51° longitudes, and is surrounded by many mountain chains. Shahrekord was part of the territories of Atabakan Fars and Luristan during the 13th century AD. A checkpoint was built on this side for controlling travelling and providing facilities for passengers, and because the soldiers of this checkpoint were Kurd, it was named "Deh Kurd" which means the village of the Kurdish people. A few decades ago, when this village was expanded, its name was changed to "Shahrekord" which means the town of the Kurdish people.

The highest humidity occurs from November to April and the lowest humidity from May to October. The highest and lowest humidity reported occur in February and June to August (66% and 31% respectively), however during some years the humidity has reached 5% and >90% in the dry and rainy seasons. The median minimum temperature in the rainy months (December to March) varies from 0°C to –12°C, the median maximum temperature in the dry months (June to September) is between 26°C to 36°C (Province, 2001).

The majority of days have sunshine and the minimum and maximum sunshine occur in January and July respectively. Between 124-136 days are at freezing or subfreezing temperature. The median temperature in the coldest months (January and

February) is between –7 °C to +5 °C and reaches 21°C to 25°C in July and August.
The average rainfall in Shahrekord is 492 mm per year (Province, 2001).
The average (median) temperatures per month is shown in table 1.15.

Table 1.15. Average (median) temperature by month in Shahrekord

Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1.9	0.2	5.4	10.9	15.2	19	23	22	20.5	13.9	7.8	3.1

1.3.8. Hajar Hospital

The Shahrekord university teaching hospital is located in Shahrekord and it is the largest hospital in the province with 485 beds. The main wards are the intensive care unit (ICU), the cardio vascular care unit, surgery, gynaecology, surgery, internal, and infectious diseases wards. The external view of the hospital is shown in figure 1.4 a & b.

Figure 1.4. External view of Hajar Hospital

(a)

(b)

Chapter 2

Material and methods

2.1. Introduction

This chapter describes the laboratory methods for processing stool and serum samples. Objectives of the study and the specific methodology for study design will be described at the beginning of each chapter.

2.2. Main objective

To describe the epidemiology of diarrhoea in Iranian children under 5 years of age.

2.2.1. Objectives

1. To establish the aetiology and characteristics of hospitalised children with acute diarrhoea in Iran.

2. To establish the risk factors for hospitalisation of children with diarrhoea.

3. To describe the prevalence of micronutrient deficiencies, parasites and viruses in children under 5 years with and without diarrhoea (Zn, Cu, Se, Vitamin A and E).

4. To describe the effect of diarrhoea- associated micronutrient deficiencies on subsequent incidence of diarrhoea.

2.3. Ethical approach

Ethical approval was obtained from the Research Ethic Committees of the Liverpool School of Tropical Medicine and Shahrekord University of Medical Sciences. During

the study, some parents wanted to know the results of the tests (rotavirus and micronutrient measurements), which only became available after testing in Liverpool. Once the results became available, they were sent to the children's hospital to provide the information to the parents during future visits. In addition, during the follow up study, children suffering from micronutrient deficiencies should have been supplemented. However the results of micronutrient concentrations were only available several months later. If the results had been available before or during the follow up, these children should have been sent to their hospital for the assessment of the micronutrient deficiencies and the doctor should have decided if the children required supplementation. The study procedures however were considered to be acceptable at the start of the study because:

a. There was no information regarding the prevalence of micronutrient deficiencies in this area prior to the current study.

b. Medical staff were allowed to supplement any child who was considered to have clinical micronutrient deficiencies.

c. The results of this study were only available several months after completion of the follow up.

d. Serum samples were collected at the beginning and not the end of episode and were therefore surrogate markers of micronutrient deficiency(as micronutrient values decrease during the acute phase and recover with cessation of symptoms).

2.4. Collection of samples

Stool and serum samples were collected from children after explaining the objectives of the study and obtaining parental consent. Serum and stool samples were collected from hospitalised children with and without acute diarrhoea, while only stool samples were collected from outpatient children with acute diarrhoea. Serum samples were collected to assess micronutrient concentration including zinc; selenium, copper, vitamin A and vitamin E. The stools were collected to identify pathogens and parasites.

All bacteria and parasites with the exception of *Cryptosporidium* were diagnosed in the hospital laboratory of Hajar Hospital in Shahrekord. Stool samples were kept at -20°C, until they were transported to Liverpool for the detection of viruses and *Cryptosporidium*. All the serum samples were also kept at -20°C until they were transported to Liverpool for micronutrient concentration analysis.
Two stool specimens were obtained either by parents or nurses from 259 children < 5 years old admitted to Hajar Hospital and 245 children attending the outpatient services of Shahrekord in Southwest Iran with a clinical diagnosis of acute diarrhoea. A further 114 children hospitalised for elective surgery from October 2001 to August 2002 were enrolled. Stool samples were processesed to identify bacteria, viruses and parasites. The first specimen was immediately processed by a trained laboratory worker using wet mounts, the formalin ether sedimentation method and bacterial cultures. The second specimen was stored at -20°C until Enzyme immunoassays (EIA), Electron Microscopy (EM), Reverse Transcription Polymerase Chain Reaction (RT-PCR) and electropherotyping were performed in Liverpool.

2.5. Laboratory tests

2.5.1. Wet mount (direct smear)

Fresh specimens were examined directly using a normal saline solution for vegetative forms of parasites, cyst and ova. The visualized amoebic trophozoites were confirmed twice by looking for the presence of red blood cells inside parasites, their movement, development of pseudopodia, and the number of protozoan nuclei. For each specimen a direct smear with iodine solution was prepared to specify the characteristics of the protozoan nuclei.

2.5.2. Formalin-Ether concentration technique

This is a sedimentation technique that concentrates helminth eggs, larvae, and protozoan cysts. The procedure is rapid and has the advantages of removing lipid and colloidal material to yield clear sediment. In addition, the presence of formalin

preserves eggs, larvae, and cysts, so the material can be examined hours or even days later.

Procedure

1. The faecal specimen was first suspended in sufficient water or 10% formalin, so that at least 10 to 12 ml of strained suspension could be recovered, which would yield 0.5 to 1.0 ml of centrifuged sediment.
2. The suspension was strained through two layers of gauze to remove particulate material.
3. The suspension was then washed twice by centrifugation in a 15 ml conical centrifuge tube (2 minutes at 2000 rpm) and the supernatant was poured off.
4. After the second centrifugation, the faecal sediment was thoroughly mixed with 10 ml of 10% formalin. At this point, the suspension can be held indefinitely if necessary.
5. The final step was to add about 3 ml ether to the 10 ml-formalinized suspension and tube was mixed vigorously for one minute, the sample was given a final centrifugation for 2 minutes. The plug of debris plus ether that forms at the top of the tube was removed with an applicator stick, and this as well as the entire supernatant was poured off, leaving only a sediment in a small volume of formalin that drains back from the sides of the tube. Debris on the sides of the tube was cleaned off with a cotton swab.
6. A drop of the concentrated sediment was mixed with a drop of 2% aqueous iodine for examination under a cover slip to diagnose cysts, ova and other forms of parasites or valuable reporting material including crystals and yeast like.

2.5.3. Enzyme immunoassays (EIA) for Cryptosporidium

A commercial plate ProSpecT® *Cryptosporidium* Micro plate Assay (Alexon. Trend 14000 Unity St. NW, Ramsey, MN 55 303, USA) was used to detect Cryptosporidium antigens. Cryptosporidium specific antigens have been found associated with *Cryptosporidium* infections and have been used as the basis of fluorescent and antigen capture immunoassays (Arrowood and Sterling, 1989; Chapman *et al.*, 1990). This procedure has used a Cryptosporidium specific antigen (CSA) that is produced by the *Cryptosporidium* organisms as they multiply within the host intestinal tract. The antigen is specific to *Cryptosporidium* and has not been found to cross-react with

other enteric parasites. The antigen is stable to transport through the host intestinal tract as well as to routine procedures used to collect and transport specimens for microscopic examination.

Procedure

ProspecT® *Cryptosporidium* Micro plate Assay is a solid phase immunoassay for the detection of CSA. Diluted stool specimens are added to break-away micro plate wells on which anti-CSA antibody is bound. If CSA is present, it is captured by the bound antibody. The wells are incubated and then washed to remove unbound material. The enzyme conjugate (monoclonal anti-CSA antibody labelled with horseradish peroxidase enzyme) is added. The wells are incubated and then washed to remove unbound conjugate. In a positive reaction, CSA binds the enzyme to the wall. The substrate for the enzyme tetra methyl benzidine (TMB) is added, in a positive reaction, the enzyme bound to the well by CSA converts the substrate to a coloured reaction product. Colour development can be detected visually or spectrophotometrically. In a negative reaction there is no CSA or an insufficient level of CSA present to bind the enzyme conjugate and no coloured reaction product develops.

Preparation of reagent and stools

Before use, all the reagents are transferred to room temperature (20°C-25°C) and mixed gently. After the test, the unused reagents can be returned to the refrigerator. All reagents with exception of the wash buffer were supplied ready-to-use in dropper bottles. The steps include dilution of wash buffer concentrate to 10x by adding 1 part concentrate to 9 parts of distilled or deionised water. The reagents are added to the test wells in the same order throughout the procedure. To avoid contamination it is important not to touch the fluid in the wells with the bottles' tips.
The tubes were labelled for each specimen, and 1 ml specimen dilution buffer (SDB) was added to each tube. For unpreserved solid specimens each tube was coated with 1 swab of specimen and was vigorously stirred into SDB. The swabs were drained of as much fluid as possible. In the next step a transfer pipette was exchanged for the swab in each well .For watery specimens instead of swab a transfer pipette was used to

draw-up 0.3 ml of specimen which were then mixed by drawing up and down once into a tube or step 2, 3, 4, below:

Procedure

The foil pouch was opened to remove the required number of microplate strips and to place then into a microplate strip holder. Specimens were added directly to the wells or pre-diluted in tubes before adding to the wells. Specimens were diluted in tubes. The following process was done to prepare the diluted samples:

1. SDB was added to each tube. The wells were charged with 1 swab of solid specimen and stirred vigorously into SDB. The swab was then changed for a transfer pipette.
2. Preserved or watery unpreserved specimen: The tubes were labelled for each specimen and 1 ml SDB was added to each tube. The specimen was shaken in the collection container. Using the transfer pipette, 0.3 ml of specimen was drawn up and the sample was expelled into SDB. The samples were mixed by drawing up and down once and the transfer pipette was left in the tube.
3. One well was used for each negative control and one well for the positive control.
4. Four drops (200μl) of the negative control were added to well A1.
5. Four drops (200μl) of the positive control were added to well B1.
6. 0.2 ml of each specimen were added to the wells using a transfer pipette.
7. The plate was covered and incubated at room temperature (20°C -25° C) for 60 minutes. The timing was started after the addition of the last specimen.
8. After decanting the contents of the wells, these were washed thoroughly by filling each well with diluted wash buffer (~350-400μl/well) and shaking out all fluid from the wells after each wash. This procedure was repeated 3 times. After the last wash the plates were inverted and tapped vigorously on clean paper towels to remove as much wash buffer as possible but were not allowed to dry out at any time.
9. Four drops (200μl) of the enzyme conjugate were added to each well.
10. The plates were then covered and incubated at room temperature (20°C -25°C) for 30 minutes.
11. After decanting and washing each well 5 times as in step 7, 4 drops (200μl) of colour substrate (TMB) were added to each well.

12. The plate was covered and incubated at room temperature (20°C -25°C) for 10 minutes.
13. One drop (50μl) of stop the solution was added to each well. Gently tapped and vortexed the wells until the yellow colour was uniform. The reactions were read within 10 minutes after adding the stop solution. The colour change was read spectrophotometrically at 450 nm.

2.5.4. Virus detection tests

2.5.4.1. Electron Microscopy (EM)

Electron microscopy was done to diagnose rotavirus, astrovirus, adenovirus, coronavirus, calicivirus and parvovirus infection. The following procedure is based on that described by Madeley 1997.

Procedure

1. Faecal specimens were resuspended in distilled water to produce a milky suspension (approximately 10% weight/ volume [w/v].
2. Carbon reinforced, formvar coated, copper specimen support grids (400 square mesh) were prepared in advance. The coated side of the grid was then further coated with a 0.1% solution (W/V) of bovine serum albumen (BSA)(Sigma, UK) to aid specimen adhesion. The surplus was drawn off using the torn edge of a piece of tweezers, used to handle the grid, leaving behind a thin layer of BSA.
3. Once the BSA layer had air-dried the grid was coated with a drop of faecal suspension. Great care was taken to cover only the coated side of the grid. Covering both sides results in poor electron beam penetration producing bad image definition. Surplus specimen was removed with filter paper to leave a thin film; hence the volume added was not known.
4. After the specimen had air-dried it was washed three times in a drop of distilled water on a microscope slide to remove crystallised salts, the surplus water was removed after each wash with filter paper.
5. Finally the specimen was negatively stained with 2% grade potassium phosphotungstate, pH 7.0 (Agar Scientific Ltd, Essex, UK). This was done by

touching the specimen side of the grid to a drop of stain on a microscope slide. The surplus was removed as before to leave a thin film of stain, which was allowed, to air dry. A fresh drop of distilled water and stain was used for each specimen and the tweezers used to handle the grids were flame sterilised between specimens to prevent cross contamination.

6. Prepared grids were viewed on a Philip 301 EM (Philips Electron optics UK Division, PYE Unicam Ltd Cambridge, UK) at a screen magnification of 45,000x. Grids were scanned horizontally, along the rows of the grid squares, from end to end, at three different locations. Each scan took approximately 3 minutes. Positive samples were generally identified in the first minute.

2.5.4.2. Enzyme immunoassays for rotavirus

The Premier ^{TM} Rotaclone© kit was used (Meridian Diagnostics, Cincinnati, OH, USA). This is an EIA for the detection of Rotavirus Antigen in Human Faecal samples. The EIA is a simple, highly sensitive method for the detection of rotavirus antigen, and is well suited for analysis of large numbers of samples.

Test method

This test utilizes monoclonal antibodies in a solid phase sandwich type EIA. Plastic microtitre wells are coated with a monoclonal antibody directed against the product of the sixth viral gene (VP6), which is the group specific antigen for all known human rotavirus. An aliquot of faecal suspension is added to the well and incubated simultaneously with an anti-rotavirus monoclonal antibody conjugated to horseradish peroxidase, resulting in the rotavirus antigen being sandwiched between the solid phase and enzyme –linked antibodies. After 60 minutes incubation at room temperature, the sample well is washed in order to remove unbound enzyme labelled antibodies. Enzyme substrate A (urea peroxide) and substrate B tetra methyl benzidine (TMB) are added to the wells and incubated for 10 minutes at room temperature. The enzyme bound in the wells converts the colourless substrate to blue colour. The intensity of the blue colour is directly proportional to the concentration of rotavirus antigen in the sample.

Specimen preparation

One ml of sample diluent was added to properly marked tube and by using a transfer pipette. Samples were added by one of the following way:

1. Solid stool- pressed sample into transfer pipette to first mark.
2. Liquid stool- aspirated sample into transfer pipette to the first mark and then mixed thoroughly.

Procedure

All the reagents were brought to room temperature (20-30° C) before use.

1. Sufficient numbers of wells were snapped off for all samples and positive control and negative control and inserted into the microtitre well holder.
2. Two drops (100μl) of each diluted faecal sample were added with positive and negative controls (sample diluent) to the bottom of separate wells.
3. Two drops (100μl) of enzyme conjugate were added to each well and mixed by gently swirling on a tabletop.
4. The microtitre plate was incubated at room temperature for 60 ± 5 minutes.
5. The liquid out of the wells was then poured into the discard vessel and the microtitre plate was tapped upside down vigorously against absorbent paper to ensure complete removal of the liquid from the wells.
6. The wells were filled with deionised water to overflowing and the liquid was then poured out as in step 5.
7. The washing procedure was repeated (step 5 and 6) four more times (for a total of 5 washes).
8. Two drops (100μl) of substrate A solution were added to each well.
9. Two drops (100μl) of substrate B solution were added to each well.
10. The wells were incubated for 10 minutes at room temperature.

Visual determinations were done after 10 minutes of incubation in step 10. Samples with blue colour greater than the negative control were considered positive and samples showing equal or less colour than the negative control were considered negative. A spectrophotometer also was used to read the colour change. Two drops (100μl) of stop solution (normal sulphuric acid) were added to each well after the

incubation in step 10. The absorbance of each well was read at 450nm using a >600 nm reference filter against an air blank within 60 minutes. Specimens with absorbance >0.150 were considered positive, specimens with absorbance equal to or less than 0.150 were considered negative. Sometimes a discrepancy occurred between the visual and spectrophotometric determinations. The manufacturer indicates that this can happen with samples containing low amounts of antigen. Spectrophotometric determination, being an objective method, is slightly more accurate and these values were used to diagnose samples as positive or negative.

2.5.5. Rotavirus genotyping

2.5.5.1. Reverse transcription polymerase chain reaction (RT-PCR)

For this purpose the extraction of rotavirus was done to use the extracted dsRNA for P and G typing and also Polyacrylamide gel electrophoresis (PAGE). The following procedures were used for rotavirus RT- PCR:

2.5.5.1.1. Extraction of rotavirus dsRNA

Extraction of rotavirus dsRNA from faecal samples was performed by using a guanidine isothiocyanate/silica glass powder extraction method (Gentsch et al., 1992) based on the method described by Boom (Boom et al., 1990). This method removes inhibitors of RT-PCR present in the stool by partially purifying rotavirus dsRNA through binding to glass powder in the presence of guanidine thiocyanate. The dsRNA is then eluted from the glass powder and can be used for RT-PCR. All centrifugations were performed in a bench- top microcentrifuge.

Reagents:

Guanidine isothiocyanate, molecular biology grade (Sigma)

Tris-HCL (Sigma)

Ethanol, 100%

Silica powder (RNAID, distributed by Stratech Scientific, Luton, UK)

Kit wash buffer (RNAID)

PLC grade water (BDH, Poole, UK

Procedure

1. 250μl of faecal supernate (10-20% supernatant in PBS) was prepared in a 1.5 ml eppendorf tube. This was done in a Class I Biosafety cabinet.
2. 500 μl of guanidine isothiocyanate (made in 50mM Tris-HCL, pH 7.5) were added to each tube and vortexed well. This was done in a Class I Biosafety cabinet. Following incubation at 65°C for 10 minutes, 8.5μl of silica powder was added to each sample. The tubes were vortexed well and rocked on a rotator for 30 minutes at room temperature.
3. The mixture was centrifuged for 60 seconds at 4000 rpm (the lowest speed that pelleted the particles was used).
4. The supernatant was removed using a separate pipette for each sample, and washed once with 700μl of guanidine wash solution (ratio of 2: 1 6M guanidine: 50mM Tris-HCL). Fresh guanidine wash solution was used for each RNA extraction procedure.
5. The tubes were centrifuged for one minute at 4000 rpm. The supernate was removed and discarded.
6. The tubes were washed three times with ethanol wash solution and after the first two washes were centrifuged for one minute at 4000 rpm; after the third wash the tubes were centrifuged for two minutes at 13000 rpm.
7. All residual liquid from the samples was removed by pipetting, and the samples were dried for 10 minutes in the centrifuge drier.
8. RNA was extracted by adding 35 μl of autoclaved, HPLC-grade H_2O and incubating for 10 minutes at 65°C, followed by centrifugation for two minutes at 1300 rpm.
9. The supernatant was aspirated with separate pipette tips and transferred to 0.5 ml microcentrifuge tubes.
10. The H_2O extraction step (step 8) was repeated. The supernates were combined to give a final RNA volume of 70 μl.
11. The tubes were centrifuged at 13000 rpm for 2 minutes to pellet any remaining silica powder and 30μl RNA was pipetted into clean 1.5 ml Microcentrifuge tubes for PAGE analysis. All RNA was stored at -80°C until used for PAGE and RT-PCR.

2.5.5.2. Polyacrylamide gel electrophoresis (PAGE) of rotavirus

Polyacrylamide gel electrophoresis was performed on the first 46 samples, which were positive for rotavirus, by EIA. The following procedures were used:

Stock solutions:

1. Laemmli sample buffer (Biorad, Hemel Hempstead, UK) Sample buffer was diluted in an equal volume of water.
2. Acrylamide: Bisacrylamide (Biorad):

A 30 g bottle of 29:1 bis-acrylamide was used. The acrylamide powder was dissolved in 73 ml H_2O using a magnetic stirrer. The solution was refrigerated in the dark. Gloves and goggles were used at all times when working with acrylamide.

3. Lower gel (separating gel) stock solution:

1.5 M Tris solution was prepared by dissolving 18.17 g of Tris base (Sigma) in 100 ml of H_2O; 37% HCL was added to give a final pH of 8.8.

4. Upper gel (stacking gel) stock solution:

0. 5 M Tris solution was prepared by dissolving 6.06 g of Tris base (Sigma) in 100 ml of H2O; 37% HCL was added to give a final pH of 6.8.

5. Ammonium persulfate (Biorad):

A 10% solution weight/volume was prepared (typically 0.1 g of ammonium persulfate per 1 ml water)

6. Temed (Biorad) was used as supplied by the manufacturer.
7. A running buffer was made up by dissolving the following in one litre of distilled water:

Tris base (Sigma)	3.03 g
Glycine (Sigma)	14.4 g

Procedure

A 10% acrylamide separating (lower) gel was made as follows

Lower gel stock (PH 8.8)	6.0 ml
Acrylamide: Bis	8.0 ml
H_2O	10.0 ml
10% ammoniun persulfate	0.12 ml
TEMED	12 μl

Everything except the TEMED was mixed in a 125 ml flask. TEMED was then added and the solution was mixed by swirling. Without delay, the gel was poured into the gel apparatus. The lower gel was overlaid with 21x diluted lower gel stock and was left to stand for 30 minutes.

A 3% acrylamide stacking (upper) gel was made as follows

Upper gel stock (PH 6.8)	2.5 ml
Acrylamide: Bis	1.5 ml
H2O	6.0 ml
10% Ammonium Persulfate	0.03 ml
TEMED	10 μl

Assembly of plates:

The Biorad Mini-Protean vertical electrophoresis system was used. Each of the pieces of apparatus was washed thoroughly using soapy water followed by alcohol. Dividing strips were placed along the lateral edges of the glass plates and secured firmly into the apparatus using bulldog clamps. Heated 1.5% agarose was poured in a line on the surface of a plastic plate and the apparatus was placed down to create a seal.

Pouring of gels:

The separating gel was poured, and layered with a small volume of 21- fold diluted lower gel stock. The gel was allowed to polymerise. After polymerisation, the 21- fold diluted lower gel stock was poured off, and the plates were rinsed with distilled water.

The apparatus was drained well, and blotting paper was used to dry between the glass plates. The stacking gel was poured and the comb was inserted, avoiding air bubble formation. After polymerisation of the stacking gel, the comb was removed. The gel plate was assembled on the electrophoresis chamber by means of bulldog clamps. To avoid leakage of reservoir buffer, the plate was sealed to the chamber with petroleum jelly. The centre of the electrophoresis chamber was filled with running buffer until the buffer overflowed into the gel.

Sample preparation and loading:

30 μl of RNA in a 1.5 ml eppendorf tube and dried in the vacuum centrifuge. 10 μl of 1x sample buffer was added per sample of dried RNA. The buffer / RNA was heated at 65°C for 10 minutes, vortexed briefly and centrifuged for a few seconds prior to use. The samples were loaded with a finely drawn Pasteur pipette (Bioquote, York, UK). The outer chamber was filled with running buffer until the lower end of the glass plate was covered. The leads were connected to a power pack and the gels were run at 150 volts for 120 minutes.

Removal of gels:

The apparatus was removed and excess buffer was poured off. The gels were removed from the apparatus and were transferred to fixing solution.

Silver staining of rotavirus genome:

The rotavirus genome was stained with silver method as described by Herring AJ et al (Herring et al., 1982).

Solutions	
A. 10% ethanol, 0.5% acetic acid	
100% ethanol	20 ml
Glacial acetic acid	1 ml
Distilled water to	200 ml
B. 0.011M silver nitrate	
Silver nitrate (Sigma)	0.4 g
Distilled water to	200 ml
C. Developer: 0.75M NaOH (Sigma); 0.1 M formaldehyde (Sigma)	

The developing solution was made at the same time as solution B, since NaOH takes at least 30 minutes to dissolve.

Developing solution	
NaOH	6.0 g
37% formaldehyde	1.5 ml (added in the safety cabinet at the time of use).
Distilled water to	200 ml
Glacial acetic acid	10 ml
Distilled water to	200 ml

Procedure

Staining was carried out in glass dishes. Step 3 was done in the safety cabinet.

The gels were detached from the plates and marked, then placed in solution A for 30 minutes. The gels were transferred to solution B for 90 minutes and rinsed briefly with distilled water. The gels were then transferred to solution C and development allowed to proceed until all bands were visible in the control lanes (strains Wa and DS1). The gels were transferred to solution D for 30 minutes. The gels were rinsed and stored in 20% ethanol /1% glycine until photographed.

2.5.5.3. RT-PCR genotyping of rotavirus

Rotavirus VP7 (G) and VP4 (P) genotypes were determined by using hemi-nested, multiplex, RT-PCR. The methods were originally described for G typing by Gouvea et al (1990) and Das et al (1993) and for P typing by Gentsch et al (1992). The dsRNA template was first denatured (to separate the strands) and each strand was then reverse transcribed to complementary DNA (cDNA). A pair of consensus primers was used in the RT-PCR reaction to amplify a fragment of the VP7 or VP4 gene. Type-specific primers representing each of the common G and P types gave products of different (type-specific) lengths when used in a second amplification reaction. The products were then resolved by electrophoresis in an agarose gel and visualised by ethidium

bromide staining. A positive control (strain Wa or DS-1 or alternatively an internal control) and a negative control (H_2o) were included in each RT-PCR experiment.

2.5.5.3.1. VP7 (G) typing

Detection of rotaviruses in clinical specimens and determination of the G-types was accomplished by extraction of the viral dsRNA from faecal specimens analysis by RT-PCR with primers specific for the VP7 genes of G serotypes 1, 2, 3, 4, 8 and 9.

Reagents

Autoclaved HPLC H_2O (BDH)

Super Reverse Transcriptase (PE Applied Biosystems, Warington, UK)

10x PCR buffer (PE Applied Biosystems, Warington, UK)

25 mM Mg CL2 (PE Applied Biosystems)

Amplitaq DNA polymerase (PE Applied Biosystems)

2.5 mM dNTP (PE Applied Biosystems)

The primers were used to detect gene 9 (VP7) typing of rotavirus strains are shown in table 2.1.

Table 2.1. Multiplex RT-PCR gene 9 (VP7) typing of rotavirus strains

Primer	Strain (ST)	NT	Sense	Sequence	Primer type
9con1	Wa (G1)	37-56	+	TAGCTCCTTTTAATGTATGG	Consensus
9con2	Wa (G1)	922-941	-	GTATAAAATACTTGCCACCA	Consensus
9T-1	Wa (G1)	176-195	-	TCTTGTCAAAGCAAATAATG	Type specific
9T-2	S2 (G2)	262-281	-	GTTAGAAATGATTCTCCACT	Type specific
9T-3P	107E (G3)	484-503	-	GTCCAGTTGCAGTGTTAGC	Type specific
9T-4	ST3 (G4)	423-440	-	GGGTCGATGGAAAATTCT	Type specific
9T-9B	116E (G9)	131-147	-	TATAAAGTCCSTTGCAC	Type specific
JRG-106	Nigeria (G8)	681-698	-	TCTTCAAAAGTCGTAGTG	Type specific
NAC-9	Malawi	176-195	-	TTTAGTTAAGGCAAATAATG	Type specific

Procedure

All PCR mixes (containing enzymes, dNTPs and MgCL2) were prepared in a clean area. These were separated from the RNA processing area, which was separated from the DNA processing area. Filter pipette tips were used throughout. All amplification reactions were conducted with a Perkin Elmer 2400 thermal cycler (PE Applied Biosystems).

A. First amplification reaction:

1. Two μl of consensus primer mixture (20μM each of 9con 1and 9con2) was added to each tube.

2. The RT and Amplitaq mixes were prepared on ice and vortexed prior to use.

1xRT(μl components)

H_20	17. 9
2.5mM dNTP	8
10x PCR buffer	5
25m M $MgCI_2$	9
RT (6-9U)	0.1= 40μl

3. The tubes with 9con1/9con2 primer mix were transferred to the RNA work area.

4. 8μl RNA was combined with the 9con1/9con2 primer mix and heated at 97°C for five minutes.

5. The tubes were cooled in ice for one minute, spun for 10 seconds at 1300 rpm and returned to the ice bath.

6. 40μl of RT mix was added to each tube, which were incubated for 60 minutes at 40°C in the thermal cycler.

7. The samples were returned to the ice bath, and 50μl of Amplitaq mix was added to each tube.

8. The tubes were centrifuged for a few seconds, returned to the ice bath and then added to the thermal cycler for 10 cycles of PCR.

PCR cycling conditions

1cycle	94°C, 1 minute
10 cycles	94°C, 0.5 minutes
	42°C, 0.5 minutes

	72°C, 1 minute
1 cycle	72°C, 7 minutes
1cycle	soak at 4°C

B. Second amplification:

1x reaction mix (μl components)

H_20	27.5
2.5mM dNTP	8
10x PCR buffer	5
25 mM $MgCL_2$	4
10 μM G9 pool*	3
Amplitaq	0.5 = 48

*Mixture at a concentration of 20 μM each of primers 9T-2, 9T-4 and 9T-9B.

Procedure

1.The first amplification product was centrifuged for 5 seconds at 1300 rpm and 2 μl of product transferred to 48 μl of 1x reaction mixture. The samples were run for 30 cycles of PCR.

PCR cycling conditions:

1 cycle	94°C, 1 minute
30 cycles	94°C, 0.5 minutes
	42°C, 0.5 minutes
	72°C, 1 minute
1 cycle	72°C, 7 minutes
1 cycle	soak at 4°C forever

2.10 μl of PCR product was electrophoresed at 150 V 60-90 minutes on a 2% agarose gel with 4 μl of ethidium bromide added. The products were visualised under ultraviolet illumination. The gels were photographed.

Interpretation of results:

The sample was positive for G types 1, 2, 3, 4, 8 or 9 if the sample gave PCR products of the appropriate size.

Limitations of the test:

This test is not a serological proof of the serotypes of the tested strains, although a strong correlation exists between the results of PCR typing and monoclonal based serotyping methods (Gouvea et al., 1990).

2.5.5.3.2. VP4 (P) typing

Detection of rotavirus in clinical specimens and determination of the P types was accomplished by extraction of the dsRNA from faecal specimens and analysis by RT-PCR with primers specific for the VP4 genes of P types 4, 6, 8,and 10.

Reagents

Autoclaved HPLC H_2O

10% PCR buffer, pH 8.3(PE Applied Biosystems)

25 mM $MgCL_2$ (Applied Biosystems)

Super Reverse Transcriptase (PE Applied Biosystems)

Amplitaq polymerase (PE Applied Biosystems)

2.5 mM dNTP solution (PE Applied Biosystems)

10x PCR buffer, pH 8.3 (PE Applied Biosystems)

25 mM MgCI2 (PE Applied Biosystems)

The primers were used to detect gene4 (VP4) typing of rotavirus strains are shown in table 2.2.

Table 2.2. Multiplex RT-PCR gene 4 (VP7) typing of rotavirus strains

Primer	Strain (ST) [GT]	NT	Sense	Sequence	Primer type
Con3	Ku (1A [8])	11-32	+	TGGCTTCGCCATTTTATAGACA	Consensus
Con2	Ku (1A [8])	868-887	-	ATTTCGGACCATTTATAACC	Consensus
1T-1	Ku (1A [8])	339-356	-	TCTACTTGGATAACGTGC	Type specific
2T-1	RV5 (1b [4])	474-494	-	CTATTGTTAGAGGTTAGAGTC	Type specific
3T-1	1076 (2A [6])	259-278	-	TGTTGATTAGTTGGATTCAA	Type specific
4T-1	K8 (3 [9])	385-402	-	TGAGACATGCAATTGGAC	Type specific
5T-1	69M (3 [9])	575-594	-	ATCATAGTTAGTAGTCGG	Type specific
NAC-10	Malawi [8]	339-356	-	TCTACTGGATTGACGTGC	Type specific

Procedure

All PCR mixes (containing enzymes, dNPTs and $MgCL_2$ were prepared in a clean area. These were separated from the RNA processing area, which was separated from the DNA processing area. Filter pipette tips were used throughout. All amplification reactions were conducted with a Perkin-Elmer 2400 thermal cycler (PE Applied Biosystems).

A. First amplification:

1. Two μl of consensus primer mixture (20 μM each of con3 and con2) was added to each tube.
2. The RT and Amplitaq mixes were prepared on ice and were vortexed prior to use.

1x RT (μl components)		1x Amplitaq (μl components)	
H_2O	17.9	H2O	36.5
2.5 mM dNTP	8	2.5 mM dNTP	8
10x PCR buffer	5	10x PCR buffer	5
25 mM $MgCL_2$	9	Amplitaq	0.5
RT (6-9U)	0.1		-
Total	40μl		50μl

3. The tubes with con3/con2 primer mix were transferred to the RNA work area.
4. 8μl RNA was combined with con3/con2 primer mix and heated at 97°C for 5 minutes.
5. The samples were cooled in an ice bath the for one minute, spun for 10 seconds at 1300 rpm and returned to the ice bath.
6. 40μl of RT mix was added to each tube, which were incubated for 60 minutes at 42°C in the thermal cycler.
7. The samples were returned to ice, and 50 μl of Amplitaq mix was added to each tube.
8. The tubes were centrifuged for five seconds, returned to the ice bath and then added to the thermal cycler for 10 rounds of PCR.

PCR cycling conditions

1 cycle 94°C, 1minute

10 cycles	94°C, 0.5 minutes
	42°C, 0.5 minutes
	72°C, 1 minute
1 cycle	72°C, 7minutes
1 cycle	soak at 4°C

B. Second amplification:

1 x Reaction mix (μl components)

H_2O	28.5	
2.5 mM dNTP	8	
10X PCR buffer	5	
25 mM $MgCL_2$	4	
10 μM G4 pool* + con3	2	(1μl Gene 4 pool + 1 μl con3)
Amplitaq	0.5	
Total	48	

*Mixture at a concentration of 20 μM each of primers 1T-1, 2T-1, 3T-1, 4T-1 and 5T-1.

Procedure

1. The first amplification product was centrifuged for 5 seconds at 13000 rpm and 2 μl of product was transferred to 48 μl of 1x reaction mixture. The samples were run for 30 cycles of PCR.

PCR cycling conditions

1 cycle	94°C, 1 minute
10 cycles	94°C, 0.5 minutes
	42°C, 0.5 minutes
	72°C, 0.75 minutes
1 cycle	72°C, 7 minutes
1 cycle	soak at 4°C

2. 10 μl of PCR product were electrophoretyped at 150 V for 60-90 minutes on a 2% agarose gel with 4 μl per 100 ethidium bromide added. The products were visualised under ultraviolet illumination. The gels were photographed.

Interpretation of results

The sample was positive for P types 4, 6, 8, 9 or 10 if the sample gave PCR products of the appropriate size.

Limitations of the test

Similar to the p types this test is not a serological proof of the serotypes of the tested strains, although a strong correlation exists between the results of PCR typing and polyclonal based G serotyping methods (Gentsch et al., 1992).

2.6. Bacteria detection tests

Each faecal sample was inoculated into 4 selective and differential media including Salmonella-Shigella agar (S.S agar), Eosin- Methylene Blue agar (E.M. B agar), Xylose- Lysine-Desoxycholate (X.L.D agar), Campylobacter blood free and one enrichment broth media (selenite F, SF). For better results each SF broth was sub cultured into another S.S.agar, E.M.B agar, X.L.D agar for 24 hours. All the media except Campylobacter blood free agar were incubated at 37°C for 24- 48 hours but Campylobacter blood free agar plates were incubated at 42°C under microaerophilic conditions for 48 hours. After 24 hours media incubated at 37°C were investigated to find suspect lactose negative and colourless or black colonies for *Salmonella*, *Shigella* and diarrhoeagenic *Escherichia coli* pathogens. Non-motile, lactose and urease negative; red colonies on XLD or colourless, small, non-lactose fermenting colonies on SS agar were examined by commercial *Shigella* antisera and visible agglutinations distinguished then as a *Shigella* species.

Non-fermenting lactose, pale, black centres colonies on SS agar or red colonies with black centres due to H_2S production on XLD were tested with *Salmonella* antisera. Those with positive reactions with agglutination were recorded as a *Salmonella* species.

Lactose fermenting, sorbitol non-fermenting, small red or pink colonies on SS agar and yellow or pale yellow colonies on XLD medium were tested with *E. coli* antisera and those giving visible agglutination with individual antisera to the different *E. coli* O-serotype species were identified as a *Escherichia coli* pathogen.
Metallic shiny colonies on EMB agar also were investigated for possible *E. coli* pathogens by inoculating the suspect colonies into differential media such as TSI, sorbitol, or citrate agar and Indole.

Campylobacter blood free media

Suitable temperature and microaerofilic conditions are essential for *Campylobacter* species. Media were incubated at 42°C with oxoid campy Gen ™ a product for the rapid generation of microaerophilic conditions which is essential for the isolation, and growth of *Campylobacter* species. When a CampyGen sachet is placed in a sealed jar, the atmospheric oxygen in the jar is rapidly absorbed with the simultaneous generation of carbon dioxide, producing the appropriate microaerophilic conditions. This method differs from those commonly used in that the reaction proceeds without hydrogen, and therefore does not require a catalyst. Furthermore, no addition of water is needed to activate the reaction.

Procedure

The inoculated media plates were placed in the appropriate jar.
The CampyGen foil sachet was opened at the tear-nick, and the CampyGen paper sachet was removed. The CampyGen paper sachet was immediately placed in the appropriate clip on the plate carrier within the jar and the jar was closed.
After 48 hours incubation the plate was removed and examined for the presence of *Campylobacter.* Suspension colonies were stained with safranin looking for the spirally curved bacilli (gull wings) o typic-1 of *Campylobacter* spp.

2.6.1. E. *coli, Salmonella spp* and *Shigella spp* antiserum test

Baharafshan Research and Producer company antiserum kits (Baharafshan Research and Producer company, Number 249, North Karghar street, Tehran, Iran) and Oxoid,

Diagnostic Reagents (*E. coli* O157 test Oxoid Ltd, Basingstoke, Hampshire. England), which are used as a screening test to identify *E. coli, Salmonella* and *Shigella* species were used in this study. Individual antisera were used for each bacteria however the principles and procedures were similar.

Principle

A suspension of bacteria in distilled water was made for each individual bacteria. In a second step, antiserum was added to the suspension. The antigen and antibody react with each other and a visible agglutination appears indicating the presence of a specific bacterium.

Procedure

1. The latex reagents were transferred to room temperature before starting the test.
2. One drop of the latex test was dispensed onto a circle on the reaction card.
3. Some loopfuls of saline were added to the circle.
4. A portion of the colony was taken with a loop emulsified in the saline drop.
5. The latex test, the saline and bacteria suspension were mixed together and spread to cover the reaction area using the loop.
6. After rocking the card in a circle motion, agglutination was observed. If invisible agglutination was seen, another non –fermented colony was tested.

If agglutination with the test reagent occurred, we tested a further portion of the colony with the control latex reagents to ensure that the isolate latex was not an auto-agglutinating strain.

2.7. Measurement of micronutrient

Two major laboratory tests, inductively coupled plasma- mass spectrometry (ICP-MS) and high-performance Liquid Chromatography (HPLC) were used to measure the Micronutritient including zinc, selenium, copper and vitamins A and E.

2.7.1. Blood collection

Blood samples were collected from hospitalised children with acute diarrhoea and hospitalised children without diarrhoea after parental consent. Three ml blood was taken with the help of trained nurse at the time of admission. Blood samples were kept at 37°C for 60 minutes then centrifuged to separate the serum. The separated serum was poured into two white and brown tubes. The white tubes were used to measure the micronutrients including zinc, selenium, copper but sera for vitamin A and E measurement were kept in brown tubes.

2.7.2. Inductively Coupled Plasma –Mass Spectrometry (ICP –MS)

Total plasma selenium, zinc and copper concentrations were measured by inductively coupled plasma –mass spectrometry (ICP-MS), using the method described by Delves et al (Delves, 1997). Briefly, plasma samples were diluted in 1:100 in Delves diluent containing a 10 ppb indium internal reference. These samples were then analysed by ICP-MS. Specific isotopes of all metals were used for determination of plasma concentrations. These were ^{63}Cu and ^{64} Cu, ^{66}Zn and ^{67} Zn and ^{82}Se. The final plasma concentration of each metal was determined by reference to standard curves generated during each series of sample runs.
A lyophilised human reference serum, Seronorm (Nycomed Pharma, Oslo, Norway) was used as a control within each series of samples.

2.7.3. High-Performance Liquid Chromatography (HPLC)

Vitamin A and E were determined in serum by High-Performance Liquid Chromatography (HPLC). The type of column used for the reverse phase assay was a Spherisorb ODS II 5 μm 250 x 4.6mm, using an isocratic mobile phase of 98% ethanol 2% water at a flow rate of 1.5ml/min. Detection was accomplished using a standard UV detector.

Principle of the method

Serum (0.2ml) was deproteinized with ethanol (0.2ml) that contained retinyl acetate as an internal standard, and the lipid fraction was extracted with hexane (1 ml) as described by Catignani & Bieri (Catignani and Bieri, 1983). After the separated 1ml aliquot of hexane was evaporated to dryness under N_2, the residue was reconstituted with 130μl methanol. 20μl of this solution was injected onto the C18 reversed-phase chromatographic column using an autosampler, and absorbance values of the vitamins and internal standards were measured at 292 nm. Peak-height ratios, calculated from comparison of the vitamins peak heights to that of the internal standard, were used to quantify each vitamin.

Sets of standards, at intervals of approximately every 15-serum samples, were included to calculate the sample concentration and to assess the accuracy of the method. The standard line range for the retinol assay was from 0.174 to 2.784 mol/L and for tocopherol from 2.32 to 37.12 μmol/L.

Chapter 3

Aetiology of acute diarrhoea among hospitalised and OPD children

3.1. Introduction

This study investigated 504 children, of whom 259 were hospitalised and 245 children seen in the Outpatient Department (OPD) of the hospital to establish the aetiology of acute diarrhoeal episodes. Stools were screened for parasites, bacteria and viruses as described in chapter 2 using ELISA, negative stain EM, selective and specific media for culture, the stool concentration method and direct stool smears.

3.2. Methodology

3.2.1. Objective

To establish the aetiology and characteristics of children with acute diarrhoea in Iran.

3.2.2. Study design

This was a cross-sectional study of children <5 years of age, admitted to the wards and the OPD of Hajar Hospital of Shahrekord, Iran. This study aimed to establish the characteristics of the children with diarrhoea and the prevalence of the viruses, bacteria and parasites in their stools. The stools of 114 children < 5 years old without diarrhoea who were also admitted to the hospital for elective surgery were also collected to compare the frequency of the viruses in asymptomatic children.

3.2.3. Selection criteria

Children <5 years of age with acute diarrhoea admitted to the hospital or attending the outpatient services were selected. Diarrhoea was defined as the passing of 3 or more liquid or semi-liquid stools or a single watery stool per day for less than 14 days. Children with persistent diarrhoea (i.e. diarrhoea >14 days duration) were excluded.

3.2.4. Sampling method

Participants were selected systematically during working days from Saturday to Thursday from October 2001 to August 2002. The sampling interval for selecting the

children was established by reviewing the number of children admitted to the hospital each day with a diagnosis of diarrhoea and the number of OPD patients with a diagnosis of diarrhoea. A sampling interval of every third patient was chosen for hospitalised children and of every fourth for OPD children.

3.2.5. Sample size

The sample size to fulfil this objective was calculated using Epi-info and aimed to enrol at least 2-3 cases per day during the 8-9 months of the field study. The sample size required was calculated with the following expected prevalence (table 3.1).

Table 3.1. Sample size by the expected prevalence of selected pathogens

Aetiology	Expected prevalence	Accepted Error (±)	Sample size required
E. coli	25%	5%	288
Rotavirus	20%	5%	245
Campylobacter	15%	5%	196
Salmonella	4%	5%	59
Shigella	4%	5%	59

Therefore a sample size of 288 hospitalised and 288 outpatients would be sufficient to establish the prevalence of the pathogens of interest with a precision of ± 5%.

3.2.6. Study procedure

After informed consent, participants were interviewed to complete a standard questionnaire with the help of a trained nurse to ascertain their general characteristics (e.g. age, gender, history of previous infections, breastfeeding, weight, height, maternal occupation, education, age, gravidity, family income, etc). Two faecal samples were collected from each child within the first 24 hours after enrolment and

OPD children were requested to bring stool samples to the wards on the following day to identify viruses, bacteria, protozoa and parasites.

3.3. Literature review

Childhood mortality constitutes a major public health problem and is one of the major challenges confronting the health systems in developing countries such as Iran. It contributes to the deaths of 4.6 million to 6 million children annually in Asia, Africa, and America (Torres et al., 2001). These deaths are 25 –30% of all deaths among children younger than 5 years of age in developing countries making diarrhoeal diseases, the fourth commonest cause of death world wide (Lundgren and Svensson, 2001).

3.3.1. Aetiology of childhood diarrhoea

Acute diarrhoea can be caused by many different agents including parasites, bacteria and viruses and more than 50 pathogens are able to cause diarrhoea. Viruses have been given particular attention in recent years with the possible introduction of vaccines. The prevalence of the different enteropathogens varies with age of the individual, how the diarrhoea is acquired, and the host's immunity. The frequency of specific pathogens also varies from community and hospital-based studies. For example during a 5-year period in Greece enteropathogens were recovered in only 14% of the stools of children with diarrhoea in the community, while in hospital-based studies enteropathogens were recovered in up to 86% of the patients (Maltezou *et al.*, 2001; Maraki *et al.*, 2003; Sethi and Khuffash, 1989; Sethi *et al.*, 1989; Youssef *et al.*, 2000). The number of pathogens recovered has also increased with the development of antigen detection methods, although many diarrhoea episodes still do not have an aetiologic agent identified. In Africa, it is estimated that between 13% and 55% of hospitalisation cases (median 24%) and 7% to 40% of outpatient cases (median 23%) of gastroenteritis in children are caused by rotavirus (Hart, 1999).

There are relatively few comprehensive studies of the viral, bacterial, and parasitic aetiology of severe acute diarrhoea in children admitted to hospital in developing

countries and studies in the last decades have established the important role that rotavirus plays in severe diarrhoea (Binka *et al.*, 2003; Youssef *et al.*, 2000). The relative importance of rotavirus and other bacteria across the world are summarised in table 3.2.

Table 3.2. Relative importance of rotavirus and other bacteria as a cause of diarrhoea

Region	N	% Pathogen isolated	HRV	*E. coli*	*Salmonella*	*Shigella*	Other pathogens	Reference
Asia								
Jordan	265	66	33	28	5	5	4	(Youssef et al., 2000)
Pakistan	16379	41	-	10	7	6	35	(Alam et al., 2003a)
Hong Kong	694	60	35	-	23	2	4	(Biswas et al., 1996)
Yemen	561	34	-	-	-	-	-	(Banajeh et al., 2001)
Kuwait	621	86	45	16	24	4	8	(Sethi et al., 1989)
	343	84	40	24	18	5	9	(Sethi and Khuffash, 1989)
Bangladesh	814	75	25	33	-	6	30	(Albert et al., 1999)
Europe								
France	1164	-	83*	-	-	-	-	(Desenclos et al., 1999)
Spain	820	60	31*	-	-	-	-	(Roman et al., 2003)
Africa								
Nigeria	315	75	22	-	-	-	59	(Ogunsanya et al., 1994)
Kenya	862	28	16	14	7	6	13	(Saidi et al., 1997)
Egypt	3513	-	3	31	1	2	44	(Zaki et al., 1986)
	6278	-	-	-	7	4	2	(Wasfy et al., 2000)
Ethiopia	630	-	-	-	12	-	14	
America								
U.S.A	763	-	31	-	-	-	-	(Staat et al., 2002)
Argentina	-	-	5	31	2	9	7	(Notario et al., 1993)

N= number, * viral gastroenteritis

3.3.2. Pattern of pathogens by age

Rotavirus is a major cause of diarrhoea in young children. Rotavirus may infect humans repeatedly from birth to old age. Neonatal infections appear to be nosocomial in origin, because they are rarely seen in babies born at home or at the village health centres. Serological surveys imply that most children have experienced a rotavirus infection by 24 months of age. Many studies have documented that rotavirus infections are more prevalent in the first year of life, however this higher frequency of rotavirus infections in young children still is under question, whereas some studies reported a higher frequency during the second year of life. Undoubtedly almost all epidemiological studies have reported that rotavirus gastroenteritis is most prevalent among children aged 6-24 months, which accounted for 80- 90 % of all rotavirus diarrhoea (Fruhwirth *et al.*, 2000; Lundgren and Svensson, 2001; Seo and Sim, 2000). It has been suggested that existing differences in its seasonality could explain the younger median age of illness among children in developing countries, compared with that among children in developed countries (6-8 month vs 14-18 month), respectively. Children in industrialised countries who are born at the end of the winter peak of rotavirus infections must wait a full year until they are next exposed to rotavirus, whereas children in the tropics can be exposed any day of the year (Nguyen et al., 2001).

3.3.3. Seasonality of diarrhoea

Epidemiological investigations of childhood diarrhoea have revealed that diarrhoea outbreaks vary in terms of its aetiology and climate. In tropical countries, rotavirus occurs year round, but winter peaks and summer troughs of infection are seen in developed countries. However the comparison of seasonality data from national laboratory surveillance systems in Europe have shown that seasonal differences occur, with the annual rotavirus peak occurring first in Spain, usually in December, followed by France in February, Northern Europe with England and Wales in February or March, and the Netherlands and Finland in March (Koopmans and Brown, 1999; Nguyen *et al.*, 2001). Seo and Sim (2000) in a 20-year study review from Korea have reported that the epidemic peak, which occurred in November of the last 15 years, was moving toward late winter and early spring in recent years. In Africa, the seasonality

varies. In northern Africa, there is a single peak in April and May corresponding with the dry season. In the rest (sub-Saharan) of Africa peaks are more likely to occur in the dry season, but this was not the sole determinant of seasonality (Hart, 1999; Mutanda *et al.*, 1984).

In contrast bacterial and protozoan diarrhoeas tend to occur in the wetter seasons in the tropics and summer in temperate countries. A hospital-based study from Greece reported that while a rotavirus-associated peak was noted in March, of the patients with bacterial infections 70% were admitted between April and September. However some outbreaks of bacterial origin such as waterborne or foodborne diarrhoea, occurs in spring or all year round (Hart, 1999; Kafetzis *et al.*, 2001).

3.4. Results

Stool samples were collected from 259 inpatients and 245 outpatients with a clinical diagnosis of diarrhoea and 114 surgical controls. The general characteristics of the participants are shown in Table 3.3. The mean (SD) age of the hospitalised and outpatient children were similar with 15.2 (12.1) and 15.6 (12.4) months respectively. Surgical controls however were older with a mean (SD) age of 30 (12.4) months. The gender distribution of the children was comparable with 143 (55%), 133 (54%) and 65 (57%) children being male among the hospitalised, outpatients and surgical controls respectively.

The highest numbers of children in a single month admitted to hospital were admitted in February (28, 11%) followed by January, June and April (27, 10%) each, May and July (26,10%) each, and the lowest number in August (6, 2%). The highest numbers of children enrolled in the OPD were seen in March, May and June (27, 11% for each of these months), and the lowest in October and August with 17 (7%) and 5 (2%) respectively. More surgical controls were admitted in April and May (15 and 14 respectively) and admissions were lower in October (2%) and August (1%). Since sampling stopped in early August, the numbers of children admitted in this month were lower than for other months, however sampling seemed to has no particular trend during the study.

Table 3.3. Characteristics of hospitalised and outpatient children with diarrhoea and surgical controls

		Children with diarrhoea		Surgical controls
		Inpatients	Outpatients	
Number		259	245	114
Male		143 (55%)	133 (54%)	65 (57%)
Age (months)	1-12	149 (58%)	135 (55%)	7 (6%)
	13-24	76 (29%)	71 (29%)	34 (30%)
	25-59	34 (13%)	39 (16%)	73 (64%)
Mean (SD)		15.2 (12.1)	15.6 (12.4)	30 (12.4)

A total of 173 viruses were detected, of which 136 were rotavirus (27%) of cases of diarrhoea, 14 coronavirus (3%), 8 calicivirus (2%), 8 adenovirus (2%) and 6 (2%) astroviruses. A total of 101 bacterial pathogens were isolated. The most frequently detected bacteria, were *E. coli* (EPEC, ETEC and EHEC) with 58 (12%) isolates, *Salmonella* with 19 (4%), *Shigell,* 13 (3%) and *Campylobacter,* 11 (2%) isolates. Sixty-three children (13%) had parasites in their stools. The most frequent parasites were *B. hominis* with 26 (5%), *G. lamblia* with 19 (4%), *Entamoeba histolytica/dispar* with 6 (1%) and *Cryptosporidium* with 12 (2%). Non-pathogenic parasites were seen in 51 (10%) stools, of which *Ent. coli* accounted for 32 (6%), *Endolimax nana* for 19 (4%) and yeasts were found in 103 (20%) of samples.

3.4.1. Pathogens in hospitalised children with diarrhoea

A total of 259 stool samples were examined, and 205 organisms; including 106 (41%) viruses, 64 (25%) pathogenic bacteria and 36 (14%) parasites were recovered. The most frequently detected organisms were rotavirus with 91 (35%), followed by EHEC (*E. coli* O157) with 15 (6%), *B. hominis,* 14 (5%), *Salmonella* and EPEC & ETEC with 22 (8%) each. Calicivirus, adenovirus and parvovirus were only rarely detected with 3, 3 and 1 positive specimen respectively. A large number of children had non-pathogenic organisms including yeasts, 71 (27%), *Ent. coli,* 22 (9%) and 10 (4%) had *End. nana.* The pathogens isolated from hospitalised children with diarrhoea are shown in table 3.4.

3.4.2. Pathogens in children attending the OPD

The pathogens isolated from outpatient children are also shown in table 3.4. Out of 245 stool samples 131 pathogenic agents were isolated. Viruses were identified in 67 (27%) specimens, bacteria in 37 (15%) and parasites in 27 (11%) episodes. The most frequent pathogens isolated were rotavirus with 45 (18%) samples, *B. hominis* with 12 (5%), EPEC and ETEC 11 (4%), coronavirus 10 (4%) and *E. coli* O157 with 9 (4%). Pathogens less frequently isolated included *G. lamblia, Campylobacter* spp, *Cryptosporidium* spp, *Salmonella* spp, calicivirus, adenovirus, and *Shigella* spp. Fifty

one non-pathogenic organisms were isolated of which yeasts were identified in 32 (14%) followed by *Ent. coli* in12 (5%) and in 7 (5%) *End. nana.*

The prevalence of rotavirus was significantly higher in hospitalised than in non-hospitalised children ($p<0.001$). Inversely the frequency of *Campylobacter* and coronavirus seemed to be higher in outpatient children, but these were not statistically significant. Among the non-pathogenic agents, yeasts were more frequently observed in hospitalised children, suggesting many children had taken antibiotics before hospitalisation ($p<0.001$).

Table 3.4. Pathogens in hospitalised and non-hospitalised children with diarrhoea

	Hospitalised (259)	OPD (245)	Surgical controls (114)	P value*
Viruses				
rotavirus	91 (35%)	45 (18%)	10 (8%)	<0.001
coronavirus	4 (2%)	10 (4%)	2 (2%)	0.1
astrovirus	4 (2%)	2 (1%)	0 (0%)	0.7
calicivirus	3 (1%)	5 (2%)	1 (1%)	0.7
adenovirus	3 (1%)	5 (2%)	0 (0%)	0.7
parvovirus	1 (%)	0 (0%)	0 (0%)	-
Bacteria				
EPEC & ETEC	22 (8%)	11(4%)	NT	0.3
E. coli O157	16 (6%)	9 (4%)	NT	0.3
Salmonella spp	13 (5%)	6 (2%)	1 (1%)	*0.2*
Shigella spp	9 (3%)	4 (2%)	1 (1%)	0.3
Campylobacter spp	4 (2%)	7 (3%)	NT	0.5
Parasites				
B. hominis	14 (5%)	12 (5%)	0 (0%)	*0.9*
G. lamblia	11 (4%)	8 (3%)	4 (4%)	0.7
E. histolytica/dispar	5 (2%)	1 (1%)	1 (1%)	0.2
Cryptosporidium spp	6 (2%)	6 (2%)	NT	0.8
Non-pathogenic				
End. nana	10 (4%)	7 (3%)	4 (4%)	0.4
Ent. coli	22 (9%)	12 (6%)	8 (7%)	*0.2*
Yeasts	71 (27%)	32 (13%)	4 (4%)	<0.001

* when hospitalised and OPD are compared NT = not tested

3.4.3. Frequency of pathogens by age

3.4.3.1. Hospitalised children by age

The frequency of pathogens in hospitalised children by age is shown in table 3.5 and figure 3.1. Rotavirus was more frequent in children less than 2 years of age and among this group, 1-2 year old children seemed to have rotavirus most frequently in their stools. After this age, the frequency of rotavirus seemed lower. Infants had a larger variety of other viruses (adenovirus, astrovirus, parvovirus) and children above 1 year old rarely had viruses other than rotaviruses. Bacterial infections had a less distinct age pattern, mostly affecting children less than 3 years of age. *E. coli* O157 and *Shigella* were more frequent in children <2 years old. Older children (36-59 months) had higher percentages of EPEC, ETEC, *B. hominis*, *Ent. histolytica/dispar*, *G. lamblia* and *Cryptosporidium* spp although these frequencies were not statistically significant.

Table 3.5. Frequency of pathogens by age in hospitalised children with diarrhoea

Age group (months)	1-12	13-24	25-36	37-59
Number of children	149	76	14	20
Viruses				
rotavirus	50 (34%)	33 (43%)	2 (14%)	6 (30%)
coronavirus	2 (1%)	2 (3%)	0 (0%)	0 (0%)
astrovirus	3 (2%)	0 (0%)	1 (7%)	0 (0%)
calicivirus	2 (1%)	0 (0%)	0 (0%)	1 (5%)
adenovirus	3 (2%)	0 (0%)	0 (0%)	0 (0%)
parvovirus	0 (0%)	0 (0%)	1 (7%)	0 (0%)
Bacteria				
EPEC & ETEC	6 (4%)	10 (13%)	2 (14%)	4 (20%)
E. coli O157	7 (5%)	8 (11%)	0 (0%)	1 (5%)
Salmonella spp	5 (3%)	6 (8%)	2 (14%)	0 (0%)
Shigella spp	3 (2%)	5 (7%)	0 (0%)	1 (5%)
Campylobacter spp	1 (1%)	1 (1%)	1 (7%)	1 (5%)
Parasites				
B. hominis	7 (5%)	4 (5%)	0 (0%)	3 (15%)
G. lamblia	2 (1%)	1 (1%)	3 (21%)	5 (25%)
Cryptosporidium spp	2 (1%)	1 (1%)	0 (0%)	3 (15%)
Ent. histolytica/dispar	0 (0%)	2 (3%)	1 (7%)	2 (10%)
Non -pathogen				
Ent. coli	3 (2%)	11(14%)	4 (29%)	4 (20%)
End. nana	3 (2%)	6 (8%)	1 (7%)	0 (0%)

The frequency of rotavirus infection was low before six months of age in hospitalised children. From this age, the incidence increased until 24 months of age when >40% of the children admitted had rotavirus. Bacteria followed a similar age pattern and were recovered from children less than 9 months of age. Numbers increased to peak in children 13-59 month of age. Other viruses however seemed to have a different pattern, with non- rotavirus, viruses being recovered more often in very young children (1-3 month old), but the numbers are too small for statistical analysis (Figure 3.1).

Figure 3.1. Pathogens in hospitalised children with diarrhoea by age (error bars =+2 SE)

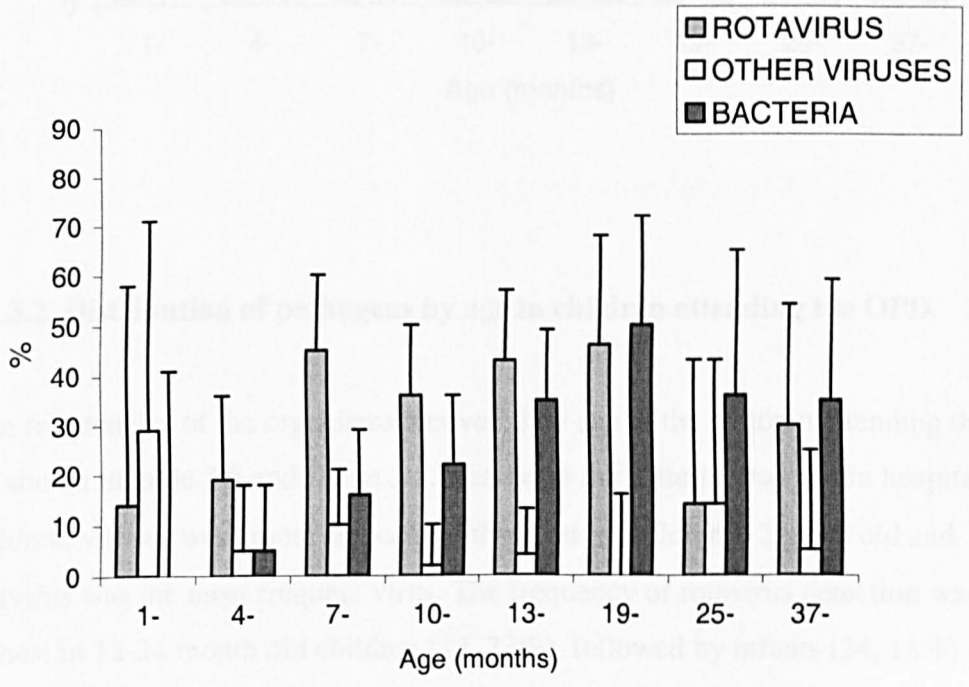
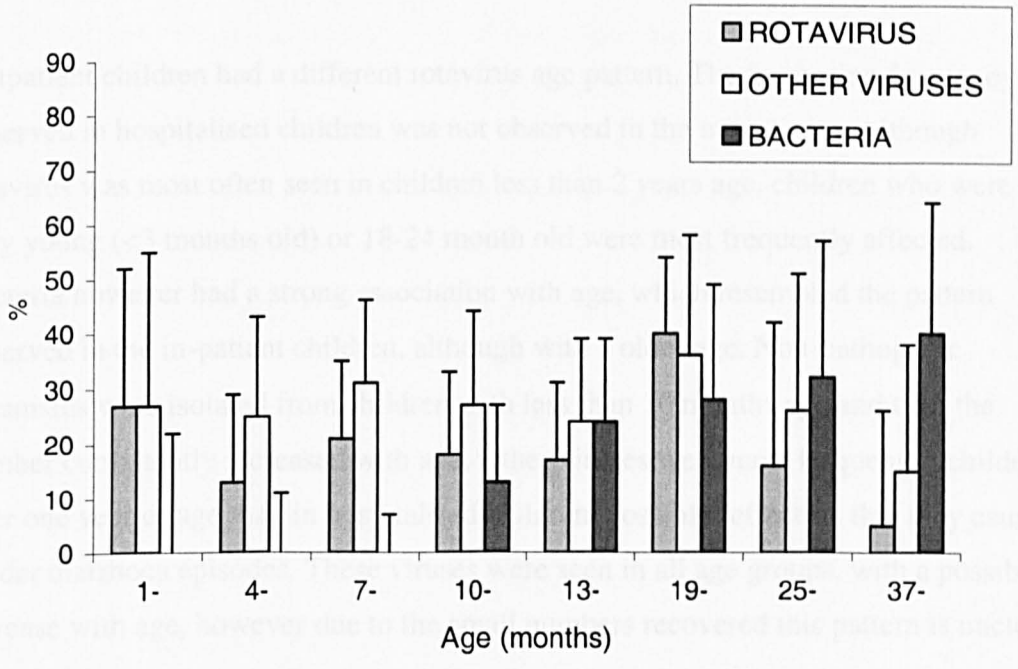


Figure 3.2. Pathogens in outpatient children by age (error bar = +2 SE)



3.4.3.2. Distribution of pathogens by age in children attending the OPD

The frequencies of the organisms recovered by age in the children attending the OPD are shown in table 3.6 and figure 3.2. Similar to the pattern observed in hospitalised children, viruses were more frequently observed in children ≤ 2 years old and rotavirus was the most frequent virus. The frequency of rotavirus detection was also highest in 12-24 month old children (17, 23%), followed by infants (24, 18%) and those 25-36 month old (3, 16%). Only one child above this age had rotavirus (1, 5%). These frequencies however were lower than those observed in hospitalised children.

Among the bacteria, EPEC/ETEC were the most frequent isolates, closely followed by *Salmonella, Shigella,* and *Campylobacter* spp. Most of these bacteria were isolated in children > 1 year old.

Crytosporidium spp, *B. hominis, End. nana* and *Ent. histolytica/dispar* were the most common parasites in children ≤ 2 years old. *G. lamblia, and Ent. coli* were most frequent in children above this age.

Outpatient children had a different rotavirus age pattern. The increasing frequency observed in hospitalised children was not observed in the outpatients. Although rotavirus was most often seen in children less than 2 years age, children who were very young (<3 months old) or 18-24 month old were most frequently affected. Bacteria however had a strong association with age, which resembled the pattern observed in the in-patient children, although with a older age. Non-pathogenic organisms were isolated from children with less than 10 months age and then the number consistently increased with age. Other viruses were most frequent in children after one year of age than in hospitalised children, possibly reflecting that they cause milder diarrhoea episodes. These viruses were seen in all age groups, with a possible increase with age, however due to the small numbers recovered this pattern is unclear (Figure 3.2).

Table 3.6. Frequency of organisms by age in non-hospitalised children

Age group (months)	1-12	13-24	25-36	37-59
Number of Children	135	71	19	20
Viruses				
rotavirus	24 (18%)	17 (23%)	3 (16%)	1 (5%)
coronavirus	5 (4%)	3 (4%)	1 (5%)	1 (5%)
astrovirus	0 (0%)	1 (5%)	0 (0%)	1 (5%)
calicivirus	2 (2%)	2 (3%)	1 (5%)	0 (0%)
adenovirus	5 (4%)	0 (0%)	0 (0%)	0 (0%)
parvovirus	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Bacteria				
EPEC& ETEC	3 (2%)	6 (8%)	1 (5%)	1 (5%)
E. coli (O157)	1 (1%)	6 (8%)	1 (5%)	1 (5%)
Salmonella spp	1 (1%)	3 (4%)	0 (0%)	2 (10%)
Shigella spp	0 (0%)	1 (1%)	2 (11%)	1 (5%)
Campylobacter spp	0 (0%)	2 (3%)	2 (11%)	3(15%)
Parasites				
B. hominis	6 (4%)	4 (6%)	1 (5%)	1 (5%)
G. lamblia	0 (0%)	3 (4%)	0 (0%)	5 (25%)
Ent. histolytica/dispar	1 (1%)	0 (0%)	0 (0%)	0 (0%)
Cryptosporidium spp	2 (2%)	3 (4%)	1 (5%)	0 (0%)
Ent. coli	1 (1%)	3 (4%)	4 (21%)	4 (20%)
End. nana	1 (1%)	4 (5%)	1 (5%)	1 (5%)

3.4.4. Pathogens among hospitalised and OPD children less than 2 years of age

The most frequent organism detected was rotavirus, responsible for 82 (36%) and 41 (20%) isolates in hospitalised and OPD children less than 2 years of age. This was followed by EPEC (32, 14%) in hospitalised children and by *B. hominis* (11, 5%), EPEC/ETEC (9, 4%) and coronavirus (8, 4%) in the outpatient.

The isolation of pathogenic bacteria, started from 4 months of age in hospitalised children, increased until 19-24 month and then slightly decreased in children over 2 years. Most of the rotaviruses were detected among children aged 7-24 months as shown in table 3.6.

Other viruses (adenoviruses, caliciviruses, coronaviruses and astroviruses) were mostly isolated in children less than 7 months and less frequent after 24 months. Pathogenic bacteria were not isolated in children less than 10 months of age. After this age, the frequency of bacterial infections increased in OPD children. No significant changes were seen for viruses.

Table 3.7. Viruses, bacteria and parasites in hospitalised and OPD children less than 2 years old

Subjects	Hospitalised	OPD
Number	225	206
Viruses		
rotavirus	82 (36%)	41 (20%)
coronavirus	2 (1%)	8 (4%)
astrovirus	3 (1%)	1 (1%)
calicivirus	2 (1%)	4 (2%)
adenovirus	3 (1%)	5 (2%)
parvovirus	1 (1%)	0 (0%)
Bacteria		
EPEC& ETEC	16 (7%)	9 (4%)
E. coli (O157)	16 (7%)	7 (3%)
Salmonella spp	11 (5%)	4 (2%)
Shigella spp	8 (4%)	1 (1%)
Campylobacter spp	2 (1%)	2 (1%)
Parasites		
B. hominis	11 (5%)	11 (5%)
G. lamblia	3 (1%)	3 (1%)
Cryptosporidium spp	5 (2%)	3 (1%)
Ent. histolytica/dispar	2 (1%)	1 (1%)
Ent. coli	14 (6%)	4 (2%)
End. nana	9 (4%)	4 (2%)

3.4.5. Results from surgical controls

One hundred and fourteen stool samples were collected from hospitalised surgical controls to examine the frequency of the viruses. Out of the 114 samples, 10 (9%) were positive for rotavirus, 2 (2%) for coronavirus, and 1 (1%) had calicivirus. In routine parasite stool examination, 8 (7%), 4 (4%) and 1 (1%) were positive for yeasts, for *G. lamblia*, *Ent. coli, and End. nana* and for *Ent. histolytica/dispar*, respectively. In routine stool culture only 1 (1%) each of the cultures were positive for *Salmonella* and *Shigella* each (table 3.8). Samples were not tested for *E. coli*, *Campylobacter* or *Cryptosporidium.* The frequency of the pathogens by age in hospitalised children without diarrhoea are shown in table 3.8. In contrast to the children with diarrhoea, rotavirus was most frequent in children more than 3 years old. However similar to the children with diarrhoea, the frequency of *G. lamblia* was higher in older children. While the frequency of *G. lamblia* and rotavirus were significant, the number of children infected with *E. nana,* and *Ent. coli* were too small to find statistical differences . The frequencies of children infected with other pathogens did not seem to have any specific affected age distribution.

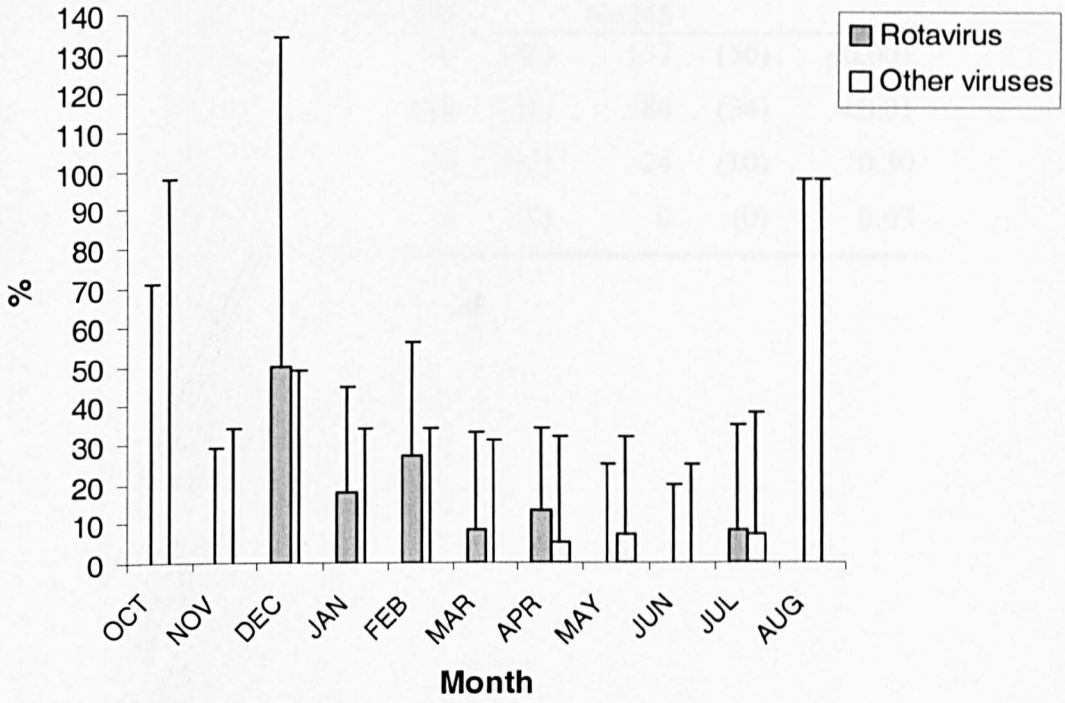
Table 3.8. Frequency of viruses, bacteria and parasites by age in surgical controls

Age group (months)	1-12	13-24	25-36	37-59
Number of children	7 (6%)	34 (30%)	46 (40%)	27 (24%)
Viruses				
rotavirus	1 (1%)	2 (2%)	1 (1%)	6 (5%)*
coronavirus	0 (0%)	0 (0%)	1 (1%)	1 (1%)
astrovirus	0 (0%)	0 (0%)	0 (0%)	0 (0%)
calicivirus	0 (0%)	1 (1%)	0 (0%)	0 (0%)
adenovirus	0 (0%)	0 (0%)	0 (0%)	0 (0%)
parvovirus	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Bacteria				
EPEC & ETEC	NT	NT	NT	NT
E. coli O157	NT	NT	NT	NT
Salmonella spp	0 (0%)	0 (0%)	1 (1%)	0 (0%)
Shigella spp	0 (0%)	0 (0%)	1 (1%)	0 (0%)
Campylobacter spp	NT	NT	NT	NT
Parasites				
B. hominis	0 (0%)	0 (0%)	0 (0%)	0 (0%)
G. lamblia	0 (0%)	0 (0%)	1 (1%)	3 (3%)*
Cryptosporidium spp	NT	NT	NT	NT
Ent. histolytica/dispar	0 (0%)	0 (0%)	1 (1%)	0 (0%)
Ent. coli	0 (0%)	2 (2%)	3 (3%)	3 (3%)
End. nana	0 (0%)	1 (1%)	2 (1%)	1 (1%)
Yeasts	0 (0%)	2 (1%)	0 (0%)	2 (2%)

NT= not tested * P <0.05

Out of the 10 rotavirus positive samples, most were recovered from December to April, coinciding with the peak rotavirus season in children with diarrhoea, suggesting that there is also transmission of the virus within the hospital or that the circulation of this virus in the community is high, with a proportion of infections being asymptomatic. Other viruses were most often detected during times when the frequency of rotavirus was low, as shown in Figure 3.3.

Figure 3.3. Frequency of viruses in surgical controls by month



3.4.6. Frequency of co-infections

The frequency of co-infections in the children with diarrhoea are shown in table 3.9. A higher number of hospitalised children with diarrhoea had at least one pathogen identified (62%) compared to OPD children (44%) ($p<0.001$). The highest number of pathogens isolated in a single child was 3. Rotavirus was the most common virus and the single-pathogen most frequently isolated (95, 70% of the specimens), followed by *E. coli* with 58 cases. Only 27 (47%) of these *E. coli* were isolated as single pathogens and (14, 24%) were isolated simultaneously with rotavirus.

Co-infections were more frequent in hospitalised than in OPD children (15% vs 10% respectively) although this was only marginally significant (P=0.07). The number of children with three pathogens was also higher in hospitalised children (6 vs. none in OPD children) (Fisher's exact test p=0.03). The list of pathogens and co-infections is shown in table 3.10.

Table 3. 9. Frequency of co-infections in cases and controls

Number of pathogens	Hospitalised N= 259	%	OPD N=245	%	P value
None	99	(38)	137	(56)	<0.001
One	120	(46)	84	(34)	<0.01
Two	34	(13)	24	(10)	0.30
Three	6	(2)	0	(0)	0.03

Table 3.10. Pathogens isolated and co-infections among the samples

			Co infected with												
	All	Alone													
rotavirus	136	95 (70%)	HRV												
coronavirus	14	6 (43%)	2	COR											
astrovirus	6	3 (50%)	1	0	AST										
calicivirus	8	5 (63%)	0	0	0	CAL									
adenovirus	8	6 (75%)	0	0	0	0	ADE								
EPEC&ETEC	33	18 (54%)	5	0	1	0	1	EPET							
E. coli O157	25	9(36)	9	1	0	2	0	0	0157						
Salmonella spp	19	14 (74%)	2	2	0	0	0	0	0	SAL					
Shigella spp	13	8 (62%)	4	0	0	0	0	0	0	0	SHI				
Campylobacter spp	11	5 (45%)	3	2	1	0	0	0	0	0	0	CAM			
B. hominis	27	7 (26%)	8	1	0	1	1	4	0	1	0	0	BHO		
G. lamblia	19	11 (58%)	3	0	0	0	0	1	2	0	1	0	1	G.L	
Cryptosporidium spp	12	3 (25%)	2	0	0	0	0	3	1	0	0	0	2	0	CRY
Ent. histolytica/dispar	6	0 (0%)	2	0	0	0	0	0	1	0	0	0	1	1	1

COR = coronavirus
AST = astrovirus
CAL = calicivirus
ADE = adenovirus
EPET = EPEC&ETEC
O157 = *E. coli* O157
HRV = human rotavirus
SHI = *Shigella* spp
CAM = *Campylobacter* spp
BHO = *B. hominis*
G.L= *G. lamblia*
CRY = *Cryptosporidium* spp
SAL = *Salmonella* spp

3.4.7. Frequency of pathogens by gender

The frequency of detection of organisms by gender is shown in table 3.11. Higher prevalences of calicivirus, coronavirus, *Shigella, Ent. histolytica/dispar* and *End.nana* were seen in male than in female children. Conversely rotavirus, adenovirus, *E. coli, Salmonella, B. hominis, Ent. coli, Cryptosporidium* spp and *Campylobacter* spp were more frequently recovered from female children. The difference for these proportion however were not statistically significant (all P values were >0.05).

Table 3.11. Frequency of organisms by gender

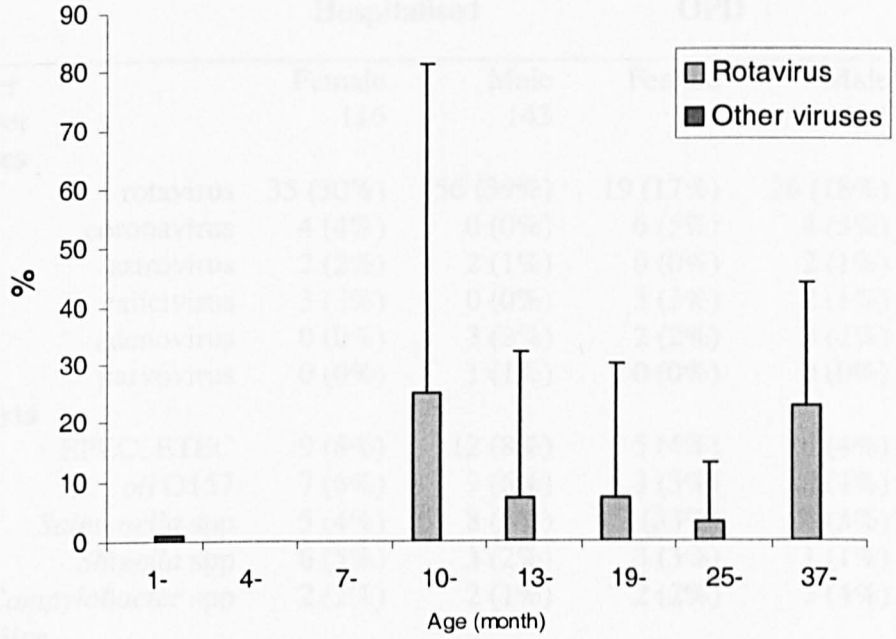
Subjects	Male	Female
Number	227	277
Viruses		
rotavirus	53 (23%)	83 (30%)
coronavirus	10 (4%)	6 (2%)
astrovirus	2 (1%)	4 (1%)
calicivirus	6 (3%)	2 (1%)
adenovirus	2 (1%)	6 (2%)
parvovirus	1 (1%)	0 (0%)
Bacteria		
EPEC, ETEC	14 (6%)	18 (7%)
E. coli O157	10 (4%)	15 (5%)
Salmonella spp	7 (3%)	12 (4%)
Shigella spp	9 (4%)	4 (1%)
Campylobacter spp	4 (2%)	7 (3%)
Parasites		
B. hominis	9 (4%)	17 (6%)
G. lamblia	8 (4%)	11 (4%)
Cryptosporidium spp	4 (2%)	8 (3%)
Ent. histolytica/dispar	5 (2%)	1 (1%)
Ent. coli	15 (6%)	19 (7%)
End. nana	8 (4%)	8 (3%)

3.4.8. Viruses in surgical controls

Children who were in the hospital for surgical reasons had an interesting age pattern of excretion of pathogens. Very few of these children were less than 9 months of old and results in this age group are difficult to interpret. However, there was no rotavirus

detected in children less than 10 months. After this age, rotavirus was isolated in all age groups (though patients were asymptomatic). There is no consistent pattern with age and the numbers of virus recovered were too small for statistical analysis. It is however interesting to see that a relatively large number of children were excreting rotavirus. The lack of an age specific pattern could be compatible with an intra-hospital transmission of rotavirus or asymptomatic infections (Figure 3.4).

Figure 3.4. Distribution of pathogens in surgical controls by age



3.4.9. Distribution of pathogens in inpatient and outpatient children

The type of pathogen by enrolment place showed that only coronavirus (4%), calicivirus (2%) and adenovirus (2%) were more often seen in outpatient than hospitalised children with diarrhoea.

Hospitalised children had higher frequencies of rotavirus, *E. coli, Salmonella, Shigella, Ent. coli, End. nana* and *Cryptosporidium.* No differences were seen for *Campylobacter, G. lamblia, Ent. histolytica/dispar, B. hominis* and astrovirus. Of

these pathogens only rotavirus was statistically higher in hospitalised children than OPD (P<0.001)

The frequency of the enteropathogens by sex and enrolment place is shown in table 3.12. The frequencies of calicivirus, coronavirus, *Shigella* and *Ent. histolytica/dispar* were higher in female children in cases and controls, but this was not statistically significant. Similarly, *E. coli* O157, rotavirus and *Salmonella* were most often seen in males in both groups, but again these proportion were not statistically significant.

Table 3.12. Frequency of organism stratified by gender (all age groups)

	Hospitalised		OPD	
Gender	Female	Male	Female	Male
Number	116	143	111	134
Viruses				
rotavirus	35 (30%)	56 (39%)	19 (17%)	26 (18%)
coronavirus	4 (4%)	0 (0%)	6 (5%)	4 (3%)
astrovirus	2 (2%)	2 (1%)	0 (0%)	2 (1%)
calicivirus	3 (3%)	0 (0%)	3 (3%)	2 (1%)
adenovirus	0 (0%)	3 (2%)	2 (2%)	3 (2%)
parvovirus	0 (0%)	1 (1%)	0 (0%)	0 (0%)
Bacteria				
EPEC, ETEC	9 (8%)	12 (8%)	5 (4%)	6 (4%)
E. coli O157	7 (6%)	9 (6%)	3 (3%)	5 (4%)
Salmonella spp	5 (4%)	8 (6%)	2 (33%)	4 (3%)
Shigella spp	6 (5%)	3 (2%)	3 (3%)	1 (1%)
Campylobacter spp	2 (2%)	2 (1%)	2 (2%)	5 (4%)
Parasites				
B. hominis	7 (6%)	7 (5%)	3 (3%)	10 (7%)
G. lamblia	5 (4%)	6 (4%)	3 (2%)	5 (4%)
Cryptosporidium spp	2 (2%)	4 (2%)	2 (2%)	4 (3%)
Ent. histolytica/dispar	4 (3%)	1 (1%)	1 (1%)	0 (0%)
Ent. coli	7 (6%)	15 (10%)	8 (7%)	4 (3%)
End. nana	6 (5%)	4 (2%)	3 (43%)	4 (3%)
Yeast	27 (23%)	44 (30%)	15 (10%)	17 (13%)

3.4.10. Pathogens by season

The seasonality of the pathogens isolated in hospitalised children by month is shown in table 3.13 and the seasonal distribution of rotavirus, bacteria and other viruses are shown in figures 3.5 and 3.6. Rotavirus was the most frequently isolated virus from November to February with more than 40% of the cases detected in each of these

months and 48 out of the 91 (53%) rotavirus identified were recovered in these four months. Between October and March (the cold season in Iran) rotavirus represented 42% of the pathogens identified in hospitalised children (62/147) and was responsible for 24% of diarrhoeal episodes among the OPD children (33/139). Outside these months, rotavirus was identified in 25% and 12% of the hospitalised and outpatient children.

The remainder of the viruses did not seem to have clear seasonal patterns and frequencies were too small to find statistical differences by month. Bacterial infections seem to be more common from April to May, although individual pathogens did not seem to cluster around any specific month.

Table 3.13. Distribution of organisms by month (hospitalised children)

Months	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug
Number of children	18	25	24	27	28	25	27	26	27	26	6
Viruses											
rotavirus	6 (33%)	11 (44%)	12 (50%)	12 (44%)	13 (46%)	8 (32%)	6 (22%)	7 (27%)	8 (29%	6 (23%)	2 (33%)
coronavirus	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (8%)	0 (0%)	1 (4%)	0 (0%)	1 (4%)	0 (0%)
astrovirus	0 (0%)	0 (0%)	0 (0%)	2 (8%)	0 (0%)	0 (0%)	2 (8%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
calicivirus	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (4%)	0 (0%)	1 (4%)	0 (0%)	1 (4%)	0 (0%)
adenovirus	1 (6%)	0 (0%)	1 (4%)	0 (0%)	0 (0%)	1 (4%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
parvovirus	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (4%)	0 (0%)	0 (0%)	0 (0%)
Bacteria											
EPEC, ETEC	1 (6%)	1 (4%)	1 (4%)	1 (4%)	2 (8%)	1 (4%)	5 (19%)	2 (8%)	3 (11%)	3 (12%)	1 (4%)
E. coli O157	2 (11%)	3 (12%)	1 (4%)	0 (0%)	2 (8%)	0 (0%)	1 (4%)	2 (8%)	2 (8%)	3 (12%)	0 (0%)
Salmonella spp	0 (0%)	1 (4%)	2 (8%)	0 (0%)	2 (8%)	3 (12%)	0 (0%)	2 (8%)	0 (0%)	2 (8%)	1 (4%)
Shigella spp	0 (0%)	1(4%)	2 (8%)	0 (0%)	2 (8%)	3 (12%)	0 (0%)	2 (8%)	0 (0%)	2 (8%)	1 (4%)
Campylobacter spp	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (8%)	1 (4%)	0 (0%)	1 (4%)	0 (0%)
Parasites											
B. hominis	2 (12%)	1 (4%)	1 (4%)	1 (4%)	2 (8%)	1 (4%)	2 (8%)	2 (8%)	0 (0%)	1 (4%)	1 (4%)
G. lamblia	2 (12%)	2 (8%)	1 (4%)	0 (0%)	0 (0%)	3 (12%)	1 (4%)	1 (4%)	0 (0%)	1 (4%)	0 (0%)
Cryptosporidium spp	0 (0%)	1 (4%)	1 (4%)	1 (4%)	0 (0%)	1 (4%)	2 (8%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Ent. histolytica/dispar	0 (0%)	0 (0%)	1 (4%)	1 (4%)	0 (0%)	2 (8%)	0 (0%)	1 (4%)	0 (0%)	0 (0%)	0 (0%)
Ent. coli	1 (6%)	2 (8%)	2 (8%)	1 (4%)	2 (8%)	4 (16%)	2 (8%)	4 (16%)	3 (11%)	1 (4%)	0 (0%)
End.. nana	0 (0%)	1 (4%)	2 (8%)	2 (8%)	0 (0%)	0 (0%)	3 (12%)	0 (0%)	1 (0%)	0 (0%)	0 (0%)

Table 3.14. Distribution of organisms by month (OPD)

Months	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June	July	Aug
Number of children	17	21	23	25	26	27	24	27	27	23	5
Viruses											0 (0%)
rotavirus	4 (24%)	4 (19%)	5 (22%)	5 (20%)	8 (31%)	7 (26%)	5 (21%)	3 (11%)	2 (7%)	2 (9%)	
coronavirus	0 (0%)	0 (0%)	0 (0%)	1 (4%)	1 (4%)	0 (0%)	1 (4%)	2 (7%)	4 (15%)	1 (4%)	0 (0%)
astrovirus	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (4%)	1 (4%)	0 (0%)	0 (0%)	0 (0%)
calicivirus	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (8%)	1 (4%)	0 (0%)	0 (0%)	2 (8%)	0 (0%)	0 (0%)
adenovirus	1 (6%)	1 (5%)	0 (0%)	1 (4%)	0 (0%)	1 (4%)	0 (0%)	0 (0%)	1 (4%)	0 (0%)	0 (0%)
parvovirus	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Bacteria											
EPEC, ETE)	2 (12%)	0 (0%)	0 (0%)	1 (4%)	1 (4%)	0 (0%)	1 (4%)	0 (0%)	4 (15%)	1 (4%)	0 (0%)
E. coli (O157)	0 (0%)	0 (0%)	1 (4%)	2 (8%)	0 (0%)	0 (0%)	1 (4%)	1 (4%)	1 (4%)	1 (4%)	0 (0%)
Salmonella spp	1 (6%)	0 (0%)	0 (0%)	0 (0%)	1 (4%)	0 (0%)	0 (0%)	2 (7%)	1 (4%)	1 (4%)	0 (0%)
Shigella spp	0 (0%)	1 (5%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (4%)	0 (0%)	1 (4%)	1 (4%)	0 (0%)
Campylobacter spp	1 (6%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (7%)	2 (8%)	1 (4%)	1 (4%)	0 (0%)	0 (0%)
Parasites											
B. hominis	1 (6%)	0 (0%)	0 (0%)	1 (4%)	3 (12%)	1 (4%)	1 (4%)	1 (4%)	2 (7%)	2 (9%)	1 (4%)
G. lamblia	0 (0%)	1 (5%)	0 (0%)	2 (8%)	0 (0%)	1 (4%)	2 (8%)	1 (4%)	0 (0%)	0 (0%)	1 (4%)
Cryptosporidium spp	0 (0%)	0 (0%)	0 (0%)	2 (8%)	0 (0%)	0 (0%)	1 (4%)	1 (4%)	1 (4%)	1 (4%)	0 (0%)
Ent. histolitica/dispar	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (4%)	0 (0%)	0 (0%)
Ent. coli	1 (6%)	1 (5%)	0 (0%)	2 (8%)	0 (0%)	0 (0%)	1 (4%)	2 (8%)	4 (15%)	1 (4%)	0 (0%)
End. nana	1 (6%)	2 (10%)	0 (0%)	2 (8%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (4%)	1 (4%)	0 (0%)

Figure 3.5. Frequency of pathogens in hospitalised children with diarrhoea by month

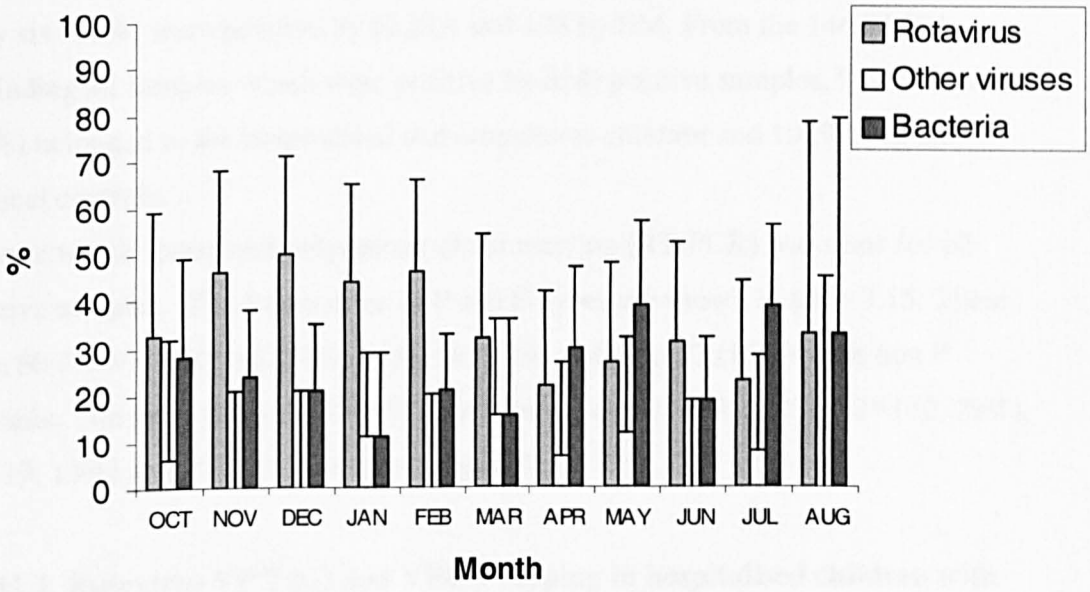
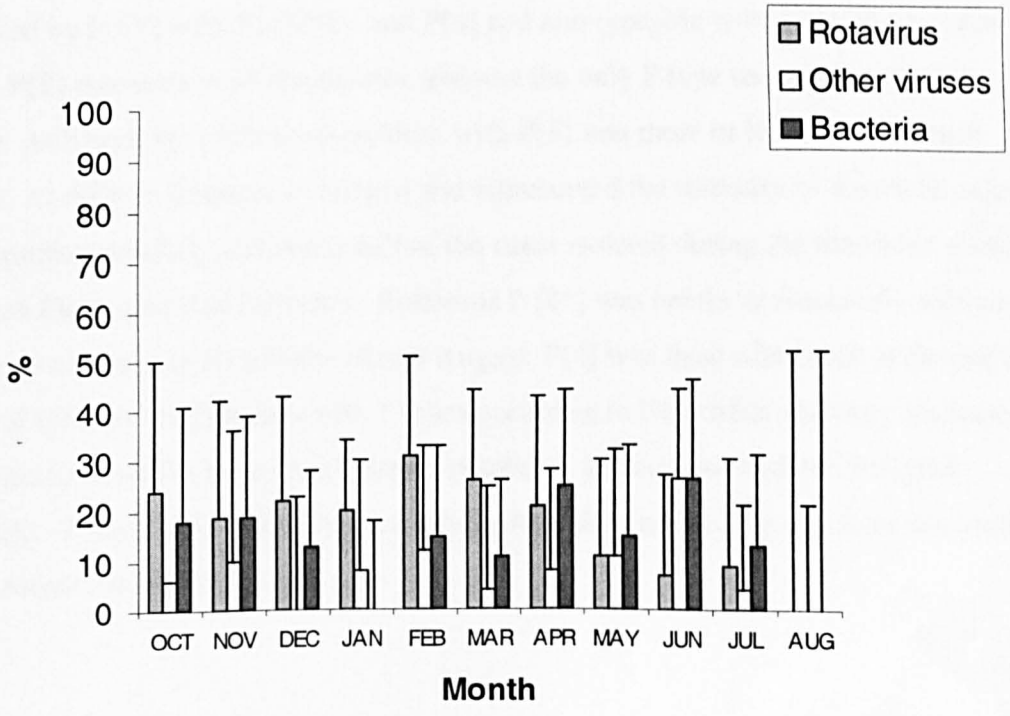


Figure 3.6. Frequency of pathogens in outpatient children with diarrhoea by month



3.4.11. Distribution of rotavirus VP 8 (G) and VP4 (P) typing

Six hundred and fourteen stool samples were tested by ELISA to identify rotavirus positive samples. Two hundred and fifty nine were from hospitalised children, 245 from the outpatients with diarrhoea and 114 from surgical controls. One hundred and forty six (24%) were positive by ELISA and 128 by EM. From the 146 ELISA (including all samples which were positive by EM) positive samples, 91 (35%) and 45 (18%) belonged to the hospitalised and outpatients children and 10 (9%) to the surgical controls.

Reverse transcription and polymerase chain reaction (RT-PCR) was done for all positive samples. The frequencies of P and G types are shown in table 3.15. There were 60 (43%) P[8]*, 48 (32%) P[8], 16 (11%) P[4] and 22 (15%) were non P typeable. The most frequent VP7 (G) genotypes were G1 (78, 53%), G1* (42, 29%), G2 (19, 13%) and 7 (5%) were non-G typeable.

3.4.11.1. Rotavirus VP 7 (G) and VP4 (P) typing in hospitalised children with diarrhoea

The frequency of rotavirus P typing in hospitalised children with diarrhoea is shown in figure 3.7. The most frequent P type was P [8] found in 37 (40%) of the isolates, followed by P[8*] with 32 (35%) and P[4] and non typeable with 11 (12%) isolates each. P[8] was seen in all the months and was the only P type seen in August (2 cases). Although the number of children with P[8] was more or less the same each month, its relative frequency changed and represented the minority of rotavirus cases in November and July and about half of the cases isolated during the diarrhoea season between December and February. Rotavirus P [8*] was nearly as frequently seen as P[8] and was seen in all months except August. P[4] was most often seen at the end of the year (October to January) with 5 cases occurring in November and only one case was seen between February to October. A total of 11 rotavirus isolates were not typeable. These rotaviruses occurred mostly from January to July and were not seen from August to December.

Figure 3.7. Frequency of rotavirus P typing in hospitalised children with diarrhoea

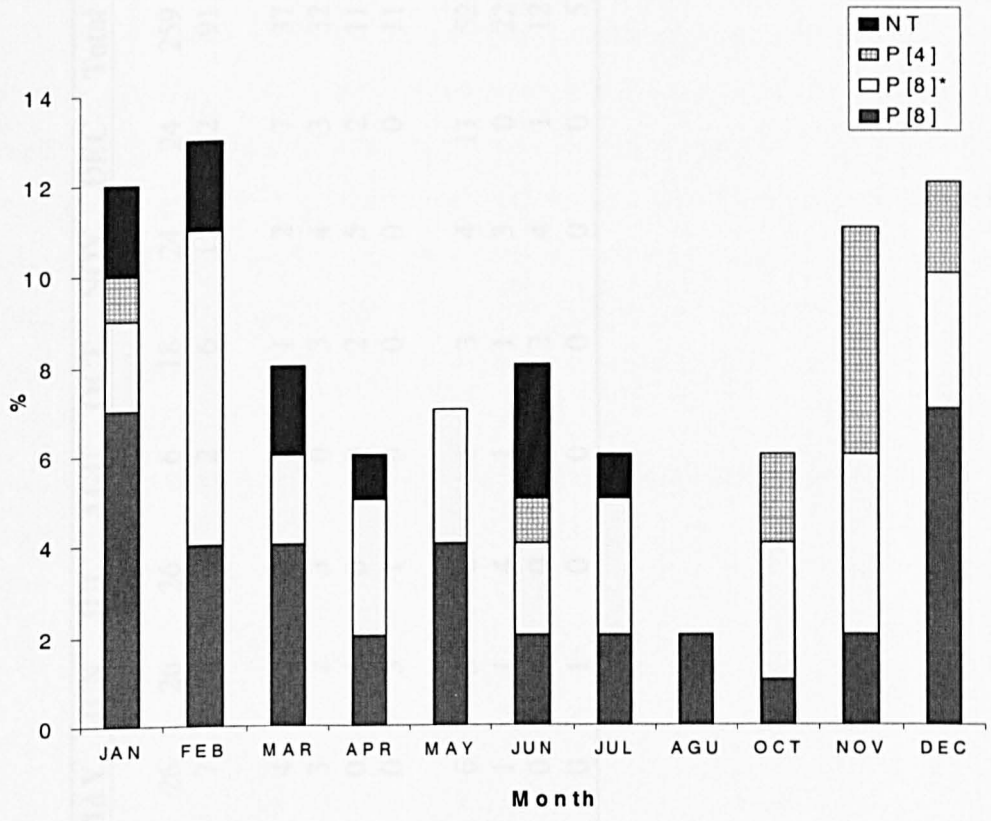


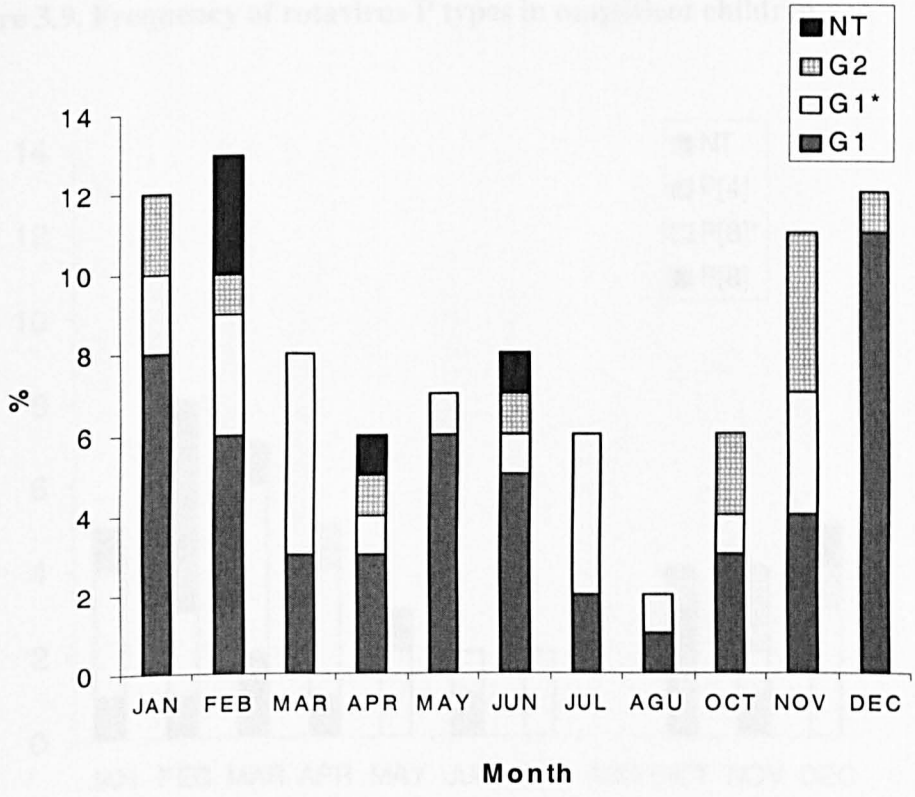
Table 3.15. Distribution of VP4 (P) and VP7 (G) typing by month in hospitalised children with diarrhoea

Months	JAN	FEB	MAR	APR	MAY	JUN	JUL	AGU	OCT	NOV	DEC	Total
Number												
Children	27	28	25	27	26	26	26	6	18	24	24	259
Rotavirus	12	13	8	6	7	8	6	2	6	11	12	91
P type												
P[8]	7	4	4	2	4	2	2	2	1	2	7	37
P[8]*	2	7	2	3	3	2	3	0	3	4	3	32
P[4]	1	0	0	0	0	1	0	0	2	5	2	11
NT	2	2	2	1	0	3	1	0	0	0	0	11
G type												
G1	8	6	3	3	6	5	2	1	3	4	11	52
G1*	2	3	5	1	1	1	4	1	1	3	0	22
G2	2	1	0	1	0	1	0	0	2	4	1	12
NT	0	3	0	1	0	1	0	0	0	0	0	5

The frequency of G typing in hospitalised children with diarrhoea is shown in figure 3.8.

The most frequent G type was G1, responsible for 52 (57%) of the rotavirus infection, followed by G1* 22 (24%) and G2 12 (13%). G non-typeable was seen in 7 (5%) in-patients. G1 was seen in all months, however its relative proportion was higher in December and May when it represented more than 80% of the viruses. No significant change was seen in other months when it was identified in about 50 of the rotavirus. G1* was seen more often in March and July and nearly half of the cases occurred in these 2 months. G2 rotavirus was most often seen at the end of the year (November to January) and less frequently from February to August. G non-typeable strains were only seen in February, April, and June, which is similar to the P pattern.

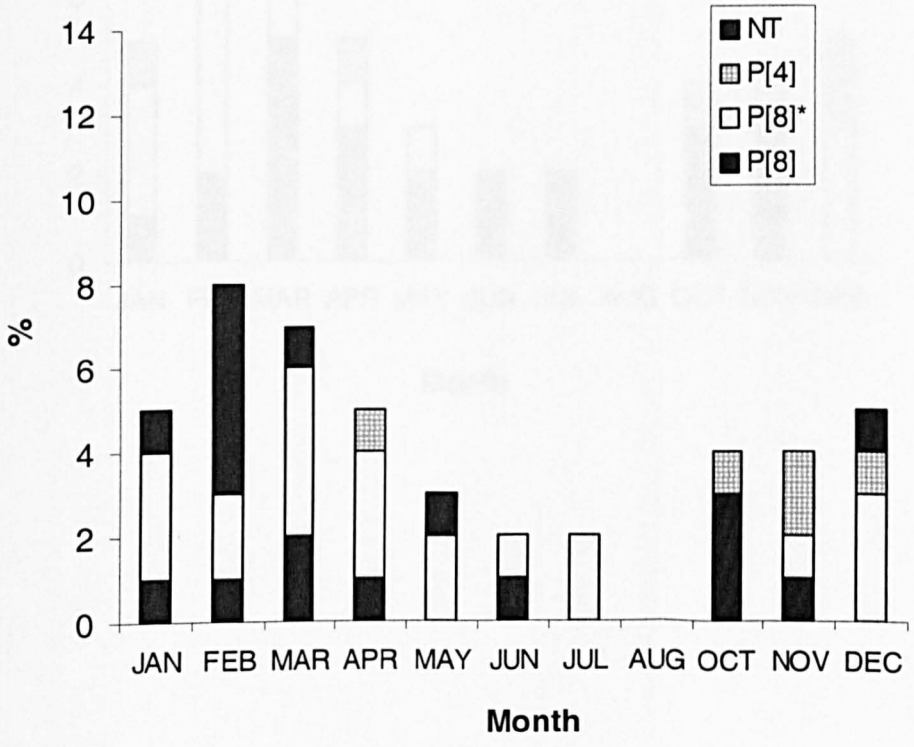
Figure 3.8. Frequency of rotavirus G types in hospitalised children with diarrhoea



3.4.11.2. Rotavirus VP 8 (G) and VP4 (P) typing in outpatient children with diarrhoea

The frequency of P types in non-hospitalised children is shown in figure 3.9. P[8*] was the most frequent P type recovered from 22 (50%) samples, followed by P[8] 8 (17%). This is the opposite to inpatients as they had a highest frequency of P [8], followed by P[8*]. Non typeable P 9 (20%) and P[4] 6 (14%).
P[8*] was seen in all months sampled except October, however all P types in July were P[8*]. P[8] was seen most frequently in October and March and was not seen in July and December or May. P[4] was most often seen in October to December, which coincided with the pattern in inpatient children, and then disappeared. P non-typeable rotaviruses were recovered from December to May, which was also the case for inpatients and was not seen from June to November.

Figure 3.9. Frequency of rotavirus P types in outpatient children



The frequencies of G types in outpatient children are shown in figure 3.10. The most frequent G types was G1 with 23 (50%), followed by G1* with 14 (31%) and G2 with

7 (16%). Only 1 child had a G non-typeable strain. G1 was seen in all months except December although with increasing relative frequency from February to July. All the cases detected in June and July belonged to this G type. G1* was identified from December to May, although with decreasing incidence and disappearing from June to December. G2 had a similar pattern to hospitalised patients, being most often found from October to April and was not seen in March and May to July.

Figure 3.10. Frequency of G types in OPD children

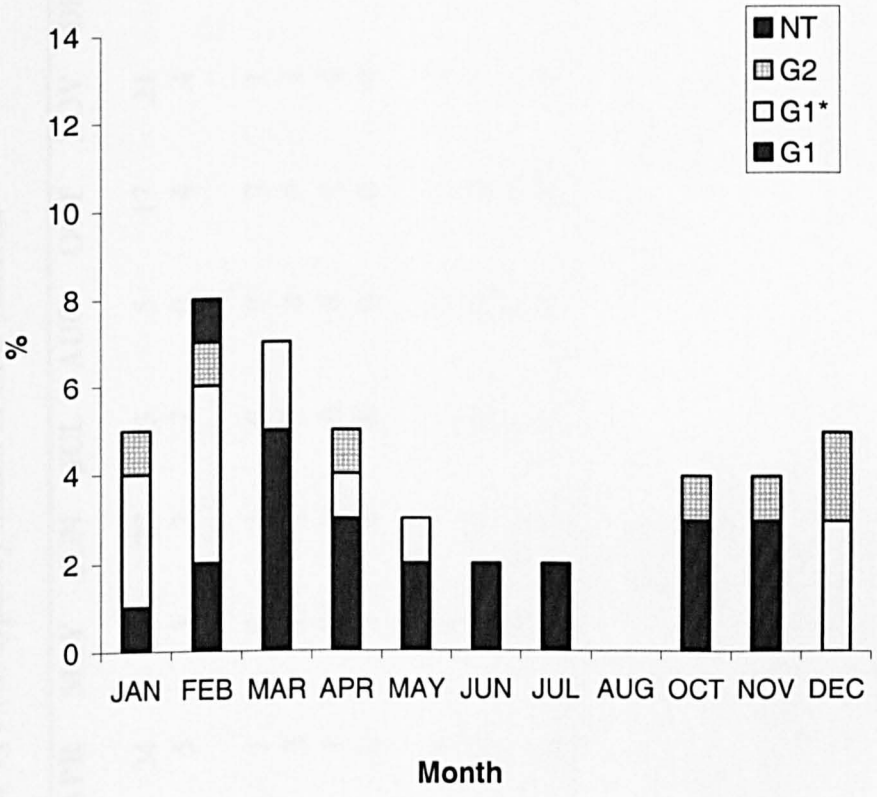


Table 3.16. Distribution of VP4 (P) and VP7 (G) types by month in OPD children

Months	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	OCT	NOV	DEC	Total
Number												
Children	25	26	27	24	27	27	23	5	17	21	23	245
Rotavirus	5	8	7	5	3	2	2	0	4	4	5	45
P type												
P[8]	1	1	2	1	0	1	0	0	3	1	0	10
P[8]*	3	2	4	3	2	1	2	0	0	1	3	21
P[4]	0	0	0	1	0	0	0	0	1	2	1	5
NT	1	5	1	0	1	0	0	0	0	0	1	9
G type												
G1	1	2	5	3	2	2	2	0	3	3	0	23
G1*	3	4	2	1	1	0	0	0	0	0	3	14
G2	1	1	0	1	0	0	0	0	1	1	2	7
NT	0	1	0	0	0	0	0	0	0	0	0	1

3.4.12. G and P non-typeable rotavirus

The RT-PCR successfully assigned 139 of the 146 (95%) rotavirus to G types and 124 (85%) to P types. The most frequent P type was P[8*] identified in 61 (42%) samples, followed by P[8] in 48 (33%), P[4] in 16 (11%) but 22 (15%) were non P-typeable. Among the G types, G1 was identified in 78 (53%), followed by G1* in 42 (29%), G2 in 19 (13%) and 7 (5%) were non G-typeable. There was no clear difference in the variety of P and G types between children with and without diarrhoea. However, The number for surgical controls with rotavirus were too small for statistical analysis. The P and G distribution among children with and without diarrhoea are shown in tables 3.17 and 3.18.

Table 3.17. Distribution of G & P types in children with diarrhoea

P	G1	G1*	G2	G-NT	Total
P[8]	39	7	0	1	47
P[8]*	29	20	0	4	53
P[4]	3	0	13		16
NT	4	9	6	1	20
Total	75	36	19	6	136

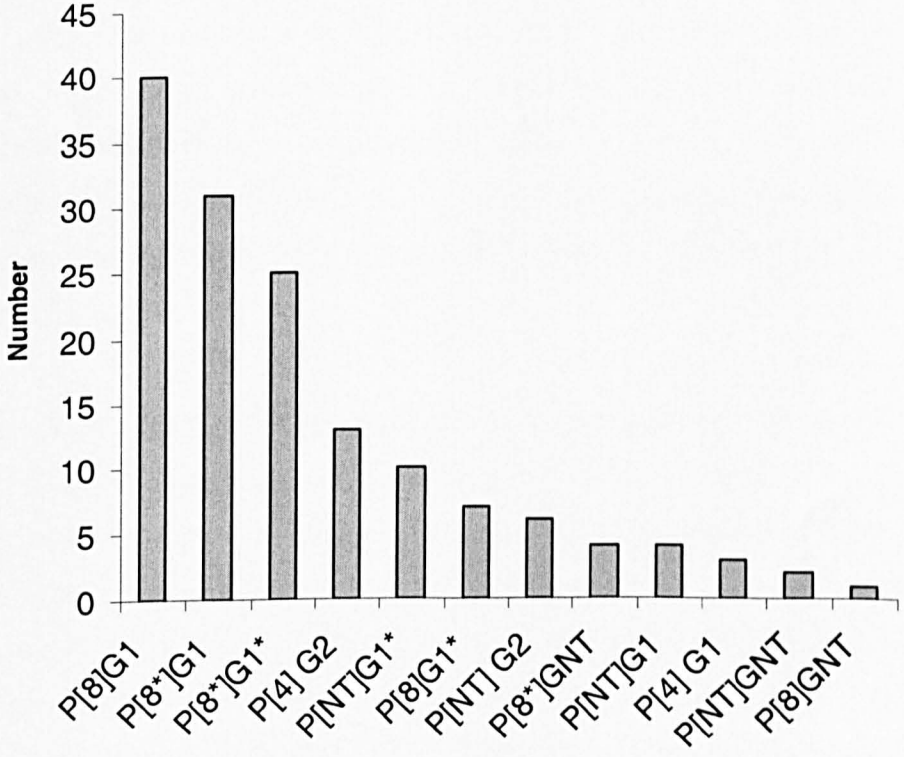
Table 3.18. Distribution of G & P types in surgical controls

P	G1	G1*	G2	G-NT	Total
P[8]	1	0	0	0	1
P[8]*	2	5	0	0	7
P[4]	0	0	0	0	0
NT	0	1	0	1	2
Total	3	6	0	1	10

3.4.13. Rotavirus P-G combinations

The predominant rotavirus P-G combinations are shown in figure 3.11. There were a total of 12 combinations with P[8] G1 accounting for 27% of the samples, followed by P[8*] G1 in 21% and P[8*] G1* in 17%.The combination of P- G typing are shown in figure 3.11. The variation of P-G combinations seen during the rest of the year suggests that there is wide strain diversity, with some strains predominating in a given year. The Iranian P[8*] strains showed 96.7% nucleotide identities to each other. Their comparison with prototype Wa showed that one strain was 93% homologous but a 2nd strain only had 89.7% nucleotide identity. Their nucleotide variation with the Malawi strain were 98% and 95% respectively. Novel G1* amplicons (118bp) were compared to a prototype Wa G1 and a Malawi G1* isolate. The Iranian strains were 99% identical to each other and showed 95% homology to both Wa and the Malawi isolate. (Malawi strain to Wa = 97.5%).

Figure 3.11. Distribution of rotavirus P –G combinations



3.4.14. Polyacrylamide gel Electrophoresis (PAGE)

Electrophoretypes were done for first 46 rotavirus positive samples. Out of 46 samples 26 (67%) were long electrophoretype, 14 (30%) were short electrophoretype and 6 (13%) of them were identified as positive samples. All the short-electrophoretypes were P[4] G2 and all long-electrophoretypes were a combination of P[8] or P[8*] with G1 or G1*.

3.5. Discussion

3.5.1. Diarrhoea and aetiology of pathogens

Diarrhoeal disease in children is one of the most common infections worldwide resulting in the death of 3 to 6 million children annually. Diarrhoeal disease is nowadays the fourth most important cause of death worldwide (Hart, 2003c).

The greatest morbidity and mortality is seen among children less than two years of age (Carroll and Reimer, 2000; Giordano *et al.*, 2001; Katyal *et al.*, 2000; Torres *et al.*, 2001). A child is estimated to experience an average 3.3 episodes of diarrhoea a year. This is higher in developing countries and in some areas the rate is up to 15-19 episodes. In contrast, a review of childhood acute diarrhoea reported that European children experience approximately 1 episode/year in the first 3 years (Baqui *et al.*, 1992; Guandalini, 1989; Torres *et al.*, 2001).

During the last decades our concept of diarrhoea and seasonality has changed from the perception that it was mostly frequent in the warm months to several peaks in a year. Rotavirus is most frequently reported in the cool seasons around the world but bacterial infections are most frequent in spring and summer especially in developing and tropical regions. However *E. coli,* one of the most frequent pathogens, has been reported all year round (Albert *et al.*, 1999; Grimprel *et al.*, 2001; Iqbal *et al.*, 2001; Tomkins, 2000).

Studies that describe the frequency of enteric pathogens have reported a wide range of aetiological factors being detected from 9 to 86% of the samples. However most studies have detected pathogens in around 40% of the children with diarrhoea (Benitez *et al.*, 1991; Biswas *et al.*, 1996; Courouble *et al.*, 2000; Madeley, 1997; Ogunsanya *et al.*, 1994). In the Middle East, one study in Kuwait isolated enteropathogenic agents in 86% of children with diarrhoea and 10% of children without diarrhoea (Sethi et al., 1989). Another study in Saudi Arabia reported that 49% of children with diarrhoea had enteric pathogens (Al-Freihi *et al.*, 1993). In our study we found that 62% and 44% of the hospitalised and non-hospitalised children with diarrhoea had enteropathogens and that 18% of hospitalised children without

diarrhoea had pathogens. This is the first report of the range of pathogens isolated in children with diarrhoea from Iran and our results are similar to other reports from this region (Al-Freihi *et al.*, 1993; El-Sheikh *et al.*, 2001; Sethi *et al.*, 1989).

3.5.2. Rotavirus

3. 5.2.1. Frequency of rotavirus

Many studies have reported that rotavirus is the most common single cause of severe dehydrating gastroenteritis in young children worldwide. In 1985, epidemiological studies estimated that rotavirus was responsible for between 20% to 25% of all diarrhoea deaths in children less than 5 years of age in developing countries (Chen *et al.*, 1991; Fruhwirth *et al.*, 2000; Hart, 2003c; Urbina *et al.*, 2003; Van der Donck *et al.*, 2003). The relative proportion of rotavirus detected in a study often reflects the characteristics of the study population. Hospital studies often report a higher prevalence of rotavirus than community based studies, reflecting the severe diarrhoea caused by this virus. The majority of studies have shown that rotavirus is responsible for between 20%- 52% of acute diarrhoea episodes in children (Albert *et al.*, 1999; Ballal and Shivananda, 2002; Biswas *et al.*, 1996; Courouble *et al.*, 2000; Cunliffe *et al.*, 2002a; Cunliffe *et al.*, 2002b; Esona *et al.*, 2003). This is still under some discussion as other studies have also reported that other pathogens like EPEC or ETEC are the most common causes of diarrhoea (Aihara, 1997; Geyid *et al.*, 1998; Mendis *et al.*, 1995; Stewien *et al.*, 1993).
Our study documented that rotavirus is responsible for 35% of the episodes of severe diarrhoea requiring hospitalisation and about 18% of the episodes in children that do not require hospitalisation, which is similar to other reports from Middle East countries (Kurugol et al., 2003). This study also confirms that rotavirus is over re-presented in hospital studies although it is still an important cause of diarrhoea in the community (Caeiro *et al.*, 1999; El-Sheikh *et al.*, 2001).

A previous study from Iran had shown that 25% of the children with diarrhoea in Tehran had rotavirus. The lower prevalence reported in this latter study might be related to the higher socio-economic status of the capital and to seasonal variation, as there was cooler weather during the months of our study than the time when the

Tehran study took place (Amini et al., 1990). These frequencies however are difficult to compare with each other due to the different study design of the studies and the number and type of laboratory tests used.

Similar to other studies, we found a strong association between rotavirus and hospitalisation and greater frequencies for other viruses in outpatient children, reflecting the milder diarrhoea caused by other virus, not requiring hospitalisation (Madeley, 1997; McIver *et al.*, 2000).

3.5.2.2. Rotavirus and seasonality

Rotavirus is highly seasonal in temperate countries, with peaks in the cooler months of December to February and fewer peaks in the summer (Maltezou et al., 2001). In tropical areas, rotavirus can be seen throughout the year, although still has a seasonal pattern. The most common pattern is for the majority of cases to occur in the cool instead than in the warm season (Biswas *et al.*, 1996; Giordano *et al.*, 2001; Hart, 2003c). This pattern however is not constant, as studies around the world have reported a peak of rotavirus in other seasons (Caeiro *et al.*, 1999; Castillo-Duran *et al.*, 1988; Courouble *et al.*, 2000; Grimprel *et al.*, 2001; Moddares, 1995). For example, in Korea the peaks of the rotavirus in the 15 years before 1990 were in October to December, however in recent years these annual peaks have been gradually delayed. Their analysis of rotavirus in hospitalised children revealed that the incidence peaked in December- January in 1993 and 1994, February –March in 1996 and March-April in 1997, 1998 and 1999 (Seo and Sim, 2000).

In Shahrekord, rotavirus was the most frequently isolated pathogen from November to February in more than 40% of the samples in each month and 53% of the rotavirus were recovered in these four months. Between October and March (the cold season in Iran) rotavirus was recovered in 42% of the hospitalised children and 24% of the non-hospitalised children. Outside these months, rotavirus represented 25% and 12% of the pathogens in hospitalised and non-hospitalised children respectively. These results are in agreement with other studies that reported a peak in the winter. Our peak incidence however was different to the study from Tehran, which reported a high

incidence in the spring. However even though our peak was in the winter, the frequency of rotavirus was also high in the spring (about 30%). Surveillance of rotavirus during several years would be necessary to clarify if these studies have shown a different pattern in the two towns, or if this was a reflection of the inter-annual variation with a lack of rotavirus peak in the year when the study in Tehran took place (Amini et al., 1990).

3.5.2.3. Rotavirus and age

Undoubtedly outbreaks of rotavirus infection are common among infants and young children. Most of the studies have documented that up to 80% of cases of diarrhoea in childhood diarrhoea can be due to rotavirus. We found that rotavirus was detected in 37% and 23% of hospitalised children less than and over 2 years old and 18.6% and 10% of the outpatient children less than and over 2 years old respectively. This is similar to reports from other countries. However, in our children a higher frequency of rotavirus detection was observed in 13- 24 month children followed by those 1-12 month of age, which is similar to the studies by Liddle et al (1997) and Roman et al (2003) which reported a higher frequency for children 6-24 month age. Studies from Iran and Turkey have reported a frequency of 19.7% rotavirus in children under 2 years and 5.1% above this age (Ballal and Shivananda, 2002; Kurugol *et al.*, 2003; Liddle *et al.*, 1997; Madeley, 1997; Moddares, 1995; Roman *et al.*, 2003).

Rotavirus is thus an important cause of diarrhoea in Shahrekord, it affects mostly children under 2 years of age and causes severe diarrhoea in the winter with an over representation of cases attending the hospital. Our age distribution is also similar to European patterns that have documented a higher frequency of rotavirus in children over 11 months old and is different to the African age pattern with a higher frequency of rotavirus in children less than 6 months (Hart., 2003).

3.5.3. Other viruses and seasonality

Similar to rotavirus, astrovirus and adenovirus are mostly isolated in the cooler semester of the year in temperate climates and during the rainy season in tropical areas (Grimprel *et al.*, 2001; Maltezou *et al.*, 2001; Mendis *et al.*, 1995; Sethi and

Khuffash, 1989; Sethi *et al.*, 1989; Van der Donck *et al.*, 2003). Other viruses, such as calicivirus and coronavirus, have been reported during all seasons, although coronavirus is more often reported during the summer (Basu *et al.*, 2003; Singh *et al.*, 1989a). Our data however did not seem to demonstrate a clear pattern for these viruses and frequencies were too small to find seasonal differences. Adenovirus however seem to coincide with the months when rotavirus is more frequent and calicivirus appeared after the rotavirus season from May to June. Individual viruses other than rotavirus did not seem to cluster around any specific month (Gonzalez *et al.*, 1997; Gonzalez *et al.*, 1989; Rahman *et al.*, 2002a; Singh *et al.*, 1989a; Singh *et al.*, 1989b).

3.5.4. Prevalence of other viruses beside rotavirus and age

Several studies have described the prevalence and association of other viruses including astrovirus, adenovirus, coronavirus and calicivirus with age. Most studies indicate that these viruses are more frequent in younger children and account for up to 10% of diarrhoea episodes among children less than 5 years old. Some studies however have reported that astrovirus affects mostly older children between 1-4 year old (Guix *et al.*, 2002; Pakianathan and McMillan, 1999). Our study showed, prevalences of 3%, 1%, 2% and 2% in all ages for coronavirus, astrovirus, calicivirus and adenovirus respectively, which is similar to other studies. In agreement with the previous study from Iran, the prevalence for adenovirus was 6.7% (Courouble *et al.*, 2000; Gonzalez *et al.*, 1997; Saderi *et al.*, 2002; Singh *et al.*, 1989a; Singh *et al.*, 1989b). However it must be remembered that electron microscopy is less sensitive than antigen detection or RT-PCR in finding Astrovirus or calicivirus, these findings might under-estimate their importance.

3.5.5. Parasites

Parasites were identified in many children with diarrhoea. The prevalence of these parasites varies widely depending on region, health facilities, socio-economic status, drinking water, toilet facilities, culture and many other factors. The prevalence of the parasites is higher in developing and tropical countries. Parasites were present in 13% of the cases of childhood diarrhoea in the present study, which is similar to other

surveys that reported parasites. The most frequent parasites in our study were *B. hominis, G. lamblia, Cryptosporidium spp* and *Ent. histolytica/dispar.* Some parasites such as *Ascaris lumbricoides, Schistosoma spp,* hookworm, *Enterobius vermicularis* and *Trichuris trichiura* seems to be extremely rare in our area. This could be the result of increasing hygiene and literacy standards in Iran over the last decades and the increase of health services coverage, which currently has reached 99% of the population.

3.5.5.1. *Blastocystis hominis*

The prevalence of *B. hominis* varies from 0.8% to 38 % diarrhoeal episodes in children (Iqbal *et al.*, 2001; Miller *et al.*, 2003; Pakianathan and McMillan, 1999; Urbina *et al.*, 2003). However most studies have reported that up to 10% of faecal samples collected from children with diarrhoea have *B. hominis.* This is the first study from Iran to examine *B. hominis* and we detected that 5 % of children with diarrhoea had *B. hominis,* (Iqbal *et al.*, 2001; Mendis *et al.*, 1995; Miller *et al.*, 2003; Pakianathan and McMillan, 1999; Urbina *et al.*, 2003).

3.5.5.2. *Cryptosporidium*

During the last 3 decades, our concept of cryptosporidiosis has changed from a rare, largely asymptomatic disease, to an important cause of diarrhoea in animals and humans worldwide. Several epidemiological studies have shown that the prevalence of this parasite has a wide range from 0% to 89% and accounts for up to 20% of all cases of childhood diarrhoea in developing countries (Mendis *et al.*, 1995; Miller *et al.*, 2003; Mosier and Oberst, 2000). Most studies however have demonstrated that its prevalence varies between 5% to 10% and between <1% to 3% in developed countries (Akyon *et al.*, 1999; Iqbal *et al.*, 2001; Molbak *et al.*, 1997a; Moles *et al.*, 1998; Nimri and Hijazi, 1994; Youssef *et al.*, 2000). Our study revealed that 2% of the children had *Cryptosporidium.* The prevalence in our study was similar to that reported by Youssef et al and Akyon et al in studies from Jordan and Turkey respectively. Outbreaks of cryptosporidiosis are reported in all seasons but it seems to be more frequent in the rainy season. Children in our study who had this parasite were most often seen during the rainy season. Interestingly though the rainy season should

start from October, there was no rain until January and we did not find any cryptosporidiosis before January (Akyon *et al.*, 1999; Youssef *et al.*, 2000).

3.5.5.3. *G. lamblia, and Ent. histolytica/dispar*

No seasonal variation has been reported for *G. lamblia*, or *Ent. histolytica/dispar*. These parasites seem to be more frequent during the summer, but can be seen all year round in all age groups. Their prevalence varies from 1% - 40 % in different societies depending on socio-economic status, access to safe drink water, weather, toilet facilities, etc. They are more common in developing than developed countries. The discovery, that *Ent. dispar* has the same morphologic appearance as *Ent. histolytica,* but that it is non-pathogenic, has thrown into question our previous knowledge on the epidemiology of the parasites as in most countries the diagnosis of amoebiasis is still based on the presence of cysts on light microscopy. Recent studies have demonstrated that the vast majority of these cysts belong to the non-pathogenic *Ent. dispar*. We were not able to distinguish between these two parasites as the ELISA kits required are expensive (> £ 2.00 per sample) and we had to report them in tandem. We found that 4% of the children had *G. lamblia* and only 1% had Ent. *histolytica/dispar*. There was no seasonal peak as reported by other authors (Iqbal *et al.*, 2001; Miller *et al.*, 2003; Nimri and Hijazi, 1994). Routine laboratory results and local unpublished surveys in Iran have shown that the overall prevalence of *G. lamblia* and *Ent. histolytica/dispar* are less than 10%.

3.5.6. Bacteria

Bacteria have an important role in diarrhoea, even though rotavirus seems to be the most common pathogen for childhood diarrhoea. Many studies have suggested that pathogens such as *E. coli* strains, *Shigella* and *Salmonella* spp are the most common causative agents of childhood bloody diarrhoea (Benitez *et al.*, 1991; Maltezou *et al.*, 2001) and bacteria can be responsible for up to 70% of the episodes of acute diarrhoea in some areas. Early studies however should be interpreted with caution as the diagnostic capacity for viruses and new pathogens has increased in recent years (Ballal and Shivananda, 2002; Ogunsanya *et al.*, 1994). We detected bacterial agents in 20% of the diarrhoea episodes, which is similar to other studies (Aihara, 1997;

Ballal and Shivananda, 2002; Geyid *et al.*, 1998; Maraki *et al.*, 2003; Stewien *et al.*, 1993). Each diarrhoeagenic bacterium is discussed below.

3.5.6.1. *Escherichia coli*

Numerous studies have reported an important role for *E. coli* strains in bloody and watery diarrhoea in children. These bacteria included ETEC, EPEC, EHEC, EIEC and EaggEC, which have been reported in up to 68% of diarrhoeal episodes (Khan *et al.*, 1988; Mendis *et al.*, 1995; Ogunsanya *et al.*, 1994; Stewien *et al.*, 1993). Many studies have reported *E. coli* as a major pathogen of childhood diarrhoea and sometimes the second most important cause of acute diarrhoea after rotavirus, especially in developing countries. We did not investigate the prevalence of EIEC and EaggEC, as we did not have specific antiserum for these species. However we detected 7% and 5% of EPEC and ETEC and *E. coli* (O157) respectively from hospitalised children with diarrhoea, while the corresponding figures were 4% each for ETEC, ETEC and *E. coli* (O157) for non-hospitalised children. The overall prevalence of *E. coli* strains was 11% which is in agreement with studies from the U.S.A (Caeiro *et al.*, 1999; Talan *et al.*, 2001), Kenya and Jordan (Aihara, 1997; Youssef *et al.*, 2000). In contrast, our prevalence was lower than reported from Bangladesh (Albert *et al.*, 1999; Torres *et al.*, 2001). In the Middle East, two studies have reported a similar prevalence of *E. coli* in Jordan (Batikhi, 2002; Na'was and Abo-Shehada, 1991) and a study from Iraq has reported a prevalence of 30% in 1991, although the higher prevalence in the later study might be related to lower standards of health facilities (Abbar et al., 1991).

3.5.6.2. *Salmonella*

Salmonella one of the most common causes of bacterial diarrhoea is responsible for up to 28% of cases in hospitalised children with diarrhoea. It is most common in the warm months of the year around the world. A few studies from Pakistan and Spain however have not reported this pathogen as an important cause of diarrhoea in childhood or a seasonal variation in the winter and the autumn and these might be important regional variations. (Benitez *et al.*, 1991; Mendis *et al.*, 1995; Oberhelman *et al.*, 2001; Saidi *et al.*, 1997; Shahid *et al.*, 1996a; Shahid *et al.*, 1996b; Urbina *et*

al., 2003; Zurawska-Olszewska *et al.*, 2002). In our study, the prevalence of children infected with *Salmonella* were 5% and 2% in the hospitalised and non-hospitalised groups respectively, which is similar to reports from Nigeria, Jordan and Kenya (Aihara, 1997; Ogunsanya *et al.*, 1994; Youssef *et al.*, 2000). *Salmonellae* were most frequently isolated in February, March and May to July, suggesting a greater frequency during the warmer months than during the cold season (Aihara, 1997; Cama *et al.*, 1999; Ogunsanya *et al.*, 1994; Youssef *et al.*, 2000).

3.5.6.3. *Shigella*

Shigella species are enteropathogens frequently causing watery or bloody diarrhoea and are responsible for 10-15% of acute diarrhoea episodes in children less than 5 years old and the most common etiologic agents of childhood dysentery. Shigellosis is common in the warm season. However studies from France, Ethiopia and Pakistan have reported no isolates of *Shigella* species from children with diarrhoea or some studies have claimed a higher prevalence in the cold season (Albert *et al.*, 1999; Khan *et al.*, 1988; Niyogi *et al.*, 1994; Oyofo *et al.*, 2002a; Oyofo *et al.*, 2002b; Urbina *et al.*, 2003; Youssef *et al.*, 2000). In our study, the overall prevalence of *Shigella* was 3% for in and outpatients and most *Shigella* spp were isolated in June, July and February.

3.5.6.4. *Campylobacter*

Campylobacter produce watery diarrhoea, which can present with blood and mucus in the stool. They are responsible for up to 17% of cases of childhood diarrhoea most often affecting children less than five years old during the warm months of the year. Though *Campylobacter* mostly affect young children, they can be seen in all age groups (Caeiro *et al.*, 1999; Lim and Tay, 1992; Maraki *et al.*, 2003; McIver *et al.*, 2001; Yoshida *et al.*, 1998). Nevertheless the seasonality and age distribution of this bacterium are under discussion as a study from Australia reported a higher susceptibility in older children with no seasonal peak during the year. In our study, we found that 2% of hospitalised and 3% of non-hospitalised children had *Campylobacter* with greater frequencies from April to July, which is similar to a study by Youssef et al (2000). One study from Iran reported the prevalence of *Campylobacter* at about 5%

with no seasonal peak (Narsis Golkarie & Mohamed Mehdi Sultandalal, 2000; Youssef *et al.*, 2000).

3.5.6.5. Bacteria and seasonality

Numerous studies have focused on bacteria and diarrhoea, but none of them has focused on seasonality of overall bacteria and childhood diarrhoea. However several epidemics have been reported by individual pathogens such as *Salmonella*, *Shigella* and *Vibrio cholera* during the summer months and it seems that bacterial diarrhoea is more common during the warm months of the year, especially in tropical and developing countries (Albert *et al.*, 1999; Maltezou *et al.*, 2001; Niyogi *et al.*, 1994; Nzeako and Okafor, 2002; Phetsouvanh *et al.*, 1999). Our study has shown that enteropathogenic bacteria were most often recovered from April to October in both in- and outpatients, although with a lower frequency in out- patients, confirming the higher frequency of bacterial diarrhoea during the warmer seasons.

3.5.7. Multiple infection

Numerous studies have reported enteric pathogens in up to 84% from children with diarrhoea. Most children had only one pathogen in their stool, although multiple infections are not rare, with reports varying from between 15% and 39% of children having multiple pathogens (Albert *et al.*, 1999; Biswas *et al.*, 1996; Caeiro *et al.*, 1999; Courouble *et al.*, 2000; El-Sheikh *et al.*, 2001; Ogunsanya *et al.*, 1994; Oyofo *et al.*, 2002b; Urbina *et al.*, 2003; Youssef *et al.*, 2000). We found that 46% and 34 % of the in- and outpatients respectively only had one pathogen, while 16% and 10% of children had more than one pathogen respectively. Studies from Saudi Arabia and Jordan have reported multiple infections, in 12% and 16% of their children respectively confirming the similar co- infection rates within the Middle East (El-Sheikh *et al.*, 2001; Youssef *et al.*, 2000). Co-infection with two viruses was less frequent than with a virus and bacterium. Combinations of rotavirus - *E. coli* O157, rotavirus -*B. hominis* and *B. hominis* – EPEC were the most frequent co-infections. *Salmonella* and rotavirus were the most frequent pathogens, which were seen as single pathogens in the stools of 75% and 70% cases respectively.

3.5.8. Molecular epidemiology and diversity of rotavirus strains

Rotavirus strains can be serotyped and genotyped on the basis of two outer capsid proteins that are the targets of neutralizing antibodies produced following natural infection. The glycoprotein VP7, which determinates G serotype, and the protease-sensitive protein VP4, which determinates P types. Fourteen rotavirus G serotype, including 9 that infect humans, and 21 P types, including 9 that infect humans, have been identified. Several studies have shown that the most frequent P types are P[8], P[4], and P[6], while the most frequent G types are G1, G2, G3, G4 and G9 (Jain *et al.*, 2001a; Jain *et al.*, 2001b; Mascarenhas *et al.*, 1997; Mascarenhas *et al.*, 1998; Van der Donck *et al.*, 2003). Serotyping and genotyping studies indicate that only five G-P combination (P[8] G1, P[4] G2, P[8] G3 P[8] G4, and P[8] G9) are common worldwide. The frequency of these combinations is different regarding to the region and year. For example in one study in Malawi, P[8] G8 and P[6] G3 were the most frequent genotypes, while G1 and G2 were not identified. A study in Libya, the most frequent genotypes were P[8] G1 and P[4] G2 , and no P[8] G9 or P[6] G1 were isolated (Cunliffe *et al.*, 1997; Cunliffe *et al.*, 2001a; Cunliffe *et al.*, 2001b). In another hospital-based study in China (1999) G1 was identified in 88% of HRV and G3 accounted for 8%. In the following year, G3 increased to 80% (Zhang et al., 2001). In our study, the most frequent P type was P [8*] (43%) followed by P[8] (32%) , P[4] (11%) and 15% were P non typeable. The most frequent G type was G1 (53%) followed by G1* (29%), G2 (13%) and 5% G non typeable. Even though in our study 15% and 5% of P and G types could not be characterized, these are at a lower prevalence than in most other studies, as for example, recent papers have characterised between 63% to 91% of the rotavirus (Cunliffe *et al.*, 1999; Jain *et al.*, 2001a; Kang *et al.*, 2001; Mascarenhas *et al.*, 1998; Villena *et al.*, 2003). In our study, the predominant rotavirus P- G combination were P[8*] G1 which accounted for 29%, followed by P[8] G1* (21%), P[8*] G1* (15%), P[4] G2 (10%) and P[8] G1* (5%). For P typing the novel P[8], P[8*] was the most commonly detected P-genotype, while for G typing, the novel variant of G1, G1* was the second most frequent G-genotype. The P[8*] and G1* amplicons were 92% to 93.4% and 88.1% to 89% similar to the corresponding sequences from the prototype P[8] G1 rotavirus, Wa. Our data are the first report of PCR from Iranian children with diarrhoea

demonstrating that the most frequent of G and P type are G1, G1* and G2 and for P type P[8*], P[8] and P[4]. These types are similar to other studies from Libya and Vietnam (Cunliffe *et al.*, 1999; Cunliffe *et al.*, 2001a; Cunliffe *et al.*, 2001b; Landaeta *et al.*, 2003) and also different to other studies from around the world reflecting the diversity and different distribution of rotavirus genotypes everywhere. These findings add to our understanding about rotavirus in Iran and contribute to our understanding of the rotavirus and should help in the development of vaccines in the future. The distribution of the G and P types by month in our study shows that the G2 and P[4] and non-typeable G and P were most often seen at the end and first month of the year and other P-G combinations were seen during the year, which illustrating the diversity of strains. The findings reported in this chapter are also reported in Journal of Medical Virology, which is included in appendix 3.

Parasites and bacteria identified during the study by the researcher and other colleagues could be subject to observer bias and the use of multiple observers could have resulted in inter-observed bias. This was addressed by appointing internal and external quality controls in Hajar Hospital to reduce this bias to a minimum. A selection of specimens with, known pathogens are sent regularly by the Quality Control of Shahrekord University Department. In addition duplicate laboratory tests are conducted weekly by each laboratory to avoid intra-observer bias.

Chapter 4

Risk factors for hospitalisation in children with diarrhoea

4.1. Introduction

This chapter describes the risk factors associated with hospitalisation in children with acute diarrhoea in Shahrekord Iran. The study used a case control approach identifying the variables that were more (or less) frequently observed among hospitalised children and comparing the characteristics of the children hospitalised with children with diarrhoea attending the outpatient and health centres. The association between the variables (confounding) was investigated using logistic regression analysis to describe the independent contribution of each variable.

4.2. Objective

The main purpose of this chapter is to establish risk factors for hospitalisation of children with acute diarrhoea in Shahrekord, Iran.

4.3. Methodology

4.3.1. Study design

The study used a case-control approach. The study compared the characteristics of children who were hospitalised with a clinical diagnosis of acute diarrhoea and two control groups of children attending other health services with acute diarrhoea. Cases were defined as children <5 years of age admitted to Hajar Hospital of Shahrekord in Iran between October 2001 and August 2002. One of the control groups comprised children of similar age who were attending three government health centres in Shahrekord with a diagnosis of acute diarrhoea but who were not hospitalised. Children with severe diarrhoea attending the heath centres and who required hospital treatment were referred to the hospitals and were not enrolled as controls. The second control group comprised children attending the OPD of Shahrekord hospital who were not hospitalised. It was decided to include 2 control groups to be able to identify confounding factors more efficiently during the analysis. The decision to hospitalise a child with diarrhoea was taken by the hospital staff and the researchers did not interfere with their decision. The term hospitalisation was taken in the widest sense

and included children admitted to the wards and children admitted for short periods to the observation wards.

4.3.2. Selection criteria

The following selection criteria were used to identify and select cases: children <5 years of age admitted to the hospital with a clinical diagnosis of acute diarrhoea. Acute diarrhoea was defined as described in chapter 3. Children with persistent diarrhoea (i.e. diarrhoea >14 days duration) were excluded as these children often have different characteristics and risk factors. For children <5 months, their parents should have stated that the child had diarrhoea and that the number of stools was different than the usual defaecation pattern.

Controls were defined as children attending the OPD services of Shahrekord hospital or one of the 3 selected primary health centres of Shahrekord, who had passed three or more liquid or semi liquid stools or a single watery stool in the day of consultation and whose main reason for consultation was diarrhoea, but who were treated as outpatients by the attending clinician.

4.3.3. Sampling and sample size

Hospitalised children with diarrhoea and controls were selected systematically during working days from October 2001 to August 2002. The sample size was calculated by identifying a number of risk factors through a literature review and selecting those that were felt to be important for the Iranian context. The likely prevalence of these selected factors in hospitalised and OPD children was calculated by reviewing the literature and making an informed guess of their likely prevalence in Iran as shown in table 4.1.

Table 4.1. Expected prevalence of risk factors for children hospitalised for diarrhoea in Iran

	Expected prevalence		Odds ratio	Sample size required
	Controls	Cases		
Low birth weight (LBW)	10%	20%	2.25	286
Malnutrition	10%	20%	2.25	286
Previous infection	20%	40%	2.67	118
Breastfeeding	80%	50%	0.25	79
Being male	50%	70%	2.33	134
Weaned	10%	30%	0.26	92

The largest number required to investigate these risk factors was 286 (LBW and malnutrition). Therefore a minimum sample size of 286 cases and 286 for each control group was selected.

After informed parental consent, the parents were interviewed with the help of a trained nurse to complete a standard questionnaire, which included details of potential risk factors as shown in appendix 1.

4.4. Literature review

4.4.1. Important factors associated with risk of hospitalisation in children with diarrhoea

Diarrhoea is the second most common disease in children after acute respiratory infections resulting in one- third of all hospitalisation and being responsible for over a quarter of all deaths in children in the world (Curtis *et al.*, 2000).

Epidemiological studies suggest that more than one billion diarrhoea episodes occur every year making diarrhoea a major cause of morbidity and mortality and one of the most important health problems in less developed countries, especially for infants and young children less than 2 years. The majority of episodes can be managed at home with the use of simple tools such as oral rehydration solution.

Despite the large number of studies related to diarrhoea, few comprehensive studies have examined the factors increasing the risk for hospitalisation of children with acute diarrhoea in developing countries.

Hospital– based case-control studies have established, that under nutrition, the presence of vomiting, fever, acute respiratory symptoms before hospital admission, the parents being illiterate, age < 12 months, lack of exclusive breast feeding, having more than 2 older siblings, having breast fed <6 months, prior diarrhoea in another household member, higher multiparity and poor personal hygiene (dirty fingernails, not using soap after defecation) or generally inadequate sanitation, distance of residence from the hospital, keeping animals inside the house and a history of previous hospitalisation or concomitant illness are risk factors for diarrhoea or hospitalisation due to severe diarrhoea (Shah *et al.*, 2003; Zodpey *et al.*, 1998).

The majority of these studies have also confirmed that there is a positive correlation between diarrhoea or hospitalisation and the frequency of vomiting and diarrhoea, dehydration, recent illness, low socio-economic status and inappropriate use of rehydration therapy (Do Carmo-Leal *et al.*, 1996; Shah *et al.*, 2003). Other less investigated factors for diarrhoea are incomplete immunisation, family structure, use of traditional healers, frequency of diarrhoea >8 days and bottle –feeding (D'Souza and Bryant, 1999; Lindtjorn, 1991).
A selection of the risk factors for hospitalisation due to diarrhoea identified by selected studies in children under 5 years is shown in table 4.2.

Table 4.2. Selected risk factors for hospitalisation by country

Country	Setting	N	Age <1 year	Recent major illness	Short breast feeding	Lack of hygiene	Malnu trition	Rota virus	Reference
France	H	725	+	-	-	-	-	+	(Moulin et al., 2002)
Bangladesh	H	-	-	-	-	-	+	+	(Dewan et al., 1998)
Kuwait	H	274	+	+	-	-	-		(Khuffash et al., 1982)
Brazil	H	406	+	+	-	-	+		(Do Carmo-Leal *et al.*, 1996)
	H	370	+	-	-	-	-	-	(Vanderlei et al., 2003)
Saudi Arabia	H	1726	-	-	+	+	-	-	(Milaat and Elassouli, 1995)
Kenya	C	1717	-	-	-	-	-	+	(Binka et al., 2003)

H = hospital, C = community, N = number

4.4.2. Malnutrition and diarrhoea

As articulated by Scrimshaw, Taylor and Gordon in their 1968 review, the relationship between malnutrition and infection is bi-directional. Malnutrition has long been associated with an increased risk of infection and lesser ability to successfully respond to the infection. Children with malnutrition often have high mortality and more prolonged recovery times than well-nourished children. Infection adversely affects nutritional status through reductions in dietary intake and intestinal absorption, increased catabolism and sequestration of nutrients that are required for tissue synthesis and growth. On the other hand, the increased severity of infection in malnourished children could be due to its general effect by impairing non-specific barriers, such as the integrity of the mucosa of the skin and the synthesis of acute phase proteins, and specific mechanisms of defence, such as the immunological responses to specific pathogens. Malnourished children are often over represented in hospital studies and they tend to develop more complications, such as persistent diarrhoea, which is associated with increased mortality.

4.4.3. Breast feeding and diarrhoea

The nutritional, immunologic, psychological, and child spacing benefits of breast-feeding are recognised universally. Beginning in the 1980s, researchers started to explore factors that can modify the nutritional impact of diarrhoea. Rowland et al (1996) discovered that a previously observed diarrhoea- induced growth deficit was absent in fully breastfed infants in an urban field site in west Africa, and they concluded that exclusive breastfeeding prevents the adverse nutritional consequences of diarrhoea (Brown, 2003; Rowland, 1986).

There is ample evidence of a positive influence of breastfeeding, especially exclusive breastfeeding, on the survival of children. In a recent meta-analysis of data from 6 developing countries, breastfeeding provided a greater degree of protection against diarrhoeal deaths than to the prevention of deaths attributable to acute respiratory infection (2000). The greatest protection is offered early in infancy and then steadily declines. Breast-fed infants younger than 2 months old are 5.8 times better protected than are their formula-fed peers, but this difference decreases to 1.4 by 9 to11 months

of age. Studies in Mexico confirmed that breast-fed infants have a decreased incidence, prevalence, and duration of both acute respiratory and diarrhoeal diseases than do formula-fed infants (Feigin, 2004). A study in Ethiopia also found a significant different for diarrhoea episodes between children who were exclusively breast-fed and children who were partially breast-fed. During this study, diarrhoea episodes were seen in only 12% (25 /217) of exclusively breast fed children compared to 40% (46/114) in those who were partially breast-fed (Ketsela et al., 1990). The incidence of diarrhoeal disease was 6 times less frequent in breast fed infants compared with bottle-fed infants. It is noteworthy that the incidence of diarrhoeal disease, even among purely breast-fed infants living in more deprived areas of the developing countries, is still higher that among bottle-fed infants living in the developed countries (Mittal, 1982).

4.4.4. Sanitation and diarrhoea

It is now widely accepted that an improvements of water supplies, sanitation, hygiene, and other environmental factors are necessary for the prevention of diarrhoeal disease in young children. Table 4.3 summarises the result of reviews of the impact of improved water, sanitation, and hygiene on diarrhoeal disease. Improving the quality of water supplies cuts the risk of diarrhoea by only about 16% (although it has other benefits) and making water more available reduces the risk by 20%. Installing adequate facilities to dispose of bodily excretions and promoting hygiene, however, are twice as effective. A recent systematic review of the impact of washing hands with soap shows that this specific practice may be almost three times as effective as improving water quality, cutting the risk of diarrhoea by 47% (Curtis, 2003).

Table 4.3. Reduction of diarrhoea by selected interventions

Intervention	Reduction in diarrhoea (%)	Reference
Improved water quality	45*	(Iijima et al., 2001)
Sanitation	50	(Messou et al., 1997)
Hygiene education	25	(Aziz et al., 1990)
Hand washing with soap	47	(Curtis and Cairncross, 2003)
	40	(Aung Myo and Thein, 1989)
	89	(Wilson et al., 1991)
Water disinfected by sun	16	(Conroy et al., 1999)
Soap distribution	27	(Peterson et al., 1998)

* odds ratio = 0.55, P =0.001

A further study from sub-Saharan Africa reported that a child's risk of diarrhoeal diseases is associated with age, water quality and sanitation, parental education and household size (Fuchs and Victora, 2002; Manun'ebo *et al.*, 1994). Messou et al (1997) from Southern Cote d'Ivoire reported a 50% reduction of the incidence rate of diarrhoea and a 85% reduction of the proportion of deaths related to diarrhoea with the use of simple interventions including changes of water supply and using oral rehydration.

4.4.5. Low birth weight and diarrhoea

A study from the USA described a significant association between birth weight and the risk for hospitalisation with very low birth weight (< 1500) babies being at the highest risk. Male gender, maternal smoking, unmarried mothers, lack of medical insurance and maternal age < 20 years were also significant factors (Newman et al., 1999).

In developing countries malnourished mothers have low birth weight children which are also more susceptible to diarrhoea and other infectious diseases due to inadequate nutrition during their gestation (Huttly et al., 1987).

4.4.6. Vomiting and diarrhoea

Vomiting is another well-known variable associated with the severity of diarrhoea and a risk factor for hospitalisation. Normal stool contains only a very small amount of fluid, however during diarrhoeal episodes the number of loose stools is increased which cause an efflux of Cl^-, Na^+ and water from the villous crypt cells. The net effect is that a large fluid load enters the colon and a voluminous stool is produced. This together with vomiting causes more water efflux resulting in mild to severe dehydration and a higher risk of hospitalisation. Epidemiological studies reveal that having vomiting in the week before or around the diarrhoeal episode is a good predictor for the severity of diarrhoea. About half of the rotavirus diarrhoea is accompanied by vomiting (Ahmed and Karim, 2002; Griffin *et al.*, 1988; Hart, 2003a).

4.5. Results

4.5.1. Characteristics of the participants

A total of 952 children were enrolled. Of these, 259 were children hospitalised with acute diarrhoea, 203 hospitalised children who had been admitted for elective surgery (surgical controls), 245 outpatient children with acute diarrhoea seen at the outpatient clinic of the hospital and 245 children with acute diarrhoea seen at 3 health centres. Children hospitalised for surgery will not be described in this chapter.

The mean age of the children with acute diarrhoea by group was similar (15.2, 15.6 and 17.2 months) for hospitalised, OPD and health centre children, respectively. The number of boys was also similar to the number of girls, with 55%, 57% and 55% of the participants in each group being male respectively. A summary of the general characteristics of the children is shown in table 4.4.

As expected the main reason for consultation was diarrhoea in all hospitalised and non-hospitalised children. The second most frequent reason for consultation was vomiting. The percentage of hospitalised children who had vomited (76%) was higher than in the outpatient children (22% and 17.6% in the OPD and health centres respectively $P<0.05$). Fever was the third reason for consultation. Acute respiratory symptoms were observed more frequently in hospitalised children (2.7%) than in outpatient children (1% for both OPD and health centre respectively, $p<0.05$). The mean birth weight was similar among the groups with 3.1, 3.0 and 3.1 Kg for hospitalised, OPD and health centre respectively.

4.5.2. Symptoms on enrolment

The mean (SD) duration of diarrhoea was higher in hospitalised children than in the OPD and health centre controls as shown in table 4.5, (4.3 (3.3), 3.7 (2.7) and 4.3 (2.9) days respectively, $p<0.05$). The number of stools in the 24 hour period before enrolment was also higher in hospitalised than OPD and health centre children (7.1 (3.8), 5.7 (2.8) and 6.2 (3.1) respectively, $p<0.05$, but the stool consistencies were similar and the percentages of children with watery stools were similar (249 (97%),

225 (92%) and 215 (88%) respectively). Hospitalised children had blood in their stools more frequently than OPD and health centre children (28 (11%), 7 (3%) and 8 (3%) respectively, $p<0.05$).

Table 4.4. General characteristics of the participants

	Group		
Subject	**Hospitalised**	**OPD**	**Heath centre**
Number	259	245	245
Age, Mean (SD)	15.2 (12.1)	15.6 (12.4)	17.2 (12.8)
Male	143 (55%)	133 (54 %)	123 (50%)
Birth weight, Kg (SD)	3.1 (5.7)	3.1 (5.2)	3.1 (4.6)
Reason for consultation			
Diarrhoea	259 (100%)	245 (100%)	245 (100%)
Vomiting	197 (76%)	54 (22%)*	43 (18%) *
Fever	33 (13 %)	5 (2%) *	6 (2%) *
Surgery	1 (0.4%)	0 (0%)	0 (0%)
ARI	10 (4%)	3 (1%)	3 (1%)

* $P<0.05$ when compared to hospitalised children with diarrhoea

Hospitalised children were less likely to have semi liquid stools than OPD and heath centre controls (128 (49%), 171 (69%) and (81%) respectively). More children requiring hospitalisation were vomiting in the week previous to consultation and the previous 24 hours before enrolment than the two control groups (174 (67%), 62 (26%) and 88 (36%) and 165 (64%), 66 (28%) and 59 (25%) respectively, $p<0.05$). The mean (SD) number of vomits in the previous week and the 24 hours preceding enrolment was also higher in hospitalised children than in children attending the OPD and health centres. Similarly, hospitalised children were more likely to have cough, wheezing and respiratory symptoms than in the two control groups, ($p<0.05$). Hospitalised children were less likely to have received ORS 83 (32%) compared to 89 (36%) and 133 (55%) in the two control groups respectively, $p<0.05$ and to have fever.

Table 4.5. Symptoms of the children before enrolment

		Group		
Subject		**Hospitalised**	**OPD**	**Heath centre**
Diarrhoea duration, days (SD)		4.3 (3.3)	3.7 (2.7)*	4.3 (2.9) *
Frequency[1]		7.1 (3.8)	5.7 (2.8)*	6.2 (3.1) *
Consistency	Watery	249 (96%)	225 (92%)	215 (88%)
	Bloody	28 (11%)	7 (3%)*	8 (3%)*
	Semi liquid	128 (49%)	170 (69%)*	198 (81%)*
Vomited [1]		165 (64%)	66 (27%)*	59 (25%)*
Mean number of vomits[1]		4.2 (3.7)	3.2 (1.8) *	2.4 (2.1) *
Vomited (previous week)		174 (67%)	63 (26%)*	88 (36%)*
Cough		15 (6%)	3 (1%) *	3 (1%) *
ARI		14 (6%)	5 (2%) *	4 (2%) *
ORS use		83 (32%)	89 (37%)*	133 (55%)*
Wheezing		18 (7%)	6 (2%) *	5 (6%)*
Fever		201 (78%)	127 (52%)*	133 (55%)*

* P <0.05 when compared with hospitalised children, [1] = previous 24 hours

Nearly all children were breast-fed, as shown in table 4.6. Weaned was interpreted as age when the child stopped breastfeeding. Although the mean weaning age was similar among the groups, hospitalised children with diarrhoea had breast fed for less time than children seen at the OPD and health centres (P<0.05).

Table 4.6. Breast-feeding pattern in participant children with diarrhoea

	Group		
Subject	**Hospitalised**	**OPD**	**Health centre**
Ever breastfed	254 (98%)	243 (99%)	244 (100%)
Duration (Months)	11.1 (9.4)	14.7 (9.1)*	15 (8)*
Currently breast feeding	184 (71%)	184 (75%)	174 (72%)
Age when weaned**	5.3 (1.4)	5.5 (1.1)	5.6 (1.1)

* P < 0.05 when compared with hospitalised children. ** = months

Hospitalised children had a larger number of illnesses in the year before enrollment than OPD and health centre children ($p<0.05$), reporting a higher frequency of diarrhoea and pneumonia ($p<0.05$) as shown in table 4.7. Other infectious diseases were also more frequent among hospitalised children than controls ($p<0.05$). History of anorexia, fever, hyperbilirubinaemia, surgery, and other non-infectious diseases however were not statistically different among the groups.

Table 4.7. Medical history among cases and controls

	Group		
Subject	**Hospitalised**	**OPD**	**Health centres**
History of previous illness	141 (54%)	86 (35%)*	99 (40%)*
Pneumonia	49 (19%)	17 (7%)*	20 (8%)*
Fever	11 (4%)	7 (3%)	6 (2%)
Neonatal jaundice	8 (3%)	10 (4%)	6 (2%)
Anorexia	17 (7%)	10 (4%)	11 (4%)
Surgery	5 (2%)	5 (2%)	5 (2%)
Diarrhoea	113 (44%)	67 (27%)*	76 (31%)*
Vomiting	14 (5%)	6 (2%)	4 (2%)*
Other infections	15 (6%)	4 (2%)	3 (1%)*
Non-infectious diseases	13 (5%)	6 (2%)	8 (3%)

*P <0.05 when compared with hospitalised children

About 44% of the children hospitalised had a history of having had diarrhoea in the previous year and the frequency of these episodes was also higher as shown in table 4.8. Hospitalised children also reported having had diarrhoea more recently than outpatient children, although the duration of the episodes reported was shorter (3.7 days) than OPD and health centre children (4.9 and 5 respectively).

The mean number of days the children had diarrhoea in the previous year was not significantly higher in hospitalised children than in non-hospitalised children. However the time interval between the previous episode of diarrhoea and the current episode was longer for OPD than hospitalised and health centre children ($p<0.05$).

Table 4.8. History of diarrhoea in the year before the current episode (mean (SD) unless specified)

	Group		
Subject	**Hospitalised**	**OPD**	**Health centre**
Had diarrhoea in Previous year, N (%)	113 (44%)	67 (27%)*	76 (31%)*
Number of episodes	2.2 (2.4)	1.8 (1.0)	1.8 (1.3)
Previous diarrhoea episode			
Weeks since previous episode	10 (8.8)	13.9 (12.3)*	7.7 (8.5)*
Duration (days)	3.7 (3)	5.1 (4.2)*	4.7 (3.5)*

* P<0.05 when compared with hospitalised children

4.5.3. Medications taken before enrolment

One hundred and seventy four (69%) hospitalised children had taken drugs before enrolment. This was higher than the frequency for ambulatory children (54% and 57% for OPD and health centre children respectively, p<0.05). The medicines most often used were cotrimoxazole, amoxycilin, ampicillin and other antibiotics. Common cold syrup was also used by all groups, reflecting the fact that many children presented with respiratory symptoms associated with diarrhoea. We did not investigate whether the diarrhoea had started before or after the use of antibiotic. The medications taken in the week before enrolment are shown in table 4.9.

4.5.4. Physical examination on enrolment

The clinical symptoms on admission are described in table 4.10. Children admitted to the hospital more often had signs of dehydration than OPD and health centre controls, reflecting the more severe presentation of the episodes. For example, 33% of children admitted had sunken eyes compared to only 11% in the OPD and health centre groups (p<0.05).

Table 4.9. Medication in the week before enrolment

	Group		
Subject	**Hospitalised**	**OPD**	**Health centre**
Taken medication	174 (69%)	131 (54%)*	147 (60%)*
Cotrimoxazole	75 (29%)	65 (27%)	58 (24%)
Amoxicillin	42 (16%)	30 (12%)	30 (12%)
Promethazine	19 (8%)	2 (1%)	3 (1%)
Ampicillin	18 (7%)	10 (4%)	5 (2%)*
Metoclopramide	14 (5%)	13 (5%)	8 (3%)
Co-Amoxyclav	10 (4%)	3 (1%)	0 (0%)*
Erythromycin	8 (3%)	3 (1%)	3 (1%)
Dycyclomidine	8 (3%)	7 (3%)	6 (2%)
Nalidixic acid	7 (3%)	2 (1%)	7 (3%)
Other antibiotics	45 (17%)	21 (9%)*	15 (6%)*
Common cold syrup	16 (6%)	22 (9%)	21 (8%)
Other drugs	33 (13)	76 (31)*	102 (42)*

* P <0.05 when compared with hospitalised children

Following the WHO classification for dehydration, children admitted to the hospital were more dehydrated than OPD and health centre controls. One hundred and thirty eight (53%) children admitted to the hospital had moderate dehydration and 20 (8%) had severe dehydration. This is higher than OPD and health centre controls as shown in table 4.11.

4.5.5. Social background

The social background of the participants is described in table 4.12. The numbers of bedrooms and of people sharing the bedroom with the child were similar among the groups. However the number of residents in the house was slightly higher (5.8) in hospitalised than in OPD and health centre children (5.3 and 5.5 in respectively, $p<0.05$).

Table 4.10. Clinical signs of dehydration on enrolment

	Group		
Subject	**Hospitalised**	**OPD**	**Health centre**
Thirst	223 (86%)	228 (93%)*	206 (84%)
Dry tongue	119 (46%)	54 (22%)*	27 (11%)*
Skin goes back slowly	102 (39%)	28 (11%)*	21 (9%)*
Restless	98 (38%)	54 (22%)	35 (14%)*
Sunken eyes	83 (32%)	27 (11%)*	27 (11%)*
Sunken fontanelle	34 (13%)	8 (3)*	8 (3)*
No tears	17 (7%)	4 (2%)*	8 (3%)
Unable to drink	12 (5%)	5 (2%)	6 (2%)
Floppy, unconscious	11 (4%)	2 (1%)*	3 (1%)*
Very dry tongue	2 (1%)	1 (1%)	1 (1%)
Very sunken eyes	1 (1%)	1 (1%)	1 (1%)
Very sunken fontanelle	1 (1%)	1 (1%)	1 (1%)

*P<0.05 when compared with hospitalised children

Table 4.11. Degree of dehydration on enrolment according to the WHO classification

	Group		
Dehydration	**Hospitalised**	**OPD**	**Health Centre**
None	97 (37%)	148 (60%)*	156 (64%)*
Moderate	138 (53%)	84 (34%)*	59 (24%)*
Severe	20 (8%)	6 (2%)*	6 (2%)*

* P <0.05 when compared with hospitalised children

Houses were classified as 'old' or 'modern'. Houses with a kitchen made of cement, brick or metal were classified as modern. The type of house or a history of travel in the two weeks before admission were not statistically different between the groups.

Not having piped water and using water from other sources (mostly rivers and springs) were more frequent in hospitalised than outpatient children (P<0.05). Similarly, hospitalised children with diarrhoea had more contact with animals; chicken, cows, goats, horses/donkeys and dogs than controls (p<0.05).

Table 4.12. Social background of the family

	Group		
Subject	**Hospitalised**	**OPD**	**Health Centre**
Number of bedrooms	3 (1.4)	3 (1.1)	2.9 (1.2)
Number of residents	5.7 (3.1)	5.3 (2.4)	5.4 (2.5)
Number sharing the bedroom	2.5 (1)	2.5 (0.8)	2.7 (0.9)*
Modern house	188 (73%)	191(78%)	195 (80%)
Travel in the previous 2 weeks	10 (4%)	4 (2%)	4 (2%)
Use springs/rivers water	17 (7%)	1 (1%) *	2 (1%) *
No piped water	10 (4%)	1 (1%) *	2 (1%) *
Keeping animals at home	69 (27%)	45 (18%) *	44 (18%) *
Cow	34 (13%)	25 (10%)	15 (6%) *
Goat	16 (6%)	6 (2%) *	2 (1%) *
Dog	12 (5%)	3 (1%) *	3 (1%) *
Sheep	25 (10%)	11 (4%)	15 (6%)
Chicken	38 (15%)	26 (11%)	19 (8%)
Horse/donkey	12 (5%)	5 (2%)	4 (2%) *

* P<0.05 when compared with hospitalised children

4.5.6. Characteristics of the family

The number of siblings and the number of pregnancies of their mothers were similar among the groups as shown in table 4.13. However, the number of people living with the child was higher in the hospitalised group ($P< 0.05$). Maternal occupation and age were similar among the groups, but fathers of children attending the health centre were more frequently unemployed (28%) than OPD and hospitalised children with diarrhoea (15% and 13% respectively), possibly reflecting a higher number of immigrants from the villages to the cities. Parents of hospitalised children with diarrhoea were also more often illiterate although the overall level of education was similar in all groups. Mothers of hospitalised children reported more often to be unhealthy, ($p<0.05$).

Table 4.13. Characteristics of the family

		Hospitalised	OPD	Health centre
Number of children**		2.1 (1.4)	1.9 (1.2)	2 (1.2)
Another person with diarrhoea**		18 (7%)	23 (9%)	28 (11%)
Mother				
Number of pregnancies**		2.0 (1.4)	2 (1.3)	2.1 (1.3)
Age **		26.1 (5.8)	27.1 (5.3)	26.9 (4.9)
Unhealthy		17 (7%)	5 (2%)*	5 (2%)*
Occupation	Household	221 (85%)	204 (83%)	220 (90%)
	Manual worker	5 (2%)	4 (2%)	4 (2%)
	Civil servant	32 (12%)	37 (15%)	21 (8%)
	Died	1 (1%)	0 (0%)	0 (0%)
Education	None	38 (15%)	11 (4%)*	13 (5%)*
	Read and write	28 (11%)	24 (10)	31 (13%)
	Primary	50 (19%)	51 (21%)	62 (25%)
	Secondary	107 (41%)	119 (49%)	113 (47%)
	University	35 (14%)	39 (16%)	25 (10%)
Father				
Occupation	Manual worker	120 (46%)	117 (47%)	94 (38%)
	Civil servant	89 (34%)	82 (33%)	74 (30%)
	Seller	16 (6%)	8 (3%)	8 (3%)
	Unemployed	33 (13%)	37 (15%)	68 (28%)*
	Died	1 (1%)	1 (1%)	1 (1%)
Education	None	29 (11%)	14 (6%)*	10 (4%)*
	Read and write	17 (6%)	13 (5%)	26 (11%)
	Primary	51 (20%)	48 (20%)	62 (25%)
	Secondary	111 (43%)	112 (46%)	102 (42%)
	University	50 (20%)	57 (23%)	45 (18%)

* $P<0.05$ when compared with hospitalised children ** Mean (SD)

4.5.7. Risk factors for hospitalisation (multivariate analysis)

Hospitalised vs OPD children

Hospitalised and OPD patients were compared using bivariate analysis to identify variables that were statistically significant as described in the previous tables of this chapter. Thirty -three variables were found to be associated with the risk of hospitalisation with a P value <0.20. These variables were then entered into a logistic regression analysis. The variables selected and the groupings used to code them are listed in table 4.14. These were, the presence of blood in the stools, having a history of vomits in the previous year, the previous week and in the preceding 24 hours, the presence of dehydration, having more than 8 stools in 24 hours, having been breastfed for less than 7 months, the presence of fever in the preceding 5 days, having been recently introduced to solid foods, the presence of rotavirus in the stools, having a history of a major illness (pneumonia, diarrhoea, or of other infections disease) in previous year, a history of hospitalisation in the previous year, having taken medications before consultation, lack of access to safe water and using river/spring water, living in an overcrowded family, having illiterate parents or an unhealthy mother and keeping animals at home.

Hospitalised vs health centre controls

Hospitalised children were also compared with children attending the health centres using bivariate analysis to identify factors statistically associated with hospitalisation. A total of twenty seven factors were found to be associated with the risk of hospitalisation with p value <0.20. These factors were then entered into a stepwise logistic regression analysis. The variables selected were the presence of watery or bloody stools, a history of vomiting in the last year, the previous week or the preceding 24 hours, the presence of dehydration, having more than 8 stools in the last 24 hours, being breast fed for less than 7 months, the presence of pneumonia, other infectious diseases, a history of hospitalisation in the previous year, using river / spring water, having a large family, illiterate parents or an unhealthy mother and keeping animals at home. The variables are listed in table 4.15.

Table 4.14. Variables selected for multivariate analysis (hospital vs OPD)

	Hospitalised	OPD	Odds Ratio	95% CI	P value
Family background					
Unhealthy mother	17	5	3.37	1.15-10.6	0.02
Maternal age <20	20	10	1.97	0.85-4.61	0.12
Illiterate mother	38	11	3.60	1.7-7.8	<0.001
Illiterate father	29	14	2.08	1.03-4.26	0.04
> 9 persons/home	34	17	2.03	1.06-3.91	0.03
Lack of piped water	10	1	9.80	1.3 - 206.2	0.01
Use river/spring water	17	1	17.14	2.39-384	<0.001
Has animals at home	69	45	1.61	1.5- 2.52	0.03
Previous year medical history					
Illness	141	86	2.21	1.52-3.21	<0.001
Pneumonia	49	17	3.13	1.69-5.85	<0.001
Vomiting	14	6	2.28	0.80-6.76	0.14
Diarrhoea	113	67	2.06	1.39-3.04	0.001
Other infectious diseases	15	4	3.70	1.13-13.40	0.02
Non infectious disease	13	6	2.11	0.73-6.32	0.20
Previous hospitalisation	88	35	3.09	1.94-4.94	<0.00 1
Clinical signs on consultation					
Stools Watery	249	225	2.21	0.96-5.19	0.06
Bloody	28	7	4.12	1.68-10.58	<0.001
Semi-liquid	128	170	0.43	0.29-0.63	<0.001
> 8 (24 hours)	74	34	2.48	1.54-4	<0.001
Vomited (previous 24 hours)	165	66	4.6	3.1-6.7	<0.001
Vomited (previous week)	174	63	5.91	3.95-8.88	<0.001
Fever (previous 5 days)	201	127	2.90	2-4.4	<0.001
Severe dehydration	20	6	3.33	1.24-9.45	0.01
Moderate dehydration	138	89	2.19	1.50-3.18	<0.001
Beast fed < 7 months	41	18	2.37	1.28-4.44	0.004
On solid food	220	185	1.83	1.14-2.94	0.01
Recently taking medications	174	131	1.78	1.22-2.60	0.002

Table 4.15. Variables selected for the multivariate analysis (hospital vs health centres)

Subject		Hospitalised	HC	OR	95%CI	P value
Family background						
	Relative with diarrhoea	18	28	0.58	0.3-1.1	0.11
	Unhealthy mother	17	5	3.37	1.2-10.6	0.02
	Maternal age < 20 years	20	9	2.19	0.9-5.3	0.07
	Illiterate mother	38	13	3.07	1.5-6.2	<0.001
	Illiterate father	29	10	2.96	1.4-6.7	0.004
	> 9 persons/home	34	18	1.91	1-3.6	0.04
	Lack of piped water	10	2	4.88	11-32.6	0.05
	Use spring/river water	17	2	8.54	1.9-54.1	0.001
	Has animals at home	69	44	1.66	1.1-2.6	0.02
Previous year medical history						
	Illness	141	99	1.76	1.2-2.6	0.002
	Pneumonia	49	20	2.63	1.5-4.7	<0.001
	Vomiting	14	4	3.44	1-12.6	0.04
	Diarrhoea	113	76	1.61	1.1-2.4	0.01
	Other infectious disease	15	3	4.96	1.3-21.1	0.01
	Previous hospitalisation	88	39	2.72	1.7-4.3	<0.001
Clinical signs on consultation						
Stools	Watery	249	215	3.47	1.6-7.8	<0.001
	Bloody	28	8	3.59	1.5-8.8	0.001
	Semi liquid	128	198	0.23	0.2-0.4	<0.001
	> 8 (24 hours)	74	42	1.93	1.2-3	0.003
	Vomited (previous 24 hours)	165	59	5.53	3.7-8.3	<0.001
	Vomited (previous week)	174	88	3.7	2.5-5.4	<0.001
	Lack of ORS use	175	109	2.57	1.8-3.8	<0.001
	Fever (previous 5 days)	201	133	2.92	2-4.37	<0.001
	Severe dehydration	20	6	3.33	1.2-9.5	0.01
	Moderate dehydration	138	59	3.60	2.4-5.4	<0.001
	Breast fed <7 (months)	41	15	2.88	1.5-5.6	<0.001
	On solid foods	220	192	1.56	1-2.5	0.07
	Recently taking medications	174	147	1.36	0.9-2.	0.11

4.5.7.1. Logistic regression analysis (hospitalised vs OPD)

The logistic regression analysis comparing hospitalised with OPD children revealed that out of the 27 variables with p<0.20, six were independently associated with an increased risk of hospitalisation. These variables included, having an illiterate or an unhealthy mother, using springs/ river water, having been hospitalised in the year before admission, the presence of blood in the stool and a history of vomits in the previous week before consultation. The adjusted odd ratios obtained are shown in table 4.16.

Table 4.16. Multivariate logistic regression comparing hospitalised vs OPD children

Variables	AOR	95% CI	P value
Use springs/river water	11.04	1.3 –93.0	0.02
Vomited in the week before enrolment	6.68	4.0 –9.2	<0.001
Blood in the stools	6.2	2.5 –15.6	0.001
Unhealthy mother	3.23	1.0 –10.3	0.04
Illiterate mother	3.02	1.4 –6.6	0.006
Hospitalised in the previous year	2.36	1.4 –3.9	<0.001

AOR = Adjusted odd ratios

4.5.7.2. Logistic regression analysis between hospitalised children and health centre controls

The logistic regression analysis comparing hospitalised with health centre controls revealed that out of the 28 variables with p<0.20, six were independently associated with increasing risk of hospitalisation. These variables included the presence of blood in the stools, having a watery stool, a history of vomiting in the week before consultation, having been hospitalised in year before admission, having been breast fed for less than 7 months and not using ORS (figure 4.1). The adjusted odd ratios obtained are shown in table 4.17 and the interim results obtained after each run of the model are shown in appendix 3.

Table 4.17. Multivariate logistic regression comparing hospitalised vs OPD health centre controls

Variables	AOR	95% CI	P value
Presence of blood in stools	124.6	5.3 –2922.0	0.002
Watery stools	31.14	2.36-4.1	<0.01
Lack of ORS use	11.78	3.90-35.2	<0.001
Vomited during the week before enrolment	6.63	2.4 –18.4	<0.001
Breast fed for less than 7 months	4.7	1.62 –14.0	0.004
Hospitalised in previous year	3.06	1.0- 9.0	0.04

AOR = adjusted odd ratios

A summary of the findings of both logistic regression analysis is shown in table 4.18. The presence of blood in the stools, a history of vomiting in the week before consultation and a history of previous hospitalisation were risk factors shared by both groups of controls. However, some risk factors such as the use of spring water and the characteristics of the mother were only seen when comparing the cases with OPD controls and, the presence of watery stools and not using ORS were only identified where comparing cases with health centre controls.

Table 4.18. Summary of both logistic regressions

	AOR (95%CI)	
Variables	**OPD**	**Health centres**
Vomited during the week before enrolment	6.7 (4-9.2)	6.6 (2.4 –18.4)
Presence of blood in stools	6.2 (2.5-15.6)	125 (5.3-2922)
Hospitalised in previous year	2.4 (1.4-3.9)	3.1 (1- 9)
Watery stools	NS	31.1 (2.36-4.1)
Lack of ORS use	NS	11.8 (3.9-35.2)
Breast fed for less than 7 months	NS	4.7 (1.6-14)
Use springs/river water	11.0 (1.3 –93)	NS
Unhealthy mother	3.2 (1.0 –10.3)	NS
Illiterate mother	3 (1.4 –6.6)	NS

NS = Not significant, AOR = adjusted odd ratios

4.6. Discussion

This study has identified that a history of vomiting in the week before consultation, the presence of bloody or watery stools, having breast fed for less than 7 months or been hospitalised in the previous year, having an illiterate mother who was not healthy, using spring or river water as the main source for drinking water and not giving ORS to the child before consultation increases the risk for hospitalisation.

Significant risk factors identified when comparing hospitalised children with both groups of controls included a history of vomiting in the week before consultation, a history of a previous hospitalisation and the presence of blood in the stools. The presence of blood in the stools however was not a frequent finding as only 11% of the children hospitalised had blood in their stools. This suggests that, although this is an important factor that should alert the health staff attending the child, it is rarely seen in clinical practice and would not be very useful to identify the majority of children who will end up in the hospital. The adjusted odd ratios were higher when comparing cases with health centre controls than when comparing them against hospital controls. This is likely to result from the characteristics of the episodes seen in these children and the background of the children. For example OPD children are more likely to have slightly more severe diarrhoea than children attending health centres. Similarly, as it is the case in other hospital based case control studies, hospital controls are more likely to have other associated pathologies or medical problems and be less different to the cases than controls selected from health centres.

Acute bloody diarrhoea is associated with *E. coli* O157 and other shiga toxin-producing *E. coli*, *Shigella*, or *Campylobacter*. Diseases in children with diarrhoea is occasionally complicated by the development of haemolytic uraemic syndrome and death when infected with *E. coli* O157 and more attention is often given to children whose mother's indicate the child has blood in the stools. In agreement with our findings, epidemiological studies in the region (e.g. Pakistan) and other parts of the world, have reported a higher frequency of blood in the stools (between 3 – 11%) in hospitalised children with acute diarrhoea (Gore and Surawicz, 2003; Mahmud *et al.*, 1993) and is likely that many of these children had bacterial diarrhoea.

The number of children who had vomited in the previous week and the preceding 24 hours before admission was also statistically higher than in the controls attending the OPD and health centres. The presence of vomiting was a risk factor independent of the severity of the diarrhoea. This is in agreement with previous studies that have indicated that the majority of children with severe diarrhoea had vomits and fever before consultation and is often seen in children with rotavirus diarrhoea (Gupta *et al.*, 1996; Hart., 2003; Vanderlei *et al.*, 2003).

The third risk factor identified by both groups was a history of hospitalisation. A large proportion (33%) of hospitalised children had a history of previous hospitalisations due to diarrhoea or other diseases possibility reflecting a higher susceptibility of these children to infectious diseases leading to hospitalisation. These finding are in agreement with other studies that have found that past hospitalisation is a risk factors for further hospitalisation (Do Carmo-Leal *et al.*, 1996).

The presence of watery stools was an independently significant variable related to the risk of hospitalisation. However this was a significant risk factor that was only identified when comparing cases with health centre controls. This probably reflected the increased severity of the diarrhoea in inpatients and OPD controls than in the health centres. This is consistent with investigations related to acute diarrhoea in developing countries that have shown that mothers complaining of watery stools is a valuable predictor of severe diarrhoea ending in hospitalisation in many cases.

Similarly, hospitalised children were less likely to use ORS, but this was not a risk factor when cases were compared to the OPD controls. Health centre controls are more likely to be given ORS in the health centres and to accept ORS because they have milder episodes, while OPD controls are likely to be children who have an intermediate degree of severity or because the health facilities use cheaper and simple interventions in the health centres than in the OPD services. This finding is in agreement with studies that have reported that a lack of awareness of the use of ORS for the treatment and prevention of dehydration increases the risk of hospitalisation. The effective use of ORS has saved millions of lives around the world, however in Iran ORS is grossly underused. The majority of deaths, hospitalisation and visits to

emergency departments due to diarrhoea could be prevented by appropriate promotion and use of ORS (Alam and Ashraf, 2003; Rao *et al.*, 1998; Santosham *et al.*, 1997).

There is ample evidence of the positive influence of breastfeeding, especially exclusive breast-feeding on the survival of the child and the negative relationship between breast - feeding and infectious diseases. For example in one cross-sectional study from Brazil (Falbo and Alves, 2002), 50% hospitalised children with diarrhoea had been breast fed for less than 2 months and nearly 20% had never been breast fed at all. This study revealed that hospitalised children with diarrhoea had been breast-fed for shorter periods than health centre controls and this variable remained significant after allowing for confounding variables. The proportion of hospitalised children who were breast fed for less than 7 months was 2 times higher (16%) than the proportion observed in health centre controls (Arifeen *et al.*, 2001; Blake *et al.*, 1993; Falbo and Alves, 2002; Fuchs *et al.*, 1996; Shahid *et al.*, 1996).

One hundred and thirty eight (53%) and 20 (8%) of the children admitted to the hospital had moderate to severe dehydration on admission, which is statistically higher than the 84 (34%) and 59 (23%) of the OPD and health centre controls with moderate dehydration. These findings are in agreement with studies that have shown that dehydration is present in the majority of hospitalised children with acute diarrhoea. However in our logistic regression we did not observe that dehydration was a risk factor. This might be due to the approach used to control for the confounding effect of the variables, as variables were subtracted from the equation on the basis of their statistical value and in no hierarchical fashion. Dehydration is strongly associated with watery diarrhoea, lack of ORS and also vomiting, and these latter factors had stronger predictive value, this dose not mean that dehydration is not an important clinical criterion (Binka et al., 2003).

Studies that focus on environmental factors associated with diarrhoea have reported that a lack of, or no access to, safe water is a risk factor for diarrhoea. The majority of the population without access to safe water belong to low socio-economic groups who also have a higher frequency of undernutrition in children. Malnourished children are more susceptible to infectious diseases and severe diarrhoea results in more hospital admissions or deaths. Consistent with these studies we found that no access to safe

water increased the risk of childhood hospitalisation and similarly using spring/ rivers or other sources of water was more frequently observed in hospitalised children (Huttly *et al.*, 1987; Nasinyama *et al.*, 2000; Yassin, 2000).

We have also shown that two maternal factors, namely being illiterate and unhealthy were significant factors with independent associations with the risk of hospitalisation. These results are in agreement with studies that have documented that parent's education, especially illiteracy is a very important factor in the management of childhood diarrhoea. While a quarter of our affected inpatients children had illiterate parents, less than 10% of children in the control groups had illiterate parents (Milaat and Elassouli, 1995; Pinto *et al.*, 1998; Shah *et al.*, 2003; Yassin, 2000).

In conclusion, the risk factors for hospitalisation due to acute diarrhoea were the presence of bloody (AOR 124.6), or watery stools (AOR 31), lack of ORS use (AOR 11.8), use of unsafe (spring/rivers) water (AOR 11), having vomited the week before consultation (AOR 6.6), having been breast fed for less than 7 months (AOR 4.7), having an unhealthy mother (AOR 3.2), having been hospitalised in the previous year (AOR 3)) and having an illiterate mother (AOR 3).

Chapter 5

Micronutrient concentrations in children with and without acute diarrhoea

5.1. Introduction

This study has compared the micronutrient concentrations in hospitalised children with diarrhoea with the micronutrient concentrations of children without acute diarrhoea. Serum samples were collected from 259 children with acute diarrhoea and 203 hospitalised to undergo elective surgery who had no acute diarrhoea. The micronutrients tested included vitamins A and E, zinc, selenium and copper. The vitamins were measured using High-Performance -Liquid Chromatography (HPLC) and the Inductively Coupled Plasma –Mass Spectrometry (ICP –MS) method was used to measure zinc, selenium and copper levels. The laboratory methods are described in detail in Chapter 2.

5.2. Methodology

5.2.1. Objective

To describe the serum concentrations of zinc, selenium, copper, vitamin A and vitamin E in children admitted to a district hospital with acute diarrhoea (cases) and without acute diarrhoea (controls).

5.2.2. Study design

This was a cross-sectional study of children <5 years of age, admitted to the wards of Hajar Hospital of Shahrekord, Iran and the children reported in this chapter are the same children described in Chapter 3. The study aimed to establish the prevalence of zinc, copper, selenium, vitamin A and E deficiencies in children with and without acute diarrhoea. For operational purposes children hospitalised for elective surgery were selected from Hajar Hospital with a few further children selected from Resallat Hospital both in Shahrekord. The characteristics of Hajar Hospital were described in Chapter 2. Resallat Hospital is a hospital in Shahrekord with similar characteristic to Hajar Hospital.

5.2.3. Selection criteria

Children <5 years of age admitted to the hospital with a clinical diagnosis of acute diarrhoea were selected. Children without diarrhoea were selected from children admitted to the surgical wards for elective surgery. Children with persistent diarrhoea (i.e. diarrhoea>14 days duration), those whose parents did not consent to blood taking or did not provide informed consent and those with a history of diarrhoea in the two preceding weeks were excluded. Children <5 years of age admitted between October 2001 and August 2002 were selected.

Parents of children admitted for surgical procedure were asked if the child had experienced an episode of acute diarrhoea in the preceding two weeks and were excluded if they had had episodes.

5.2.4. Sample size

The sample size to investigate the objective of this study was calculated by using Epi-info. The sample size required was calculated with the expected prevalences described in table 5.1.

Table 5.1. Expected prevalence of micronutrients and required sample size.

Micronutrient	Expected deficiency		Accepted Error (±)	Sample size required/ group
	With diarrhoea	Without diarrhoea		
Vitamin A	30%	10%	2.5%	92
Vitamin E	30%	10%	2.5%	92
Zinc	40%	25%	2.5%	**253**
Selenium	35%	15%	2.5%	106
Copper	35%	15%	2.5%	106

The sample size required to describe zinc deficiencies was the largest sample size required. Therefore a sample size of 253 children with diarrhoea and 253 without diarrhoea would be sufficient to compare the prevalence of the micronutrients of

interest with a precision of ± 5 %. Participants with and without acute diarrhoea were selected with every second patient attending the hospital being invited to participate.

5.2.5. Laboratory procedures

Blood samples (3 ml) were collected from each of the children enrolled. The blood was left to clot, centrifuged at 1500 rpm for 5 minutes and the serum was stored -20°C until transported to Liverpool.

Serum samples were kept in dark- colour safety tubes for the detection of vitamins using the High-Performance -Liquid Chromatography method, as described by Catignani and Bieri (1983) and zinc, selenium and copper concentrations were measured using Inductively Coupled Plasma –Mass Spectrometry.

5.2.6. Statistical analysis

Micronutrient concentrations in children with and without diarrhoea were compared using parametric tests. Values for most micronutrients had skewed distributions. For this reason, arithmetical values were log transformed, when appropriate, to calculate geometrical means. Two-sample t-tests were used to compare the values in children with diarrhoea with those of the surgical controls. In addition, the micronutrient concentrations of the children with diarrhoea were described according to selected characteristics of the children, including the duration and severity of the episodes before admission, the presence of vomiting and the aetiology of the episodes. The data are presented in summary tables and as scatter plots to facilitate interpretation. P values <0.05 were considered to be statistically significant.

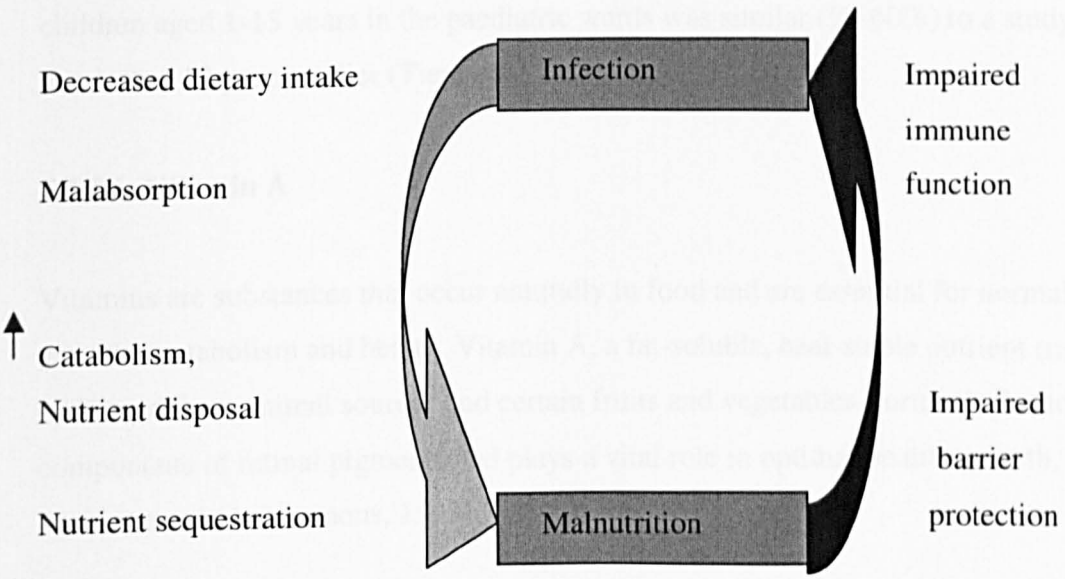
5.3. Literature review

5.3.1. Relationship between diarrhoea and micronutrient deficiencies

Nutrition is an emerging issue on the national agendas of many countries. South Asian countries and Southeast Asian populations now comprise about 56% of Asia's total population and about 33% of the world population. Most South and Southeast Asian countries rank in the medium and low levels of the Human Development Index. Only Singapore, Thailand, and Malaysia have a high ranking. An unfortunate feature of the nutrition situation in South-Asian countries is that the incidence of low birth weight is as high as 34% (1990), ranging from 25% in Sri Lanka to 50% in Bangladesh (as against less than 7% in the countries of Europe and North America). Even in countries of Africa where the overall food and nutrition situation is worse than in South Asia, the incidence is often below 20% (Gopalan, 1996).
Micronutrient deficiencies are also known as ' the hidden hunger', and these deficiencies are determining and aggravating factors for the health status of the population and quality of life. The nutritional problems that have serious health consequences are iron, vitamin A and iodine deficiencies. In spite of recent efforts in the prevention and control of these deficiencies, recent estimates indicate that globally over two billion people are at risk of vitamin A, iodine, zinc and/or iron deficiency (Ramakrishnan and Martorell, 1998; Ramakrishnan, 2002).

Each day of illness due to diarrhoea produces a weight deficit of 20-40 grams and poor nutrition is associated with more serious and prolonged diarrhoea (Patwari, 1999). 'Catch-up growth' often described in children born at low birth weight does not occur in malnourished children. Malnutrition, particularly wasting, is a strong predictor of diarrhoeal disease duration and prolonged illness could exacerbate by itself nutritional faltering, thereby increasing the subsequent risk of death. Poor appetite, vomiting, deliberate withholding of food resulting in poor intake, malabsorption of macro and micro-nutrients, hastening of intestinal transit time, disturbance of metabolic and endocrine functions and direct loss of protein and other nutrients into the gastrointestinal tract are some of the known mechanisms which have an impact on nutrition during an episode of diarrhoea (Patwari, 1999; Stephenson *et al.*, 2000). The relationship between nutrition and diarrhoea is shown in figure 5.1.

Figure 5.1. Relationship between nutrition and diarrhoea



5.3.2. Prevalence of malnutrition

Malnutrition can be seen in several forms, but the four most important forms of malnutrition are protein-energy malnutrition (PEM), iron deficiency anaemia, iodine deficiency disorders and vitamin A deficiency (Stephenson et al., 2000).

PEM affects an unacceptably large proportion of children under 5 years in developing countries and approximately 27% of under 5 year olds are considered underweight, about 32% and 9% are stunted and wasted respectively. The results of a descriptive analysis from 191 countries in 2003 showed that the highest rate of childhood malnutrition was found in South Asia (57 children per 100), while the lowest rate was found in Europe (just 1 child per 100) (El-Ghannam, 2003; Tumwine and Barugahare, 2002; Umeta *et al.*, 2003). Approximately 70% of the world's malnourished children live in Asia, resulting in the region having the highest concentration of childhood malnutrition. Rates of malnutrition in preschool children range from 16% in the Republic of China to 64% in Bangladesh. The prevalence of stunting and underweight is especially high in South Asia where one in every two preschool children is stunted (Khor, 2003). Despite improvements in the socio-economic status of many countries

in this region in the last few decades the prevalence of malnutrition in children has still not changed. For example in Thailand, the prevalence of hospital malnutrition in children aged 1-15 years in the paediatric wards was similar (50-60%) to a study conducted 10 years earlier (Tienboon, 2002).

5.3.2.1. Vitamin A

Vitamins are substances that occur naturally in food and are essential for normal growth, metabolism and health. Vitamin A, a fat-soluble, heat-stable nutrient (retinol) is derived from animal sources and certain fruits and vegetables, forms the basic components of retinal pigments and plays a vital role in optimal health, growth, and development (Anonymous, 1999).

Vitamin A influences growth, survival, and resistance to infection. Vitamin A deficiency can reduce the T- cells' ability to fight infection and decrease mucus production resulting in more bacteria being able to attach themselves to the respiratory mucosa. Thus vitamin A deficiency increases the body's susceptibility to respiratory infections (Pinnock, 1991). Vitamin A deficiency is the single most important cause of blindness in children in developing countries.

The WHO classifies vitamin A deficiency as a major micronutrient deficiency in the world. More than 250 million of the world's children suffer from vitamin A deficiency and as many as 3- 5 million Asian children may develop xerophthalmia every year. One-tenth of the children with xerophthalmia have severe corneal involvement, and half of them become blind. Although vitamin A-related blindness is declining, sub-clinical vitamin A deficiency still remains unchanged in developing countries (Donnen *et al.*, 1996; Fiedler, 2000; Hatun and Tezic, 1995; Hatun *et al.*, 1995; Kucukbay *et al.*, 1997; Oso *et al.*, 2003; Pinnock, 1991; Stephenson *et al.*, 2000). Nearly half of all vitamin A deficiency and xerophthalmia in the world occurs in South and Southeast Asia, with large numbers of cases in India (35.3 million), Indonesia (12.6 million) and China (11.4 million) (Khor, 2003).
The prevalence of vitamin A deficiency varies from 1% to 70% across the world (Faber and Benade, 1999; Faber *et al.*, 2000; Mehra *et al.*, 1994; Tan *et al.*, 2002). In some areas however its prevalence can be very high and for example, one community

based study in Sudan only found 3 (0.004%) children who had vitamin A equal or more than 3.0 mg/dL (the recommended cut-off point for normal values) among 1360 children (El Bushra *et al.*, 1994; Harrison, 1992). The prevalence of vitamin A deficiency in selected countries is shown in table 5.2.

Table 5.2. Prevalence of vitamin A deficiency in selected countries

Country	Number	Prevalence	Reference
Without diarrhoea			
Africa			
Cameroon	279	72%	(Gouado et al., 1998)
Ethiopia	4770	63%	(Haidar et al., 2003)
	147	32%*-49%	(Tafesse et al., 1996)
Nigeria	358	8%	(Akinyinka et al., 2001)
South Africa	115	37%	(Faber and Benade, 1999)
Sudan	1441	>99%	(El Bushra *et al.*, 1992)
Asia			
Cambodia	10942	1%*	(Semba et al., 2004)
China	8669	12%	(Tan et al., 2002)
India	308	36%	(Khandait et al., 2000)
Philippines	1715	47%	(Solon et al., 1978)
Pakistan	3074	32%	(Paracha et al., 2000)
Turkey	160	16*-42%	(Kurugol et al., 2000)
	21	51%	(Hatun et al., 1995)
	960	12%	(Wetherilt et al., 1992)
With diarrhoea			
Asia			
Bangladesh	400	3%-10%	(Ahmed et al., 2000)
India	125	0%	(Agarwal et al., 1996)
	25	68%	(Buyukgebiz et al., 1990)
	1987	87%	(Rahman et al., 1995)
Africa			
Katana (Congo)	415	74%-79%**	(Donnen et al., 1996)
South Africa	189	92%	(Hussey and Klein, 1990)

* = severe vitamin A deficiency only, ** = malnourished children

Vitamin A supplementation can lower a deficient child's risk of dying of infection by an estimated 23% (Khan *et al.*, 2002; Stephenson *et al.*, 2000). Xerophthalmia, due to

vitamin A deficiency is a major preventable cause of childhood blindness in many countries, particularly in the Middle East and South East Asia (Diniz Ada and Santos, 2000; Gilbert and Awan, 2003; Underwood, 2004). In hospital based studies, vitamin A supplementation has reduced overall case mortality by 23%, measles mortality by 50% and diarrhoea mortality by 50% (D'Souza and D'Souza, 2002; Glasziou and Mackerras, 1993).

According to one report from Iran's Ministry of Health and Medical Education in some parts of Iran, the prevalence of blindness in children is still around 15 per 1000 children confirming that vitamin A deficiency is still a serious problem in Iran (Isna, 2003). The effect of Vitamin A supplementation in children in selected studies is shown in table 5.3.

Table 5.3. Effect of vitamin A supplementation in children in selected studies

Country	Number	ARI	Diarrhoea incidence	Mortality	Duration of diarrhoea	Xerpohthalmia	Measles	Reference
Asia								
China	172	+	+	NT	NT	NT	NT	(Lie et al., 1993)
Nepal	1972	NT	NT	NT	+	NT	NT	(Strand et al., 2002)
India	174	NC	NC	NT	NT	NT	NT	(Biswas et al., 1994)
	108	NT	NC	NT	NT	NT	NT	(Dewan et al., 1995)
	7691	+	NT	NC	NT	NT	NT	(Vijayaraghavan et al., 1990)
Indonesia	3225	*	NT	NT	NT	NT	NT	(Kartasasmita et al., 1991)
Bangladesh	1772	+	+	NT	NT	NT	NT	(Bloem et al., 1990)
America								
Brazil	1240	NT	+	NT	+	NT	NT	(Barreto et al., 1994)
Africa								
Ethiopia	4770	NT	+	NT	NT	NT	+	(Haidar et al., 2003)
Ghana	1500	NT	NT	+	NT	NT	NT	(Kirkwood et al., 1996)
	21,906	NC	NT	NC	NC	NT	NT	(Anonymous, 1993)
Tanzania	687	*	+	NT	NT	NT	NT	(Fawzi et al., 2000)
Sudan	1441	NT	+	NT	NT	NT	NT	(El Bushra *et al.*, 1992)2)
	28,753	+	+	+	NT	NT	NT	(Fawzi et al., 1994)

ARI = acute respiratory infection, NT = not tested, NC = not changed, * ARI increased

5.3.2.2. Vitamin E

Vitamin E is a fat-soluble vitamin that exists in eight different forms. Each form has its own biological activity, measure of potency or functional use in the body. Alpha-tocopherol is the most active form of vitamin E in humans. There are three specific situations when a vitamin E deficiency is likely to occur, namely persons who cannot absorb dietary fat, premature children with very low birth weight (birth weights less than 1500 grams) and individuals with rare disorders of fat metabolism. Vitamin E deficiency is usually characterized by neurological problems due to poor nerve conduction. Vegetable oils especially palm oil (*Elaeis guineensis*), nuts, wheat germ oil, almonds, dry roasted, safflower oil, corn oil and soybean oil are rich in vitamin E. Vitamin E is a major anti-oxidant nutrient that retards cellular aging resulting from oxidation and supplies oxygen to the blood (Annoymous, 2004). Human trials of varying doses of vitamin E, including low, supplemental, and pharmacological doses, have found that vitamin E may improve immunity, vascular function, and brain performance (Martin *et al.*, 2002; Sundram *et al.*, 2003; Wattanapenpaiboon and Wahlqvist, 2003). In recent years, vitamin E has been investigated as a cardioprotective agent and experimental studies have identified potential mechanisms by which vitamin E may inhibit the development of arteriosclerosis, and observational studies of individuals without coronary disease suggest that vitamin E intake may protect future cardiovascular events (Manson et al., 2003).

Vitamin E improves neutrophil function *in vitro* in patients with glutathione synthetase deficiency by protecting the plasma membrane and or cysto- skeletal components from toxic oxygen species. However, sufficient clinical experience to document a beneficial effect of vitamin E in this or other disorders for which antioxidant therapy has been provided, is still lacking. Deficiencies of protein and vitamins A and E are associated with reduced immunocompetence. In contrast, excessive intake of fat, in particular polyunsaturated fatty acids (e.g. linoleic and arachidonic acids), iron and vitamin E are immunosuppressive (Baehner and Boxer, 1979; Boxer *et al.*, 1979a; Boxer *et al.*, 1979b; Chandra and Chandra, 1986). Diets rich in vitamin E have been shown to significantly delay the development of pathologic processes, including neurodegenerative disorders. Palm vitamin E (30% tocopherol, 70% tocotrientols) has been extensively researched for its nutritional and

health promoting properties, including antioxidant, cholesterol lowering and anti-cancer activities and protection against arteriosclerosis.

Epidemiological studies have mostly investigated serum vitamin E levels and its deficiency in subjects with cancer or health problems. However two community - based studies reported vitamin E deficiencies in 22 % and 66% in populations from Turkey and Cameroon respectively (Gouado *et al.*, 1998; Wetherilt *et al.*, 1992). Granot *et al* (2000) have reported that duration of diarrhoea does not affect vitamins A and E concentrations and serum vitamins were similar in infants with acute or persistent diarrhoea. In that study non-malnourished infants with persistent diarrhoea did not exhibit plasma antioxidant depletion nor enhanced lipid peroxidation. In these infants, oxidative stress, as reflected in plasma, did not play a role in the pathogenesis of persistent diarrhoea (Lindley et al., 1994). Experimental studies have suggested that the concentration of lipid peroxidion (thiobarbituric acid reactive substances and malondialdehyde) increase in vitamin E deficient subjects and that small intestinal brush border membranes from vitamin E deficient animals displayed changes in both static and dynamic components of membranes fluidity as measured by steady state fluorescence polarography (Lindley et al., 1993).

5.3.2.3. Zinc

The history of the recognition of the importance of zinc in nutritional and even more so, in clinical medicine and public health is remarkably brief (Hambidge, 2000). Zinc deficiency syndrome has been described in the Middle East and is based on observations of subjects exhibiting hypogonadal dwarfism, hepatosplenomegaly and anaemia during their late teens and early adulthood. The first case relates to subjects from the Fars provinces of Iran where zinc deficiency was associated with geophagia. A second case series described subjects from Egypt who were infested with heavy parasitic loads of *Ancylostoma duodenale, Schistosoma mansoni* and *S. haematobium* (Hambidge, 2000; Hambidge *et al.*, 2000; Prasad, 2001a, b). Since the early 1960s there has been recurring interest in the possible occurrence of zinc deficiency or disturbed zinc metabolism as a factor in a wide range of disease states from the common cold to wound healing in surgical patients.

Zinc deficiency appears to be widespread in low-income countries because of a low dietary intake of zinc-rich animal-source food and a high consumption of cereal grains and legumes, which contain inhibitors of zinc absorption (Penny et al., 2004). In experimental models, zinc has been shown to be necessary for cellular metabolism. Also it is critical for cellular and humoral immune function and physical growth, supplementation in such individuals improves immune function including delayed cutaneous hypersensitivity and increases the number of CD4 (helper) lymphocytes (Hosea et al., 2004). Zinc deficiency has direct effects on the gastrointestinal tract, such as impaired intestinal brush border integrity, increase in the mucosal mass and in the absorption of the water and sodium secretion in response to bacterial enterotoxins (Polat et al., 2003). Zinc is most frequently evaluated by measuring plasma levels, even though plasma zinc is an acute phase reactant that may change in response to metabolic alterations. Plasma zinc levels react to dietary intake in a rapid although difficult to measure manner during the acute phase of infection. Low plasma levels of zinc have been observed in congenital diseases such as sickle cell anaemia, Down's syndrome and acrodermatis enterophathica and acquired conditions, including malabsorbtion syndrome and alcoholism (Albert *et al.*, 2003; Baqui *et al.*, 2002; Black, 2003).

Because there is a strong association between protein and zinc contents in virtually all types of foods, insufficient protein intake may often be the cause of zinc deficiency. A typical instance of the link between a low protein intake and zinc deficiency has been documented in Vietnamese children, of whom 50% experience protein energy malnutrition during infancy (Polat *et al.*, 2003; Thu *et al.*, 1999). Protein-energy malnutrition results in a decrease in immunologic defence mechanisms and leads to greater susceptibility to infection, especially diarrhoea.

Acute stress including major operations decreases the serum zinc level temporarily and zinc deficiency may be the result of chronic zinc depletion caused by other conditions (Albert et al., 2003).

A nationwide survey of people 6 months to 74 years of age in the United States documented that serum zinc concentrations were higher in the morning fasting samples than in the morning non-fasting samples, although the magnitude of this difference varied with age. For young adults (≅20 –30 years of age) little difference in

serum zinc concentrations between the morning fasting and the morning non- fasting samples was seen. The association between serum zinc concentration and gender is more controversial as some studies reported higher serum zinc levels in females. Studies from India have reported a significantly higher serum zinc in babies than in females (Hotz *et al.*, 2003; King *et al.*, 1994; Sriniwas *et al.*, 2003). The cut-off point for zinc deficiency is still controversial. Most studies have defined zinc deficiency when the serum zinc concentration is less than 60 mg/dl (9.18μmol/L), however the National Health and Nutrition Examination survey (NHANES) have defined zinc deficiency as a serum level of < 65 mg/dl (9.94μmol/L) for children < 3-9 years of age (Hotz et al., 2003). Numerous studies have investigated the relationship between diarrhoea and zinc (Castillo-Duran *et al.*, 1994; Roy, 1991; Sazawal *et al.*, 1997; Sur *et al.*, 2003), these studies have found that zinc supplementation reduces the duration, incidence and severity of diarrhoea episodes. A community- based study from India showed significant differences between the zinc supplemented and placebo groups in linear growth and weight-for-age (Sur et al., 2003). Children in this study were defined as zinc deficient if the serum zinc concentration of individual children was less than 9.94 μmol/L. The majority of studies that have investigated the relationship between diarrhoea and zinc, have compared the serum zinc concentrations between children with and without diarrhoea or investigated the differences in zinc serum concentrations between subsequent samples in individual cases, for example, serum zinc on admission and after recovery. Also many clinical trials have investigated the changes in serum zinc before and after zinc supplementation. There is now enough evidence demonstrating the efficacy of zinc supplementation on the clinical course of acute diarrhoea. However, effectiveness studies to assess different strategies for delivering zinc supplementation to children with diarrhoea should be undertaken. These studies should investigate the feasibility, sustainability, and cost-effectiveness of different zinc-delivery mechanisms, and monitor variables, such as consumption of ORS, antibiotic-usage rate, non-diarrhoea morbidity, and overall mortality (Bahl et al., 2001).The prevalence of zinc deficiency observed in children with and without diarrhoea in selected studies is shown table 5.4.

Table 5.4. Prevalence of zinc deficiency in selected studies

Trial	N	Prevalence	Cut-off point**	Reference
With diarrhoea				
Bangladesh	301	15%	<9.18	(Osendarp et al., 2002)
	148	22%	<9.18	(Osendarp et al., 2002)
India	462	34%	<9.18	(Sazawal et al., 1995)
Brazil	45	7%	1.40	(Fisberg et al., 1984)
Uganda	96	48%	-	(Bitarakwate et al., 2003)
Without diarrhoea				
India	15,000	44%	<9.18	(Bhandari et al., 2002)
Indonesia	549	78%	<9.18	(Lind et al., 2003)
Iran	881	65%	-	(Mahmoodi and Kimiagar, 2001)
Pakistan	3074	54%	_	(Paracha and Jamil, 2001)
Chile	-	55%	<12.3	(Torrejon et al., 2004)
Burkina Faso	685	72%	-	(Muller et al., 2003)
Mexico	1,363	34%	<9.94	(Villalpando et al., 2003)
		19-24%	<9.94	(Villalpando et al., 2003)
		60%	<9.18	(Villalpando et al., 2003)
Australia	364	24-67%	<10.8	(Holt et al., 1980)
U.K*	1,193	13%	-	(Thane et al., 2004)

N= number, * = subjects were children 4-14 years, ** = μmol/L,

5.3.2.4. Selenium

Selenium is a trace element that is an essential component of a number of proteins, including glutathione peroxidase, glutathione reductase and thioredoxine reductase. Selenium seems to play a vital role in antioxidant protection due to its incorporation in the form of selenocysteine into several antioxidant enzymes where it affects all components of the immune system, i.e., the development and expression of non-specific, humoral, and cell-mediated responses (Arthur et al., 2003). A deficiency of selenium in China was found to lead to a cardiomyopathy known as Keshan disease

(Beck, 2001; Kiremidjian-Schumacher and Stotzky, 1987; Levander, 1982; Schwarz and Foltz, 1958; Schwarz *et al.*, 1959).

The main physiological role of selenium was established 2 decades ago, by the discovery that selenium is an essential structural component of the mammalian enzyme, glutathione peroxidase which is involved in the defence systems of the cell against reactive oxygen species (Bunker, 1992; Wasowicz *et al.*, 2003). In addition, selenium is required for the proper functioning of the immune system and its deficiency has been hypothesised to have a negative impact on immune functions, to increase the risk of the occurrence, virulence, or disease progression of some viral infections and also has been associated with dilated cardiomyopathy, skeletal muscle myopathy, osteoarthropathy and cretinism (in iodine – deficient populations). Skeletal muscle disorders manifested by muscle pain, fatigue, proximal weakness, and serum creatine kinase elevation have been reported in patients with selenium deficiency (Chariot and Bignani, 2003). Marginal deficiencies may contribute to reduce immune functions against some cancers and viral diseases (Jackson *et al.*, 2003; Olmez *et al.*, 2004; Sempertegui *et al.*, 2003).

Selenium deficiency has been shown to affect neutrophil function, antibody production, proliferation of T and B-lymphocytes in response to mitogens, and cytodestruction by T lymphocytes and natural killer cells. In contrast supplementation with selenium has been shown to stimulate the function of neutrophils, production of antibodies, proliferation of T and B-lymphocytes in response to mitogens, production of lymphokines, NK- cell-mediated cytodestruction, delayed-type hypersensitivity reactions and allograft rejection, and the ability of a host to reject transplanted malignant tumors. The mechanism(s) whereby selenium affects the immune system is speculative and its antioxidant role and ability of selenium to interact with cell membranes, probably represent only a few of many regulatory mechanisms (Kiremidjian-Schumacher and Stotzky, 1987).

Nowadays the increasing population of children with HIV-infection has made the important role of selenium more clear, as a low plasma level of selenium is an independent predictor of mortality in HIV children, and appears to be associated with

faster disease progression (Campa et al., 1999). Recent studies have demonstrated that not only the host immune response is affected by a deficient diet, but the viral pathogen can also be altered. Dietary deficiency that leads to oxidative stress in the host, e.g. selenium deficiency can alter the viral genome such that a normally benign or mildly pathogenic virus becomes highly virulent in the deficient oxidatively stressed host. Influenza and Coxsackie virus infections in selenium deficient hosts has changed their genomes and the virulence of the virus increased (Beck et al., 2003).

A review of human blood selenium concentrations worldwide reveals very large differences in the apparent dietary status of individuals in different areas. The question has been raised as to whether blood selenium measurement is a reliable index of actual selenium status in term of bioavailability and function (Diplock, 1993) Similarly to zinc, many studies have described serum selenium concentrations in children with and without diarrhoea, but the cut-off point to define selenium deficiency is under question, as selenium intake varies by region. Some studies however have suggested that serum selenium concentrations <0.80 μmol/L or 0.90 μmol/L could be used as cut off points for selenium deficiency (Sammalkorpi et al., 1988). A study from Turkey reported a very tight range of serum selenium (0.69 – 0.93 μmol/L) in 1,918 school children (Piechotowski et al., 2002). Among the very few studies that have focused on selenium deficiency, a community study from Spain documented a prevalence of selenium deficiency (below 0.57 mol/L) of 7% in representative samples (Diaz Romero et al., 2001).

5.3.2.5. Copper

Many studies have reported that copper with selenium, zinc and molybdenum are involved in many biochemical processes such as cellular respiration, cellular utilization of oxygen, DNA and RNA reproduction, maintenance of cell membrane integrity, and sequestration of free radicals. These elements are involved in destruction of free radicals through cascading enzyme systems (Chan et al., 1998).

Copper appears to be an essential element playing a crucial role in the immune system and has many important functional roles related to the maintenance of immune

function, bone health and haemostasis. Some workers have suggested a role of long-term marginal copper deficiency in the aetiology of a number of degenerative diseases. Copper containing enzymes, such as copper-zinc superoxide dismutase, cytochrome C oxidase and diamine oxidase may be involved but evidence to date is not conclusive (Bonham *et al.*, 2002; Ciftci *et al.*, 2003).

Copper deficiency occurs principally in infancy, although it has been reported in older children and young adults. Deficiency in normal infants is rare before the age of 5-6 months. It is encountered in protein-energy malnutrition and copper deficiency has been reported in prematurity and after prolonged total parental nutrition, when anaemia, neutropaenia, metaphyseal changes and periosteal reactions may occur (Mino, 1993; Mino *et al.*, 1993).

In a study from Brazil, 49% of malnourished children had copper levels below 90 micrograms/dl (the minimum normal limit) and children with prior diagnosis of acute diarrhoea and hospitalisation had lower copper levels than those who did not have a history of acute diarrhoea or previous hospitalisation. Breast feeding was associated with higher copper levels (Fisberg et al., 1984). Another study from India has reported that the mean serum copper level in children with acute diarrhoea is significantly greater than in the serum of children with chronic diarrhoea and apparently healthy control groups. Epidemiological studies have suggested that serum copper seems to increase with the duration of infectious diseases (Sachdev et al., 1989).

5.4. Results

5.4.1. Concentration of micronutrients in cases and controls

Two hundred and fifty six and 196 serum samples from hospitalised children with and without acute diarrhoea were tested to analyse their micronutrient status. The serum geometrical mean vitamin A, E and selenium concentrations were significantly lower in children with diarrhoea than in controls. Serum concentrations of zinc were slightly higher in cases than controls (p= 0.06). The geometrical mean serum copper was not different in children with and without diarrhoea (table 5.5).

5.4.2. Vitamin A

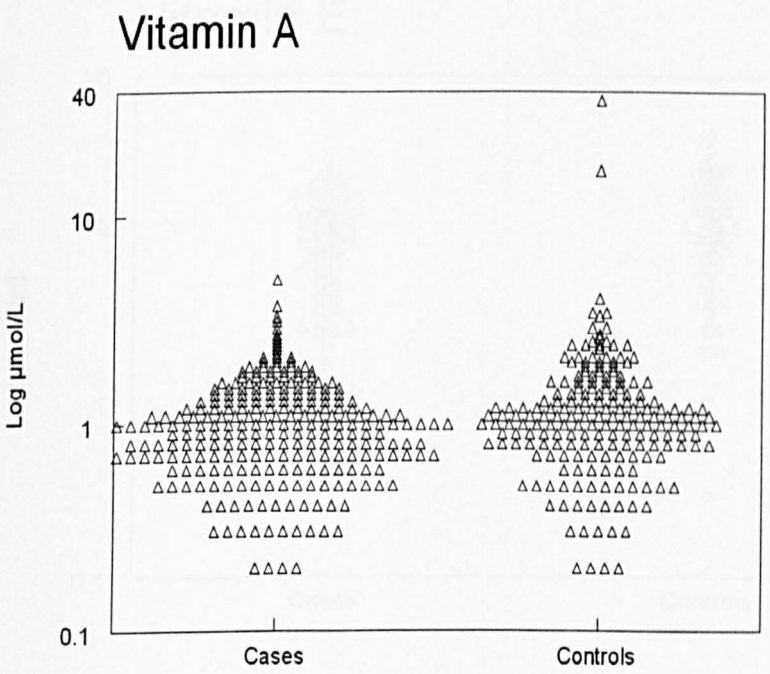
The geometrical mean (SD) serum vitamin A concentration of children with diarrhoea was lower (0.91μmol/L (1.8) than in controls without diarrhoea (1.03 (2), P= 0.03) with ranges of 0.04 to 4.88 μmol/L for cases and 0.02 to 36.3 μmol/L for controls. The proportion of children with severe vitamin A deficiency was higher in cases than in controls (15 (6%) vs 9 (5%)), but this was not statistically significant. However the proportion of children with diarrhoea who had severe/ moderate vitamin A deficiency (71(28%)) was significantly higher than for children without diarrhoea (38 (19%)) (OR=1.6, 95% CI 1 - 2.6, P= 0.04). The serum vitamin A concentrations in children with and without diarrhoea and dotplots with all the values are shown in table 5.5 and figure 5.2.

Table 5.5. Concentration of micronutrients in cases and controls

Micronutrient	Cases			Controls			P value
	Geometrical mean (SD) μmol/L	Range μmol/L	Number deficient*	Geometrical mean (SD) μmol/L	Range μmol/L	Number deficient	
Vitamin A	0.91(1.8)	0.1-4.9	71 (28%)	1.03 (2)	0.1-36.3	38 (19%)	0.03
Vitamin E	8.91(1.7)	1.3-24.4	205 (86%)	9.77 (1.9)	0.3-67.5	136 (79%)	0.02
Zinc**	6.8 (7.9)	0.9-78.7	186 (82%)	7.7 (11.6)	0.3-157.8	151 (81%)	0.06
Selenium	0.75 (1.6)	0.1-9.3	164 (66%)	0.97 (1.8)	0.1-4.1	87 (45%)	<0.01
Copper	1.47 (1.8)	0.3-2.9	112 (47%)	1.47 (1.9)	0.2-11.4	86 (49%)	0.95

P values: students T test, cases vs controls, * Cut off point <0.70μmol/L, <16 μmol/L, 9.94μmol/L, 0.90μmol/L and <1.5 μmol/L for vitamin A, E, zinc, selenium and copper deficiency respectively, ** arithmetic mean

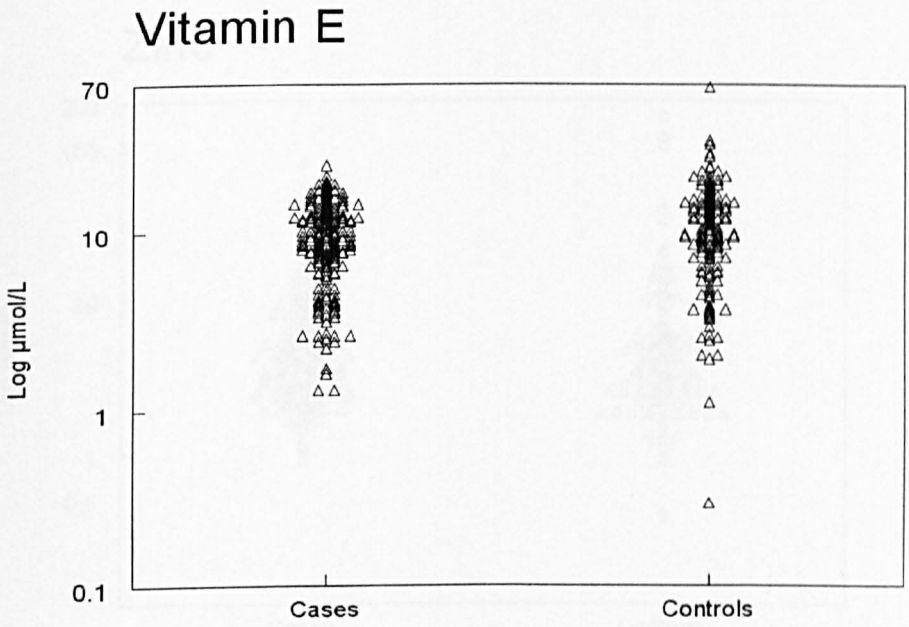
Figure 5.2. Serum vitamin A concentrations in cases and controls



5.4.3. Vitamin E

Two hundred and thirty five and 175 serum samples from cases and controls were tested to measure vitamin E concentrations. Children with diarrhoea had a significantly lower mean (SD) (8.9μmol/L (1.7)) serum vitamin E than controls (9.8 μmol/L (1.94)), (P= 0.02), with a range of 1.3 to 24.4 μmol/L for cases and 0.3 to 67.5 μmol/L for controls. The frequency of severe vitamin E deficiency (<16 μmol/L) was higher (141, 60%) in cases than in controls (88, 50%) (OR =1.5, 95 CI 0.98 - 2.47, p= 0.06). Mild to severe vitamin E deficiency (11.5μmol/L) was also significantly more frequent (205, 87 %) in cases than in controls (136, 78%) (OR = 2, 95 CI 1.1 –3.4, P = 0.01). The serum vitamin E concentrations in children with and without diarrhoea are described in table 5.5 and figure 5.3.

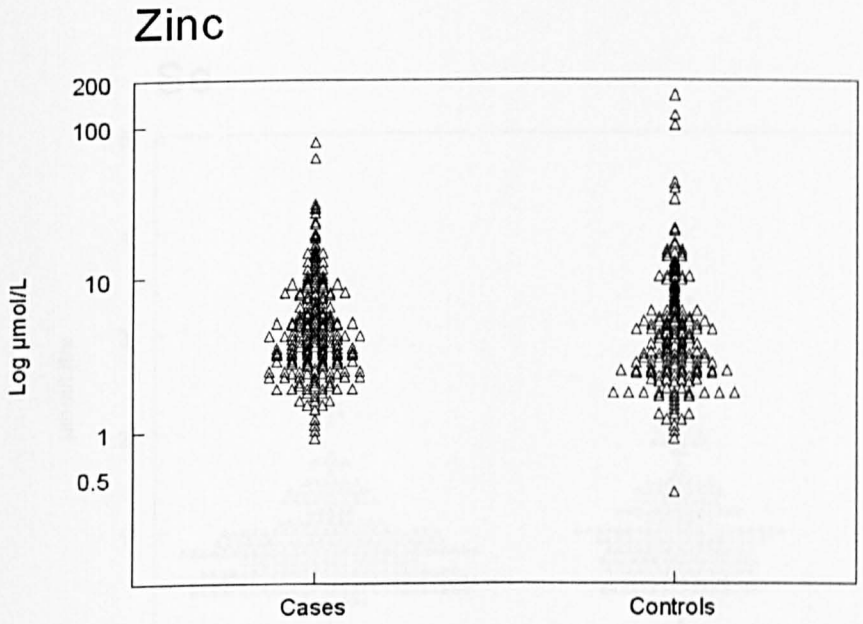
Figure 5.3. Serum vitamin E concentrations in cases and controls



5.4.4. Zinc

Serum zinc concentrations were measured in 228 serum samples from the cases and in186 controls. Cases had a lower arithmetical mean concentration (6.8μmol/L) than the controls (7.7μmol/L), P = 0.06. With more than three quarter of the diarrhoeic children being zinc deficient (<9.94 μmol/L). The frequency of zinc deficiency was not different between cases and controls at the time of admission to the hospital. One hundred and eighty six (82%) of the cases and 151 (81%) of the controls were zinc deficient respectively (<9.94 μmol/L), (OR =0.95, 95%CI 0.6 – 1.6, P=0.94). The cases had a range zinc concentration of 0.9 to 78.7μmol/L and the controls 0.35 to 157.8 μmol/L. The serum zinc concentrations in children with and without diarrhoea are described in table 5.5 and figure 5.4.

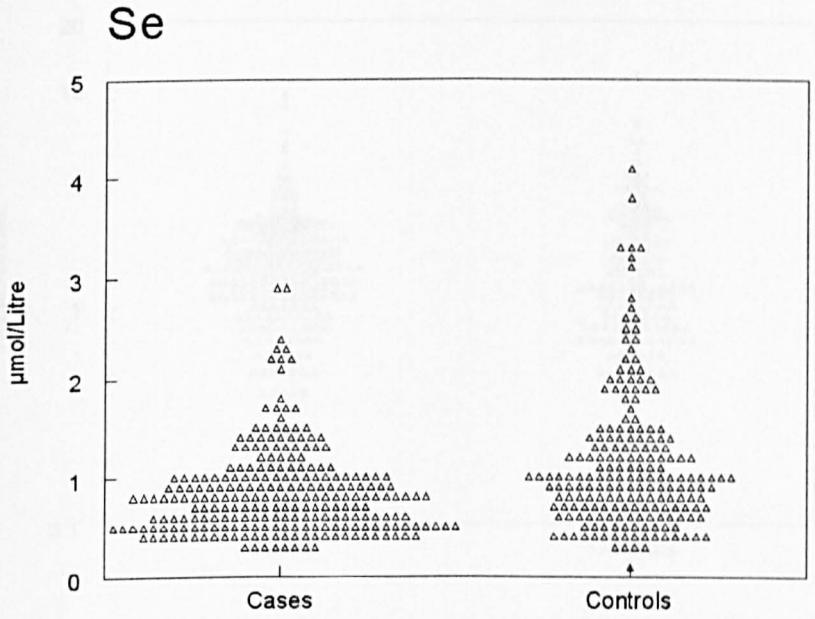
Figure 5.4. Serum zinc concentrations in cases and controls



5.4.5. Selenium

Selenium serum concentrations were measured in 251 children with diarrhoea and 196 controls. The geometrical mean (SD) serum selenium of children with diarrhoea was significantly lower (0.75 μmol/L (1.6)) than in controls without diarrhoea (0.97 μmol/L (1.8)), ($p<0.01$), and the values ranged from 0.29 to 2.88 μmol/L for cases and 0.07 to 4.13 μmol/L for controls. The frequency of selenium deficiency (<0.90 μmol/L) was significantly higher (164, 66%) in cases, than in controls (87, 47%), ($p<0.01$). The serum selenium concentrations of the children with and without diarrhoea are shown in table 5.5 and figure 5.5.

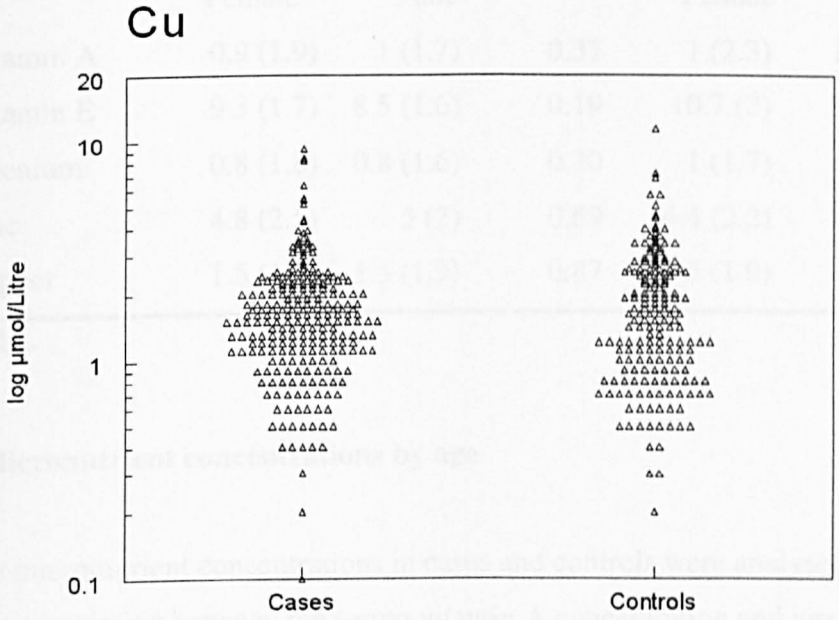
Figure 5.5. Serum selenium concentrations in cases and controls



5.4.6. Copper

Two hundred and forty one and 178 serum samples of cases and control were examined to describe their serum copper concentrations. The geometrical mean (SD) serum copper was no different in the children with and without diarrhoea, (1.47 (1.8) µmol/L vs (1.47 (1.9) µmol/L, (p =0.95)) .The copper concentration was lower in cases than in controls with a range of 0.11 to 9.34 µmol/L for cases and 0.24 to 11.41µmol/L for controls. The frequency of serum copper deficiency (< 1.5 µmol/L) was not different (112, 47%) in cases than in controls (86, 48%). The serum copper concentrations of the children with and without diarrhoea are shown in table 5.5 and figure 5.6.

Figure 5.6. Serum copper concentrations in cases and controls



5.5. Micronutrient concentrations by gender

The concentrations of all micronutrients in cases and controls by gender are shown in table 5.6. The geometrical mean serum concentrations of vitamin A and zinc in cases were higher in males than in females. In contrast, serum vitamin E concentrations were higher in females than males. However these differences were not statistically significant. Serum selenium and copper concentrations were equal in males and females.

Serum micronutrient concentrations of vitamin E and copper in controls were slightly higher in females than in males. In contrast serum zinc and vitamin A concentrations were higher in males than in females. The concentration of selenium was equal in males and females. However serum vitamin E concentrations were significantly higher in females than in males (p= 0.04).

Table 5.6. Concentration of micronutrients by gender in cases and controls

Subject*	Cases		P value	Controls		P value
	Female	Male		Female	Male	
Vitamin A	0.9 (1.9)	1 (1.7)	0.37	1 (2.3)	1.1 (1.7)	0.19
Vitamin E	9.3 (1.7)	8.5 (1.6)	0.19	10.7 (2)	9.3 (1.8)	0.04
Selenium	0.8 (1.8)	0.8 (1.6)	0.30	1 (1.7)	1(1.9)	0.78
Zinc	4.8 (2.5)	5 (2)	0.69	4.4 (2.2)	4.5 (2.5)	0.97
Copper	1.5 (1.7)	1.5 (1.9)	0.87	1.5 (1.9)	1.4 (2)	0.46

*μmol/L

5.6. Micronutrient concentrations by age

Serum micronutrient concentrations in cases and controls were analysed by age. There was no association between the serum vitamin A concentration and age in cases and controls. The highest mean vitamin A values (1 μmol/L) were seen in children <12 months of age in cases and in >36 months of age in controls (1.1μmol/L). Similarly, there was no trend in the geometrical mean (SD) serum vitamin E concentrations by age in cases. Older children (> 36months) had lower vitamin E levels, but this was not statistically significant. A positive trend was observed between the geometrical mean serum selenium concentrations and age in both cases and controls, with younger having lower concentrations than older children ($p<0.05$). Similarly, there was a negative association between the geometrical mean zinc concentrations and age in cases, with higher levels observed in children <13 months than older children ($p=0.05$). In controls serum zinc was highest in children < 13 months, slightly lower in infants and children with 13-24 month of age. The lowest serum zinc was seen in older children.

In controls serum copper concentrations increased with age to reach 1.8 μmol/L in children 24 – 36 months. However this was not statistically significant. In contrast, in the control group, the geometrical mean serum copper concentration increased with age from 1.1μmol/L in infants to 1.9 μmol/L in children >36 months of age, ($p=0.01$). The geometrical means of the micronutrients by age are shown in table 5.7.

Table 5.7. Mean (SD) of micronutrient concentrations (μmol/L) by age in cases and controls

Micronutrient*	Cases					Controls				
Age (m)	**1-12**	**13-24**	**25-36**	**37-59**	**P**	**1-12**	**13-24**	**25-36**	**37-59**	**P**
Number	147	76	14	19		19	72	71	41	
Vitamin A	0.95 (1.7)	0.87 (1.7)	0.8 (1.6)	0.9 (2.6)	0.39	0.9 (1.8)	1 (2.1)	1 (1.7)	1.1 (1.9)	0.73
Vitamin E	9.1 (1.7)	9.1 (1.6)	9.5 (1.5)	6.3 (2.2)	0.28	11 (1.9)	9.1 (2.3)	10.2 (1.7)	9.8 (1.8)	0.71
Selenium)	0.79 (1.5)	0.81(1.5)	1.1 (1.8)	1.3 (1.9)	0.05	0.8 (1.6)	0.9 (1.7)	1 (1.9)	1.2 (1.8)	0.05
Zinc	5.3 (2.1)	4.9 (2.1)	3.6 (2.2)	4.1 (1.7)	0.13	4.7 (2.1)	4.4 (2.3)	4.9 (2.6)	3.6 (2.2)	0.72
Copper	1.4 (1.8)	1.8 (1.7)	1.8 (2.3)	1.1 (1.7)	0.33	1.1 (2.1)	1.3 (1.9)	1.5 (1.9)	1.9 (1.8)	0.01

* μmol/L

5.7. Association between micronutrient concentrations and clinical sings on consultation, previous year medical history, family background and laboratory results of children with acute diarrhoea

5.7.1. Vitamin A concentrations

The association between vitamin A concentrations and the characteristics of the children with diarrhoea was explored by analysing the geometrical mean vitamin A concentration of children with and without each characteristic. The factors described here were described in detail in the chapter of risk factors for hospitalisation. Table 5.8 describes the geometrical means of vitamin A by each risk factor. Children with fever in the 5 days before admission, those with signs of dehydration or vomiting in the week before consultation and children who had a history of illness in the previous year had lower vitamin A concentrations than children without these risk factors. Among the family background characteristics children who had mothers > 20 years had higher geometrical mean vitamin A than children of older mothers. However this was not statistically significant (p=0.09).

There was no association between diarrhoea duration, number of stools in the 24 hours before consultation, characteristics of the of stools, having an unhealthy mother, use of ORS, keeping animals at home, piped water and a history of hospitalisation and serum vitamin A concentration.

The pathogens causing the diarrhoea episode were associated with the serum vitamin A concentrations. The geometrical mean of serum vitamin A concentration was lower in children infected by viruses than children without viruses. However these differences were not statistically significant. However children with coronavirus in their stools had a marginally significant higher serum vitamin A than children without coronavirus (p= 0.07). There was also a positive association between vitamin A and shigellosis and children with *Shigella* had lower serum vitamin A concentration than children without *Shigella* (p=0.002).

Table 5.8. Mean (SD) serum vitamin A concentration by variable

Variable	Geometrical mean vitamin A (μmol/L) If variable present	If variable absent	P value
Family background			
Mother unhealthy	0.9 (1.8)	0.9 (1.5)	0.53
Mother age<20	0.91 (1.5)	1.09 (1.8)	0.09
Use spring/river water	0.8 (1.8)	1.1 (1.8)	0.19
Lack of piped water	0.9 (1.8)	1.2 (1.7)	0.60
Ownership of animals	1(1.5)	1 (1.8)	0.64
Previous year medical history			
Illness	0.9 (1.7)	1 (1.8)	0.03*
Other infections	0.7 (1.7)	1 (1.7)	0.04*
Non- infectious-diseases	0.8 (1.6)	1 (1.7)	0.13
Diarrhoea	0.9 (1.7)	1 (1.9)	0.11
Hospitalised	0.9 (1.9)	1 (1.7)	0.53
Clinical signs on consultation			
Stools Watery	0.9 (1.8)	1.1 (1.5)	0.30
< 7 times/day	0.9 (1.8)	1 (1.7)	0.67
Mild dehydration	0.9 (1.7)	1 (1.7)	0.02*
Vomits in previous week	0.9 (1.8)	1 (1.7)	0.02*
Vomits in previous 24 hours	0.9 (1.7)	1 (1.9)	0.55
Fever in previous 5 days	1.2 (1.8)	1.2 (1.5)	0.001*
Diarrhoea >12 days	1 (1.8)	0.6 (1.9)	0.04
Birth weight <2500 (gram)	0.8 (1.9)	0.9 (1.8)	0.35
Pathogens			
Viruses**	0.6 (1.5)	1 (1.8)	0.007
Bacteria	0.8 (2)	1 (1.7)	0.003
Parasites	1 (2.1)	0.9 (1.7)	0.43
Rotavirus	1 (1.7)	1 (1.7)	0.69

* P <0.05, * * rotavirus excluded

5.7.2. Vitamin E concentrations

A similar approach was used to investigate the association between vitamin E concentrations and the presence or absence of risk factors for hospitalisation, as shown in table 5.9.

There was no association between most of the risk factors for hospitalisation and the geometrical mean vitamin E concentrations of the children with diarrhoea.

Children who had vomited in the week before consultation however had higher serum vitamin E concentrations than children who had not vomited (p= 0.02). Children who had watery stools in the 24 hours before admission had marginally lower serum vitamin E concentration than children without watery stools (p=0.08).

Considering the pathogens isolated from the stools, children with shigellosis seemed to have lower concentrations of vitamin E. However the differences were not statistically significant (p = 0.07).

Table 5.9. Mean (SD) serum vitamin E by variable (μmol/L)

Variable	Geometrical mean vitamin E (μmol/L) If variable present	If variable absent	P value
Family background			
Mother unhealthy	8.9 (1.7)	10.1 (1.4)	0.71
Mother age <20	8.3 (1.8)	8.9 (1.7)	0.57
Lack of piped water	8.9 (1.7)	11.5 (1.6)	0.14
Use spring/river water	8.3 (2.1)	8.9 (1.7)	0.85
Ownership of animals	8.7 (1.7)	8.9 (1.7)	0.45
Previous year medical history			
Illness	8.7 (1.7)	9.1 (1.8)	0.56
Other infections	9.3 (1.5)	8.9 (1.7)	0.94
Non-infectious diseases	7.9 (1.5)	8.9 (1.7)	0.19
Diarrhoea	8.9 (1.5)	8.9 (1.7)	0.89
Hospitalised	8.3 (1.8)	9.1 (1.7)	0.25
Clinical signs on consultation			
Stools Watery	8.9 (1.7)	11.5 (1.6)	0.08**
>7 times/day	8.7 (1.7)	9.1 (1.8)	0.31
Dehydration	8.9 (1.7)	9.1 (1.8)	0.55
Vomits in previous week	9.5 (1.7)	7.8 (1.9)	0.02*
Vomits in preceding 24 hours	9.1 (1.7)	8.7 (1.7)	0.56
Fever in last 5 days	8.9 (1.7)	8.9 (1.9)	0.77
Diarrhoea >7 days	8.7 (1.7)	10.5 (1.6)	0.14
Birth weight <2500 (gram)	9.8 (1.6)	8.9 (1.7)	0.68
Pathogens			
Viruses ***	8.3 (1.6)	8.9 (1.7)	0.41
Bacteria	8.7 (1.6)	8.9 (1.8)	0.17
Parasites	8.3 (2.1)	8.9 (2.1)	0.91
Rotavirus	8.5 (1.7)	9.1 (1.7)	0.40
Shigella	6.5 (1.9)	8.9 (1.7)	0.07*

* $P < 0.05$, ** $P < 0.10$, *** rotavirus excluded

5.7.3. Serum zinc concentration

The analysis of the association between zinc and the risk factors, identified that most of the factors reducing zinc serum concentrations were related to the characteristics associated with the acute episode as shown in table 5.10. There is a strong association between the presence of dehydration on admission and serum zinc concentrations and dehydrated children had lower concentrations (4.3 μmol/L) than non –dehydrated children (6 μmol/L, p= 0.007). Similarly children who had shorter diarrhoea episodes and lower number of stools in the 24 hours preceding admission had higher serum zinc concentrations than children with more prolonged diarrhoea episodes and higher stool frequency ($p < 0.05$). However children who were vomiting in the week before enrolment had higher mean serum zinc concentrations than children who were not vomiting ($p<0.05$). Children with watery stools had higher zinc serum concentrations than children without watery stools, however the number of children who did not have watery stools was too small to analysis these results.

The children's characteristics (birth weight, age, gender, duration of breast feeding) were not associated with alterations in serum zinc concentrations.

The mother's characteristics did not affect the mean serum zinc concentrations in the children. However in nearly all children with pathogens, children who had a pathogen identified in their stools had lower mean serum zinc concentrations than children without pathogen. However none of these differences was significant.

Table 5.10. Mean (SD) serum zinc concentrations by variable (μmol/L)

Variable	Zinc μmol/L		P value
	If variable present	If variable absent	
Family background			
Mother unhealthy	5 (2.1)	4.4 (1.9)	0.57
Mother age<20	4.1 (2.4)	5 (2.1)	0.43
Lack of piped water	4.9 (2.1)	5.2 (2)	0.66
Use spring/river water	5.8 (2)	4.9 (2.1)	0.28
Ownership of animals	4.8 (2.2)	5 (2)	0.69
Previous year medical history			
Illness	5 (2.1)	4.8 (2.1)	0.95
Other- infections e	4 (1.7)	4.9 (2.1)	0.31
Non-infectious diseases	4(2.1)	4.9(2.1)	0.34
Diarrhoea	5.1 (2.2)	4.7 (1.9)	0.61
Hospitalised	5.1 (2.1)	4.8 (2.1)	0.59
Clinical signs on consultation			
Stools Watery	5 (2.1)	2.7 (2.2)	0.01*
< 8 times in 24 hours	5.5 (2.1)	4.3 (1.9)	0.05*
Dehydration	4.3 (2)	6 (2.4)	0.007*
Vomits in previous week	5.4 (2.1)	4.2 (1.9)	0.03*
Vomits in preceding 24 hours	5 (2.1)	4.8 (2)	0.59
Fever in last 5 days	5 (2.1)	4.6 (2.1)	0.38
Diarrhoea <6 days	5.2 (2.1)	3.8 (2.1)	0.01
Birth weight <2500 (gram)	4.8 (1.9)	4.9 (2.1)	0.91
Pathogens			
Viruses**	4 (2.1)	5 (2.1)	0.20
Bacteria	4.8 (2.1)	4.9 (2.1)	0.88
Parasites	5.4 (1.9)	4.9 (2.1)	0.47
Rotavirus	5 (2.1)	4.8 (2.1)	0.50

* P<0.05, ** rotavirus excluded

5.7.4. Serum selenium concentration

The association between serum selenium concentrations and the risk factors are shown in table 5.11. Most of the factors associated with lower selenium concentrations were factors associated with the biological characteristics of the child. There was a negative association between serum selenium concentrations and the presence of dehydration, having breast-fed for < 6 months, being < 13 months old and not having piped water at home ($p<0.05$).
None of the factors related to the medical history in the previous year or the clinical signs on consultation besides dehydration were associated with the concentration of selenium in serum.

Regarding the pathogens isolated, serum selenium concentrations of children who had viruses identified in their stools were lower than other children ($p <0.01$). Children who had parasites in their stools had higher mean serum selenium concentrations than children without parasites but this was not statistically significant.

Table 5.11. Geometrical mean (SD) serum selenium concentrations by variable

Subjects	Selenium (μmol/L)		P value
	If variable present	If variable absent	
Family background			
Mother unhealthy	0.76 (1.7)	0.8 (1.5)	0.31
Mother age<20	0.68 (1.4)	0.8 (1.6)	0.24
Lack of piped water	0.76 (1.6)	0.98 (1.5)	0.03*
Use spring/river water	0.8 (1.6)	0.76 (1.6)	0.58
Ownership of animals	0.78 (1.6)	0.76 (1.6)	0.72
Previous year medical history			
Illness	0.78 (1.5)	0.74 (1.6)	0.34
Other infections	0.68 (1.4)	0.8 (1.6)	0.38
Non -infectious diseases	0.66 (1.4)	0.8 (1.6)	0.18
Diarrhoea	0.78 (1.5)	0.76 (1.6)	0.36
Hospitalised	0.8 (1.7)	0.76 (1.5)	0.65
Clinical signs on consultation			
Stools Watery	0.76 (1.6)	0.74 (1.7)	0.85
< 8 times/days	0.76 (1.6)	0.74 (1.6)	0.81
Dehydration	0.70 (1.5)	0.87 (1.7)	0.01*
Vomits previous week	0.74 (1.5)	0.86 (1.7)	0.23
Vomits in preceding 24 hours	0.78 (1.5)	0.72 (1.7)	0.12
Fever in previous 5 days	0.74 (1.5)	0.78 (1.7)	0.27
Birth weight <2500 (gram)	0.74 (1.6)	0.78 (1.6)	0.49
Age <13 months	0.72 (1.5)	0.78 (1.7)	0.01*
Pathogens			
Viruses**	0.58 (1.7)	0.78 (1.6)	0.005*
Bacteria	0.78 (1.6)	0.76 (1.6)	0.93
Parasites	0.9 (1.7)	0.76 (1.5)	0.11
Rotavirus	0.7 (1.7)	0.81 (1.6)	<0.009*

* P<0.05 ** rotavirus excluded

5.7.5. Serum copper concentration

Among risk factors for hospitalisation, children having an illness in the previous year, especially non-infectious diseases and children who were vomiting or had fever in previous 5 days before admission had lower serum copper concentrations. In contrast children who had watery stools had significantly higher copper serum concentrations. There was an association between serum copper concentrations and dehydration ($p = 0.003$). These finding are similar to those described for zinc, as the characteristics of the acute episode seemed to affect zinc and copper concentrations simultaneously.

Children who had parasites, bacteria and viruses except rotavirus had similar serum copper concentrations than children without these pathogens. However children with rotavirus had lower serum copper levels than children with other pathogens ($p=0.04$).

The serum copper concentrations by the selected variables are shown in table 5.12.

Table 5.12. Geometrical mean (SD) serum copper concentrations by selected variables

Subjects	Copper (μmol)/L		P value
	If variable present	If variable absent	
Family background			
Mother unhealthy	1.47 (1.8)	1.7 (1.8)	0.17
Mother age<20	1.58 (1.6)	1.47 (1.9)	0.53
Lack of piped water	1.47 (1.9)	1.28 (1.5)	0.39
Use spring/river water	1.38 (1.6)	1.47 (1.9)	0.76
Ownership of animals	1.51(1.8)	1.44 (1.9)	0.90
Previous year medical history			
Illness	1.41 (1.8)	1.58 (1.9)	0.08**
Other infections	0.93 (1.9)	1.51 (1.8)	0.007*
Non-infectious diseases	1.1 (1.9)	1.51 (1.8)	0.13
Diarrhoea	1.38 (1.7)	1.51(1.9)	0.12
Hospitalised	1.58 (1.9)	1.44 (1.8)	0.41
Clinical signs on consultation			
Stools Watery	1.51 (1.8)	0.78 (2.9)	0.04*
< 8 times/day	1.2 (1.8)	1.44 (1.9)	0.53
Dehydration	1.34 (1.9)	1.73 (1.8)	0.003*
Vomits in previous week	1.38 (1.8)	1.73 (1.8)	0.03*
Vomits in previous 24 hours	1.44 (1.8)	1.54 (1.9)	0.50
Fever in last 5 days	1.38 (1.9)	1.90 (1.7)	0.01*
Diarrhoea <6 days	1.47 (1.9)	1.51 (1.5)	0.81
Birth weight <2500 grams	1.47 (1.7)	1.47 (1.9)	0.80
Pathogens			
Viruses***	1.58 (2)	1.47 (1.8)	0.75
Bacteria	1.62 (1.8)	1.44 (1.9)	0.35
Parasite	1.58 (2.1)	1.47 (1.8)	0.35
Rotavirus	1.28 (1.9)	1.58 (1.8)	0.04*

* P<0.05, ** P <0.10, *** rotavirus excluded

A summary of the risks factors examined and their association with vitamin A and E, selenium, zinc and copper is shown in table 5.13.

Table 5.13. Risks factors and their association with micronutrients

Subjects	Vitamin A	Vitamin E	Selenium	Zinc	Copper
Family background					
Mother unhealthy	-	-	-	-	-
Mother age<20	-	-	-	-	-
Lack of piped water	-	-	+	-	-
Use spring/river water	-	-	-	-	-
Ownership of animals	-	-	-	-	-
Previous year medical history					
Illness	+	-	-	-	±
Other infections	+	-	-	-	+
Non-infectious diseases	-	-	-	-	+
Diarrhoea	-	-	-	-	+
Hospitalised	-	-	-	-	+
Clinical signs on consultation					
Stools Watery	-	±	-	+	+
< 8 times/day	-	-	-	+	-
Dehydration	+	-	+	+	+
Vomits in previous week	+	+	-	+	-
Vomits in previous 24 hours	-	-	-	-	-
Fever in last 5 days	+	-	-	-	+
Diarrhoea <6 days	+	-	-	-	-
Age <13 months	-	-	+	-	-
Birth weight <2500 (gram)	-	-	-	-	-
Pathogens					
Viruses*	+	-	+	-	-
Bacteria	+	-	-	-	-
Parasite	-	-	-	-	-
Rotavirus	-	±	+	-	+

+ P< 0.05, ± P<0.10, * rotaviruses excluded

5.8. Micronutrients and clinical signs of the diarrhoea episode

The concentration of micronutrients in serum in children with and without watery stools is shown in table 5.14 and the association between the characteristics of the acute diarrhoea episode and the micronutrient concentrations was analysed as shown in table 5.15.

Children who had watery stools in the preceding 24 hours of consultation had a higher geometrical mean serum copper, zinc and selenium concentrations than children who did not have watery stools, however serum selenium concentrations was not statistically significant (p=0.85). In contrast, children who had watery stools in the 24 hours before admission had a lower serum vitamins E and A than children who did not have watery stools. However only vitamin E was marginally significant (p =0.08). The number of children who did not have watery stools was small, it is then surprising zinc and copper concentrations still reached statistical significance.

5.8.1. Duration of the diarrhoea episodes

The mean (SD) serum micronutrients concentrations by duration of diarrhoea are shown in table 5.15 and figure 5.7. The mean serum vitamin A was higher in children without diarrhoea than children with diarrhoea. However there was no association between the duration of the episode and vitamin A concentrations. The values observed for all the children are shown in figure 5.7. Similarly children without diarrhoea had higher mean serum vitamin E concentrations than children with diarrhoea, but this was not statistically significant. The mean serum vitamin E concentrations did not change with the duration of the episodes.

Among the children with diarrhoea, the mean was also associated with the duration of the diarrhoea episode before enrolment. The mean serum zinc concentration was highest (5.8 μmol/L) in children who had diarrhoea for between 24 and 72 hours, slightly lower (5.4 μmol/L) in children with diarrhoea less than 24 hours and lowest (4.1 μmol/L) in children whose episodes had lasted for more than 72 hours ($p< 0.01$). The distribution of the zinc concentration values by diarrhoea duration is shown in figure 5.7.

Children with diarrhoea had lower selenium concentration than children without diarrhoea ($p<0.01$). However, the geometrical mean of selenium in children with short episodes (<24 hours) was lower than the mean of children with more prolonged diarrhoea duration. The mean serum selenium concentration was highest (0.9 μmol/L) in children with diarrhoea lasting for between 24 and 72 hours followed by children with diarrhoea lasting for more than 72 hours.

The mean serum copper was lower in children with diarrhoea than in children without diarrhoea, but it was not statistically significant. There was no association between the duration of the acute diarrhoea episode and the level of copper as shown in figure 5.7.

Table 5.14. Serum concentrations of micronutrients in children with and without watery stools

	Watery stools		Non watery stools		
	N	Mean (SD)μmol/L	N	Mean (SD) μmol/L	P value
Vitamin A	246	0.91(1.8)	9	1.1 (1.5)	0.30
Vitamin E	226	8.9 (1.7)	9	11.5 (1.6)	0.08**
Selenium	241	0.76 (1.6))	9	0.74 (1.7))	0.85
Zinc	220	5 (2.1)	8	2.7 (2.2)	0.01*
Copper	233	1.51 (1.8)	8	1.19 (1.16)	0.04*

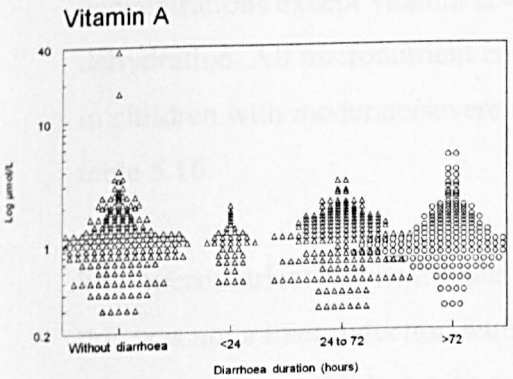
N = number, * P<0.05, ** P < 0.10

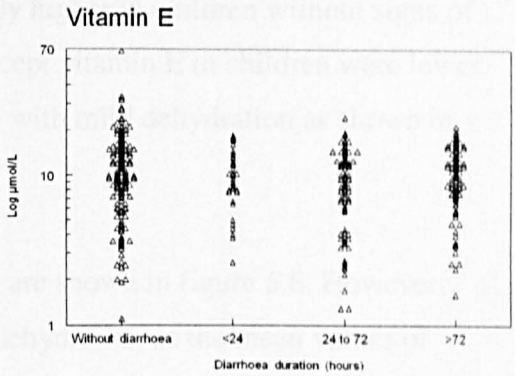
Table 5.15. Concentration of micronutrients by duration of the diarrhoeal episode

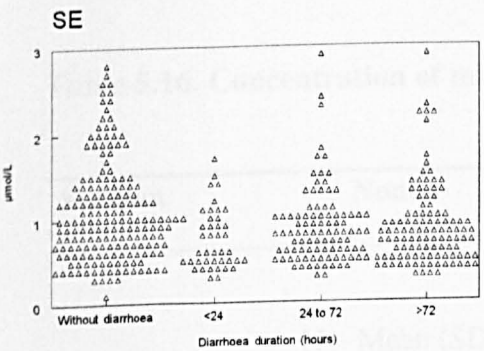
	<24 hours		24 to 72 hours		> 72 hours		Without diarrhoea		P value
	N	Mean (SD)	N	Mean (SD)	N	Mean SD)	N	Mean (SD)	
Vitamin A	41	0.97 (1.5)	98	0.91 (1.9)	113	0.93 (1.8)	196	1 (2)	0.16
Vitamin E	37	9.1 (1.6)	95	8.3 (1.9)	100	9.5 (1.7)	175	9.8 (1.9)	0.11
Selenium	41	0.7 (1.5)	96	0.90 (1.5)	118	0.77 (1.6)	196	1 (1.8)	<0.01
Zinc	37	5.6 (2.2)	89	5.8 (1.3)	101	4.1 (1.6)	186	4.3 (1.4)	<0.01
Copper	39	1.28 (2.1)	93	1.4 (1.7)	109	1.62 (1.8)	178	1.47 (1.9)	0.60

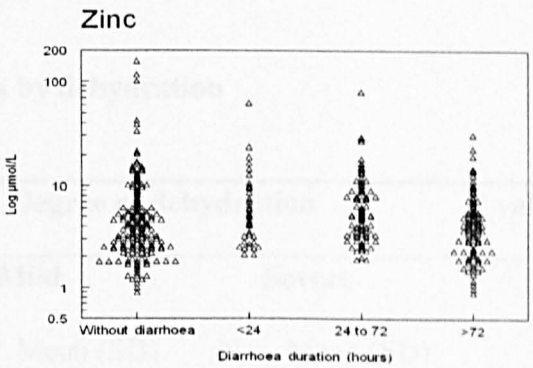
N= number

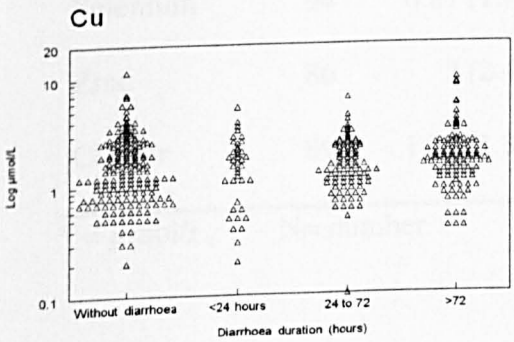
Figure 5.7. Serum vitamins A and E, selenium, zinc and copper concentrations by diarrhoea duration











5.8.2. Dehydration

The micronutrient concentrations were also modified by the degree of dehydration at the time of admission to the hospital. The geometrical mean of all micronutrient concentrations except vitamin E were significantly higher in children without signs of dehydration. All micronutrient concentrations except vitamin E in children were lower in children with moderate/severe than in children with mild dehydration as shown in table 5.16.

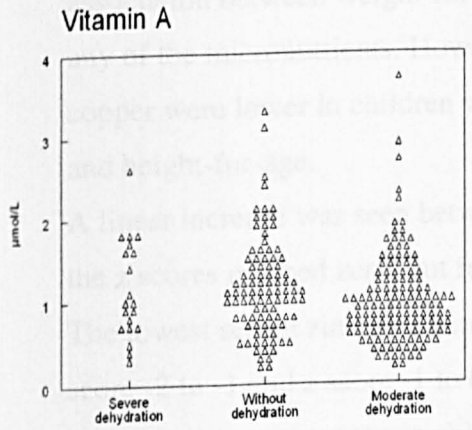
The micronutrient concentrations by dehydration are shown in figure 5.8. However, this was not a liner reduction with the degree of dehydration as the mean values of micronutrients in children with moderate dehydration were similar to those with severe dehydration.

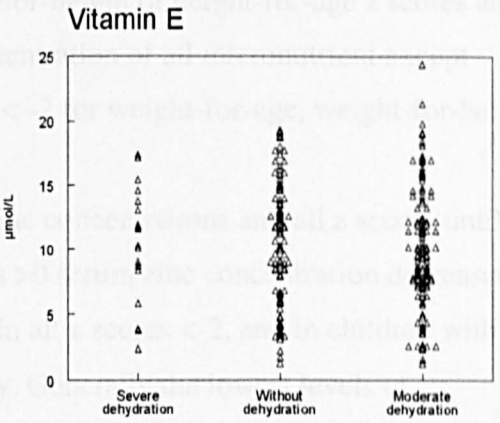
Table 5.16. Concentration of micronutrients by dehydration

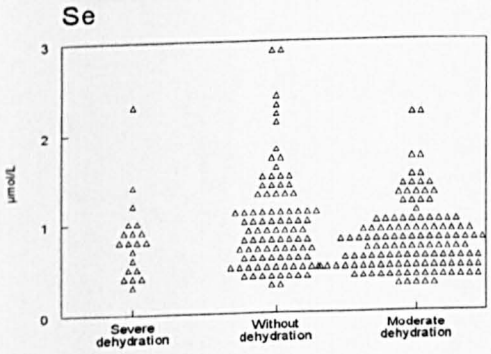
Subject	None		Degree of dehydration				P value
			Mild		Severe		
	N	Mean (SD)*	N	Mean (SD)	N	Mean (SD)	
Vitamin A	96	1 (1.7)	136	0.87 (1.8)	20	1 (1.7)	0.02
Vitamin E	90	9.1 (1.8)	123	8.9 (1.7)	19	9.3 (1.7)	0.55
Selenium	94	0.87 (1.7)	133	0.7 (1.5)	20	0.78 (1.5)	0.01
Zinc	86	2 (2.4)	121	4.3 (2)	18	5.1 (1.7)	0.007
Copper	89	1.73 (1.8)	130	1.34 (1.9)	19	1.31 (1.7)	0.003

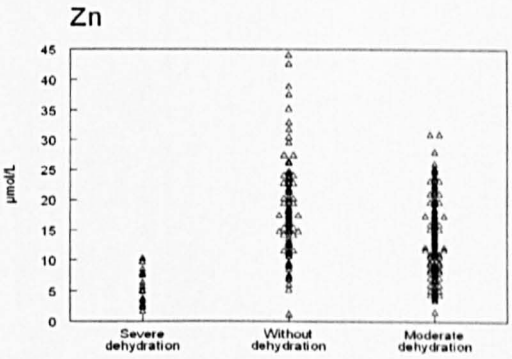
* = μmol/L, N= number

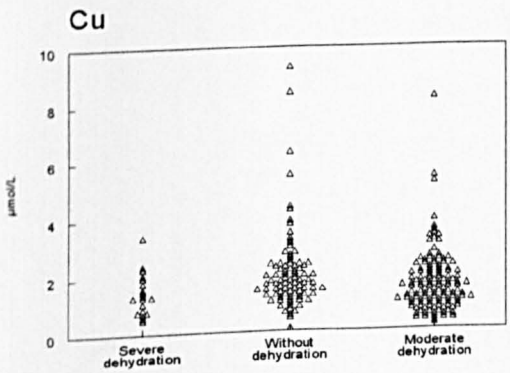
Figure 5.8. Serum vitamin A and E, copper, selenium and zinc concentrations by dehydration











5.9. Micronutrient and anthropometry

The mean concentrations of the micronutrients by weight-for-age, weight-for-height and height-for-age z scores are shown in table 5.17. Surprisingly, there was no clear association between weight-for-age, weight-for-height or height-for-age z scores and any of the micronutrients. However the concentration of all micronutrient except copper were lower in children with z scores < -2 for weight-for-age, weight-for-height and height-for-age.

A linear increase was seen between serum zinc concentrations and all z scores until the z scores reached zero, but for all z scores >0 serum zinc concentration decreased. The lowest serum zinc concentrations were in all z scores <-2, and in children with z score -2 to -1 and z score -1 to 0 respectively. Generally the lowest levels of micronutrients were seen in children with z score <-2. Serum vitamin E concentrations was lower in children with z score between 1 to 0 and >0, however this was not statistically significant.

Table 5.17. Serum concentrations of micronutrients in children by nutritional z scores

	Z score <-2		Z score -2 to -1		Z score -1 to 0		Z score > 0		P value
Weight-for-height	N	Mean (SD)	N	Mean (SD)	N	Mean (SD)	N	Mean (SD)	
Vitamin A	62	0.87 (2)	45	1 (1.7)	42	1 (1.7)	106	0.91 (1.7)	0.44
Vitamin E	53	9.8 (1.7)	45	9.1 (1.9)	39	9.8 (1.6)	98	8.1 (1.7)	0.17
Selenium	61	0.76 (1.5)	45	1.23 (1.7)	41	0.85 (1.6)	103	0.72 (1.5)	0.25
Zinc	58	4.4 (2.2)	42	4.9 (2)	37	5.6 (2.2)	90	5 (1.9)	0.35
Copper	50	1.6 (1.6)	41	1.6 (2.2)	40	1.7 (1.7)	97	1.28 (1.8)	0.15
Weight-for-age									
Vitamin A	70	0.85 (2)	77	0.97 (1.7)	60	1 (1.7)	48	0.93 (1.7)	0.54
Vitamin E	62	10 (1.5)	72	9.3 (1.6)	57	8.7 (1.9)	44	7.2 (1.8)	0.06
Selenium	69	0.76 (1.6)	76	0.85 (1.6)	59	0.70 (1.5)	46	0.74 (1.6)	0.11
Zinc	65	4.7 (2)	68	4.9 (2.2)	54	6 (2.1)	40	4.1 (1.7)	0.06
Copper	67	1.5 (1.9)	71	1.54 (1.7)	58	1.44 (1.7)	44	1.41 (2.1)	0.89
Height-for-age									
Vitamin A	51	0.8 (1.7)	60	1 (2.1)	79	1 (1.6)	66	0.91 (1.7)	0.12
Vitamin E	48	9.3 (1.5)	56	9.3 (1.7)	70	8.5 (1.8)	61	8.5 (1.9)	0.94
Selenium	50	0.78 (1.6)	59	0.76 (1.6)	77	0.76 (1.6)	65	0.78 (1.6)	0.91
Zinc	45	4.6 (1.9)	56	4.9 (2.1)	68	6.2 (2)	59	4.1 (2.1)	0.01
Copper	49	1.28 (2)	57	1.51 (1.7)	71	1.54 (1.7)	64	1.5 (2)	0.74

N= number

The proportion of children who were below the accepted cut off point for vitamin A and zinc by z scores is shown in table 5.18.

Table 5.18. Proportion of children who were below the accepted micronutrient cut off point by z score

	Children with deficiency/Children in the group (%)			
	< -2	**-2 to-1**	**-0.99 to 0**	**>0**
		Weight-for-age z score		
Vitamin A < 0.70 μmol/L	24/69 (35%)	16/61 (30%)	18/60 (30%)	13/48 (27%)
Zinc <9.94 μmol/L	56/65 (86%)	55/68 (81%)	39/54 (72%)	39/40 (97%)
		Weight-for-height z score		
Vitamin A < 0.70 μmol/L	20/62 (32%)	11/44 (25%)	9/42 (19%)	32/106 (30%)
Zinc <9.94 μmol/L	50/66 (76%)	34/50 (68%)	29/45 (64%)	76/104 (73%)
		Height-for-age z score		
Vitamin A < 0.70 μmol/L	17/50 (34%)	15/50 (25%)	21/79 (27%)	18/66 (27%)
Zinc <9.94 μmol/L	40/45 (89%)	46/56 (82%)	51/68 (75%)	53/59 (89%)

The proportion of children who were zinc or vitamin A deficient in children with z scores <-2 for weight-for-age, weight-for-height and height-for- age were higher than children with z scores between -2 and -1 and those who had z scores between -1 to 0. Among all z scores, there was only a negative linear growth between vitamin A deficiency and weight-for-age z score.

5.10. Micronutrient concentrations by pathogen

The concentration of the micronutrients was examined according to the pathogen identified in the stool samples of the children with diarrhoea. The mean concentration

of the micronutrients in children who had rotavirus, other viruses besides rotavirus, bacteria and parasites are shown in table 5.19.

Children, who had diarrhoea due to viruses other than rotavirus, had the lowest mean serum zinc, selenium and vitamin A concentrations compared to children with bacteria, parasites, rotavirus and those without pathogens in their stools. In contrast the highest mean serum copper concentrations was seen in this group of children.

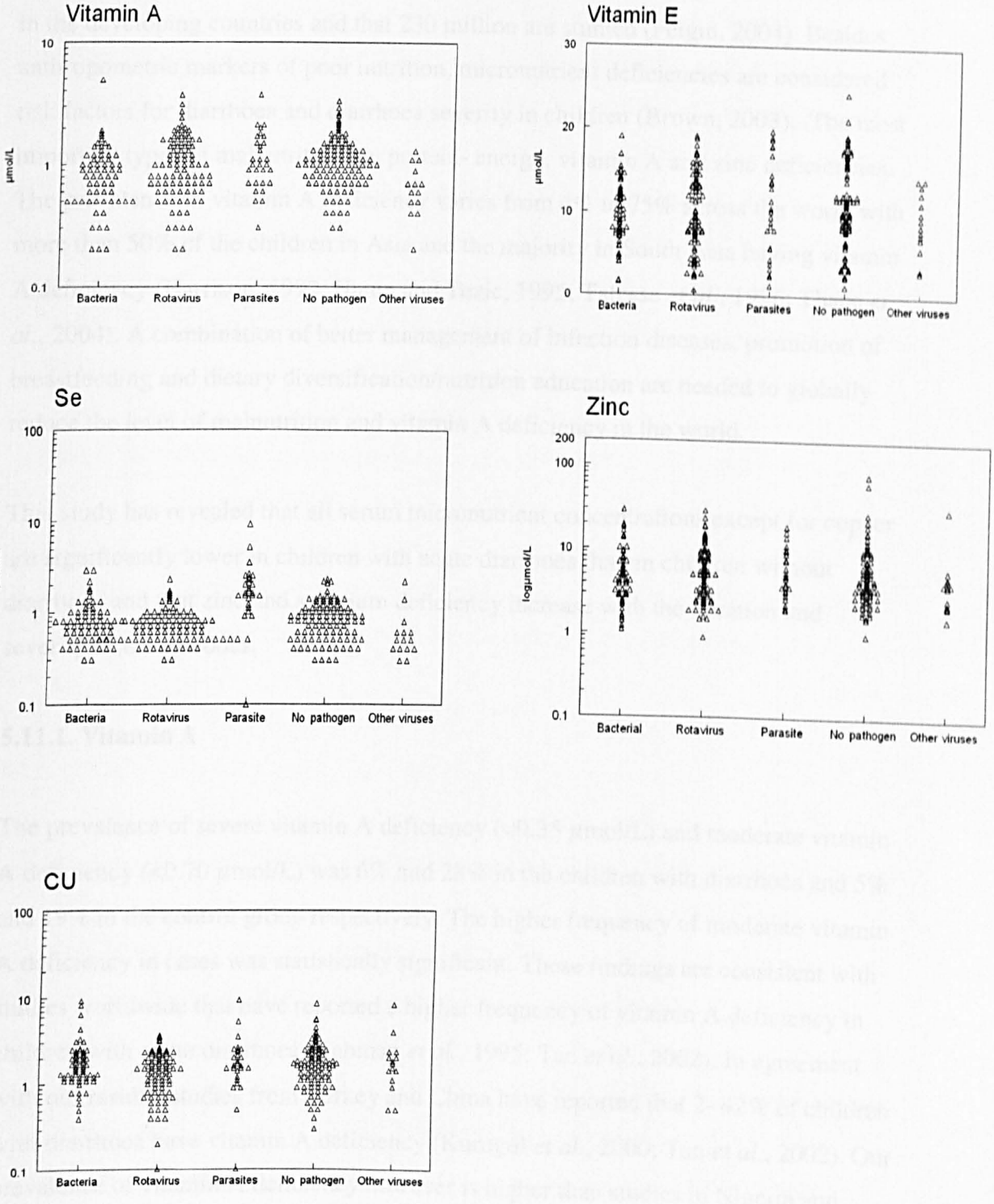
Children infected with parasites had the highest concentrations of all the micronutrients when compared to children with diarrhoea due to rotavirus. The lowest mean serum vitamin E was seen in children with diarrhoea due to bacteria. The distribution of vitamins A and E, zinc, selenium and copper are shown in figure 5.9.

Table 5.19. Mean serum micronutrient concentrations (μmol/L) by pathogens

	Parasites		Bacteria		Rotavirus		Other viruses		No pathogen	
	N	Mean (SD)	N	Mean (SD)	N	Mean (SD)	N	Mean (SD)	N	Mean (SD)
Vitamin A	31	1 (2.1)	63	0.8 (2)	90	0.93 (1.8)	14	0.64 (1.5)	103	1 (1.6)
Vitamin E	26	8.3 (2.1)	58	8.7 (1.6)	84	8.5 (1.7)	14	8.3 (1.6)	92	9.3 (1.7)
Zinc	25	5.4 (1.9)	57	4.8 (2.1)	82	5 (2.1)	13	4 (2.1)	91	5.1 (2.1)
Selenium	30	0.9 (1.7)	63	0.78 (1.6)	89	0.7 1.5)	15	0.65 (0.49)	91	0.81 (1.6)
Copper	27	1.58 (2.1)	59	1.62(1.8)	85	1.28 (1.9)	15	1.58 (2)	98	1.6 (1.8)

N= number

Figure 5.9. Micronutrients concentrations by pathogens



5.11. Discussion

The WHO estimates that 174 million children below 5 years of age are malnourished in the developing countries and that 230 million are stunted (Feigin, 2004). Besides anthropometric markers of poor nutrition, micronutrient deficiencies are considered risk factors for diarrhoea and diarrhoea severity in children (Brown, 2003). The most important types of malnutrition are protein- energy, vitamin A and zinc deficiencies. The prevalence of vitamin A deficiency varies from 4% to 75% across the world with more than 50% of the children in Asia and the majority in South Asia having vitamin A deficiency (Harrison, 1992; Hatun and Tezic, 1995; Tafesse *et al.*, 1996; Thane *et al.*, 2004). A combination of better management of infection diseases, promotion of breastfeeding and dietary diversification/nutrition education are needed to globally reduce the level of malnutrition and vitamin A deficiency in the world.

This study has revealed that all serum micronutrient concentrations except for copper are significantly lower in children with acute diarrhoea than in children without diarrhoea and that zinc and selenium deficiency increase with the duration and severity of the episodes.

5.11.1. Vitamin A

The prevalence of severe vitamin A deficiency (<0.35 μmol/L) and moderate vitamin A deficiency (<0.70 μmol/L) was 6% and 28% in the children with diarrhoea and 5% and 19% in the control group respectively. The higher frequency of moderate vitamin A deficiency in cases was statistically significant. These findings are consistent with studies worldwide that have reported a higher frequency of vitamin A deficiency in children with acute diarrhoea (Rahman *et al.*, 1995; Tan *et al.*, 2002). In agreement with our results, studies from Turkey and China have reported that 2- 42% of children with diarrhoea have vitamin A deficiency (Kurugol *et al.*, 2000; Tan *et al.*, 2002). Our prevalence of vitamin A deficiency however is higher than studies in Nigeria and Bangladesh that have reported prevalences of between 0% and 3-10% in children with diarrhoea (Agarwal *et al.*, 1996; Akinyinka *et al.*, 2001). However consistent with the

majority of studies, the mean serum vitamin A concentration was significantly lower than that in children without diarrhoea (Ashour et al., 1999).

Several factors were associated with an increased risk for vitamin A deficiency. These included the presence of dehydration, a history of vomiting in the previous week, illness in the previous year, fever before consultation, diarrhoea for more than 12 days duration and having diarrhoea due to viruses other than rotavirus and bacteria. For example the mean serum vitamin A concentrations of children with diarrhoea due to viruses other than rotavirus was 0.64 μmol/L which was significantly lower than mean of children with diarrhoea due to other pathogens (1μmol/L). These findings are in agreement with a study from China that reported a higher prevalence of vitamin A deficiency in children with acute respiratory infections, fever and diarrhoea before the blood collection. However, not all studies have reported these associations and studies from Brazil and India documented that no association was seen between serum vitamin A depletion and a history of previous hospitalisation and duration of diarrhoea (Agarwal *et al.*, 1996; Ferraz *et al.*, 2004; Tan *et al.*, 2002). These apparently contradictory studies may reflect the prevalence of vitamin A in the general population and the severity of the acute episodes. In consistency with studies worldwide, we did not find any association between gender and age of our children and serum vitamin A.

5.11.2. Zinc

Numerous studies have investigated the association between diarrhoea and low serum zinc concentrations. Despite numerous studies however, there is no universally accepted cut off point for zinc deficiency. Epidemiological studies have reported a prevalence of zinc deficiency (defined as serum zinc deficiency <9.14 μmol/L or <9.94 μmol/L) from 7% to 78% of the children across the world (Fisberg *et al.*, 1984; Lind *et al.*, 2003). The cut off point used in our study was <9.94 μmol/L following the recommendation of Hotz et al (2003). The prevalence of zinc deficiency was 82% in cases and this prevalence was very similar to the controls (81%). The very high prevalence of zinc deficiency in our study is one of the highest prevalence of deficiency reported in the region and the world. However, consistent with of our study, two studies from Burkina Faso and Indonesia have reported prevalence rates of 72% and 78% zinc deficiency in children without diarrhoea respectively. A recent

study in Turkey reported a prevalence of zinc deficiency in 50% of the children with diarrhoea but that zinc deficiency was also highly prevalent in healthy controls (Polat et al., 2003).There is only one study from Iran previous to our study. This study reported a prevalence of 65% zinc deficiency in junior high school girl students in the capital, Tehran (Mahmoodi and Kimiagar, 2001). However more studies are required to approach the impact of zinc deficiency in Iran and the region. One of the possibilities for the high prevalence of zinc deficiency in Iran might be related to the geographic characteristics and mountains, which surround the country. Iodine deficiency for example is also one of the most important health problems in Iran. Zinc deficiency may be due to low consumption of animal products in low socio-economic sectors and poor content of this micronutrient in soil and food sources. These patterns could be attributed to differences in zinc content of soil and/or differences in dietary habits of the families. The higher mean serum zinc in controls than the cases, is also consistent with studies elsewhere that have reported lower serum zinc in children with diarrhoea than in children without diarrhoea. Furthermore, several studies have documented that low serum zinc concentration is a risk factor for diarrhoea and diarrhoea severity (Bitarakwate *et al.*, 2003; Van Biervliet *et al.*, 2003; Villalpando *et al.*, 2003). The mean serum zinc concentrations in our children was higher than mean serum zinc concentrations in children with persistent diarrhoea in Bitarakwate's study (2003) and the mean serum zinc concentrations in controls was lower than the mean serum zinc of healthy controls in the same study and to the serum zinc concentration of children with *G. lamblia* in Turkey (Bitarakwate *et al.*, 2003; Ertan *et al.*, 2002; Van Biervliet *et al.*, 2003). While there was no association between gender and age of children and serum zinc concentrations in children with and without diarrhoea, a negative association was observed between age and serum zinc in cases, with children younger than 13 months of age having lower serum zinc than children > 13 months. Children with watery stools had significantly higher zinc serum than children without watery stools. However the number of children who did not have watery stools was too small to interpret the results.

In contrast to our study, Strand *et al* reported (2004) that children with signs of dehydration in Nepal had higher mean serum zinc concentrations than children without signs of dehydration (Strand et al., 2004). Children with signs of dehydration had lower mean serum zinc concentrations. However, consistent with other findings of the Nepal study the mean serum zinc concentration was lower in children who had

bloody diarrhoea than children who did not have bloody diarrhoea. In Brazil, children with signs of dehydration had lower serum zinc concentrations. This was similar to our findings, confirming that diarrhoea might leads to zinc depletion. Studies have also reported lower serum zinc concentrations in children with persistent diarrhoea than in children with acute diarrhoea (Sachdev *et al.*, 1988; Strand *et al.*, 2004). Children with diarrhoea for more than 3 days have higher mean serum zinc than children with diarrhoea lasting for 24 –72 and <24 hours. This association of the serum zinc concentrations with diarrhoea duration in our study is in agreement with this study from Brazil (Castillo-Duran et al., 1988) which documented a higher micronutrient depletion in children with longer diarrhoea episodes.
Our study revealed that serum zinc concentrations was significantly lower in children with a history of vomiting in the week before consultation and in children who had watery diarrhoea ≥ 6 days.

5.11.3. Vitamin E

Two hundred and thirty five and 175 serum samples were tested from cases and controls respectively. Children with diarrhoea had a significantly lower mean serum vitamin E than controls. The frequency of severe vitamin E deficiency was significantly higher in cases than in controls (OR =1.48, 95 CI 0.98 - 2.47, p= 0.06). Mild to severe vitamin E deficiency was also significantly higher in cases than in controls (OR = 1.96, 95 CI 1.3 –3.42), p= 0.01). The lower mean serum vitamin E concentrations in cases is in agreement with studies from Pakistan and Egypt that have reported lower vitamin E concentrations in malnourished children than in healthy controls, however the study design of those studies was not similar to our study (Ashour *et al.*, 1999; Smit *et al.*, 1997). The serum concentrations of vitamin E was not affected by gender, although female controls had a slightly higher mean than males. Two community-based studies from Turkey and Cameroon have reported that vitamin E deficiency was present in 22% and 66% of their subjects respectively. In the latter study the prevalence of vitamin E deficiency varied from one village to another and the result of a high prevalence of vitamin E deficiency in our study is understandable (Gouado *et al.*, 1998; Wetherilt *et al.*, 1992). In children with diarrhoea, the lowest and highest mean serum vitamin E were seen in children > 36

and < 13 months respectively. In controls there was a negative association between the mean serum vitamin E concentrations and age, but this was not significant. However this lack of clear pattern of vitamin E concentration and gender and age was also seen in Venezuela and Thailand (Brunetto *et al.*, 1999; Sripanidkulchai *et al.*, 2003). Children who had watery stools, a history of vomiting in the week before consultation and children who were infected with *Shigella* had lower mean vitamin E than children without these factors. However the only significant difference was seen in children who were vomiting in the previous week ($p<0.05$) and the two other factors were marginally significant ($p= 0.07$ and 0.08). The mean of serum vitamin E concentrations in our study was less than half of the mean serum vitamin E reported in samples from Finland in children with juvenile chronic arthritis and healthy controls. In Iran, normal serum vitamin E was reported from 15 volunteer medical students in Tehran (Honkanen *et al.*, 1990; Meraji *et al.*, 1997). There are very few studies that have focused on diarrhoea and vitamin E. In Singapore, no vitamin E deficiency was observed in the general population (Hughes et al., 1998). Despite the vitamin E deficiency observed in our children there is scanty information to interpret the clinical relevance of these findings.

5.11.4. Selenium

The mean serum selenium concentrations of the children with diarrhoea 251was significantly lower than in the 196 controls ($p<0.01$). The prevalence of selenium deficiency was also significantly higher in cases, (66%) than in controls (47%). There was no association between serum selenium and gender and selenium concentrations consistently increased with age in both children with and without diarrhoea. This relationship between age and selenium that we observed is in agreement with reports of serum selenium of 1010 healthy children in Germany which observed a similar pattern (Muntau et al., 2002).This increase may be due to the increased availability of selenium in the diet of older children. Considering effect of the duration of diarrhoea on serum selenium, we observed a positive effect between serum selenium and diarrhoea duration. This has been reported in children with prolonged diarrhoea in Brazil (Castillo-Duran et al., 1988). However our study did not find a consistent trend, although children with shorter diarrhoeal duration had lower selenium concentrations

than children with 24-72 hours duration. Children with diarrhoea for > 72 hours also had low selenium.

In Turkey there was no association between vomiting and dehydration and serum selenium in children with diarrhoea (Olmez et al., 2004), and our selenium values in children with diarrhoea was similar to the mean serum selenium of school children in Turkey. However our controls' mean serum selenium was higher than the target population of that study (Piechotowski et al., 2002). Selenium levels in the population vary by geographical areas. For example, selenium levels have decreased over several decades in Europe and a study in Ecuador found high background levels, probably reflecting a high environmental and dietary intake (Koyanagi et al., 2004).

Although the effect of selenium on diarrhoea and other diseases has been investigated by many studies, no study has focused on the association between selenium status and particular viruses. Only one experimental study revealed that the host selenium status selectively influences susceptibility to experimental viral myocarditis (Gomez et al., 2001) and the clinical relevance of our findings is still unclear. The mean serum selenium concentrations of our children who had viruses and notably children infected with rotavirus were significantly lower than mean serum selenium of children who had diarrhoea due to other pathogens and of children in whom pathogens were not identified. The lack of studies about the effect of enteropathic viruses and selenium makes it difficult to contextualise our data and more studies are needed to investigate change of selenium in gastroenteritis of viral and bacterial origin (Sammalkorpi et al., 1988).

5.11.5. Copper

Two hundred and forty one and 178 serum samples were examined to compare serum copper concentrations in cases and controls respectively. The mean serum copper concentration was similar in both groups. No study has previously investigated the difference between serum copper concentrations in children with and without diarrhoea. A few studies from Egypt and Brazil have described copper concentrations in children. We observed higher serum copper in children with higher z scores for weight-for-age, weight-for-height and height-for-age which is consistent with the study from Egypt that reported higher serum copper in apparently healthy controls than malnourished children or children with bronchitis and asthma (Ashour et al.,

1999). Similarly, the study from Brazil concluded that acute diarrhoea leads to copper depletion and lower plasma levels, although this study had a different study design, making the comparison of these studies difficult (Castillo-Duran et al., 1988). Serum copper levels did not alter by gender which is different to the Benes and Alarcon studies in the Czech and Venezuelan populations who reported higher serum copper in adolescent females than males (Alarcon *et al.*, 1997; Benes *et al.*, 2000). Furthermore, we observed an association between the serum copper concentration and age in our controls. Conversely in Benes' and Alarcon's studies, they observed a negative association between serum copper and age in adult males. However their study design and target populations were dissimilar and again this makes comparisons difficult to interpret. The mean serum copper concentrations in cases and controls were lower than the mean serum copper of the Turkey study on children with Giardiasis and apparently healthy controls (3.1μmol/L and 2.35 μmol/L respectively). There was a positive association between serum copper and the duration of diarrhoea, with the lowest mean serum copper concentrations occurring in children with diarrhoea < 24 hours and the highest in children with diarrhoea > 3 days which is different to an Indian study (Sachdev et al., 1989) that reported higher serum copper concentrations in children with acute diarrhoea than children with chronic diarrhoea and healthy controls. The mean serum copper concentration was lower in children with a history of non-infectious diseases, vomiting or fever in the previous week, those with rotavirus and children with signs of dehydration. We could not find any study that had investigated the association between rotavirus and serum micronutrients to compare our results. No clear trends in the serum copper concentrations were observed with the duration of diarrhoea, history of hospitalisation and a history of diarrhoea in the previous year.

Overall, the factors more often associated with micronutrient deficiencies were related to the characteristics of the clinical episode. For example, the presence of watery diarrhoea was associated with changes in vitamin E, zinc and copper concentration. The presence of dehydration modified selenium, zinc and copper levels and a history of vomits in the week before consultation modified vitamin A and E and zinc concentrations. Viruses were associated with low vitamin A and selenium, while rotavirus specifically modified selenium and copper. These associations have not been described before and merit further study.

The measurement of micronutrients during an acute infectious event can be misleading as many of these elements behave like acute phase reactants. However, several studies have described that children who are deficient (or have low serum levels due to their redistribution to other organs) are at a higher risk of subsequent diarrhoea in the ensuing months. Most of these studies however have only measured one micronutrient at a time, and the cross sectional study presented in this chapter indicates that these deficiencies often occur at the same time. The hypothesis that micronutrient deficiencies resulting from an acute episode predispose to further diarrhoea episodes will be examined in the next chapter.

Some studies have reported an association between micronutrient concentrations and age, especially for zinc and selenium. In our study, hospitalised children with acute diarrhoea had a lower mean (SD) age 15.2 (12.1) than hospitalised children without diarrhoea 27.6 (12.2), ($p < 0.01$). The higher mean age of these controls could be a possible confounder and should be considered a limitation of the study requiring further analysis. In a subsequent study, the mean (SD) age in hospitalised children with diarrhoea and controls should be similar.

Chapter 6

The effect of diarrhoea -associated micronutrient deficiencies on the risk of subsequent diarrhoea in children

6.1. Introduction

The introduction to the WHO report on nutrition and infection, published in 1965, stated:

> The concept that nutrition could make man more susceptible to infection disease and also alter the course and outcome of the resulting illness has long been current in the history of medicine and public health. Circumstantial evidence is plentiful, principally based on clinical experience. Well-controlled observations have been few, and hence clear proof in support of the concept has been slow to accumulate. It has been much easier to demonstrate that infection is often directly responsible for lowering the state of nutrition.

Nonetheless, globally, infectious diseases are responsible for the death of 10 million children younger than 5 years of age each year, and more than one half of these deaths are associated with malnutrition. This problem has been termed the "silent genocide" by WHO and is the most under reported health problem facing humans (Feigin, 2004).

6.2. Methodology

6.2.1 Objective

To describe the effect of diarrhoea -associated micronutrient deficiencies on the risk of subsequent diarrhoea in children.

6.2.2. Study design

This was a longitudinal prospective cohort to describe the effect of micronutrient deficiencies during an acute episode of diarrhoea on the subsequent incidence of diarrhoea. Participants included children <5 years old with diarrhoea attending Hajar Hospital in Shahrekord Iran. Children were assessed on admission to the hospital as

described in chapter 2. A blood sample was collected at the time of admission to measure various micronutrients (zinc, selenium, copper, vitamin A and vitamin E). Children were then followed every fortnight for 14 weeks to establish the incidence of diarrhoea in these weeks.

6.2.3. Selection criteria

Children <5 years old admitted to the hospital with an episode of acute diarrhoea defined as (≥ 3 liquid or semi liquid stools or a single watery stool per day) were selected. For children <5 months, it was sufficient if the parents stated that the child had diarrhoea. Children were identified through the cross-sectional study described in chapter 1 and all children who lived in Chaharmahal and Bakhtiari province who were permanent residents were enrolled after informed parental consent.

6.2.4. Sample size

The sample size was calculated with Epi-info defining as the main outcome the expected cumulative incidence of acute diarrhoea episodes in zinc depleted and zinc replete participants in the 14 weeks follow up.

It was expected that 40% of the children would have low serum zinc concentrations (< 9.94 μmol/L) on enrollment. The expected cumulative incidence of diarrhoea for deficient and non-deficient children was expected to be 8 and 1.6 episodes per 12 months/child respectively with a relative risk of subsequence diarrhoea in zinc deplete children of 5. The sample size required to establish this incidence with 80% power was 162 zinc deficient and 243 zinc replete children.

6.2.5. Study variables

After informed parental consent, the parents were interviewed with the help of a trained nurse to complete a standard questionnaire, which included details of their socio-economic background, family characteristics, medical history and clinical presentation on admission. The study variables are listed in appendix 1. After discharge from the hospital, children were followed up every fortnight by telephone,

post or household visits to assess if the child had further diarrhoea episodes. The following data were collected during each visit: presence of diarrhoea or presence of vomiting and present management of the episode, duration of the new diarrhoea episode and the number of stools per day.

Parents not available during the first telephone contact or visit were given new appointments to establish the outcome. Children were dropped from the study if we were unable to make contact after 3 consecutive attempts and were classified as defaulters. In the postal follow up, a second letter was sent to the family if there was no reply to the first letter within 7 days. Home visits were made for 7% of the participants. If they were not at home at the time of the visit a message was left with a pre-stamped envelope asking to report any event of diarrhoea or vomiting.

6.2.6. Laboratory procedures

Three-ml blood samples were collected from the children whose parents had agreed to participate in the follow- up study. The blood was left to clot, centrifuged at 1500 rpm for 5 minutes and the serum was stored -20°C until transported to Liverpool.
Sera were kept in dark- coloured safety tubes for the detection of vitamins using the HLPC method, as described by Catignani et al (1983). Zinc, selenium and copper were measured by the ICP-MS method, as described by Delves et al (1997) and explained in chapter 2.

6.3. Literature review

6.3.1. Micronutrient deficiencies in children

Malnutrition increases morbidity and mortality and affects physical growth and development. Some of these effects result from specific micronutrient deficiencies (Bhan et al., 2001). Malnutrition is a major factor in the aetiology, management and prognosis of diarrhoeal disease in young children (Hambidge, 1992). Vitamin A supplementation studies conducted in Nepal and Indonesia resulted in significant reductions in the rate of childhood deaths. Aside from reducing the death rate from illnesses such as measles associated pneumonia and diarrhoea, vitamin A also

decreases the severity of the symptoms related to infectious diseases of viral origin (Barber, 1998; Malvy, 1999).

Protein -energy malnutrition and micronutrient deficiencies increase the risk of death from common diseases such as acute gastroenteritis, pneumonia and measles

It has been estimated that 12 million children <5 years old die annually due to infection and malnutrition (Caballero, 2002; Checkley *et al.*, 2002; Moore *et al.*, 2000; Popa *et al.*, 1998; Roy *et al.*, 1997; Umeta *et al.*, 2003; Youssef *et al.*, 2000). WHO has estimated that malnutrition was associated with over half of all childhood deaths in developing countries in 1995 and many several community cross sectional studies have reported that up to 65% of the children are undernourished. For example, Majumdar et al reported that nearly 50% of Pakistani children under five were stunted (Black and Sazawal, 2001; Black, 2003; Majumdar *et al.*, 1997; Roy, 1995; Tan *et al.*, 2002; Tumwine and Barugahare, 2002).

Several studies have reported a correlation between the nutritional status of the child and the risk of subsequent diarrhoea. Children with low weight-for-height, height-for-age and weight-for-age have higher frequencies of diarrhoea (Kossmann *et al.*, 2000a; Kossmann *et al.*, 2000b; Wierzba *et al.*, 2001). One review study on infection in malnourished children reported that 20% of malnourished children were affected by infections acquired in the hospital, a 25% measles fatality rate and more frequent parasites than wellnourished children (Reinert, 1993).

6.3.2. The immune system and the malnourished host

6.3.2.1. Mucosal immunity

The first barrier to potential pathogens is the physical integrity of the skin and mucous membranes. The interstitial space in the submucosa of the respiratory and gastrointestinal tracts contains IgA- secreting plasma cells. Secretory IgA, binds to the mucosa and serves as a proteolytic resistant "antiseptic paint". Diminished secretory IgA has been noted in malnutrition, which may increase host susceptibility to infection by permitting an increased penetration of infectious agents into the circulation. In addition, deficiencies in protein, vitamin A, B complex, ascorbic acid,

and zinc are associated with tissue changes that contribute to a decrease in host resistance to infection.

6.3.2.2. Humoral and cellular immunity

Numerous studies of B- cell function have been performed in patients with protein-calorie malnutrition. Serum immunoglobulins may be normal or elevated, and antibody affinity is decreased, which may explain the higher frequency of antigen complexes found in malnourished patients.

The cellular immune system appears to be the component of the immune system most affected. These children are more susceptible to tuberculosis, measles, disseminated *herpes simplex*, hepatitis and *Pneumocystis jiroveci*, which are diseases whose prevention require optimal function of the cellular immune system. In addition there is a decrease in circulating lymphocytes bearing mature T- cell markers, and an increase in null cells. Histological studies of lymphoid tissues show severe depletion of T-cells suggesting that the defect involves a failure of maturation of T- cells.
As early as 1977, Simon *et al* recognised that the thymus was an early critical barometer of nutrition (Simon et al., 1977) and severe nutritional deficiency leads to thymic atrophy and fibrotic changes with the cortex being affected earlier than the medulla (Feigin, 2004).

6.3.2.3. Phagocytosis and the complement system

Defects in phagocytosis and killing have been identified in malnourished individuals but these deficits are subtle. Research has shown that neutrophils from malnourished children have enhanced baseline adhesion; however, after stimulation with chemotactic factors, the adherence response is decreased and neutrophil chemotaxis is diminished. After nutrition recovery, the abnormal adherence is reversed and the chemotactic activity improved. Schopfer and Douglas (1977) investigated the neutrophil function of children with kwashiorkor. Chemotactic response was reduced at early intervals (30, 60, and 120 minutes) and reached values achieved by controls only after 180 minutes, which suggested an early migration defect (Schopfer and Douglas, 1977).

The proteins of the complement system appear to be sensitive to nutritional stress. Seth and Chandra (1972) noted low serum complement in patients with protein-calorie malnutrition (Seth and Chandra, 1972). Sirisinha and associates also reported low serum concentration of all complement components except C4, in malnourished children (Sirisinha et al., 1973).

6.3.3. Risk of subsequent diarrhoea after acute diarrhoea

Nutritional risk factors for diarrhoea in the developing world can be grouped as those that can be measured by anthropometry, infant and child feeding practices and micronutrient status. Children in developing countries are often observed to have both diarrhoea and malnutrition. For example in Egypt low weight-for-age and a history of suffering one episode of diarrhoea were associated with an increased risk of subsequent diarrhoea (RR= 2.1) (Wierzba et al., 2001). Severe to moderate malnutrition and cell mediated immunodeficiency are risk factors for subsequent diarrhoea after hospital discharge (Baqui *et al.*, 1993a; Baqui *et al.*, 1993b; Caballero, 2002; Fagundes-Neto and de Andrade, 1999) and up to 75% of undernourished children suffer from subsequent diarrhoea after hospital discharge (Roy et al., 1997). Similarly, there is an association between anthropometric indicators of nutritional status (weight-for-age and for-height) and the duration of illness, the severity of faecal purging and most importantly, the case- fatality rate (Kossmann *et al.*, 2000a; Kossmann *et al.*, 2000b; Wierzba *et al.*, 2001).

In addition infectious diarrhoea causes cytokine-induced malnutrition, which results from the action of pro-inflammatory cytokines such as tumour necrosis factor and interleukins 1, 6 and 8 (Chandra, 2002; Chandra and Chandra, 1986; Patwari, 1999). Considering the usually scanty resources available in less developed countries a reduction in diarrhoea – related mortality may be possible by identifying high- risk subjects and targeting them for intensive intervention.

6.3.4. Zinc, vitamin A, and selenium deficiencies and risk of subsequent diarrhoea

Micronutrients and particularly vitamin A, zinc, selenium, folic acid and iron deficiencies are very important to maintain health and their deficiencies do not only

contribute to delays in growth and development, but are also important factors in the transmission and progression of infection diseases. Trace mineral deficiencies may affect several biological functions in humans, including physical growth, psychomotor development and immunity (Abrams *et al.*, 2003; Castillo Duran *et al.*, 1982; Castillo-Duran *et al.*, 1983; Castillo-Duran *et al.*, 1987; Castillo-Duran and Uauy, 1988; Castillo-Duran *et al.*, 1988, 1990; Castillo-Duran *et al.*, 1994; Castillo-Duran *et al.*, 1995; Fisberg *et al.*, 1984; Rodriguez *et al.*, 1985; Uauy *et al.*, 1985).
A follow –up study showed that children with vitamin A deficiency had a four-fold greater risk of respiratory disease in the next 3 months than children who were not vitamin A deficient ($P < 0.01$). No relation was found however between vitamin A deficiency and diarrhoea (Bloem et al., 1990).

Vitamin A supplementation can lower a deficient child's risk of dying of infectious diseases by an estimated 23% (Pinnock, 1991). Vitamin A deficiency reduces the T-cells' ability to fight infection and decreases mucus production resulting in more bacteria being able to attach to the respiratory mucosa and in an increased susceptibility to respiratory infections (Hu *et al.*, 2001; Khan *et al.*, 2002; Pinnock, 1991; Stephenson *et al.*, 2000). Most childhood deaths in low-income settings are attributable to infections such as measles and it is reasonable to assume that the effect of vitamin A supplementation in reducing mortality in children could be attributable to a reduced incidence of infections. Despite the apparent logic of this assumption, most studies of this relationship found no effect of vitamin A supplementation on the incidence of diarrhoea. However, researchers in Ghana discovered that clinic visits and hospital admission for diarrhoea decreased in vitamin A supplemented children, even thought diarrhoeal incidence rates reminded unchanged. Thus, it appeared that vitamin A reduced the severity of illness without affecting the overall attack rate (Brown, 2003).

Epidemiological studies have reported a strong correlation between low serum concentrations of zinc or disturbed zinc metabolism and the incidence and severity of a wide range of diseases, from a common cold to wound healing in surgical patients (Black and Sazawal, 2001; Black, 2003; Hambidge and Krebs, 1995; Hambidge, 2000; Hambidge *et al.*, 2000). Zinc deficiency has been shown to be associated with atrophy of lymphoid tissues, reduced lymphocyte count and the proportions of CD4

helper T cells, (Sazawal et al., 2001), decreased lymphocyte proliferation, impaired delayed cutaneous hypersensitivity, reduced cytotoxic activity of lymphocytes and reduced natural killer cell activity (Chandra, 2002; Chandra and Chandra, 1986; Sazawal *et al.*, 2001).

A recently published pooled analyses of studies focusing on the effect of zinc on the incidence of diarrhoea observed that all studies consistently resulted in a reduction in diarrhoeal incidence of nearly 20% among zinc- supplemented children (Brown, 2003). Zinc given during an acute episode of diarrhoea reduced the duration and severity of the current episode and, if given for 14 days during and after an acute episode, reduced the incidence of diarrhoea and acute lower respiratory infections in the subsequent two to three months (Baqui *et al.*, 1993a; Baqui *et al.*, 1993b; Baqui *et al.*, 2002). The mechanisms for this protective effect could be the result of a restoration of the immune function in zinc- deficient children. However, this has not been elucidated.

6.3.5. Zinc supplementation on community based studies

Randomised double-blind community-based interventions to assess the effect of zinc supplementation on the incidence diarrhoea have been conducted. In India children aged between 6 and 41 months receiving daily doses of 10 mg of zinc for 5 days a week and children who received one dose of 50 mg zinc once a week had significantly lower diarrhoea episodes (15.8% in daily and 16.5% in the weekly groups respectively) than the placebo group (30.8%). And also the incidence of diarrhoea in the daily and weekly zinc-supplemented groups was 0.68 and 0.69 episodes child, and 3 times higher (1.67) in the placebo group (relative risk 0.41, 95% CI 0.24-0.71). Diarrhoeal incidence of < 4 day was significantly lower in the supplemented groups (Gupta et al., 2003). A summary of the effect of zinc supplementation in selected clinic trial studies is shown in table 6.1.

Table 6.1. Effect of zinc supplementation in selected clinical trials

Country	Number	ARI	Diarrhoea incidence	Duration of diarrhoea	Mortality	Stool output	Gain weight	Reference
Asia								
Pakistan	*	-	-	-	+	-	-	(Bhutta et al., 2000)
	287	-	-	+	-	+	-	(Bhatnagar et al., 2004)
Bangladesh	11881	+	+	+	+	-	-	(Baqui et al., 2002)
	-	-	-	-	-	-	+	(Khatun et al., 2001)
	301	+	+	+	-	-	-	(Roy, 1991)
	65	+	+	+	-	-	-	(Roy et al., 1999)
	111	-	-	+	-	+	-	(Roy et al., 1997)
Vietnam	146	+	+	-	-	-	+	(Ninh et al., 1996)
Africa								
Burkina Faso	685	-	+	-	NC	-	-	(Muller et al., 2003)
Ethiopia	200	+	_	-	-	-	+	(Umeta et al., 2003)
America								
Brazil	1240	-	+	+	-	-	-	(Barreto et al., 1994)
	81	-	+	+	-	-	-	(Al-Sonboli et al., 2003)
	215	+	+	-	-	-	-	(Lira et al., 1998)
Mexico	219	-	+	-	-	-	-	(Rosado et al., 1997)
Guatemala	89	+	-	+	-	-	-	(Ruel et al., 1997)
Peru	246	-	+	+	-	-	-	(Penny et al., 2004)

* Pooled analyses, ARI = acute respiratory infection, NC = not changed

6.3.6. Risk of subsequent diarrhoea in cohorts of children with nutritional deficiencies

Low birth weight (LBW <2500 mg) is now recognized as a major cause of stunting in childhood, and affects an estimated 17 million infants (16% of newborns) across the world. LBW is particularly common in south central Asia (28%), middle and Western Africa (21% and 17% respectively) (Stephenson et al., 2000). Mortality rates in children under 5 years are 2.5 times higher in those moderately underweight and 5 times higher in the severely underweight. About 50% of deaths among these children are associated with malnutrition. A review paper by Rice et al (2000) found that the strongest and most consistent relationship between malnutrition and an increased risk of death was observed for diarrhoea and acute respiratory infections (Rice et al., 2000). It was suggested that in 2000, in developing countries, 32.5% and 27% of children are stunted and underweight respectively (Stephenson et al., 2000).

Cohort studies have provided evidence that malnutrition is a major determinant of diarrhoea mortality in young children, Yoon (2000) and Moore (1997) reported that lower height-for-age, height-for-weight and weight-for-age were significant risk factors for diarrhoea and mortality in Philippino and Brazilian children (Moore *et al.*, 2000; Yoon *et al.*, 1997). In Sudan, the same anthropometric indicators were significantly and inversely associated with the risk of subsequent diarrhoea with the risk being between 1.75 to 2 times higher among children with weight-for-age z scores <-3 compared with children having a weight-for-age z score ≥ 1 and their attained height was on average 17 mm lower for children with diarrhoea (Kossmann *et al.*, 2000a; Kossmann *et al.*, 2000b). Moore et al (2000) also reported that, with nutritional improvement in children, the number of episode/child/year and duration of diarrhoea episodes decreased. For example the incidence of diarrhoea decreased from 30.8-day/child/years at the beginning of the study to 8.5 days/child/year and the incidence of diarrhoea decreased from 8.5 to 2.5 episodes per year (Moore et al., 2000).

6.3.7. Clinical trials to study the effect of micronutrient supplementation on the risk of subsequence diarrhoea

Clinical trials to assess if zinc supplementation reduces the incidence and duration of subsequent diarrhoea after an initial episode have been conducted in Asia and Latin America. Zinc supplemented children on average had a 10-24% lower mean duration of the diarrhoea episodes and 28% decrease in stool output than children who were not supplemented (Al-Sonboli *et al.*, 2003; Black and Sazawal, 2001; Black, 2003; Roy, 1995; Roy *et al.*, 1997; Rukgauer *et al.*, 1997). In several clinical trials from India, Vietnam and Mexico, zinc supplemented children had 7– 71% reduction in the incidence of diarrhoea and between 17- 35% reduction in prevalence of diarrhoea (Ninh *et al.*, 1996; Rosado *et al.*, 1997; Sazawal *et al.*, 1995).

In one double –blind randomised trial, children with zinc < 9.18 μmol/l at the time of enrollment who received zinc supplements, had a 17% lower incidence of diarrhoea than not supplemented children, whereas in children with plasma zinc < 50 μmol/l the zinc- supplemented children had a 33% lower incidence of diarrhoea than the not supplemented group. Also some studies have reported that zinc supplemented children have been found to have lower rates of persistent diarrhoea, dysentery and, pneumonia (Black and Sazawal, 2001; Black, 2003; Sazawal *et al.*, 1995; Sazawal *et al.*, 1996a; Sazawal *et al.*, 1996b; Sazawal *et al.*, 1997).

A randomised double blind placebo controlled trial from India randomly assigned patients to one of four intervention groups; 20 mg zinc once daily for 14 days, A single 200 000 IU dose of vitamin A on day 14, both zinc and vitamin A and placebo. The study found that combined zinc and vitamin A synergistically reduced the prevalence of persistent diarrhoea and dysentery (Rahman et al., 2002b). Similarly in a double-blind randomised controlled clinical trial on moderately malnourished Bangladeshi children, the effect of zinc and/or vitamin A supplementation on the clinical outcome of persistent diarrhoea revealed that zinc, but not vitamin A, supplementation in persistent diarrhoea reduces stool output, prevents weight loss and promotes earlier recovery (Khatun et al., 2001).

Recent information confirms earlier conclusions that improvements in the vitamin A status result in a 23% reduction in young child mortality. Supplemented children had less malnutrition, diarrhoea, malaria and acute respiratory infections than unsupplemented children (Faruque *et al.*, 1999; Grubesic and Selwyn, 2003; Ramakrishnan and Martorell, 1998). However, randomised clinical trials of vitamin A supplementation have produced mixed results on the nutritional gains, ranging from improved ponderal or linear growth to little or no discernible effects (Rahman *et al.*, 2001; Rahman *et al.*, 2002a; Rahman *et al.*, 2002b; Ramakrishnan and Martorell, 1998).

Several clinical studies have established the importance of selenium in animal and human nutrition (Levander, 1982; Schwarz and Foltz, 1958; Schwarz *et al.*, 1959). Human selenium deficiency has been hypothesised to have a negative impact on the immune function and to increase the risk of infection and more marginal deficiencies may contribute to reduce immune function and an increase in some cancers and viral diseases (Jackson *et al.*, 2003; Sempertegui *et al.*, 2003).

6.4. Results

6.4.1. Characteristics of the participants at the start of study

Two hundred and fifty nine children with acute diarrhoea were invited to participate in the study and 211 children with an age range from 2 to 59 months were recruited. The 211 children had all been hospitalised, were under 5 years of age, and had been admitted with a clinical diagnosis of acute diarrhoea and all parents had agreed to take part in study.

One hundred and eighty four (87%) of the 211 participants completed the follow up. Twenty-seven (13%) children missed appointments and were followed for between 2 to 12 weeks of the study. Eighty-nine (42%) participants were female and 122 (58%) male. The mean (SD) birth weight of the participants was 3.11 (0.56) kg and their mean (SD) age on enrollment was 15.6 (12.5) months. One hundred and thirty eight (65%) of the participants had a history of vomiting in the week before enrolment. The initial diarrhoea episode lasted for between 1 to 13 days with most children having diarrhoea for 2 (20%) and 3 days (18%). Fourteen percent of the children had diarrhoea that had lasted for only one day and only 5% had diarrhoea episodes that lasted for 13 days. The characteristics of the children on enrolment are shown in table 6.2.

6.4.2. Completeness of follow up

Out of the 211 children enrolled into the study, 184 (87%) completed the follow- up and data for 27 children were not available for between 2 and 12 weeks. The number of children who were followed and the number of weeks missed is shown in table 6.3.

Table 6.2. Characteristics of the children on enrollment

Subject		
Characteristics of the children		
Female	89	42%
Mean age (SD)	15.6	(12.5)
Mean birth weight (SD)	3.11	(0.596)
Weight for height z score < -1	87	41%
Weight for age z score < -1	121	57%
Background characteristics		
Keep animals at home	56	27%
Mean mother's age (SD), years	26.1	(5.6)
Father has a manual job	99	47%
Diarrhoea on enrolment		
Fever in the previous 5 days	162	77%
Vomiting in the previous week	138	65%
Severe dehydration	15	7%
Mild dehydration	108	52%
Diarrhoea duration (SD), days	4.3	(3.1)
Mean number of stools in last 24 hours (SD)	7.1	(3.9)
Previous medical history		
Illness in the previous year	115	55%
Diarrhoea in the previous year	92	44%
Laboratory tests		
Selenium <90 μmol/l	135	64%
Vitamin A <0.70 μmol/l	57	27%
Zinc<9.94μmol/l	158	75%
Presence of pathogens in stool	142	67%
Metrical mean (SD) zinc μmol/l	6.8	(7.9)
Geometrical mean (SD) vitamin A μmol/l	0.91	(1.8)
Geometrical mean (SD) selenium μmol/l	0.75	(1.6)
Geometrical mean (SD) copper μmol/l	1.47	(1.8)
Geometrical mean (SD) vitamin E μmol/l	8.9	(1.7)

Table 6.3. Distribution of the children were followed by weeks of follow up

Number/s of visits missed	Frequency	Percent	Cum %
0	184	87%	87
1	3	2%	89
2	6	3%	92
3	7	3%	95
4	5	2%	97
5	4	2%	99
6	2	1%	100
Total	211	100%	100

* Cum %= cumulative percent

6.4.3. Comparison of the children followed with the 48 children with incomplete follow up

A comparison between the children who had completed follow up and those who defaulted was made to compare if the children lost to follow up were different to those followed. The medical history in the year before enrollment including diarrhoea, the clinical aspects of the last episode of diarrhoea such as the degree of dehydration, presence of fever, number of stools and diarrhoea duration were not different between the two groups as shown in table 6.4 (all $p > 0.05$). The nutritional characteristics of the children were also similar. These included the weight-for-age and weight-for-height z scores, vitamin A, zinc and selenium levels ($p>0.05$).

The family background characteristics were also similar, and these included the mother's age, birth weight and frequency with which they kept animals at home. The frequency of pathogens detected in the stools was also similar, in total 142 (66%) pathogens were detected in the group that was followed compared to 27 (56%) in the group not followed. Considering the micronutrient concentrations between children who were followed and those who defaulted, zinc, copper, vitamins A and E and selenium were not different; however the geometrical mean (SD) zinc of children who were followed was slightly lower and marginally significant (4.7 (2) vs 5.8 (2.1) μmol/l respectively, $p = 0.05$).

Table 6.4. Characteristics of the children followed and not followed

Subject	Children		P value
	Followed	Not followed	
Characteristics of the children			
Female	89 (42%)	27 (56%)	0.10
Mean age (SD) month	15.6 (12.5)	13.6 (10.3)	0.31
Mean birth weight (SD) grams	3111 (596)	2997 (552)	0.20
Weight-for-height z score < -1	87 (42%)	20 (42%)	0.94
Weight-for-age z score <-1	121 (57%)	32 (67%)	0.23
Background characteristics			
Keep animals at home	56 (27%)	13 (27%)	0.91
Mean mother's age (SD) years	26.1 (5.6)	26.5 (6.4)	0.90
Father has a manual job	99 (47%)	21 (44%)	0.79
Diarrhoea on enrolment			
Fever in previous 5 days	162 (77%)	38 (72%)	0.87
Vomiting in previous week	138 (65%)	36 (75%)	0.28
Sever dehydration	15 (7%)	5 (10%)	0.54
Mild dehydration	108 (51%)	30 (62%)	0.25
Diarrhoea duration (SD) day	4.3 (3.1)	4 (8%)	0.12
Mean number of stools in last 24 hours	7.1 (3.9)	7 (3.8)	0.90
Previous medical history			
Illness in the previous year	115 (55%)	26 (54%)	0.95
Diarrhoea in the previous year	92 (44%)	21 (44%)	0.87
Laboratory tests			
Selenium <0.90 μmol/l	131 (69%)	31 (65%)	0.97
Vitamin A <0.70 μmol/l	57 (27%)	14 (29%)	0.88
Zinc <9.94μmol/l	158 (75%)	32 (80%)	0.69
Presence of pathogens in stool	142 (67%)	27 (56%)	0.25
Geometrical mean (SD) zinc μmol/l	4.7 (2)	5.8 (2.1)	0.05*
Geometrical mean (SD) vitamin A μmol/l	0.93 (1.8)	0.89 (1.6)	0.47
Geometrical mean (SD) selenium μmol/l	0.77 (1.6)	0.69 (1.5)	0.11
Geometrical mean (SD) copper μmol/l	1.51 (1.8)	1.34 (1.9)	0.36
Geometrical mean (SD) vitamin E μmol/l	8.7 (1.8)	9.5 (1.6)	0.64

* Marginally significant

6.4.4. Frequency of subsequent diarrhoea episodes

Out of the 211 children, 153 (73%) of the children had no diarrhoea during the 14 weeks of follow up and 58 (27%) developed a new episode.

Forty-four (76%) children had one episode, 12 (21%) children had 2 episodes, 1 (1%) had 3 and another child had 4 episodes. In total 75 diarrhoea episodes were identified

and the children had an average of 0.3 episodes per child. Children who had diarrhoea had an average of 1.3 episodes per child. The frequency of the diarrhoea episodes per child is shown in table 6.5.

Table 6.5. Frequency of diarrhoea episodes

Time/s with diarrhoea	Frequency	Percent	Cum%
0	153	72%	72
1	44	21%	93
2	12	6%	99
3	1	0.5%	99
4	1	0.5%	100
Total	211	100%	100

Cum% = cumulative percent

6.4.5. Diarrhoea duration in the subsequent diarrhoea episodes

Children who had subsequent diarrhoea episodes had diarrhoea for a total of 109 days, which represents 7.4% of the time of the study period. Thirty-four (59%) of these episodes only lasted for one day, 12 (21%) lasted for 2 and 8 (14%) for 3 days. None of the children developed persistent diarrhoea and the longest diarrhoea duration was 10 days. While the mean (SD) duration of the initial hospitalised diarrhoea episode was 4.3 (3.3) days, the mean (SD) for subsequent diarrhoea episodes was 1.9 (1.6) days. The mean (SD) age of the children who had subsequent diarrhoea episode was higher than the age of the children who did not have diarrhoea (19.6 (15.3) vs 14.1 (10.9) months $p<0.05$). Of the 17 (29%) children who had rotavirus during their first episodes, the duration of subsequent diarrhoea was 1.7 days while children without rotavirus in their stools had a slightly longer duration (1.95 days). However these differences were not statistically different. The duration of the diarrhoea episodes are shown in table 6.6.

Table 6.6. Frequency of days with diarrhoea

Day/s with diarrhoea	Frequency	Percent	Cum%
1	34	59	59
2	12	21	79
3	8	14	93
4	1	1.5	95
6	1	1.5	97
7	1	1.5	98
10	1	1.5	100
Total	58	100	100

Cum % = Cumulative percent

6.4.6. Frequency of stools per day in children with subsequent diarrhoea

The number of stools per 24 hours in children who had subsequent episodes ranged from 1 to 6. Mean (SD) number of stools per day was 3.2 (1.2), with the highest frequency of children having four stools per 24 hours (18, 31%). This was followed by 15 (26%) of children with three stools and only 2 (4%) children with six stools per day. The frequencies of stool per day are shown in table 6.7.

Table 6.7. Frequency of stools per day

Stool/stools/day	Frequency	Percent	Cum %
1	6	10	10
2	6	10	20
3	15	26	46
4	18	31	77
5	4	7	84
6	2	4	88
NA*	7	12	100

Cum % = cumulative percent, NA = Not available

6.4.7. Children that required medical consultations

Out of the 96 further consultations for 58 children who had subsequent diarrhoea episodes during the 14 weeks follow up, 48 (83%), 40 (69%) and 9 (16%) of consultations were in the health centres, private clinics and hospitals, respectively. The majority of them consulted because of a new episode of diarrhoea and/or vomiting. The majority of the children who had subsequent diarrhoea (57%) were seen in health centres and private clinics. Nine children were further hospitalised. Of these, 5 were referred by a private clinic or health centre and 4 came spontaneously to hospital during the night or the weekend.

6.4.8. Variables associated with increased risk of subsequent diarrhoea

To identify the characteristics and clinical factors associated with an increased risk for subsequent diarrhoea, we classified children as having experienced or not subsequent diarrhoea in the follow up period and described the characteristics of these groups using univariate analysis. The analysis showed that paternal occupation (e.g. being a manual worker), having vitamin A deficiency at the time of the first diarrhoea episode, age less than 7 months, keeping animals at home, maternal age less than 20 years, low weight-for-age and weight-for-height z scores and zinc deficiency were significantly associated with a higher risk of experiencing subsequent diarrhoea during the follow up period as shown in table 6.8. Among these criteria, age less than 7 months had an OR < 1 possibly reflecting the protective role of maternal immunity.

Serum zinc levels <9.94 μmol/ L (an accepted cutoff point for zinc deficiency) (Hotz et al., 2003), and having a mother <20 years old or a father who was a manual worker were marginally significant.

Children with fathers doing manual work were more likely to have subsequent diarrhoea, although this was marginally significant (p=0.06). Similarly, children who were young (≤ 6 months) and had young mothers (<20 years) had a lower risk of having subsequent diarrhoea, but again these were marginally significant. The frequency of children with weight-for-height z score was higher in children with subsequent diarrhoea than children without subsequent diarrhoea.

There was a positive association between keeping animals at home and the risk of subsequent diarrhoea (OR=2.4 95% CI =1.2- 4.8%). Similarly weight-for-age and weight-for-height z scores less than -1 were associated with increased risk, as children with weight-for-age and height z score < -1 had OR of 2.2 and 2.5 respectively (p< 0.05 for both). In addition the selected cutoff points for vitamin A and zinc deficiency were good markers to identify children at risk of subsequent diarrhoea (vitamin A, OR =2.9 and zinc OR=2.8). The concentration of the micronutrients were also associated with malnutrition and the concentration of the micronutrients in children with weight-for-age and weight-for-height z score <-1 in children with and without subsequent diarrhoea are shown in figure 6.3 and 6.4.

Table 6.8. Characteristics of children with and without subsequent diarrhoea

Variable	With diarrhoea	Without diarrhoea	OR (95 CI)	P value
Index diarrhoea episode	N = 58	N = 153		
Presence of vomiting in last 24h	39 (67%)	94 (61%)	1.27 (0.6- 2.5)	0.57
Presence of vomiting in last week	41 (71%)	97 (63%)	1.37 (0.7- 2.8)	0.43
Fever in previous 5 days	48 (83%)	114 (75%)	1.60 (0.7- 2.4)	0.31
Bloody stool	4 (7%)	19 (12%)	0.52 (0.1 -1.7)	0.35
Semi liquid stool	26 (44%)	75 (49%)	0.83 (0.4- 1.6)	0.66
ORS use	16 (28%)	55 (36%)	0.69 (0.3-1.4)	0.34
Given medication	37 (64%)	101 (66%)	0.92 (0.5-1.8)	0.91
Dehydration	36 (62%)	87 (57%)	1.24 (0.6-2.4)	0.60
Stool number <5	16 (28%)	51 (33%)	0.77 (0.4-1.6)	0.55
Diarrhoea duration <5 days	35 (60%)	73 (48%)	1.65 (0.9-3.2)	0.15
Previous history				
Illness in previous year	36 (62%)	79 (52%)	1.53 (0.8-3)	0.22
Hospitalised in previous year	25 (43%)	46 (30%)	1.80 (0.9-3.5)	0.09
Diarrhoea in previous year	32 (55%)	60 (39%)	0.64 (0.3-1.2)	0.20
Family background				
Family with diarrhoea	4 (7%)	12 (8%)	0.86 (0.2-3.1)	1
Father education ***	49 (85%)	118 (77%)	1.57 (0.7-3.8)	0.36
Spring, pool, etc water use	4 (7%)	9 (6%)	1.19 (0.3- 4.5)	0.75
Father occupation****	34 (59%)	24 (16%)	1.90 (1- 3.7)	0.06**
No piped water	2 (3%)	5 (3%)	1.06 (0.14-6.4)	1
Age ≤ 6 months	5 (9%)	30 (20%)	2.59 (0.9-8.1)	0.08**
Keep animal at home	23 (38%)	33 (22%)	2.39 (1.2-4.8)	0.01*
Unhealthy mother	4 (7%)	8 (5%)	1.37 (0.3-5.9)	1
Mother age <20	1 (2%)	14 (9%)	0.18 (0.1-1.3)	0.07**
Child's characteristics				
Female	23 (40%)	66 (43%)	0.87 (0. 5-1.7)	0.76
Breast fed < 13 months	11 (19%)	27 (18%)	0.58 (0.2-1.8)	0.42
Weight-for-age z score <-1	41 (71%)	80 (52%)	2.20 (1.1-4.4)	0.02*
Weight-for-height z score< -1	33 (57%)	54 (35%)	2.50 (1.3-4.9)	0.005*
Height-for-age z score <-1	21(36%)	70 (46%)	0.67 (0.3-1.3)	0.27
Laboratory results				
Vitamin A <0.70 μmol/l	25 (43%)	32 (21%)	2.91 (1.4-5.9)	0.001*
Vitamin E <11.6μmol/l	32 (55%)	83 (54%)	1.22 (0.6-2.5)	0.67
Vitamin E<16 μmol/l	42 (72%)	125 (82%)	0.64 (0.2-1.7)	0.47
Selenium <0.90 μmol/l	35 (60%)	96 (63%)	0.87 (0.4-1.7)	0.78
Zinc<9.18 μmol/l	48 (83%)	106 (69%)	2.78 (1-8.6)	0.06**
Zinc <9.94 μmol/l	49 (84%)	109 (71%)	2.92 (0.9-10.7)	0.07**
Copper < 1.5 μmol/l	28 (52%)	62 (43%)	1.37 (0.7-2.6)	0.38
Child with other viruses	3 (5%)	8 (5%)	0.99 (0.2-4.3)	1
Child with rotavirus	17 (29%)	59 (39%)	0.65 (0.3-1.3)	0.24
Child with bacteria	17 (29%)	35 (22%)	1.40 (0.7-2.9)	0.42
Child with parasite	5 (9%)	20 (13%)	1.75 (0.5-5.6)	0.34

* P< 0.05, ** P<0.10, *** illiterate father or only able to read, **** father doing a manual work

The weight-for-height z score and concentrations of vitamin A, vitamin E, zinc, selenium, and copper in children with and without subsequent diarrhoea are shown in figures 6.1 to 6.3.

Figure 6.1. Weight-for-height z scores in children with and without subsequent diarrhoea

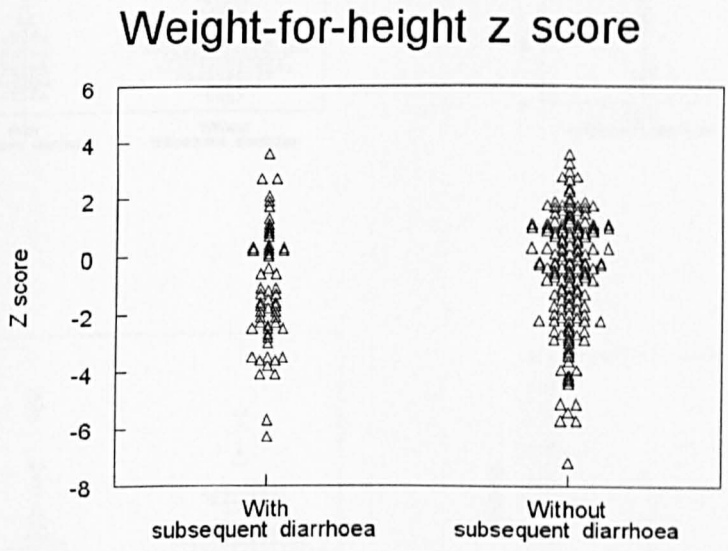
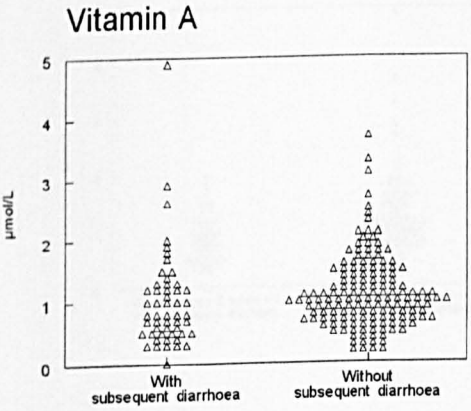
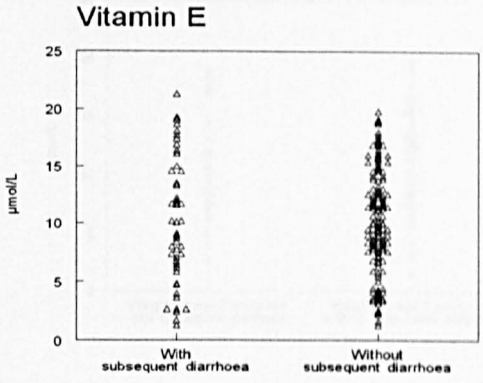
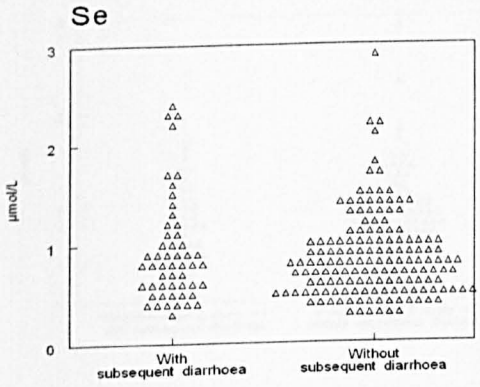
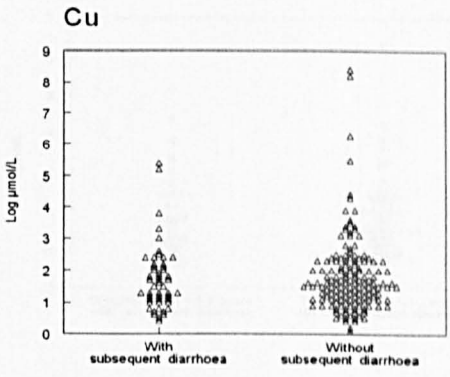


Figure 6.2. Concentration of vitamin A*, vitamin E, zinc*, selenium, and copper in children with and without subsequent diarrhoea (* = statistically significant)









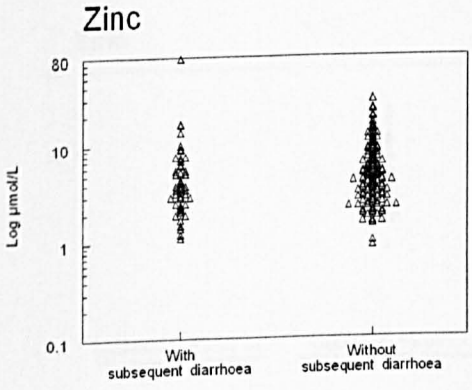


Figure 6.3. Concentration of vitamin A, vitamin E, zinc, selenium and copper in children with weight-for-age z score <-1 by subsequent diarrhoea

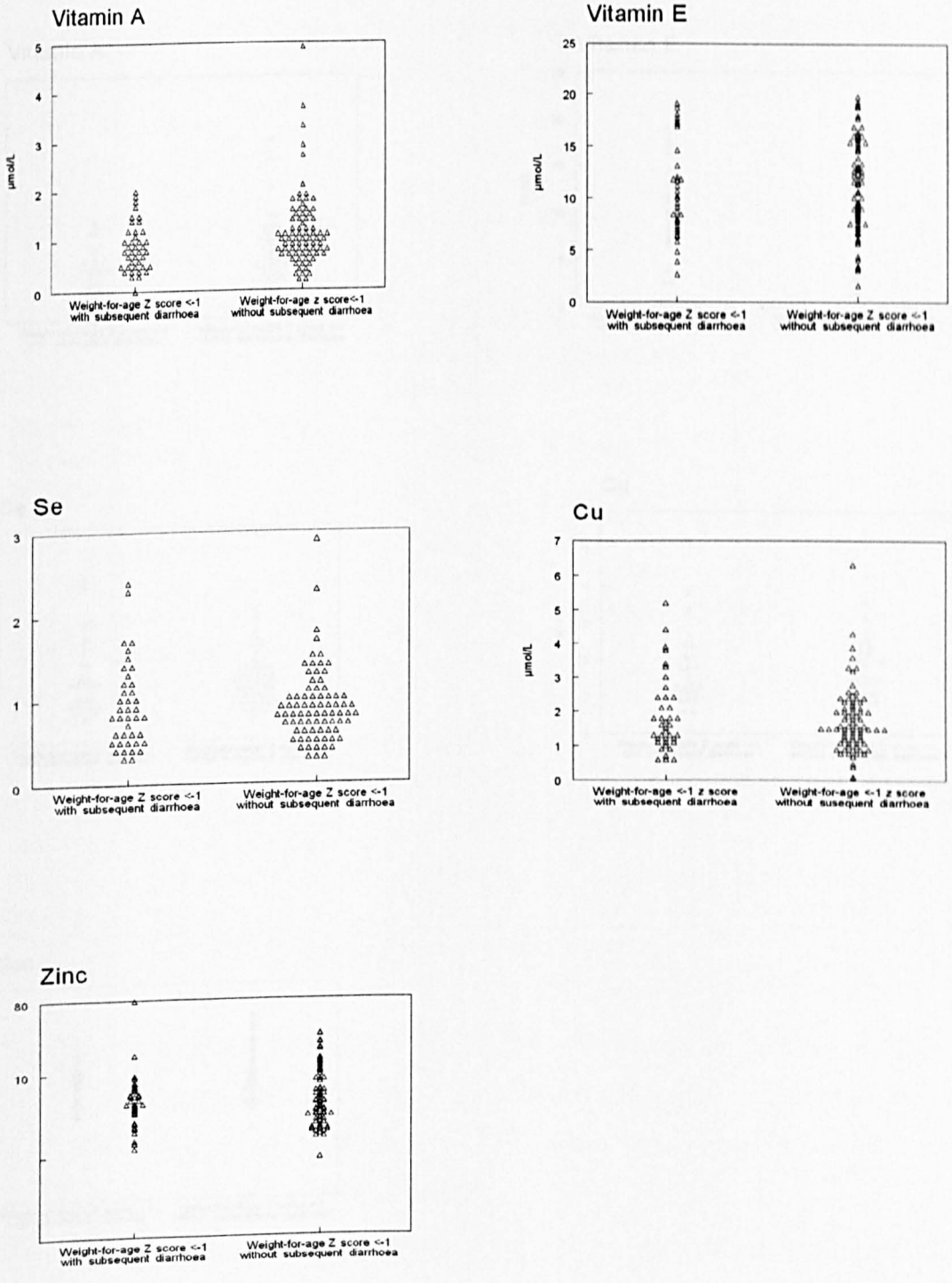
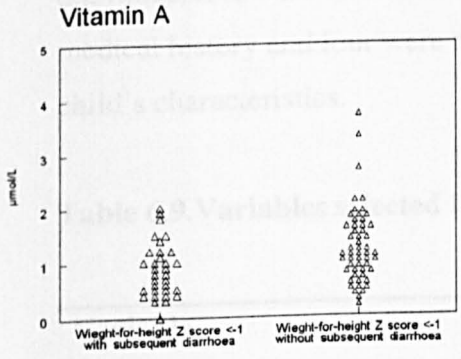
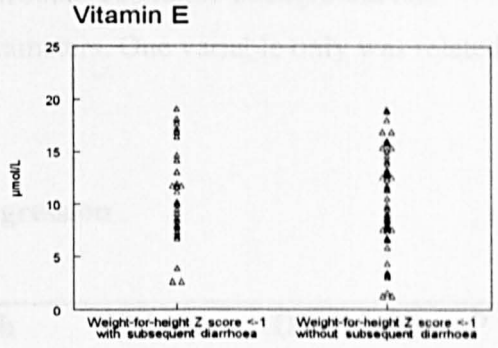
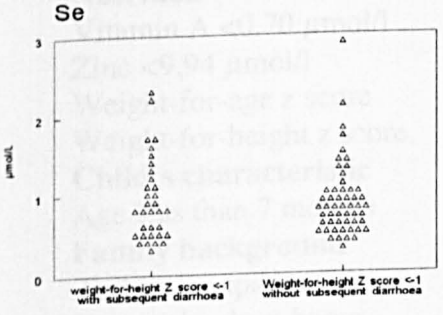
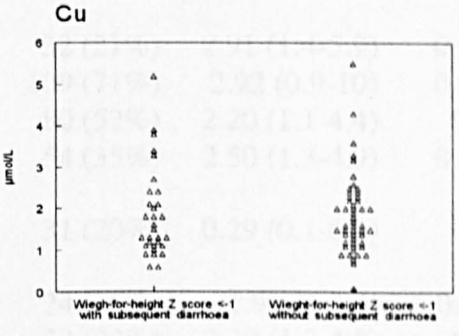


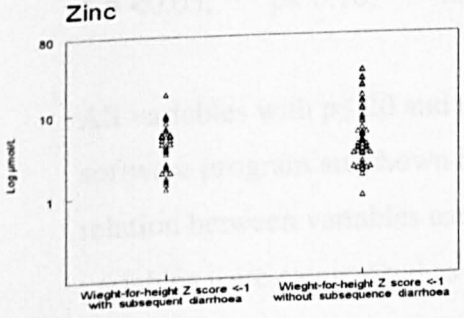
Figure 6.4. Concentration of vitamin A, vitamin E, zinc, selenium and copper in children with weight-for-height z score <-1 by subsequent diarrhoea











4.6.9. Multivariate analysis

A summary of the variables with P values <0.20 is shown in table 6.9. A total of 11 variables were selected. Of these only one was a clinical feature of the acute diarrhoea, five were variables associated with socio-economic background and medical history and four were nutritional parameters. One variable only was related to child's characteristics.

Table 6.9.Variables selected for logistic regression

Variable	With subsequent diarrhoea	Without subsequent diarrhoea	OR (95 CI)	P value
Index diarrhoea episode				
Duration <5 days	35 (60%)	73 (48%)	1.65 (0.9-3.2)	0.15
Nutrition				
Vitamin A <0.70 μmol/l	25 (43%)	32 (21%)	2.91 (1.4-5.9)	0.001*
Zinc <9.94 μmol/l	49 (84%)	109 (71%)	2.92 (0.9-10)	0.07**
Weight-for-age z score	41 (71%)	80 (52%)	2.20 (1.1-4.4)	0.02*
Weight-for-height z score	33 (57%)	54 (35%)	2.50 (1.3-4.9)	0.005*
Child's characteristic				
Age less than 7 months	4 (0.7)	31 (20%)	0.29 (0.1-0.9)	0.03*
Family background				
Father occupation*	34 (59%)	24 (16%)	1.90 (1-3.7)	0.06**
Keep animals at home	23 (38%)	33 (22%)	2.39 (1.2-4.8)	0.01*
Mother age <20 years	1 (2%)	14 (9%)	0.18 (0.1-1.3)	0.07**
Previous medical history				
Hospitalised in previous year	25 (41%)	46 (31%)	1.80 (0.9-3.5)	0.09
Diarrhoea in previous year	32 (55%)	60 (38%)	0.64 (0.3-1.2)	0.20

* p <0.05, ** p< 0.10, *** father doing a manual work

All variables with p≤ 20 and their original name with categories used in Epi 2002 software program are shown in table 6.10. Different categories were used to find any relation between variables and subsequence diarrhoea. Except mothers age all variables were categorised as bi-nominal.

Variables with p values ≤0.20 were then entered into a multivariate logistic regression to establish their independent association after allowing for confounding effects. The procedures for the analysis and interim results are attached in appendix 6.

Table 6.10. Variables original name with categories used in Epi 2002 program

Variable	Original name	Categories used
Duration < 5 days	forhowlong	< 4 days = 0, > 4 days= 1
Vitamin A < 0.70 μmol/l	Vitamin A	< 0.70 μmol = 0, > 0.70 μmol = 1
Zinc < 9.94 μmol/l	Zinc	< 9.94 μmol= 0, > 9.94 μmol = 1
Father occupation	Fatheroccupation	Manual worker= 0, others = 1
Weight-for-age z score	FldCDCWAZ	WAZ <-1 = 0, WAZ >-1 = 1
Weight-for-height zscore	FldCDCWHZ	WHZ<-1 = 0, WHZ>-1 = 1
Age less than 7 months	age	≤6 =0, >6= 1
Keep animals at home	DoYouKeepAnimals	Yes =1, no= 0
Mother age <20years	Agem	17-19 =0, ≥20 =1
Hospitalised in previous year	Hasyourchildever	Yes-= 1, no= 0
Diarrhoea in previous year	hasyourchildhad	Yes=1, no =0

After entering the variables into the multivariate logistic regression, the variables that remained significant are shown in table 6.11. Interim results obtained while processing the data are shown in appendix 6.

Vitamin A<0.70 μmol/l, zinc<9.94 μmol/l, and weight-for-age z score were independent variables statistically significant (AOR = 2.8, 3.9, and 2.5 respectively). There was a negative association between duration of diarrhoea of the initial episode and the risk of subsequent diarrhoea and children who had shorter diarrhoea episode were more likely to have subsequent diarrhoea episodes (AOR =2.7). Of the variables related to the environment and family background, the ownership of animals remained significant (AOR= 2.7).

The mothers whose children had subsequent diarrhoea were also significantly older than mothers of children without subsequent diarrhoea (27.8 vs 25.4 years respectively), (p<0.05).

This multivariate logistic regression thus revealed that micronutrient deficiencies at the time of the initial acute episode and the child's nutritional status (as measured by anthropometry) are the main factors associated with an increased risk of having further diarrhoea episodes.

Table 6.11. Adjusted odds ratios obtained through the logistic regression

Variable	OR	AOR	OR (95-CI)	P value
Duration <5 days*	1.7	2.7	1.2-6.1	0.01
Vitamin A <0.70 μmol/l	2. 9	2.8	1.3-5.8	0.006
Zinc <9.94 μmol/l	2.9	3.9	1.2-12.8	0.04
Weight-for-age z score	0.7	2.5	1.3-5.3	0.01
Keep animal at home	2.4	2.7	1.3- 5.5	0.01

* of the initial episode

6.4.10. Combination of risk factors

The odds ratios of the variables that were significantly associated with increased risk of subsequent diarrhoea are shown in table 6.12. The combination of these risk factors increased the ORs and children who had three risk factors (zinc <9.94 μmol/l, kept animals at home and vitamin A < 0.70 μmol/l) had the highest risk of subsequent diarrhoea (OR = 9.2)

Table 6.12. Odds ratios of factors significantly associated with increased risk of subsequent diarrhoea

Subject	Diarrhoea N= 58	Without diarrhoea= 153	Odd ratio (95% CI)	P value
Zinc <9.94 μmol/L	49	109	2.92 (1-10.7)	0.02
WAZ<-1	41	80	2.20 (1.3-4.4)	0.005
Keep animal at home	23	33	2.39 (1.2-4.8)	0.01
Vit.A<0.70 μmol/L	25	32	2.91 (1.4-5.9)	0.001
Vit.A & animal	11	5	6.93 (2.1–4.3)	2tail <0.001
Animal & zinc	19	22	2.90 (1.3-6.3)	0.004
Zinc & vit. A	21	25	2.86 (1.4-6.0)	0.003
WAZ<-1 & zinc	28	38	2.82 (1.4-5.6)	0.001
WAZ <-1& animal	13	12	3.39 (1.3-8.7)	0.001
W & vit. A	13	9	4.62 (1.7 –13)	0.001
WAZ<-1 & vit.A & animal	6	2	6.9 (2.1-2.4)	2tail <0.001
WAZ <-1& vit.A & zinc	11	5	6.9 (2.1-2.4)	2tail <0.001
WAZ <-1 & animal & zinc	11	7	4.88 (1.6-14.9)	2tail <0.001
Vit.A & zinc & animal	9	3	9.18 (2.2 –45)	2tail <0.001
WAZ<-1& animal&zinc & vit.A	5	0	Undefined	2tail <0.001

WAZ =Weight-for-age z score, zinc = zinc <9.94 μmol/L, animal =keep animals at home, vit.A = vitamin A

6.4.11. Subsequent vomiting

During follow up the presence or absence of vomiting was investigated. In a logistic regression, variables that increased the risk of subsequent vomiting were, the presence of mild to severe dehydration and diarrhoea episodes lasting more than 5 days during the initial episode. Vomiting occurred only for short periods and its origin and characteristics were poorly defined. This study therefore cannot emphasise this result until more studies confirm our findings. A summary of the significant variables associated with an increased risk of subsequent diarrhoea and vomiting is shown in table 6.13.

Table 6.13. Variables independently associated with an increased risk of subsequent diarrhoea or vomiting

Variables	Subsequent diarrhoea	Subsequent vomiting
Mild or dehydration	-	+
Diarrhoea >5 days	+	+
Vitamin A deficiency	+	-
Zinc deficiency	+	-
Weight-for-age z score <-1	+	-
Keeping animal at home	+	-

6.5. Discussion

Uncertain living conditions, inadequate food intake, and poor medical facilities in developing countries enhance unnecessary morbidity and mortality especially in infants and young children. In addition to protein-calorie malnutrition, deficiencies in essential micronutrients have a specific health impact (Malvy, 1999). It has been estimated that malnutrition is associated with up to two-thirds of all deaths among children and 12 million children <5 years old die annually due to infection and malnutrition (Rice *et al.*, 2000; Roy *et al.*, 1997; Umeta *et al.*, 2003; Wekell *et al.*, 1997). Micronutrients disturb several biological functions such as gene expression, protein synthesis, immunity, skeletal growth and maturation (Roy *et al.*, 1997; Umeta *et al.*, 2003). Iran is no exception, protein-energy malnutrition and infectious diseases are still the leading health problems (Lankarani and Musaiger, 1991).

Improving vitamin A and zinc status has reduced mortality among older infants and young children and the prevalence of severe illness and clinic attendance among children of developing countries (Tomkins, 2000). Although all studies focusing on the effect of vitamin A supplementation have reported a reduction in childhood mortality, its effect on morbidity is less clear. In a follow up study from Ghana there was no significant difference in the prevalence of diarrhoea or acute respiratory infections in vitamin A supplemented children and non vitamin A supplemented children. Some researchers have speculated that vitamin A supplementation is less effective in the presence of zinc deficiencies and the lack of efficacy in these studies prompted the study of micronutrient combinations (Anonymous, 1993).

Nutritional studies have reported that the highest rate (57%) of children with malnutrition is seen in South Asia and the lowest rate (1%) in Europe. Furthermore, some community based studies have reported that the prevalence of malnutrition can be above 70% (Saleh and El Sherif, 1993).

Our study revealed that 153 (73%) of the 211 children recruited experienced no further diarrhoea episodes after hospital discharge and 58 (27%) had a total of 109 days with diarrhoea ranging from 1 to 4 episodes per child. The most frequent number of days with diarrhoea per child was one-day (59%), followed by 2 and 3 days. The mean duration of subsequent diarrhoea was significantly shorter than the initial

episode (1.9 vs 4.3), ($p < 0.05$), and these shorter episodes required less hospitalisations. In England, the sequel of infectious intestinal disease in a population based sample of cases and matched controls, cases were six times more likely than controls to have diarrhoea and 10% of cases consulted their GP in the 3 months following an initial episode with 2.3% being referred to the hospital. Our prevalence was higher than the UK study. However as our study is hospital- based, it is likely that our children were more susceptible to diarrhoea. It is expected that hospitalised children with diarrhoea come from disadvantaged populations and that these children would have a higher incidence of diarrhoea when they return to the community than children selected through a community based study. A community study from Nigeria reported that 33% and 37% of the children followed had diarrhoea during the 3 months of the study, which is higher than our incidence. This might possibly reflect a more organised public health system and better sanitation in our environment which could explain our lower incidence (Cumberland *et al.*, 2003; Omokhodion *et al.*, 1998; Ruuska and Vesikari, 1991).

Forty-eight (22%), 40 (19%) and 9 (4%) of the children consulted the health centres, private clinics and the hospital respectively. Our results are therefore similar to studies that investigated the number of consultations of children after discharge and children with a acute severe episode are risk of further episodes (Cumberland *et al.*, 2003; Ruuska and Vesikari, 1991).

Many studies have reported that nutritional factors including anthropometric factors, and micro-nutritional status are risk factors for diarrhoea (Bhandari *et al.*, 1994; Saleh and El Sherif, 1993; Tomkins, 2000; Wierzba *et al.*, 2001). Our study revealed that vitamin A and zinc deficiency, weight-for-age, weight-for- height z scores were significant risk factors for subsequent diarrhoea, this is in agreement with almost all of these studies and there is a body of evidence that supplementation with micronutrients reduces the incidence and duration of diarrhoea in children (Anand *et al.*, 1994; Brown, 2003; Pinnock, 1991). Few studies however have described the association between multiple factors in one child and risk of diarrhoea. Our study therefore documented that more than one factor is often implicated in a child and that this aggregation of factors can have an additive effect on the risk of subsequent diarrhoea. It is therefore conceivable that multiple micronutrient supplementation

could have a better effect than single micronutrient supplementation. This approach is currently being investigated by UNICEF and our findings provide further evidence for its use.

Considering the association between subsequent diarrhoea and age, this study showed that children with <7 months of age are less likely to suffer subsequent diarrhoea episodes, suggesting that maternal antibodies can protect children in their early months of life, an alternative explanation is that younger children are less bottle fed and are therefore less exposed to organisms that cause diarrhoea. In addition younger children may not have been exposed at the start of the rotavirus season and they will have contact in the following year. In addition, if a child had a mother < 20 years of age the child had a reduced risk of subsequent diarrhoea (p=0.07). This might be due to over reaction of younger mothers to the first diarrhoea episodes of their children (as children of younger mother tend to be the first or second child) and children from younger mothers had milder episodes (therefore less micronutrient deficiencies).

Our cut off points for vitamin A and zinc deficiency were good markers to identify children at risk of subsequent diarrhoea (vitamin A, OR =2.9 and zinc OR=2.8). The prevalence of zinc deficiency was higher (84%) in children who later experienced subsequent diarrhoea than in children without subsequent diarrhoea (71%), however this was also marginally significant (p = 0.07). Although our study was not a clinical trial, our results are in agreement with reports that have demonstrated lower stool output and a decrease of duration of diarrhoea in children supplemented with zinc (Brown, 2003; Roy *et al.*, 1997).

There was an inverse association between weight-for-age and weight-for-height z scores and diarrhoea. Children with weight-for-height and weight-for-age z scores < -1 had more diarrhoea episodes during the follow up period than children with z scores >-1. This finding confirms the evidence from many other studies, that have demonstrated that anthropometry and especially low weight-for-height and weight-for-age z scores are good markers to identify children with an increased risk of subsequent diarrhoea (Kossmann *et al.*, 2000a; Kossmann *et al.*, 2000b; Moore *et al.*, 2000). Our study showed that nutritional status (weight-for-age z score <-1, vitamin A and zinc deficiency as observed in the initial episode) were the most significant independent variables for subsequent diarrhoea during 3 months after hospital

discharge. These findings are in agreement with other studies worldwide, which have documented that there is an association between malnutrition, diarrhoea and other infectious diseases (Brown, 2003; Rice *et al.*, 2000; Uysal *et al.*, 2000; Wekell *et al.*, 1997).

Among the other variables investigated keeping animals at home had a strong positive association with subsequent diarrhoea episode. This association was kept even after multivariate analysis. There are however no specific studies that have investigated the effect of contact with animals and keeping animals at home with diarrhoea. Although it is possible that animals transmit infections, keeping animals at home is also likely to be a marker for a particular life style (e.g. poor farmers) and represent a risky set of behavioural patterns. In keeping with this hypothesis, many studies worldwide have reported a correlation between hygienic behaviour or an unclean domestic environment and the incidence of diarrhoea and diarrhoea epidemics after farm visits (Haque *et al.*, 2003; Huttly *et al.*, 1987; Muhe *et al.*, 1995; Strina *et al.*, 2003) and this area merits further investigation.

General discussion

Introduction

Diarrhoeal diseases are the second most important cause of death in children worldwide resulting in the death of 3 to 6 million children annually (Hart, 2003b). The greatest morbidity and mortality of diarrhoea is seen among children less than two years of age. The highest incidence of episodes of diarrhoea are often reported from Asia with up to 15-19 episodes per year in some areas (Giordano *et al.*, 2001; Torres *et al.*, 2001). In contrast, to this high incidence European children experience approximately 1 episode per year (Guandalini, 1989). Several factors such as living conditions, inadequate food intake, and lack of access to medical facilities in developing countries increase its avoidable morbidity and mortality especially among infants and young children.

Acute diarrhoea is caused by a wide range of pathogens, and more than 50 parasites, bacteria and viruses have been associated with diarrhoea in children. Viruses have been given particular attention in recent years, with the possible introduction of vaccines. The prevalence of these enteropathogens often varies with age of the individual, how the diarrhoea is acquired, and the host's immunity. Rotavirus is the most frequently reported pathogen causing severe diarrhoea in the cool season of temperate countries while bacterial infections are most frequent in the spring and summer (Albert *et al.*, 1999; Grimprel *et al.*, 2001).

Aetiology of childhood diarrhoea

Two hundred and fifty nine children under 5 years old admitted to Hajar Hospital, 245 children with acute diarrhoea attending the hospital's OPD and 114 children without diarrhoea hospitalised for elective surgery were recruited from October 2001 to August 2002. Stool samples were screened for enteropathogens using ELISA, negative stain EM, selective and specific media for culture, the stool concentration method and direct stool smears. Enteropathogens were detected in 62% and 44% of the hospitalised and OPD children with diarrhoea and 18% of hospitalised children without diarrhoea. This is the first report of the range of pathogens isolated in children

with diarrhoea from Iran and our results are similar to other reports from this region (Al-Freihi *et al.*, 1993; El-Sheikh *et al.*, 2001; Sethi *et al.*, 1989). Viruses were the most frequent pathogens detected in 41% and 27% of the children hospitalised and those at OPD; followed by bacteria in 25%, 15% and parasites in 14% and 11% respectively. The frequency of rotavirus was significantly higher in inpatient than in outpatient children. In contrast, the frequency of coronavirus was significantly higher in outpatient children than in inpatients. The frequency of other pathogens was not significantly different between inpatients and outpatient children. Co-infections however were more frequent in hospitalised children (15% vs 10% in hospitalised and OPD respectively), however this was only marginally significant (p=0.07). The number of children with three pathogens was also higher in hospitalised children (6 vs none in OPD children) (Fisher's exact test p=0.03).

The most frequently detected bacteria were ETEC & EPEC in 7% of cases of childhood diarrhoea followed by *E. coli* O157 in 5%, *Salmonella* in 4%, *Shigella* in 3% and *Campylobacter* in 2%. These findings are in agreement with studies from Kuwiat and Jordan that have reported a similar aetiology of childhood diarrhoea (Sethi and Khuffash, 1989; Youssef *et al.*, 2000). In contrast, our prevalence is lower than studies in Kenya and Bangladesh (Albert *et al.*, 1999; Torres *et al.*, 2001).

Parasites were detected in 13% of the cases of childhood diarrhoea, which is similar to other surveys that have reported diarrhoea due to parasites. The most frequent parasites were *B. hominis, G. lamblia, Cryptosporidium* spp and *Ent. histolytica/dispar.*

Our study revealed that rotavirus is responsible for 35% of the episodes of severe diarrhoea requiring hospitalisation and about 18% of the episodes in children that do not require hospitalisation. This is similar to other reports from Middle East countries (Kurugol et al., 2003). A previous study from Iran had shown that 25% of the children with diarrhoea in Tehran had rotavirus.
Rotavirus was most frequently isolated from November to February and was present in more than 40% of the samples. It also affected mostly children < 2 years and the highest frequency of rotavirus detection was observed in 13- 24 month children followed by those 1-12 month of age. These findings are similar to the studies by

Liddle et al (1997) and Roman et al (2003) which reported a higher frequency for children 6-24 month age, and to studies from Iran and Turkey that reported a frequency of 20% rotavirus in children under 2 years and 5% above this age.

The seasonal peak of rotavirus in our study is consistent with almost all of the studies that have reported a highest frequency of rotavirus in the cool season of temperate countries (Kurugol *et al.*, 2003; Liddle *et al.*, 1997; Roman *et al.*, 2003).
The (RT-PCR) successfully typed 95% of G types and 85% P types. The most frequent P type was P[8] in 108 samples (including P[8] in 48 and P[8*] in 60) of rotavirus cases, followed by P[4] in 16, and was P non-typeable in 22 cases. Among the G types, G1 was identified in 120 (including G1 in 78 and G1* in 42) of rotavirus cases, G2 in 19, and was G non-typeable in 7 cases. The most frequent G and P types: G1, G2, P[8] and P[4] are similar to those reported from around the world.

Risk factors for hospitalisation

Hospital based case-control studies have established that malnutrition, the presence of vomiting, fever, acute respiratory symptoms before hospital admission, parents being illiterate, age < 12 months, lack of exclusive breast feeding, having more than 2 older siblings, having breast fed <6 months, higher multiparity and generally inadequate sanitation, distance to the hospital, and a history of previous hospitalisation or concomitant illness are risk factors for hospitalisation due to severe diarrhoea (Shah *et al.*, 2003; Zodpey *et al.*, 1998).

To identify risk factors for hospitalisation a total of 749 children with acute diarrhoea were enrolled. Of these, 259 were children who were hospitalised, 245 children seen as outpatients and 245 children attending 3 health centres. The general characteristics including age and gender of children were similar.

The risk factors were identified by comparing hospitalised children with both groups of controls (OPD and health centres) included, a history of vomiting in the week before consultation, a history of a previous hospitalisation and the presence of blood in the stools. The adjusted odd ratios were higher when comparing cases with health centre controls than when comparing them against hospital controls. This is likely to

result from the characteristics of the episodes seen in these children and the background of the children. For example OPD children are more likely to have slightly more severe diarrhoea than children attending health centres. Similarly, as it is the case in other hospital based case-control studies, hospital controls are more likely to have other associated pathologies or medical problems and be less different to the cases than controls selected from health centres.

The presence of vomiting was a risk factor for hospitalisation independent of the severity of the diarrhoea. This is in agreement with previous studies that have indicated that the majority of children with severe diarrhoea had vomits and fever before consultation and is often seen in children with rotavirus diarrhoea (Gupta *et al.*, 1996; Hart, 2003a; Vanderlei *et al.*, 2003).

Having a history of previous hospitalisation was a risk factor identified by both groups. Thirty three percent of inpatient children had a history of previous hospitalisations possibly reflecting a higher susceptibility of these children to infectious diseases leading to hospitalisation. These findings are in agreement with other studies that have found that past hospitalisation is a risk factor for further hospitalisation (Do Carmo-Leal *et al.*, 1996).

The presence of watery stools was an independently significant variable related to the risk of hospitalisation. However this was a significant risk factor that was only identified when comparing cases with health centre controls. This probably reflected the increased severity of the diarrhoea in inpatients and OPD controls than in the health centres. Similarly, hospitalised children were less likely to use ORS, but this was not a risk factor when cases were compared to the OPD controls. Health centre controls are more likely to be given ORS in the health centres and to accept ORS because they have milder episodes; while OPD controls are likely to be children who have an intermediate degree of severity or because the health facilities use cheaper and simple interventions in the health centres than in the OPD services.

In agreement with studies worldwide that have reported an effect of breast-feeding on diarrhoeal disease, children who were breast-fed for shorter periods were at higher

risk and this factor remained significant after allowing for the effect of other confounding variables (Arifeen et al., 2001).
As expected, the frequency of dehydration was higher in children admitted to the hospital than in OPD and health centre controls. This finding is in agreement with studies that have shown that dehydration is present in the majority of hospitalised children with acute diarrhoea. However in our logistic regression we did not observe that dehydration was a risk factor. Dehydration is strongly associated with watery diarrhoea, lack of ORS and vomiting; and these latter factors had stronger predictive values. This however does not mean that dehydration is not an important clinical criterion for management (Binka et al., 2003).

In conclusion, the risk factors for hospitalisation due to acute diarrhoea were the presence of bloody (AOR, 124.6) or watery stools (AOR, 31), lack of ORS use (AOR, 11.8), use of unsafe (spring/river) water (AOR, 11), having vomited the week before consultation (AOR, 6.6), having been breast fed for less than 7 months (AOR, 4.7), having an unhealthy mother (AOR, 3.2), having been hospitalised in the previous year (AOR, 3) and having an illiterate mother (AOR, 3).

Micronutrient status in cases and controls

It has been estimated that malnutrition is associated with up to two-thirds of all deaths among children and 12 million children under 5 years old die annually due to infection and malnutrition (Rice et al., 2000). In addition to protein-energy malnutrition, iron deficiency anaemia, vitamin A deficiency, zinc deficiency, selenium and Vitamin E are another important micronutrient deficiencies that play important roles in human immunity to fight infectious diseases (Stephenson et al., 2000).

The prevalence of vitamin A deficiency varies from 1% to 70% (Faber and Benade, 1999; Mehra *et al.*, 1994; Tan *et al.*, 2002). For vitamin E, most epidemiological studies have investigated serum vitamin E levels and its deficiency in subjects with cancer or health problems. However, two community based studies have reported vitamin E deficiencies in 22 % and 66% among populations in Turkey and Cameroon respectively (Gouado *et al.*, 1998; Wetherilt *et al.*, 1992).

Numerous studies have found that zinc supplementation reduces the duration, incidence and severity of diarrhoea episodes (Sazawal *et al.*, 1997; Sur *et al.*, 2003). Selenium and copper are also essential elements playing a crucial role in the immune system and have many important functional roles related to the maintenance of immune functions, bone health and haemostasis (Bonham *et al.*, 2002; Ciftci *et al.*, 2003).

Two hundred and fifty six and 196 serum samples from children hospitalised with and without acute diarrhoea were tested to analyse their micronutrient status. The serum geometrical mean vitamin A, E and selenium concentrations were significantly lower in children with diarrhoea than in controls. Concentrations of zinc serum were slightly higher in cases than controls (p= 0.06). The geometrical mean serum copper concentrations were not different in children with and without diarrhoea.

There was no clear pattern between micronutrient concentration and gender. However serum vitamin E concentrations were significantly higher in females than in males (P= 0.04). The prevalence of severe to moderate vitamin A deficiency (<0.70 μmol/L) was 28% in the children with diarrhoea and 19% in the control group respectively. In agreement with our results, studies from Turkey and China have reported that 2- 42% of children with diarrhoea have vitamin A deficiency (Kurugol *et al.*, 2000; Tan *et al.*, 2002) . Our prevalence of vitamin A deficiency however is higher than studies in Nigeria and Bangladesh that have reported prevalences of between 0% and 3-10% in children with diarrhoea (Agarwal *et al.*, 1996; Akinyinka *et al.*, 2001).

The prevalence of zinc deficiency was 82% in cases and this prevalence was very similar to the controls (81%). The very high prevalence of zinc deficiency in our study is one of the highest prevalences ever reported in the region and the world. However, consistent with our study, two studies from Burkina Faso and Indonesia have reported prevalence rates of zinc deficiency of 72% and 78% in children without diarrhoea respectively. A recent study in Turkey reported that the prevalence of zinc deficiency was 50% in children with diarrhoea and that zinc deficiency was also highly prevalent in healthy controls (Polat et al., 2003). There is only one study in Iran that examined zinc deficiency. In this study the authors reported a prevalence of 65% of zinc deficiency in junior high school female students in Tehran (Mahmoodi

and Kimiagar, 2001). One of the possibilities for this high prevalence of zinc deficiency in Iran might be related to the geographical characteristics and mountains, which surround the country. Zinc deficiency may be due to low consumption of animal products in low socio-economic sectors and poor content of this micronutrient in soil and food sources. This picture could be attributed to differences in zinc content of soil and/or differences in dietary habits of the families. The higher mean serum zinc in controls than the cases is also consistent with studies elsewhere that have reported lower serum zinc in children with diarrhoea than in children without diarrhoea.

Overall, the factors more often associated with micronutrient deficiencies were related to the characteristics of the clinical episode. For example, the presence of watery diarrhoea was associated with changes in vitamin E, zinc and copper concentrations. The presence of dehydration modified selenium, zinc and copper levels and a history of vomits in the week before consultation modified vitamin A and E and zinc concentrations. Enteropathic viruses were associated with low vitamin A and selenium, while rotavirus specifically modified selenium and copper. These associations have not been described before and merit further study.

The measurement of micronutrients during an acute infectious event however can be misleading as many of these elements behave like acute phase reactants. However, several studies have described that children who are deficient (or have low serum levels due to their redistribution to other organs) are at a higher risk of subsequent diarrhoea in the ensuing months. Most of these studies however have only measured one micronutrient at a time, and the cross sectional study presented in chapter 5 indicates that these deficiencies often occur simultaneously.

Effect of diarrhoea -associated micronutrient deficiencies on the risk of subsequent diarrhoea in children

To study the effect of micronutrient deficiencies on the risk of subsequent diarrhoea, this study follow a cohort of 211 children for 3 months. Our study revealed that, 153 (73%) of the 211 children recruited experienced no further diarrhoea episodes after hospital discharge and 58 (27%) had a total of 109 days with diarrhoea ranging from 1

to 4 episodes per child. The most frequent number of days with diarrhoea per child was one-day (59%), followed by 2 and 3 days. The mean duration of subsequent diarrhoea was significantly shorter than the initial episode (1.9 vs 4.3), ($P < 0.05$).

The multivariate logistic regression showed that nutritional parameters such as weight-for-height z score <-1, vitamin A and zinc deficiencies identified during the initial diarrhoea episode were the most significant variables associated with a child experiencing a subsequent episode of diarrhoea. These findings are in agreement with other worldwide studies which documented that there is an association between malnutrition and diarrhoea and other infectious diseases (Brown, 2003; Rice *et al.*, 2000; Uysal *et al.*, 2000; Wekell *et al.*, 1997). In addition children who had micronutrient concentrations below selected cut off points for vitamin A and zinc deficiency had a high risk of subsequent diarrhoea (vitamin A deficiency, OR =2.9 and zinc deficiency OR=2.8). Weight-for-age z score was also a statistically significant independent variable (AOR = 2.5).
There was a negative association between the duration of the initial episode of diarrhoea and the risk of subsequent diarrhoea and children with shorter initial diarrhoea episodes were more likely to have subsequent diarrhoea episodes (AOR =2.7). The mothers whose children had subsequent diarrhoea were also significantly older than mothers of children without subsequent diarrhoea (27.8 vs 25.4 years respectively).

This multivariate logistic regression analysis thus revealed that micronutrient deficiencies at the time of the initial acute episode and the child's nutritional status (as measured by anthropometry) are the main factors associated with an increased risk of having further diarrhoea episodes. Among the other variables investigated, keeping animals at home had a strong positive association with subsequent diarrhoea episodes. This association remained significant even after multivariate analysis. There are however no specific studies that have investigated the effect of contact with animals or of keeping animals at home with diarrhoea. Although it is possible that animals transmit infections, keeping animals at home is also likely to be a marker for a particular life style (e.g. poor farmers), which represents a risky set of behavioural patterns. In keeping with this hypothesis, many studies worldwide have reported a correlation between hygienic behaviour or an unclean domestic environment and the

incidence of diarrhoea and diarrhoea epidemics after farm visits (Haque *et al.*, 2003; Huttly *et al.*, 1987; Muhe *et al.*, 1995; Strina *et al.*, 2003) and this area merits further investigation.

Conclusion

This study has investigated the epidemiology of childhood diarrhoea in Shahrekord, Iran. Pathogens were detected in 62%, 44% and 18% of children hospitalised with diarrhoea, OPD and children hospitalised without diarrhoea respectively. Viruses were the most frequent pathogens detected followed by bacteria and parasites. Rotavirus was an important cause of severe diarrhoea and is over represented in the hospital. Rotavirus was most frequently detected in the cool season and was responsible for 35% and 18% of diarrhoea episodes in hospitalised and OPD children. The most frequent P and G rotavirus genotypes were P[8], P[4], G1, G2 .

In a multivariate analysis, the risk factors for hospitalisation due to acute diarrhoea were the presence of bloody (AOR, 124.6), or watery stools (AOR, 31), lack of ORS use (AOR, 11.8), use of unsafe (spring/river) water (AOR, 11), having vomited in the week before consultation (AOR, 6.6), having been breast fed for less than7 months (AOR, 4.7), having an unhealthy or illiterate mother (AOR, 3.2 and 3 respectively), and having been hospitalised in the previous year (AOR, 3).

The frequency of vitamins A and E and selenium deficiencies were statistically higher in children with diarrhoea than in controls. The proportion of children who were zinc and copper deficient (< 9.94 and 1.5 μmol/L respectively) were similar in children with and without diarrhoea. More than 75% of the children were vitamin E and zinc deficient.

Factors associated with micronutrient deficiencies were related to the characteristics of the clinical episode. Watery diarrhoea was associated with changes in vitamin E, zinc and copper concentrations. The presence of dehydration modified selenium, zinc and copper concentrations and a history of vomiting in the week before consultation modified vitamin A and E and zinc concentrations. Viruses were associated with low vitamin A and selenium, while rotavirus modified selenium and copper. Nutritional parameters (weight-for-age z score <-1, vitamin A and zinc deficiency as observed in the initial episode) were the most significant independent variables associated with an increased risk of a child having subsequent diarrhoea episode during the 3 months after hospital discharge. Children who had micronutrient concentrations below selected cut off points for vitamin A and zinc had a higher risk of subsequent diarrhoea (vitamin A deficiency, OR =2.9 and zinc deficiency OR=2.8).

Recommendations

There are very few studies that have investigated the epidemiology of rotavirus in childhood diarrhoea in Iran. Studies that describe its epidemiology over consecutive seasons are required to better understand its characteristics. Rotavirus is an important cause of childhood diarrhoea. However our observation that rotavirus was present in 9% of asymptomatic children, should be further investigated, as this could be an important source of transmission. The role that rotavirus plays in hospitalised children should be highlighted to medical staff to reduce unnecessary antibiotic therapy. Despite recent progress in laboratory techniques, rotavirus diagnostic tests are not routinely used in Iran, use of such tests would be helpful to prevent unnecessary use of antibiotic therapy. The distribution of P and G types in our study and the high morbidity in children < 2 years of age will support the introduction of the rotavirus vaccines once they become available. Further studies should be conducted to monitor P and G genotype changes. Unfortunately in Iran many parents and medical staff believe that antibiotic therapy is the best management for diarrhoea. This attitude needs to be changed and this study highlights the importance of rotavirus in the aetiology of diarrhoea. The future role of rotavirus screening tests needs to be discussed for operational purposes. The treatment of a child with diarrhoea is based on symptomatic and supportive treatment in the vast majority of cases. Most of the tests described in this thesis therefore are not necessary for the appropriate treatment of an individual patient and should only be available in specialised reference laboratories or research centres. These tests however are very useful to monitor the predominant genotypes and pathogens responsible for diarrhoea and would be appropriate for use in a surveillance centre for diarrhoea, which would inform the introduction and development of potential rotavirus vaccines.

Risk factors for hospitalisation due to acute diarrhoea identified variables that are modifiable. These included lack of ORS use (AOR, 11.8), use of unsafe (spring/river) water (AOR, 11), breast-feeding for less than 7 months (AOR, 4.7) and having an unhealthy or illiterate mother (AOR, 3.2 and 3). The presentation of these factors would prevent a high proportion of hospitalisation and intervention studies should be conducted to assess their efficiency. It is necessary to improve the attitude of the

parents about ORS and its role for the management of acute diarrhoea. The maternal factors and the association between short breast-feeding and higher risk of diarrhoea in our study are good guidelines for the government to improve the mothers' knowledge about their important roles in childhood diarrhoea and its management.

The frequency of vitamin A and E and selenium deficiencies were statistically higher in children with diarrhoea than in controls. More than 75% of children were vitamin E deficient and zinc deficient. It seems that childhood micronutrient deficiencies in Iran are highly prevalent and national surveys should be conducted to assess their prevalence in asymptomatic children.

Our study documented that more than one factor is often implicated in a child and that an aggregation of factors can have an additive effect on the risk of subsequent diarrhoea. It is therefore conceivable that multiple micronutrient supplementation could have a better effect than single micronutrient supplementation. This approach is currently being investigated by UNICEF and our findings provide further evidence for its use in developing countries, these interventions should be tested in Iran.

Children with micronutrient concentrations below selected cut off points for vitamin A and zinc deficiency had a higher risk of subsequent diarrhoea. These cut off points are good markers for parents and medical staff of hospitalised children with micronutrient deficiency to improve their micronutrient status and reduce diarrhoea episodes in the ensuing months after hospital discharge.

Keeping animals at home had a strong positive association with subsequent diarrhoea episodes. This association remained even after multivariate analysis. It is possible that animals transmit some infections. Keeping animals at home is also likely to be a marker for a particular lifestyle and to represent a risky behavioural pattern. This observation may help the government to encourage farmers to change their traditional mode of farming whereby animals are kept in residential areas and their own houses and move to the countryside. Farmers should be made aware of this risk for the benefit of their family and other people. Alternatively in the long-term, legislation should be required to remove farmers from residential areas. The association between

diarrhoea or hospitalisation due to diarrhoea and environmental risk factors such as keeping animal at home and using unsafe sources of water for drinking purposes need further explanation and studies that focus this specific question should be encouraged.

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Appendix 1:

Epidemiology of acute diarrhoea in Iranian children

Cross sectional yes =1, no=2 ☐

Case control (case =1, hospital control =2,clinic control =3, health centre control=4) ☐ follow up yes =1, no =2 ☐

Study number ☐☐☐

Hospital number ☐☐☐

Place of enrolment_____

First name _____ **Surname** _____

Address: _____

Telephone number (if follow up) _____

d d m m y y

1-Sex: (female =1, male=2) ☐ 2-Date of admission: ☐☐ ☐☐ ☐☐

d d m m y y

3-Age: (months) 4-Date of discharge: ☐☐ ☐☐ ☐☐

5-place of the birth: (hospital=1, health centre=2, home=3)

6--Reason for consultation: 1-_____

2-_____

3-_____

History of current episode

7--For how long has the child had diarrhoea? Days ☐☐

8-Was the stool watery in the last 24 hours? (Yes=1, no=2) ☐

9-Was the stool bloody in the last 24 hours? (Yes=1, no=2) ☐

10-Was the stool semi-liquid? (Yes=1, no=2) ☐

11-How many stools had the child in the last 24 hours? ☐

12- has the child vomited in the last week? (Yes=1, no=2) ☐

13-If yes, for how many days? ☐☐

14-Does the child has vomited in the last 24 hours? ☐

15-If yes, how many times? ☐

16- have you given ORS to your child? (Yes=1, no=2) ☐

17-Has the child had fever in the pervious 5 days? (Yes =1, no=2) ☐

Previous medical history:

18-Has your child ever breast-fed? (Yes=1, no=2) ☐

19-If yes, is your child breast-feeding now? (Yes=1, no=2) ☐

20-If no breast feeding, how old was the child when he/she stopped B.F? ☐

21-is the child receiving complemeny foods now? (Yes =1, No, BF only=2) ☐

22-If yes, would you tell us the age when he/she started to eat other foods? (Months) ☐☐

23- do you know what was your child birth weight? (grams) ☐☐☐☐

Not known ☐

24-Has your child ever been hospitalised before? (Yes=1, no=2) ☐

25-If yes, how many times? ☐☐

27-Does your child have any long term disease? (Yes=1,no=2,don't know=3) ☐

-If yes, which one/s? _____

28-Has your child had diarrhoea in the last year? (Yes=1, no=2) ☐

29-If yes, how many times? ☐

30-How many days did it last the last your child diarrhoea? (day) ☐☐

31-Approximately how long age was last diarrhoea episode?

Months ☐ weeks ☐☐

32-Has your child had measles in last 6 months? (Yes=1, no=2) ☐

33-How many days did it last the last time your child had diarrhoea (days) ☐☐

34-How many times has your child attended a health centre in the last month? ☐

35-How many times has your child attended a private doctor in the last month? ☐

36-Have you given any medication to your child in the last week? (Yes=1, no=2) ☐

If yes, which one/s _____ ☐

Physical exam:

37- How is the child's alertness? (Well, alert=1, restless-irritable =2, sleepy, floppy or uncounscious=3) ☐

38-are the eyes normal/ sunken or very sunken?(Normal=1,sunken=2, very sunken=3) ☐

39-Does the child has tears when he/she cries? (Yes=1,no=2,not known=3) ☐

40-Is the child able to drink liquid? (Yes=1,no=2) ☐

41-If so, is the child thirstier than normal? (Yes=1,no=2) ☐

42-Is the child's tongue wet, dry or very dry? (Wet=1,dry=2,very dry=3) ☐

43-Skin pinch: (Goes back quickly =1, goes back slowly=2, goes back very slowly=3) ☐

44- is the child's fontanel sunken? (Yes=1, no=2,not ☐

applicable=3)

45-Weight (gr) ☐☐☐☐

46- Height (cm.) ☐☐

47-Mid-upper arm circumference (MUAC) (cm) ☐☐

48-Respiratory rate ☐☐

49-Heart rate ☐☐

50-Temperature on admission: ☐☐

Family characteristics:

Mother's name _____

51-Mother's age: years ☐☐

52-Mother's education: (reads and writes =1, primary =2, secondary=3,

University =4, none =5)

53-Number of the pregnancies? ☐

54-Is the mother's child healthy? (Yes=1, no=2) ☐

-If no, what is the major disease: _____

55-Which child number is this one in your family? ☐

56-Father's education: (reads and writes =1, primary =2, secondary=3, ☐

University =4, none =5)

57-Father's occupation: (farmer=1, manual worker=2, official desk work=3, unemployment=4,

shopper=5) ☐

58-How many bedrooms/ rooms do you have at home? ☐

59-How many people live in your house? ☐

60-How many people sleep in the same room with your child? ☐

61-Has anybody else had diarrhoea at your home during in past 2 weeks ☐

(Yes=1, no=2) ☐

62-If so, how many people had diarrhoea? ☐

☐

63- do you keep animals in your home? (Yes=1, no=2)

64-If yes, which one/s do you have? ☐

cow ☐

goat ☐

sheep ☐

horse or donkey ☐

chicken ☐

dog

65-What kind is your living house? (Modern=1, old=2) ☐

66-Do you have piped water in your home? (Yes=1, no=2) ☐ 67-If no which one do you use? (Spring=1, river=2, lake=3) ☐

68-do you use other water sources for cloths or washing dishes? (Yes=1, no=2) ☐

69-Have you travelled in last two weeks with your child? (Yes=1, no=2) ☐

Only for objective 4 (after discharge from hospital)
(Visit 1):

Respondent: (mother/father=1, other relative =2, neighbours =3. Not followed =4) ☐

Date of planned follow up: d d m m y y ☐☐ ☐☐ ☐☐

Date followed: d d m m y y ☐☐ ☐☐ ☐☐

Has the child had diarrhoea in last 2 weeks? (Yes=1, no=2) ☐

If yes, for how many days? (days) ☐

How many liquid- stools per day does the child in last 24 hours?

Has the child had vomiting in last 2 weeks (Yes=1, no=2) ☐

If yes, for how many days? (days) ☐

(Visit 2):

Respondent: (mother/father=1, other relative =2, neighbours =3. Not followed =4) ☐

Date of planned follow up: d d m m y y ☐☐ ☐☐ ☐☐

Date followed: d d m m y y ☐☐ ☐☐ ☐☐

Has the child had diarrhoea in last 2 weeks? (Yes=1, no=2) ☐

If yes, for how many days? (days) ☐

How many liquid- stools per day does the child in last 24 hours?

Has the child had vomiting in last 2 weeks (Yes=1, no=2) ☐

If yes, for how many days (days

Visit number: 1- 2- 3- 4- 5- 6-

7- 8- 9- 10- 11- 12-

13- 14-

Respondent: (mother/father=1, other relative =2, neighbours =3. Not followed =4) d d m m y y

Date of planned follow up: ☐☐ ☐☐ ☐☐

Date followed: ☐☐ ☐☐ ☐☐

Has the child had diarrhoea in last 2 weeks? (Yes=1, no=2) ☐

If yes, for how many days? (days) ☐

How many liquid- stools per day does the child in last 24 hours?

Has the child had vomiting in last 2 weeks (Yes=1, no=2) ☐

If yes, for how many days (days) ☐

Laboratory investigation:

Name _____

Study number ☐☐☐

Parasite:

Was faeces sample taken? (Yes=1, no=2)

Wet mount _____

Zn Stain for Cryptosporidium: (Pos=1,neg=2, not done =3) ☐

PCR foe Cryptosporidium: (Pos=1,neg=2, not done =3) ☐

Bacteria:

Culture:

E.coli ☐

Salmonella ☐

Shigella ☐

Campylobacter ☐

others ☐

Serology (yes=1, no=2) ☐ salmonella ☐

(yes=1, No=2) Shigella ☐

(yes=1, no=2) E.coli

Was sample saved for Liverpool ? (yes=1, no=2)

Viruses: (positive=1, negative 2)

If positive which one was?

(HRV=1, Adenovirus =2,Astrovirus=3,Calicivirus=4)

For prevalence of micronutrient

Was blood sample taken? (yes=1, no=2)

Vitamin A: _____

Vitamin E: _____

Se: _____

Cu: _____

Zn: _____

Appendix 2: questionnaire translated into Farsi

بررسی وضعیت اپیدمیولوژیکی اسهال حاد در کودکان زیر ۵ سال

نام و نام خانوادگی

شماره مطالعه

سن بیمار:

توصیفی - مقطعی

کنترل موردی - بیمار = ۱، نمونه کنترل بیمارستان = ۲، کلینیک = ۳، مرکز بهداشتی = ٤ ☐

آیا برای مطالعهٔ کوهورت انتخاب شده است (بلی = ۱ و خیر = ۲ ☐

شماره پرونده بیمارستانی

محل انتخاب

آدرس:

تلفن

تاریخ پذیرش: تاریخ ترخیص:

جنسیت - دختر = ۱، پسر = ۲ ☐

محل تولد - بیمارستان = ۱، مراکز بهداشتی = ۲، خانه = ۳ ☐

علّت بستری شدن

۱ - .....

۲ - .....

۳ - .....

تاریخچهٔ اسهال و بیماری کنونی

- چه مدت اسهال دارد (روز) ☐☐

آیا مدفوع ٢٤ ساعته گذشته آبکی بوده؟ بلی = ۱، خیر = ۲ ☐

آیا مدفوع ٢٤ ساعته گذشته خونی بوده؟ بلی = ۱، خیر = ۲ ☐

آیا مدفوع ٢٤ ساعت گذشته، نیمه مایع بوده؟ بلی = ۱، خیر = ۲ ☐

چه تعداد مدفوع در ٢٤ ساعت گذشته دفع کرده است؟ ☐

در ماه گذشته به پزشک خصوصی مراجعه کرده است ؟بلی = ۱ ، خیر = ۲ ☐

آیا کودک هرگز در بیمارستان بستری شده است ؟ بلی = ۱ ، خیر = ۲ ☐

اگر جواب آری است چند بار بستری شده است ؟ ☐

آیا کودک هیچگونه دارویی در طول هفته گذشته دریافت کرده است بلی = ۱ ، خیر = ۲ ☐

اگر جواب آری است کدام دارو را دریافت کرده است .

۱ - .....

۲ - .....

۳ - .....

معاینه فیزیکی

- آیا کودک هوشیار است ؟ هوشیار=۱ ، بی قرار=۲ ، خواب آلود=۳ ، غیرهوشیار (بی هوش)=٤ ☐

- آیا کودک در زمان گریه اشک دارد ؟ بلی = ۱ ، خیر = ۲ ، نامعلوم = ۳ ☐

- اگر جواب آری است آیا کودک تشنه است ؟ بلی = ۱ ، خیر = ۲ ☐

- وضعیت تورگر پوست بیمار چگونه است ؟

سریعا به حالت اول بر می گردد=۱ آهسته بر می گردد = ۲ ، خیلی آهسته بر می گردد = ۳ ☐

- آیا وضعیت چشمهای کودک نرمال است ؟ نرمال = ۱ ، فرو رفته = ۲ ، خیلی فرورفته = ۳ ☐

- آیا کودک قادر به نوشیدن است ؟بلی = ۱ ، خیر = ۲ ☐

- آیا زبان کودک مرطوب است ؟ مرطوب = ۱ ، خشک = ۲ ، خیلی خشک = ۳ ☐

- آیا فونتانل فرو رفته است ؟بلی = ۱ ، خیر = ۲ ، نامعلوم = ۳ ☐

- وزن کودک gr. تعداد ضربان .....

- قد کودک cm ..... درجه حرارت .....

- تعداد تنفس ..... دور بازو.....

خصوصیات فامیلی

آیا در طول دو هفته گذشته در خانواده شما کسی اسهال داشته است ؟ بلی = ۱ ، خیر = ۲ ☐

اگر جواب آری است چند نفر اسهال داشته‌اند ☐

آیا بچه در هفته گذشته استفراغ داشته است ؟ بلی = ۱ ، خیر = ۲ ☐

اگر جواب آری است چند روز استفراغ داشته است ؟ ☐

آیا در ۲٤ ساعت گذشته کودک استفراغ داشته است ؟ بلی = ۱ ، خیر = ۲ ☐

اگر جواب آری است چند بار استفراغ داشته است ؟ ☐

آیا کودک با او.آر.اس تغذیه شده است ؟ بلی = ۱ ، خیر = ۲ ☐

آیا کودک در ٥ روز گذشته تب داشته است ؟ بلی = ۱ ، خیر = ۲ ☐

سابقهٔ پزشکی

آیا کودک با شیر مادر تغذیه شده است ؟ بلی = ۱ ، خیر = ۲ ☐

اگر جواب آری است آیا اکنون با شیر مادر تغذیه می‌شود ؟ بلی = ۱ ، خیر = ۲ ☐

اگر با شیر مادر تغذیه نمی‌شود ، در چه سنی تغذیه با شیر مادر متوقف شده است ؟ ماه ☐☐

آیا در حال حاضر با غذای کمکی تغذیه می‌شود ؟ بلی = ۱ ، خیر = ۲ ☐

اگر جواب آری است در چه سنی شروع به خوردن غذای کمکی کرده ؟ ماه ☐☐

آیا وزن کودک را در بدو تولد می‌دانید گرم ☐☐☐☐ نامعلوم ☐

آیا در طول یک سال گذشته کودک بیماری داشته است ؟ بلی = ۱ ، خیر = ۲ و نامعلوم = ۳ ☐

اگر جواب آری است کدام بیماری را داشته است ؟

۱ - .....

۲ - .....

۳ - .....

آیا در طول دو هفته گذشته با کودک خود مسافرتی داشته‌اید ؟ بلی = ۱ ، خیر = ۲ ☐

آیا کودک در سال گذشته مبتلا به اسهال بوده است ؟ بلی = ۱ ، خیر = ۲ ☐

اگر جواب آری است چند مرتبه اسهال داشته است ؟ ☐

آخرین باری که اسهال داشته چند وقت پیش بود ☐

آخرین مرتبه اسهال چند روز طول کشید ؟ ☐

در ماه گذشته به مرکز بهداشتی مراجعه کرده است ؟ بلی = ۱ ، خیر = ۲ ☐

مادر . سن مادر (سال) □□

تحصیلات مادر : بیسواد = ۱ . خواندن و نوشتن = ۲ ، ابتدایی = ۳ ، دبیرستان = ٤ ، دانشگاهی = ٥ □

شغل مادر ....

آیا مادر کودک سالم است بلی = ۱ ، خیر = ۲ □

اگر جواب خیر است بیماری چیست ؟ ۱ -

۲ -

این کودک چندمین کودک در خانواده است شماره □

شغل پدر

تحصیلات پدر : بیسواد = ۱ ، خواندن و نوشتن = ۲ ، ابتدایی = ۳ ، دبیرستان = ٤ ، دانشگاهی = ٥ □

خصوصیات منزل مسکونی

- منزل مسکونی شما چند اتاق دارد؟ □□

- چند نفر در خانهٔ شما با هم زندگی می‌کنند ؟ □

- چند نفر در یک اتاق با این کودک زندگی می‌کنند □

- آیا در منزل حیوان نگهداری می‌کنید ؟ بلی = ۱ ، خیر = ۲ □

اگر جواب آری است کدام یک از حیوانات را دارید ؟

گاو = ۱ ، بز = ۲ ، گوسفند = ۳ . جوجه = ٤ ، سگ = ٥ ، اسب و یا خر = ٦ □

منزل مسکونی شما چه نوع است ؟ جدید (کریدوری) = ۱ و قدیمی = ۲ □

آیا در منزل مسکونی خود آب لوله‌کشی دارید ؟ بلی = ۱ ، خیر = ۲ □

اگر جواب خیر است چه نوع آب مصرفی دارید ؟ آب چشمه = ۱ ، رودخانه = ۲ ، دریاچه = ۳ . □

آیا از منابع آبی دیگری مانند رودخانه ، دریاچه ، چشمه برای مصارف دیگری مانند شستن لباسها و یا شستشوی ظروف استفاده می‌کنید ؟ بلی = ۱ ، خیر = ۲ □

Journal of Medical Virology 73:309–312 (2004)

Epidemiology of Rotavirus Diarrhoea in Iranian Children

B. Khalili,[1,2] L.E. Cuevas,[1]* N. Reisi,[3] W. Dove,[2] N.A. Cunliffe,[2] and C.A. Hart[2]
[1]*Liverpool School of Tropical Medicine, Liverpool, United Kingdom*
[2]*Department of Medical Microbiology and Genitourinary Medicine, Liverpool, United Kingdom*
[3]*Faculty of Medicine, Shahrekord University of Medical Science, United Kingdom*

Human rotavirus is the most important cause of severe diarrhoea in infants and young children worldwide. We describe the aetiology of viral diarrhoea and the characteristics of rotavirus infection in Shahrekord, Iran. Two hundred and fifty nine children <5 years old admitted to Hajar Hospital, 245 children with acute diarrhoea attending primary health centres in Shahrekord, and 114 children hospitalised for elective surgery were selected from October 2001 to August 2002. Stool samples were screened for enteric viruses using EM. Rotaviruses were characterised using ELISA, reverse transcription-polymerase chain reaction (RT-PCR), and electropherotyping. One hundred and eighty six viruses were identified, of which 146 (78%) were rotavirus. The second most frequent virus was coronavirus, followed by calicivirus. Rotaviruses exhibited a marked seasonal variation, being most frequently isolated from November to February (50% of rotavirus recovered) and affected mostly children <2 years old. The RT-PCR successfully typed 139 of the 146 (95%) rotavirus G types and 124 (85%) P types. The most frequent P type was, P[8] in 108 (74%), P[4] in 16 (11%), and was P non-typeable in 22 (15%). Among the G types, G1 was identified in 120 (82%), G2 in 19 (13%), and was G-non-typeable in 7 (5%). Our results are the first report of rotavirus genotypes affecting Iranian children. The most frequent G and P types (G1, G2, P[8], and P[4]) are similar to those reported from around the world and will be covered by existing rotavirus vaccines targeting G types G1–G4. ***J. Med. Virol. 73:309–312, 2004.***

KEY WORDS: rotavirus; epidemiology; diarrhoea; children; Iran

INTRODUCTION

Diarrhoea is one of the most common diseases in children resulting in the death of more than 5 million children every year. The greatest morbidity and mortality is seen among children less than 2 years of age [Carroll and Reimer, 2000; Hart, 2003].

Human Rotavirus is responsible for a large proportion of these deaths and 20–52% of acute diarrhoea episodes [Cunliffe et al., 2002a; Hart, 2003]. Rotavirus is a double stranded RNA virus with a genome comprising 11 linear segments, each encoding one or two virus proteins (VPs 1, 2, 3, 4, 6, 7). VP4 and VP7, the outer capsid proteins encoded by respectively genome segments 4 and 9 are both involved in attachment to and entry into enterocytes and are the major rotavirus-neutralizing antigens. Epitopes on VP7 define the G (for glycoprotein) types whereas those on VP4 define the P (for protease-sensitive) types. Currently 14 G and 20 P types have been described. Globally, G1–G4 are the most common G types although there is a tremendous diversity as, for example, while G8 has caused 30% of gastro-enteritis in Malawi [Cunliffe et al., 2001a], G5 was the G type most often isolated in South America [Gouvea et al., 1994], and other novel serotypes (e.g., G9) have been reported worldwide recently [Ramachandran et al., 2000; Cunliffe et al., 2002b].

Diarrhoea is still one of the main health problems in Iranian children. There is no information on the pathogens most often involved, nevertheless many hospitals beds are still occupied by children with acute diarrhoea. This study describes the aetiology of viral diarrhoea and the characteristics of rotavirus in children with acute diarrhoea in Shahrekord, Iran.

MATERIALS AND METHODS

Children under 5 years old admitted to Hajar Hospital (inpatients) or attending three primary health centres (outpatients) in Shahrekord, Iran with a clinical diagnosis of acute diarrhoea were selected systematically

*Correspondence to: Dr. L.E. Cuevas, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA.
E-mail: lcuevas@liv.ac.uk
Accepted 23 February 2004
DOI 10.1002/jmv.20092
Published online in Wiley InterScience (www.interscience.wiley.com)

during working days from October 2001 to August 2002. In addition, a group of children hospitalised for elective surgery in Hajar Hospital (surgical controls) were selected for comparison. After informed consent, participants were interviewed to ascertain their demographic and social background and clinical history. For the purpose of this study, acute diarrhoea was defined as the presence of three or more liquid or semi-liquid stools or a single watery stool per day of less than 14-days duration. A minimum of five children were enrolled every week to obtain a representative sample of the pathogens for the duration of the study.

Stool samples were collected in plastic containers for light microscopy, rotavirus enzyme immunoassays (EIA), negative stain electron microscopy (EM), rotavirus reverse transcription polymerase chain reaction (RT-PCR), and electropherotyping. EM was performed using a Philip 301 electron microscope (Philips Electron optics UK Division, PYE Unicam, Ltd, Cambridge, UK) [Madeley, 1997] at a screen magnification of 45,000×. Grids were scanned horizontally along the rows of the grid squares from end to end at three different locations. Rotavirus antigens were detected using the Rotaclone EIA kit (Meridian Diagnostics, Cincinnati, OH) as described by the manufacturer. EIA positive samples were suspended in phosphate buffered saline at a concentration of 10% for dsRNA extraction. Suspensions were clarified by centrifugation and dsRNA was extracted using a guanidine isothiocyanate/silica method developed by Boom et al. and modified by Gentsch et al. [1992]. Extracted RNA was eluted in 70 µl of RNase free water and used directly for polyacrylamide gel electrophoresis (PAGE) and RT-PCR. For PAGE, 30 µl of purified dsRNA was heated at 65°C for 10 min and electrophoresed for 120 min at 150 V on 10% polyacrylamide gel. Controls of known long and short electropherotypes were included on each gel. After silver nitrate staining [Herring et al., 1982], the gels were photographed to determine the electropherotypes. Rotavirus G- and P-genotypes were determined using a multiplex RT-PCR as described by Gouvea et al. [1990] and Gentsch et al. [1992].

Amplicons were visualised under UV light after electrophoresis on a 2% agarose gel stained with ethidium bromide. Samples co-migrating with reference strains of known genotypes were assigned a genotype. Samples failing to G or P type were repeated with additional G1 and P[8] typing primers nac 9 and nac 10 respectively [Cunliffe et al., 2001b].

RESULTS

Stool samples were collected from 259 inpatients and 245 outpatients with a clinical diagnosis of diarrhoea and 114 surgical controls. The general characteristics of the participants are shown in Table I. The mean (SD) age of the hospitalised and outpatient children were similar with 15.2 (12) and 15.6 (12.3) months, respectively. Surgical controls however were older with a mean (SD) age of 30 (12.4) months. The gender distribution of the children was comparable with 143 (55%), 133 (54%), and 65 (57%) children being male among the hospita-

TABLE I. Characteristics of Hospitalised and Outpatient Children With Diarrhoea and Surgical Controls

		Children with diarrhoea		
		Inpatients	Outpatients	Surgical controls
Number		259	245	114
Male		143 (55%)	133 (54%)	65 (57%)
Age (months)	1–12	149 (58%)	135 (55%)	7 (6%)
	13–24	76 (29%)	71 (29%)	34 (30%)
	25–59	34 (13%)	39 (16%)	73 (64%)
Age (SD)		15.2 (12.)	15.6 (12.3)	30 (12.4)
Reason for consultation	Diarrhoea	259 (100%)	245 (100)	NA
	Vomiting	196 (76)	54 (23%)	NA
	Fever	33 (13%)	5 (2%)	NA
	Surgery	1 (1%)	0 (0%)	114 (100%)
Birth weight in kg (SD)		3.1 (5.7)	3.1 (5.2)	3.1 (5.1)
Diarrhoea duration (days)		4.3 (3.3)	3.7 (2.7)[a]	NA
Number of episodes in previous 24 hr		7.1 (3.8)	5.7 (2.8)[a]	NA
Consistency	Watery	249 (97%)	225 (92%)	NA
	Semi liquid	128 (49%)	170 (69%)[a]	NA
	Bloody	28 (11%)	7 (3%)[a]	NA
Vomited in previous 24 hr		165 (64%)	66 (27%)[a]	NA
ARI		14 (6%)	5 (2%)[a]	NA
Fever		201 (78%)	127 (54%)[a]	NA
Viruses	Rotavirus	91 (35%)	45 (18%)	10 (8.4%)
	Coronavirus	4 (1.5%)	10 (4%)	2 (1.9%)
	Astrovirus	4 (1.5%)	2 (2%)	0 (0%)
	Calicivirus	3 (1.2%)	5 (2%)	1 (0.5%)
	Adenovirus	3 (1.2%)	5 (2%)	0 (0%)
	Parvovirus	1 (1%)	0 (0%)	0 (0%)

[a] $P < 0.05$ when compared to hospitalised children with diarrhoea, NA = not applicable.

lised, outpatients, and surgical controls, respectively. The duration of the diarrhoea episode before consultation was higher in hospitalised than outpatient children with a mean (SD) of 4.3 (3.3) and 3.7 (2.7) days respectively and they had higher stool frequencies before enrolment ($P < 0.05$ for both). Vomiting was the second most frequent complaint and again, hospitalised children were vomiting more frequently than outpatients (76 and 23%, respectively) ($P < 0.05$). Fever was present in the majority of hospitalised children (78%) and acute respiratory symptoms were associated with the diarrhoea episodes in a smaller number (6%).

A total of 186 episodes of virus excretion were identified and rotavirus was identified in 146 (78%). Ten (8.4%) of the rotaviruses were identified in children without diarrhoea as shown in Table I. The second virus detected most frequently was coronavirus (16), followed by calicivirus (all norovirus-like) (9). Other viruses included adenovirus (8), astrovirus (6), and parvovirus (1). Each of the other viruses except rotavirus was recovered from children with diarrhoea.

Rotavirus was identified more frequently in children <2 years old (37 and 19% in hospitalised and outpatient children, respectively) than children >2 years old (23 and 10% for hospitalised and outpatients, respectively), with the highest frequency observed in 1 to 2 year old children followed by children <1 year old. More than half of the rotaviruses identified in hospitalised children were isolated from November to February, with lower numbers occurring during the hot summer months between June and August. Rotavirus infection had a similar pattern in outpatient children as most cases were detected from February to March (the cold winter season) and was rare during the hot summer months between June and August. Rotavirus, however, was responsible for a lower proportion of cases of diarrhoea in the health centres where other viruses (mainly calicivirus and coronavirus) were also identified frequently. Most asymptomatic rotavirus infections identified in surgical controls occurred at the peak of the rotavirus season. Our results did not demonstrate a clear seasonal pattern for other viruses besides rotavirus, as frequencies were too small to show any differences. Astrovirus and adenovirus, however, were mostly identified in the cooler semester of the year.

RT-PCR assigned G types to 139 of 146 (95%) of rotaviruses and P types to 124 (85%) (Fig. 1). The most frequent P type was P[8] in 108 (74%) samples, P[4] in 16 (11%), and 22 (15%) were P-non-typeable. Among the G types, G1 was identified in 120 (82%) samples, G2 in 19 (13%), and 7 (5%) were G-non-typeable. Sixty one strains required the alternative P[8] typing primer nac 10 and 42 strains required the alternative G1 typing primer nac 9. The type designations of strains requiring alternative primers were selectively confirmed by nucleotide sequencing (data not shown).

Electrophoretypes were done for the first 46 rotavirus positive samples. All the short-electrophoretypes were P[4] G2 and all long-electrophoretypes were a combination of P[8]G1and P[8]G2. P[4] had similar monthly

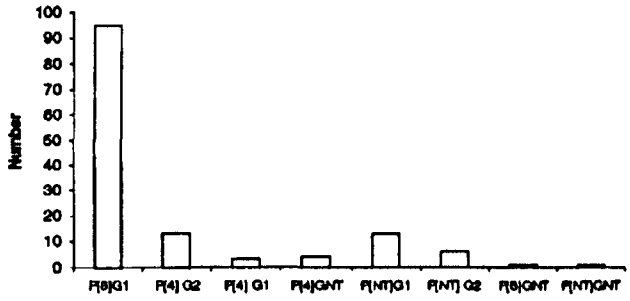

Fig. 1. Distribution of P–G combinations.

distribution patterns to the G and P non-typeable specimens and these were most frequent in the last and first months of the year, the time when rotavirus was most frequent. The variation of P–G combinations seen during the rest of the year suggests that there is wide strain diversity, with some strains predominating in a given year (data not shown). P[8] rotaviruses were seen in all months. P[4] was seen only as a cluster at the end of the year and P-non-typeable strains were mostly seen from January to July.

DISCUSSION

This report confirms that rotavirus is an important cause of paediatric diarrhoea in Southwestern Iran. Our findings are in agreement with reports world wide and a previous study from Iran [Moddares, 1995; Kelkar et al., 1999] which reported that rotavirus is the most important cause of severe diarrhoea in children. In our study, rotavirus was responsible for 35% of diarrhoea episodes in hospitalised children and 18% of episodes in the children attending the health centres. This over representation in hospitalised children is the result of the more severe clinical episode resulting from rotavirus as compared to other viruses and is a well-recognised feature of rotavirus infection globally [el-Sheikh et al., 2001]. The frequency of rotavirus infection was strongly associated with age and more than 90% of rotavirus cases occurred in children <2 years old. The highest frequency was observed in 13–24 month old children and the second most affected group were infants.

Rotavirus has a marked seasonal variation in this region and was isolated most frequently from November to February with 50% of rotaviruses recovered in these months. These results are in agreement with studies reporting a peak of rotavirus in winter months in temperate regions. The peak incidence, however, is different to a previous study from Tehran [Amini et al., 1990], which reported a higher incidence in the spring. Although the peak incidence occurred in the winter, the incidence of rotavirus was still high in the spring. We would need to continue surveillance for rotavirus to elucidate whether the two studies have demonstrated a different seasonal pattern in two towns, or variations in the annual incidence with a higher rotavirus incidence in the year when our study took place.

The frequency of detection of other viruses, besides rotavirus, was higher in outpatient children. This is likely to result from the milder diarrhoea caused by these viruses. Similar to rotavirus, astrovirus and adenovirus were identified mostly in the cooler semester of the year. This is the most frequent season for these viruses in temperate climates and varies from tropical areas, when these viruses often occur during the rainy season [Sethi et al., 1989]. The data, however, did not demonstrate a clear pattern, as the calicivirus and coronavirus frequencies were too small to find statistical differences. In addition, although coronavirus have been reported as a cause of diarrhoeal disease, their role is unclear [Zhang et al., 1994]. Adenoviruses however seemed to coincide with the rotavirus season and calicivirus appeared after this season from May to June. However it is stressed that the prevalence of calicivirus reported here is likely to be an underestimate as EM is not the most sensitive method for detecting the virus [Kirkwood and Bishop, 2001]. Viruses other than rotavirus however did not seem to cluster around any specific month.

The results in the first report on the genotypes of rotavirus affecting Iranian children. It is noteworthy that a large number of rotaviruses could not be G or P typed with the conventional primers described by Gouvea et al. [1990] and Gentsch et al. [1992] and additional primers were required that substantially increased the number of typable strains in our collection. The monthly distribution of the G and P types shows that the G2 and P[4] and non-typeable G and P were most often seen at the last and first months of the year and other P–G combinations were seen during the year, reflecting that there is a large diversity of rotavirus strains during the year. G1, G2, P[8], and P[4] are among the most common global rotavirus serotypes and should be covered by existing rotavirus vaccines [Hoshino and Kapikian, 2000].

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Appendix 4: Interim results from the logistic regression for clinical signs risk factors for hospitalisation (hospitalised vs OPD)

EpiInfo 2002

Results Library
Current View: **C:\ALLDEF~1\database\IRAN2c.MDB:IRAN2**
Record Count: **952** *Date:* **29/06/2004 10:20:09**

LOGISTIC CC3 = BRELESSTHAN7CC3 dehydration HasFewerThe HasTheChild HasTheChildVomited HaveYouGiven IsTheChild STOOL WasTheStool WasTheStoolBloody WasTheStooLSemiliquid

Next Procedure

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
BRELESSTHAN7CC3	2.5847	0.8814	7.5794	0.9496	0.5489	1.7301	0.0836
dehydration (b.moderate/a.none)	1.4228	0.4830	4.1910	0.3526	0.5512	0.6398	0.5223
dehydration (c.severe/a.none)	0.0000	0.0000	>1.0E12	-12.9004	320.0878	-0.0403	0.9679
HasFewerThe	1.8123	0.5312	6.1827	0.5946	0.6261	0.9496	0.3423
HasTheChild	5.1765	1.4778	18.1329	1.6441	0.6396	2.5705	0.0102
HasTheChildVomited	1.1206	0.3286	3.8216	0.1139	0.6259	0.1819	0.8556
HaveYouGiven	2.3273	0.7962	6.8026	0.8447	0.5473	1.5435	0.1227
IsTheChild	2.7429	0.3790	19.8530	1.0090	1.0099	0.9992	0.3177
STOOL	0.7252	0.1877	2.8012	-0.3213	0.6895	-0.4660	0.6412
WasTheStool	14.6481	1.0394	206.4402	2.6843	1.3499	1.9886	0.0467
WasTheStoolBloody	5.9732	0.6407	55.6857	1.7873	1.1390	1.5691	0.1166
WasTheStooLSemiliquid	1.0685	0.3305	3.4541	0.0662	0.5987	0.1106	0.9119
CONSTANT	*	*	*	-13.7658	4.3921	-3.1343	0.0017

Convergence:	Converged
Iterations:	14
Final -2*Log-Likelihood:	98.4862

Test	Statistic	D.F.	P-Value
Score	31.8446	12	0.0015
Likelihood Ratio	37.1410	12	0.0002

LOGISTIC CC3 = BRELESSTHAN7CC3 HasFewerThe HasTheChild HasTheChildVomited HaveYouGiven IsTheChild STOOL WasTheStool WasTheStoolBloody WasTheStooLSemiliquid

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Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
BRELESSTHAN7CC3	2.6527	0.9146	7.6941	0.9756	0.5433	1.7956	0.0726
HasFewerThe	2.0549	0.6287	6.7168	0.7202	0.6043	1.1919	0.2333
HasTheChild	4.9424	1.4383	16.9830	1.5978	0.6298	2.5371	0.0112
HasTheChildVomited	0.9556	0.2892	3.1578	-0.0454	0.6099	-0.0745	0.9406
HaveYouGiven	2.0966	0.7559	5.8152	0.7403	0.5205	1.4224	0.1549
IsTheChild	3.3378	0.4978	22.3812	1.2053	0.9709	1.2415	0.2144
STOOL	0.6743	0.1793	2.5367	-0.3941	0.6760	-0.5829	0.5599
WasTheStool	14.2901	1.1373	179.5602	2.6596	1.2913	2.0596	0.0394
WasTheStoolBloody	5.0187	0.5933	42.4502	1.6132	1.0894	1.4808	0.1387
WasTheStooLSemiliquid	1.1048	0.3580	3.4098	0.0997	0.5750	0.1734	0.8623
CONSTANT	*	*	*	-13.2260	4.1123	-3.2162	0.0013

Convergence:	Converged
Iterations:	6
Final -2*Log-Likelihood:	103.1147

Test	Statistic	D.F.	P-Value
Score	31.6922	10	0.0005
Likelihood Ratio	35.4248	10	0.0001

LOGISTIC CC3 = BRELESSTHAN7CC3 HasFewerThe HasTheChild HaveYouGiven IsTheChild STOOL WasTheStool WasTheStoolBloody WasTheStooLSemiliquid

Previous Procedure Next Procedure Current Dataset

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
BRELESSTHAN7CC3	2.6552	0.9172	7.6872	0.9765	0.5424	1.8005	0.0718
HasFewerThe	1.9885	0.6101	6.4807	0.6874	0.6028	1.1403	0.2542
HasTheChild	4.9609	1.7132	14.3647	1.6016	0.5425	2.9524	0.0032
HaveYouGiven	2.0500	0.7415	5.6675	0.7178	0.5188	1.3835	0.1665
IsTheChild	3.9509	0.6268	24.9041	1.3739	0.9394	1.4626	0.1436
STOOL	0.6645	0.1767	2.4992	-0.4087	0.6759	-0.6047	0.5454
WasTheStool	13.9807	1.1665	167.5653	2.6377	1.2672	2.0815	0.0374
WasTheStoolBloody	5.1088	0.6100	42.7857	1.6310	1.0843	1.5041	0.1325
WasTheStooLSemiliquid	1.0916	0.3537	3.3691	0.0877	0.5750	0.1525	0.8788
CONSTANT	*	*	*	-13.3699	4.0990	-3.2617	0.0011

Convergence:	Converged
Iterations:	6
Final -2*Log-Likelihood:	103.5543

Test	Statistic	D.F.	P-Value
Score	32.9246	9	0.0001
Likelihood Ratio	36.9500	9	0.0000

LOGISTIC CC3 = BRELESSTHAN7CC3 HasFewerThe HasTheChild HaveYouGiven IsTheChild STOOL WasTheStool WasTheStoolBloody

Previous Procedure Next Procedure Current Dataset

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
BRELESSTHAN7CC3	2.6332	0.9150	7.5776	0.9682	0.5393	1.7953	0.0726
HasFewerThe	2.0651	0.7023	6.0719	0.7252	0.5503	1.3178	0.1876
HasTheChild	4.8789	1.7272	13.7817	1.5849	0.5298	2.9914	0.0028
HaveYouGiven	2.0281	0.7404	5.5554	0.7071	0.5141	1.3754	0.1690
IsTheChild	3.9463	0.6227	25.0115	1.3728	0.9421	1.4571	0.1451
STOOL	0.6835	0.1910	2.4458	-0.3805	0.6505	-0.5850	0.5586
WasTheStool	13.7747	1.1545	164.3548	2.6228	1.2649	2.0735	0.0381
WasTheStoolBloody	4.8994	0.6328	37.9353	1.5891	1.0443	1.5217	0.1281
CONSTANT	*	*	*	-13.1704	3.8696	-3.4036	0.0007

Convergence:	Converged
Iterations:	6
Final -2*Log-Likelihood:	103.5775

Test	Statistic	D.F.	P-Value
Score	32.9195	8	0.0001
Likelihood Ratio	36.9268	8	0.0000

LOGISTIC CC3 = BRELESSTHAN7CC3 HasFewerThe HasTheChild HaveYouGiven IsTheChild WasTheStool WasTheStoolBloody

Previous Procedure Next Procedure Current Dataset

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
BRELESSTHAN7CC3	2.7031	0.9436	7.7440	0.9944	0.5370	1.8518	0.0641
HasFewerThe	1.9924	0.6827	5.8141	0.6893	0.5464	1.2615	0.2071
HasTheChild	5.3353	1.9534	14.5720	1.6743	0.5126	3.2661	0.0011
HaveYouGiven	2.0507	0.7502	5.6059	0.7182	0.5131	1.3997	0.1616
IsTheChild	3.6466	0.5889	22.5791	1.2938	0.9302	1.3908	0.1643
WasTheStool	15.9571	1.3767	184.9499	2.7699	1.2501	2.2157	0.0267
WasTheStoolBloody	5.4559	0.7248	41.0707	1.6967	1.0299	1.6474	0.0995
CONSTANT	*	*	*	-14.0528	3.6069	-3.8960	0.0001

Convergence:	Converged
Iterations:	6
Final -2*Log-Likelihood:	103.9269

Test	Statistic	D.F.	P-Value
Score	32.7015	7	0.0000
Likelihood Ratio	36.5774	7	0.0000

LOGISTIC CC3 = BRELESSTHAN7CC3 HasTheChild HaveYouGiven IsTheChild WasTheStool WasTheStoolBloody

Previous Procedure Next Procedure Current Dataset

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
BRELESSTHAN7CC3	2.8160	1.0080	7.8670	1.0353	0.5242	1.9752	0.0482
HasTheChild	6.9360	2.6466	18.1774	1.9367	0.4916	3.9399	0.0001
HaveYouGiven	2.2616	0.8499	6.0185	0.8161	0.4994	1.6342	0.1022
IsTheChild	3.9957	0.6318	25.2708	1.3852	0.9411	1.4720	0.1410
WasTheStool	15.9749	1.3429	190.0301	2.7710	1.2634	2.1934	0.0283
WasTheStoolBloody	7.9755	1.1108	57.2650	2.0764	1.0058	2.0644	0.0390
CONSTANT	*	*	*	-14.5294	3.6061	-4.0292	0.0001

Convergence:	Converged
Iterations:	6
Final -2*Log-Likelihood:	107.5820

Test	Statistic	D.F.	P-Value
Score	32.5971	6	0.0000
Likelihood Ratio	36.7596	6	0.0000

LOGISTIC CC3 = BRELESSTHAN7CC3 HasTheChild HaveYouGiven WasTheStool WasTheStoolBloody

Previous Procedure Next Procedure Current Dataset

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
BRELESSTHAN7CC3	2.3395	0.9949	5.5013	0.8499	0.4363	1.9483	0.0514
HasTheChild	6.2895	2.7456	14.4077	1.8389	0.4229	4.3482	0.0000
HaveYouGiven	1.6446	0.7319	3.6955	0.4975	0.4131	1.2044	0.2284
WasTheStool	5.1563	0.7403	35.9138	1.6402	0.9903	1.6563	0.0977
WasTheStoolBloody	5.2850	1.2635	22.1071	1.6649	0.7301	2.2803	0.0226
CONSTANT	*	*	*	-10.1288	2.5882	-3.9134	0.0001

Convergence:	Converged
Iterations:	5
Final -2*Log-Likelihood:	143.4707

Test	Statistic	D.F.	P-Value
Score	31.7280	5	0.0000
Likelihood Ratio	34.7529	5	0.0000

LOGISTIC CC3 = BRELESSTHAN7CC3 HasTheChild WasTheStool

WasTheStoolBloody

Previous Procedure Next Procedure Current Dataset

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
BRELESSTHAN7CC3	2.1222	0.9152	4.9211	0.7524	0.4291	1.7534	0.0795
HasTheChild	6.9074	3.0411	15.6891	1.9326	0.4186	4.6172	0.0000
WasTheStool	4.5722	0.6749	30.9731	1.5200	0.9761	1.5572	0.1194
WasTheStoolBloody	5.0284	1.1950	21.1594	1.6151	0.7332	2.2029	0.0276
CONSTANT	*	*	*	-9.1923	2.4387	-3.7694	0.0002

Convergence:	Converged
Iterations:	5
Final -2*Log-Likelihood:	146.2214

Test	Statistic	D.F.	P-Value
Score	31.4578	4	0.0000
Likelihood Ratio	34.1583	4	0.0000

LOGISTIC CC3 = BRELESSTHAN7CC3 HasTheChild wasTheStoolBloody

Previous Procedure Next Procedure Current Dataset

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
BRELESSTHAN7CC3	1.8573	0.8201	4.2066	0.6191	0.4171	1.4844	0.1377
HasTheChild	6.8476	3.0427	15.4107	1.9239	0.4139	4.6486	0.0000
wasTheStoolBloody	3.6294	0.9560	13.7781	1.2891	0.6806	1.8939	0.0582
CONSTANT	*	*	*	-6.7486	1.7565	-3.8422	0.0001

Convergence:	Converged
Iterations:	5
Final -2*Log-Likelihood:	148.7294

Test	Statistic	D.F.	P-Value
Score	29.5494	3	0.0000
Likelihood Ratio	31.6503	3	0.0000

LOGISTIC CC3 = HasTheChild wasTheStoolBloody

Previous Procedure Next Procedure Current Dataset

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
HasTheChild	6.6858	4.4844	9.9679	1.9000	0.2038	9.3240	0.0000
wasTheStoolBloody	6.4597	2.6377	15.8197	1.8656	0.4570	4.0823	0.0000
CONSTANT	*	*	*	-6.5856	0.9934	-6.6297	0.0000

Convergence:	Converged
Iterations:	5
Final -2*Log-Likelihood:	585.9728

Test	Statistic	D.F.	P-Value
Score	104.1431	2	0.0000
Likelihood Ratio	110.9972	2	0.0000

Interim results from the logistic regression for medical history for risk factors for hospitalisation

LOGISTIC CC3 = HasYourChild HasYourChildEver HasYourChildHad

IfWhichPneMONIA OtherInDis OtherNonIn YesVomitinG

Previous Procedure Next Procedure Current Dataset

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
HasYourChild	0.6282	0.2775	1.4220	-0.4649	0.4168	-1.1153	0.2647
HasYourChildEver	2.2400	1.2964	3.8702	0.8065	0.2790	2.8905	0.0038
HasYourChildHad	2.1188	1.0021	4.4797	0.7508	0.3820	1.9656	0.0493
IfWhichPneMONIA	2.1393	0.9972	4.5891	0.7605	0.3894	1.9528	0.0508
OtherInDis	3.7469	0.9626	14.5843	1.3209	0.6934	1.9050	0.0568
OtherNonIn	1.8561	0.5856	5.8832	0.6185	0.5886	1.0507	0.2934
YesVomitinG	1.0497	0.3600	3.0607	0.0486	0.5460	0.0889	0.9291
CONSTANT	*	*	*	-7.3591	2.2021	-3.3418	0.0008

Convergence:	Converged
Iterations:	5
Final -2*Log-Likelihood:	631.4910

Test	Statistic	D.F.	P-Value
Score	36.9517	7	0.0000
Likelihood Ratio	40.1173	7	0.0000

LOGISTIC CC3 = HasYourChild HasYourChildEver HasYourChildHad IfWhichPneMONIA OtherInDis OtherNonIn

Previous Procedure Next Procedure Current Dataset

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
HasYourChild	0.6370	0.2822	1.4377	-0.4510	0.4154	-1.0859	0.2775
HasYourChildEver	2.2948	1.3418	3.9244	0.8306	0.2738	3.0339	0.0024
HasYourChildHad	2.0799	0.9918	4.3616	0.7323	0.3778	1.9382	0.0526
IfWhichPneMONIA	2.1018	0.9834	4.4921	0.7428	0.3875	1.9167	0.0553
OtherInDis	3.8036	0.9791	14.7768	1.3359	0.6924	1.9294	0.0537
OtherNonIn	1.5414	0.5207	4.5632	0.4327	0.5537	0.7814	0.4345
CONSTANT	*	*	*	-6.9229	1.9625	-3.5275	0.0004

Convergence:	Converged
Iterations:	5
Final -2*Log-Likelihood:	636.2031

Test	Statistic	D.F.	P-Value
Score	36.6208	6	0.0000
Likelihood Ratio	39.6445	6	0.0000

LOGISTIC CC3 = HasYourChild HasYourChildEver HasYourChildHad IfWhichPneMONIA OtherInDis

Previous Procedure Next Procedure Current Dataset

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
HasYourChild	0.6816	0.3070	1.5129	-0.3834	0.4068	-0.9423	0.3460
HasYourChildEver	2.3071	1.3521	3.9365	0.8360	0.2726	3.0665	0.0022
HasYourChildHad	2.0472	0.9805	4.2745	0.7165	0.3756	1.9075	0.0565
IfWhichPneMONIA	1.9739	0.9371	4.1577	0.6800	0.3801	1.7890	0.0736
OtherInDis	4.1332	1.0805	15.8105	1.4191	0.6845	2.0731	0.0382
CONSTANT	*	*	*	-6.2148	1.5899	-3.9089	0.0001

Convergence:	Converged
Iterations:	5
Final -2*Log-Likelihood:	641.4913

Test	Statistic	D.F.	P-Value
Score	36.7463	5	0.0000
Likelihood Ratio	39.7523	5	0.0000

LOGISTIC CC3 = HasYourChildEver HasYourChildHad IfWhichPneMONIA OtherInDis

Previous Procedure Next Procedure Current Dataset

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
HasYourChildEver	2.1506	1.2899	3.5857	0.7657	0.2608	2.9360	0.0033
HasYourChildHad	1.5213	1.0210	2.2669	0.4196	0.2035	2.0620	0.0392
IfWhichPneMONIA	1.6942	0.8716	3.2934	0.5272	0.3391	1.5546	0.1200
OtherInDis	3.5989	0.9922	13.0542	1.2806	0.6574	1.9480	0.0514
CONSTANT	*	*	*	-5.6344	1.4206	-3.9661	0.0001

Convergence:	Converged
Iterations:	5
Final -2*Log-Likelihood:	642.3914

Test	Statistic	D.F.	P-Value
Score	36.4101	4	0.0000
Likelihood Ratio	38.8522	4	0.0000

LOGISTIC CC3 = HasYourChildEver HasYourChildHad OtherInDis

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Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
HasYourChildEver	2.5934	1.6394	4.1024	0.9530	0.2340	4.0725	0.0000
HasYourChildHad	1.5290	1.0275	2.2753	0.4246	0.2028	2.0936	0.0363
OtherInDis	3.7328	1.0352	13.4592	1.3172	0.6544	2.0129	0.0441
CONSTANT	*	*	*	-5.0547	1.3545	-3.7317	0.0002

Convergence:	Converged
Iterations:	5
Final -2*Log-Likelihood:	644.8701

Test	Statistic	D.F.	P-Value
Score	34.3535	3	0.0000
Likelihood Ratio	36.3735	3	0.0000

Interim results from the logistic regression for family background risk factors for hospitalisation

LOGISTIC CC3 = DoYouUseOther fathereduccc3 lakeofsafewater morethan9personscc3 motherageless20cc3 mothereduccc3 unhealthymother doyoukeepanimals

Previous Procedure Next Procedure Current Dataset

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
DoYouUseOther	7.4393	0.8832	62.6620	2.0068	1.0873	1.8457	0.0649
fathereduccc3	0.6858	0.2519	1.8665	-0.3772	0.5109	-0.7384	0.4603
lakeofsafewater	2.8332	0.2816	28.5060	1.0414	1.1779	0.8841	0.3766
morethan9personscc3	0.6370	0.3286	1.2347	-0.4511	0.3377	-1.3356	0.1817
motherageless20cc3	1.8262	0.8068	4.1336	0.6022	0.4168	1.4448	0.1485
mothereduccc3	3.7881	1.3817	10.3858	1.3319	0.5146	2.5883	0.0096
unhealthymother	3.1593	1.1032	9.0477	1.1504	0.5368	2.1429	0.0321
doyoukeepanimals	1.2232	0.7617	1.9642	0.2014	0.2417	0.8336	0.4045
CONSTANT	*	*	*	-11.2219	3.1954	-3.5119	0.0004

Convergence:	Converged
Iterations:	6
Final -2*Log-Likelihood:	644.0874

Test	Statistic	D.F.	P-Value
Score	33.3496	8	0.0001
Likelihood Ratio	38.8993	8	0.0000

LOGISTIC CC3 = DoYouUseOther lakeofsafewater morethan9personscc3 motherageless20cc3 mothereduccc3 unhealthymother doyoukeepanimals

Previous Procedure Next Procedure Current Dataset

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
DoYouUseOther	7.4720	0.8888	62.8134	2.0112	1.0862	1.8515	0.0641
lakeofsafewater	2.8273	0.2809	28.4547	1.0393	1.1781	0.8822	0.3777
morethan9personscc3	0.6411	0.3312	1.2407	-0.4446	0.3369	-1.3198	0.1869
motherageless20cc3	1.8706	0.8286	4.2226	0.6262	0.4154	1.5075	0.1317
mothereduccc3	2.9940	1.3930	6.4351	1.0966	0.3904	2.8090	0.0050
unhealthymother	3.1192	1.0908	8.9195	1.1376	0.5361	2.1221	0.0338
doyoukeepanimals	1.1847	0.7400	1.8966	0.1695	0.2401	0.7058	0.4803
CONSTANT	*	*	*	-11.4734	3.1824	-3.6053	0.0003

Convergence:	Converged
Iterations:	6
Final -2*Log-Likelihood:	647.6172

Test	Statistic	D.F.	P-Value
Score	32.6802	7	0.0000
Likelihood Ratio	38.1439	7	0.0000

LOGISTIC CC3 = DoYouUseOther lakeofsafewater morethan9personscc3 motherageless20cc3 mothereduccc3 unhealthymother

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Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
DoYouUseOther	7.7564	0.9226	65.2107	2.0485	1.0863	1.8858	0.0593
lakeofsafewater	2.8768	0.2877	28.7686	1.0567	1.1748	0.8994	0.3684
morethan9personscc3	0.6164	0.3215	1.1818	-0.4839	0.3321	-1.4570	0.1451
motherageless20cc3	1.9743	0.8869	4.3947	0.6802	0.4083	1.6661	0.0957
mothereduccc3	3.0600	1.4269	6.5622	1.1184	0.3893	2.8732	0.0041
unhealthymother	3.1463	1.1005	8.9952	1.1462	0.5360	2.1386	0.0325
CONSTANT	*	*	*	-11.3996	3.1637	-3.6033	0.0003

Convergence:	Converged
Iterations:	6
Final -2*Log-Likelihood:	648.1154

Test	Statistic	D.F.	P-Value
Score	32.2624	6	0.0000
Likelihood Ratio	37.6457	6	0.0000

LOGISTIC CC3 = DoYouUseOther morethan9personscc3 motherageless20cc3 mothereduccc3 unhealthymother

Previous Procedure Next Procedure Current Dataset

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
DoYouUseOther	10.8066	1.3854	84.2974	2.3802	1.0481	2.2710	0.0231
morethan9personscc3	0.6235	0.3251	1.1957	-0.4724	0.3322	-1.4220	0.1550
motherageless20cc3	2.0126	0.9054	4.4738	0.6994	0.4076	1.7161	0.0861
mothereduccc3	3.0607	1.4278	6.5610	1.1186	0.3890	2.8754	0.0040
unhealthymother	3.3703	1.1909	9.5386	1.2150	0.5308	2.2890	0.0221
CONSTANT	*	*	*	-10.1438	2.5966	-3.9066	0.0001

Convergence:	Converged
Iterations:	6
Final -2*Log-Likelihood:	649.6802

Test	Statistic	D.F.	P-Value
Score	32.3066	5	0.0000
Likelihood Ratio	37.4056	5	0.0000

LOGISTIC CC3 = DoYouUseOther motherageless20cc3 mothereduccc3 unhealthymother

Previous Procedure Next Procedure Current Dataset

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
DoYouUseOther	10.8019	1.3850	84.2448	2.3797	1.0480	2.2708	0.0232
motherageless20cc3	2.0323	0.9152	4.5133	0.7092	0.4071	1.7422	0.0815
mothereduccc3	3.4937	1.6642	7.3344	1.2509	0.3784	3.3060	0.0009
unhealthymother	3.2462	1.1482	9.1775	1.1775	0.5302	2.2206	0.0264
CONSTANT	*	*	*	-10.8609	2.5576	-4.2465	0.0000

Convergence:	Converged
Iterations:	6
Final -2*Log-Likelihood:	651.7377

Test	Statistic	D.F.	P-Value
Score	30.2427	4	0.0000
Likelihood Ratio	35.3481	4	0.0000

Conclusion of significant risk factors identified from family background, clinical signs and medical history from logistic regression

LOGISTIC CC3 = DoYouUseOther mothereduccc3 unhealthymother

Previous Procedure Next Procedure Current Dataset

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
DoYouUseOther	12.1473	1.5702	93.9723	2.4971	1.0438	2.3922	0.0167
mothereduccc3	3.1049	1.5174	6.3535	1.1330	0.3653	3.1014	0.0019
unhealthymother	3.2217	1.1418	9.0901	1.1699	0.5292	2.2106	0.0271
CONSTANT	*	*	*	-9.4648	2.4133	-3.9220	0.0001

Convergence:	Converged
Iterations:	6
Final -2*Log-Likelihood:	658.9193

Test	Statistic	D.F.	P-Value
Score	27.1799	3	0.0000
Likelihood Ratio	32.3906	3	0.0000

LOGISTIC CC3 = HastheChild HasYourChildEver HasYourChildHad mothereduccc3 OtherInDis unhealthymother WasTheStoolBloody DoYouUseOther

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Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
HastheChild	6.0555	3.9362	9.3158	1.8010	0.2198	8.1947	0.0000
HasYourChildEver	2.2513	1.3415	3.7780	0.8115	0.2641	3.0722	0.0021
HasYourChildHad	1.0656	0.6715	1.6910	0.0636	0.2356	0.2698	0.7873
mothereduccc3	2.9185	1.3208	6.4489	1.0711	0.4045	2.6478	0.0081
OtherInDis	1.5720	0.4072	6.0685	0.4523	0.6892	0.6563	0.5116
unhealthymother	3.1477	0.9879	10.0297	1.1467	0.5913	1.9394	0.0525
WasTheStoolBloody	5.9988	2.3596	15.2504	1.7916	0.4761	3.7633	0.0002
DoYouUseOther	10.7343	1.2586	91.5484	2.3734	1.0936	2.1703	0.0300
CONSTANT	*	*	*	-17.7338	3.1131	-5.6965	0.0000

Convergence:	Converged
Iterations:	6
Final -2*Log-Likelihood:	528.6350

Test	Statistic	D.F.	P-Value
Score	127.0913	8	0.0000
Likelihood Ratio	144.2808	8	0.0000

LOGISTIC CC3 = HasTheChild HasYourChildEver mothereduccc3 OtherInDis unhealthymother WasTheStoolBloody DoYouUseOther

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
HasTheChild	6.1169	3.9997	9.3550	1.8111	0.2168	8.3550	0.0000
HasYourChildEver	2.2890	1.3839	3.7860	0.8281	0.2567	3.2256	0.0013
mothereduccc3	2.9240	1.3238	6.4586	1.0730	0.4043	2.6537	0.0080
OtherInDis	1.6031	0.4180	6.1479	0.4720	0.6858	0.6882	0.4913
unhealthymother	3.1490	0.9905	10.0111	1.1471	0.5901	1.9438	0.0519
WasTheStoolBloody	6.0359	2.3757	15.3354	1.7977	0.4757	3.7788	0.0002
DoYouUseOther	10.9198	1.2840	92.8662	2.3906	1.0922	2.1889	0.0286
CONSTANT	*	*	*	-17.7629	3.1137	-5.7047	0.0000

Convergence:	Converged
Iterations:	6
Final -2*Log-Likelihood:	528.7077

Test	Statistic	D.F.	P-Value
Score	126.9321	7	0.0000
Likelihood Ratio	144.2081	7	0.0000

LOGISTIC CC3 = HasTheChild HasYourChildEver mothereduccc3 unhealthymother WasTheStoolBloody DoYouUseOther

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Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
HasTheChild	6.0892	4.0084	9.2502	1.8065	0.2133	8.4682	0.0000
HasYourChildEver	2.3647	1.4330	3.9022	0.8606	0.2556	3.3677	0.0008
mothereduccc3	3.0029	1.3639	6.6117	1.0996	0.4027	2.7306	0.0063
unhealthymother	3.2398	1.0234	10.2563	1.1755	0.5880	1.9993	0.0456
WasTheStoolBloody	6.2420	2.4594	15.8424	1.8313	0.4752	3.8536	0.0001
DoYouUseOther	11.0408	1.2991	93.8369	2.4016	1.0918	2.1996	0.0278
CONSTANT	*	*	*	-17.0600	2.8581	-5.9691	0.0000

Convergence:	Converged
Iterations:	6
Final -2*Log-Likelihood:	538.7023

Test	Statistic	D.F.	P-Value
Score	128.4598	6	0.0000
Likelihood Ratio	145.7303	6	0.0000

Appendix 5: Interim results from the logistic regression for clinical signs risk factors for hospitalisation (hospitalised vs Health centre)

LOGISTIC cc4 = BRELESSTHAN7CC3 dehydration HasFewerThe HasTheChild HasTheChildVomited HaveYouGiven noORSUSE IsTheChild STOOL WasTheStool WasTheStoolBloody WasTheStooLSemiliquid

Previous Procedure Next Procedure Current Dataset

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
BRELESSTHAN7CC3	2.7222	0.7078	10.4689	1.0014	0.6872	1.4572	0.1451
dehydration (b.moderate/a.none)	0.4004	0.1108	1.4474	-0.9153	0.6556	-1.3960	0.1627
dehydration (c.severe/a.none)	2.4662	0.2242	27.1275	0.9027	1.2234	0.7378	0.4606
HasFewerThe	2.1397	0.4376	10.4627	0.7607	0.8098	0.9393	0.3476
HasTheChild	7.8809	1.7893	34.7104	2.0644	0.7564	2.7291	0.0063
HasTheChildVomited	1.1560	0.2956	4.5212	0.1450	0.6958	0.2084	0.8350
HaveYouGiven	1.1494	0.2842	4.6480	0.1393	0.7129	0.1953	0.8451
noORSUSE	11.4193	2.6686	48.8640	2.4353	0.7417	3.2833	0.0010
IsTheChild	1.0299	0.0901	11.7702	0.0295	1.2429	0.0237	0.9811
STOOL	2.8860	0.6034	13.8034	1.0599	0.7985	1.3273	0.1844
WasTheStool	52.4678	2.3085	1192.4786	3.9602	1.5937	2.4849	0.0130
WasTheStoolBloody	59.4000	0.9890	3567.5337	4.0843	2.0895	1.9547	0.0506
WasTheStooLSemiliquid	0.3542	0.0721	1.7399	-1.0379	0.8121	-1.2780	0.2013
CONSTANT	*	*	*	-22.0813	7.3398	-3.0084	0.0026

Convergence: Converged
Iterations: 7
Final -2*Log-Likelihood: 74.2408

Test	Statistic	D.F.	P-Value
Score	50.2662	13	0.0000
Likelihood Ratio	63.3644	13	0.0000

LOGISTIC cc4 = BRELESSTHAN7CC3 dehydration HasFewerThe HasTheChild HasTheChildVomited HaveYouGiven noORSUSE STOOL WasTheStool WasTheStoolBloody WasTheStooLSemiliquid

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
BRELESSTHAN7CC3	3.8272	1.1893	12.3164	1.3421	0.5963	2.2506	0.0244
dehydration (b.moderate/a.none)	0.4971	0.1614	1.5310	-0.6990	0.5739	-1.2179	0.2233
dehydration (c.severe/a.none)	2.2006	0.2569	18.8470	0.7887	1.0958	0.7198	0.4717
HasFewerThe	2.1854	0.6222	7.6756	0.7818	0.6410	1.2198	0.2226
HasTheChild	6.4652	1.9392	21.5549	1.8664	0.6144	3.0379	0.0024
HasTheChildVomited	1.3972	0.4373	4.4641	0.3345	0.5927	0.5643	0.5725
HaveYouGiven	0.5887	0.1805	1.9193	-0.5299	0.6030	-0.8788	0.3795
noORSUSE	11.0404	3.3133	36.7889	2.4016	0.6141	3.9106	0.0001
STOOL	2.6125	0.6876	9.9262	0.9603	0.6811	1.4100	0.1585
WasTheStool	39.6125	1.7997	871.9170	3.6791	1.5774	2.3325	0.0197
WasTheStoolBloody	172.0912	3.1915	9279.3402	5.1480	2.0345	2.5304	0.0114
WasTheStooLSemiliquid	0.3370	0.0930	1.2212	-1.0877	0.6569	-1.6558	0.0978
CONSTANT	*	*	*	-23.0757	6.7066	-3.4407	0.0006

Convergence:	Converged
Iterations:	7
Final -2*Log-Likelihood:	97.6141

Test	Statistic	D.F.	P-Value
Score	61.0815	12	0.0000
Likelihood Ratio	79.5150	12	0.0000

LOGISTIC cc4 = BRELESSTHAN7CC3 dehydration HasFewerThe HasTheChild HaveYouGiven noORSUSE STOOL WasTheStool WasTheStoolBloody WasTheStooLSemiliquid

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
BRELESSTHAN7CC3	3.7068	1.1706	11.7376	1.3102	0.5881	2.2279	0.0259
dehydration (b.moderate/a.none)	0.4429	0.1470	1.3340	-0.8145	0.5626	-1.4478	0.1477
dehydration (c.severe/a.none)	1.8273	0.2185	15.2830	0.6028	1.0837	0.5563	0.5780
HasFewerThe	2.0939	0.6045	7.2533	0.7390	0.6339	1.1658	0.2437
HasTheChild	6.9068	2.1522	22.1649	1.9325	0.5949	3.2484	0.0012
HaveYouGiven	0.6430	0.2050	2.0166	-0.4416	0.5832	-0.7573	0.4489
noORSUSE	11.6961	3.5379	38.6659	2.4593	0.6101	4.0311	0.0001
STOOL	2.7120	0.7668	9.5918	0.9977	0.6445	1.5479	0.1216
WasTheStool	38.0218	1.8512	780.9075	3.6382	1.5420	2.3594	0.0183
WasTheStoolBloody	164.9433	3.1353	8677.5548	5.1056	2.0219	2.5251	0.0116
WasTheStooLSemiliquid	0.3123	0.0874	1.1165	-1.1638	0.6500	-1.7905	0.0734
CONSTANT	*	*	*	-22.4967	6.5668	-3.4258	0.0006

Convergence:	Converged
Iterations:	7
Final -2*Log-Likelihood:	99.2886

Test	Statistic	D.F.	P-Value
Score	61.1856	11	0.0000
Likelihood Ratio	79.5505	11	0.0000

LOGISTIC cc4 = BRELESSTHAN7CC3 HasFewerThe HasTheChild HaveYouGiven noORSUSE STOOL WasTheStool WasTheStoolBloody WasTheStooLSemiliquid

Previous Procedure Next Procedure Current Dataset

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
BRELESSTHAN7CC3	4.4735	1.4700	13.6142	1.4982	0.5678	2.6384	0.0083
HasFewerThe	1.9100	0.6313	5.7785	0.6471	0.5648	1.1456	0.2520
HasTheChild	7.4147	2.3871	23.0306	2.0035	0.5783	3.4646	0.0005
HaveYouGiven	1.0947	0.3972	3.0173	0.0905	0.5173	0.1749	0.8611
noORSUSE	12.2081	3.7741	39.4900	2.5021	0.5990	4.1774	0.0000
STOOL	2.7062	0.8103	9.0379	0.9955	0.6153	1.6181	0.1056
WasTheStool	47.5126	2.8340	796.5487	3.8610	1.4384	2.6842	0.0073
WasTheStoolBloody	129.0671	3.9074	4263.3138	4.8603	1.7845	2.7237	0.0065
WasTheStooLSemiliquid	0.4372	0.1400	1.3654	-0.8273	0.5810	-1.4239	0.1545
CONSTANT	*	*	*	-24.0194	6.1712	-3.8922	0.0001

Convergence:	Converged
Iterations:	7
Final -2*Log-Likelihood:	108.2889

Test	Statistic	D.F.	P-Value
Score	62.4240	9	0.0000
Likelihood Ratio	79.9878	9	0.0000

LOGISTIC cc4 = BRELESSTHAN7CC3 HasFewerThe HasTheChild noORSUSE STOOL WasTheStool WasTheStoolBloody WasTheStooLSemiliquid

Previous Procedure Next Procedure Current Dataset

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
BRELESSTHAN7CC3	4.0502	1.3727	11.9500	1.3988	0.5520	2.5338	0.0113
HasFewerThe	1.9829	0.6757	5.8192	0.6846	0.5493	1.2463	0.2127
HasTheChild	8.0600	2.6162	24.8313	2.0869	0.5741	3.6352	0.0003
noORSUSE	11.3480	3.6200	35.5740	2.4290	0.5830	4.1668	0.0000
STOOL	3.0197	0.9138	9.9783	1.1052	0.6098	1.8122	0.0700
WasTheStool	45.9693	2.7503	768.3368	3.8280	1.4369	2.6641	0.0077
WasTheStoolBloody	125.8440	3.7383	4236.3672	4.8350	1.7941	2.6949	0.0070
WasTheStooLSemiliquid	0.3788	0.1259	1.1396	-0.9707	0.5619	-1.7274	0.0841
CONSTANT	*	*	*	-23.7012	6.1131	-3.8771	0.0001

Convergence: Converged
Iterations: 7
Final -2*Log-Likelihood: 109.4796

Test	Statistic	D.F.	P-Value
Score	63.0839	8	0.0000
Likelihood Ratio	81.1309	8	0.0000

LOGISTIC cc4 = BRELESSTHAN7CC3 HasTheChild noORSUSE STOOL WasTheStool WasTheStoolBloody WasTheStooLSemiliquid

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
BRELESSTHAN7CC3	4.4359	1.5267	12.8889	1.4897	0.5442	2.7374	0.0062
HasTheChild	8.9775	2.9308	27.4995	2.1947	0.5712	3.8426	0.0001
noORSUSE	9.9089	3.3077	29.6845	2.2934	0.5598	4.0969	0.0000
STOOL	3.2805	1.0011	10.7495	1.1880	0.6056	1.9618	0.0498
WasTheStool	42.1356	2.6994	657.6949	3.7409	1.4020	2.6683	0.0076
WasTheStoolBloody	158.3323	5.1673	4851.5324	5.0647	1.7461	2.9005	0.0037
WasTheStooLSemiliquid	0.3957	0.1330	1.1773	-0.9270	0.5562	-1.6665	0.0956
CONSTANT	*	*	*	-23.4180	5.9506	-3.9354	0.0001

Convergence:	Converged
Iterations:	7
Final -2*Log-Likelihood:	111.0505

Test	Statistic	D.F.	P-Value
Score	62.3817	7	0.0000
Likelihood Ratio	79.5601	7	0.0000

LOGISTIC cc4 = BRELESSTHAN7CC3 HasTheChild noORSUSE STOOL WasTheStool WasTheStoolBloody

Previous Procedure Next Procedure Current Dataset

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
BRELESSTHAN7CC3	5.1476	1.8095	14.6437	1.6385	0.5334	3.0717	0.0021
HasTheChild	10.2017	3.3895	30.7046	2.3226	0.5622	4.1313	0.0000
noORSUSE	12.1037	4.1020	35.7145	2.4935	0.5521	4.5166	0.0000
STOOL	2.5055	0.8365	7.5046	0.9185	0.5597	1.6410	0.1008
WasTheStool	64.5353	4.0671	1024.0173	4.1672	1.4104	2.9547	0.0031
WasTheStoolBloody	280.9600	8.9461	8823.7874	5.6382	1.7587	3.2059	0.0013
CONSTANT	*	*	*	-26.6097	5.8570	-4.5433	0.0000

Convergence:	Converged
Iterations:	7
Final -2*Log-Likelihood:	113.8847

Test	Statistic	D.F.	P-Value
Score	59.2627	6	0.0000
Likelihood Ratio	76.7259	6	0.0000

LOGISTIC cc4 = BRELESSTHAN7CC3 HasTheChild noORSUSE WasTheStool WasTheStoolBloody

Previous Procedure Next Procedure Current Dataset

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
BRELESSTHAN7CC3	4.8957	1.7597	13.6206	1.5884	0.5221	3.0425	0.0023
HasTheChild	7.5041	2.7825	20.2377	2.0154	0.5062	3.9817	0.0001
noORSUSE	11.5932	4.0421	33.2505	2.4504	0.5376	4.5582	0.0000
WasTheStool	40.3912	2.8885	564.8033	3.6986	1.3459	2.7481	0.0060
WasTheStoolBloody	143.6295	5.5076	3745.6585	4.9672	1.6639	2.9854	0.0028
CONSTANT	*	*	*	-22.9877	5.1203	-4.4895	0.0000

Convergence:	Converged
Iterations:	7
Final -2*Log-Likelihood:	116.7003

Test	Statistic	D.F.	P-Value
Score	58.6376	5	0.0000
Likelihood Ratio	73.9103	5	0.0000

Interim results from the logistic regression for medical history risk factors for hospitalisation

LOGISTIC cc4 = HasYourChild HasYourChildEver HasYourChildHad IfWhichPneMONIA OtherInDis YesVomitinG

Previous Procedure Next Procedure Current Dataset

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
HasYourChild	0.4519	0.2115	0.9656	-0.7943	0.3874	-2.0504	0.0403
HasYourChildEver	2.0500	1.2279	3.4227	0.7179	0.2615	2.7449	0.0061
HasYourChildHad	2.1485	1.0694	4.3164	0.7648	0.3559	2.1486	0.0317
IfWhichPneMONIA	2.4262	1.1758	5.0061	0.8863	0.3696	2.3983	0.0165
OtherInDis	4.4845	1.1755	17.1082	1.5006	0.6831	2.1967	0.0280
YesVomitinG	2.3344	0.6788	8.0285	0.8478	0.6302	1.3451	0.1786
CONSTANT	*	*	*	-7.7260	2.0117	-3.8404	0.0001

Convergence:	Converged
Iterations:	5
Final -2*Log-Likelihood:	622.7059

Test	Statistic	D.F.	P-Value
Score	29.6674	6	0.0000
Likelihood Ratio	33.2027	6	0.0000

LOGISTIC cc4 = HasYourChild HasYourChildEver HasYourChildHad IfWhichPneMONIA OtherInDis

Previous Procedure Next Procedure Current Dataset

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
HasYourChild	0.4668	0.2205	0.9883	-0.7618	0.3827	-1.9907	0.0465
HasYourChildEver	2.1739	1.3090	3.6104	0.7765	0.2588	3.0003	0.0027
HasYourChildHad	2.1788	1.0898	4.3561	0.7788	0.3535	2.2032	0.0276
IfWhichPneMONIA	2.2828	1.1202	4.6520	0.8254	0.3632	2.2726	0.0231
OtherInDis	4.4923	1.1880	16.9874	1.5024	0.6786	2.2138	0.0268
CONSTANT	*	*	*	-6.1108	1.5521	-3.9370	0.0001

Convergence:	Converged
Iterations:	5
Final -2*Log-Likelihood:	630.0891

Test	Statistic	D.F.	P-Value
Score	28.8641	5	0.0000
Likelihood Ratio	31.6706	5	0.0000

Interim results from the logistic regression for family background risk factors for hospitalisation

LOGISTIC cc4 = DoYouKeepAnimals DoYouUseOther fathereduccc3 HasAnybody lakeofsafewater morethan9personscc3 motherageless20cc3 mothereduccc3 unhealthymother

Previous Procedure Next Procedure Current Dataset

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
DoYouKeepAnimals	1.3574	0.8357	2.2048	0.3056	0.2475	1.2347	0.2170
DoYouUseOther	3.9442	0.7889	19.7191	1.3723	0.8211	1.6712	0.0947
fathereduccc3	2.0634	0.7146	5.9577	0.7244	0.5410	1.3389	0.1806
HasAnybody	0.4388	0.2251	0.8554	-0.8237	0.3406	-2.4187	0.0156
lakeofsafewater	1.2537	0.2098	7.4904	0.2261	0.9120	0.2479	0.8042
morethan9personscc3	0.7543	0.3847	1.4790	-0.2820	0.3435	-0.8208	0.4118
motherageless20cc3	2.4934	1.0071	6.1734	0.9136	0.4626	1.9752	0.0482
mothereduccc3	2.0074	0.8391	4.8021	0.6968	0.4450	1.5658	0.1174
unhealthymother	2.6641	0.9147	7.7591	0.9799	0.5454	1.7965	0.0724
CONSTANT	*	*	*	-8.4256	2.6457	-3.1846	0.0014

Convergence:	Converged
Iterations:	6
Final -2*Log-Likelihood:	607.6068

Test	Statistic	D.F.	P-Value
Score	33.8497	9	0.0001
Likelihood Ratio	38.0901	9	0.0000

LOGISTIC cc4 = DoYouKeepAnimals DoYouUseOther fathereduccc3 HasAnybody morethan9personscc3 motherageless20cc3 mothereduccc3 unhealthymother

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
DoYouKeepAnimals	1.3625	0.8389	2.2129	0.3093	0.2474	1.2501	0.2113
DoYouUseOther	4.2025	0.9114	19.3776	1.4357	0.7798	1.8410	0.0656
fathereduccc3	2.0609	0.7128	5.9589	0.7232	0.5417	1.3350	0.1819
HasAnybody	0.4381	0.2246	0.8544	-0.8253	0.3408	-2.4216	0.0155
morethan9personscc3	0.7556	0.3858	1.4797	-0.2803	0.3429	-0.8173	0.4138
motherageless20cc3	2.5109	1.0144	6.2146	0.9206	0.4624	1.9910	0.0465
mothereduccc3	2.0314	0.8505	4.8517	0.7087	0.4442	1.5955	0.1106
unhealthymother	2.9036	1.0091	8.3545	1.0659	0.5392	1.9768	0.0481
CONSTANT	*	*	*	-8.3097	2.3599	-3.5211	0.0004

Convergence:	Converged
Iterations:	6
Final -2*Log-Likelihood:	609.6331

Test	Statistic	D.F.	P-Value
Score	34.5469	8	0.0000
Likelihood Ratio	38.8495	8	0.0000

LOGISTIC cc4 = DoYouKeepAnimals DoYouUseOther fathereduccc3 HasAnybody motherageless20cc3 mothereduccc3 unhealthymother

Previous Procedure Next Procedure Current Dataset

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
DoYouKeepAnimals	1.4155	0.8792	2.2787	0.3475	0.2429	1.4303	0.1526
DoYouUseOther	4.2221	0.9136	19.5108	1.4403	0.7810	1.8443	0.0651
fathereduccc3	2.1875	0.7651	6.2541	0.7828	0.5360	1.4605	0.1442
HasAnybody	0.4371	0.2242	0.8521	-0.8277	0.3406	-2.4300	0.0151
motherageless20cc3	2.5247	1.0212	6.2418	0.9261	0.4618	2.0053	0.0449
mothereduccc3	2.0952	0.8820	4.9775	0.7397	0.4415	1.6754	0.0939
unhealthymother	2.8367	0.9867	8.1551	1.0426	0.5388	1.9352	0.0530
CONSTANT	*	*	*	-8.8316	2.2839	-3.8668	0.0001

Convergence:	Converged
Iterations:	6
Final -2*Log-Likelihood:	610.3074

Test	Statistic	D.F.	P-Value
Score	33.7870	7	0.0000
Likelihood Ratio	38.1753	7	0.0000

LOGISTIC cc4 = DoYouUseOther fathereduccc3 HasAnybody motherageless20cc3 mothereduccc3 unhealthymother

Previous Procedure Next Procedure Current Dataset

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
DoYouUseOther	4.5514	0.9910	20.9040	1.5154	0.7778	1.9483	0.0514
fathereduccc3	2.2717	0.8052	6.4091	0.8205	0.5292	1.5505	0.1210
HasAnybody	0.4491	0.2311	0.8726	-0.8005	0.3389	-2.3622	0.0182
motherageless20cc3	2.7208	1.1080	6.6813	1.0009	0.4584	2.1836	0.0290
mothereduccc3	2.1480	0.9086	5.0779	0.7645	0.4390	1.7416	0.0816
unhealthymother	2.7841	0.9676	8.0106	1.0239	0.5392	1.8990	0.0576
CONSTANT	*	*	*	-8.6401	2.2661	-3.8127	0.0001

Convergence:	Converged
Iterations:	6
Final -2*Log-Likelihood:	612.3711

Test	Statistic	D.F.	P-Value
Score	31.9858	6	0.0000
Likelihood Ratio	36.1116	6	0.0000

LOGISTIC cc4 = DoYouUseOther HasAnybody motherageless20cc3 mothereducc3 unhealthymother

Previous Procedure Next Procedure Current Dataset

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
DoYouUseOther	4.6740	1.0251	21.3118	1.5420	0.7741	1.9919	0.0464
HasAnybody	0.4884	0.2553	0.9342	-0.7167	0.3310	-2.1655	0.0303
motherageless20cc3	2.7284	1.1086	6.7153	1.0037	0.4595	2.1842	0.0289
mothereducc3	3.0858	1.4634	6.5071	1.1268	0.3807	2.9602	0.0031
unhealthymother	2.8114	0.9815	8.0529	1.0337	0.5369	1.9252	0.0542
CONSTANT	*	*	*	-7.9826	2.2063	-3.6181	0.0003

Convergence:	Converged
Iterations:	6
Final -2*Log-Likelihood:	616.2523

Test	Statistic	D.F.	P-Value
Score	29.9751	5	0.0000
Likelihood Ratio	33.4594	5	0.0000

LOGISTIC cc4 = DoYouUseOther HasAnybody motherageless20cc3 mothereducc3

Previous Procedure Next Procedure Current Dataset

Conclusion of significant risk factors identified from family background, clinical signs and medical history from logistic regression

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
DoYouUseOther	5.2561	1.1648	23.7174	1.6594	0.7688	2.1584	0.0309
HasAnybody	0.5050	0.2654	0.9609	-0.6831	0.3282	-2.0816	0.0374
motherageless20cc3	2.3598	1.0015	5.5604	0.8586	0.4373	1.9633	0.0496
mothereduccc3	2.7181	1.3227	5.5858	0.9999	0.3675	2.7209	0.0065
CONSTANT	*	*	*	-5.7057	1.9084	-2.9898	0.0028

Convergence:	Converged
Iterations:	6
Final -2*Log-Likelihood:	629.9416

Test	Statistic	D.F.	P-Value
Score	24.7521	4	0.0001
Likelihood Ratio	27.2093	4	0.0000

LOGISTIC cc4 = HasYourChild HasYourChildEver HasYourChildIlad IfWhichPneMONIA otherindis DoYouUseOther HasAnybody motherageless20cc3 mothereduccc3 BRELESSTHAN7CC3 HasTheChild noORSUSE WasTheStool WasTheStoolBloody

Previous Procedure Next Procedure Current Dataset

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
HasYourChild	0.4009	0.0257	6.2461	-0.9139	1.4010	-0.6523	0.5142
HasYourChildEver	5.0092	1.1118	22.5695	1.6113	0.7680	2.0979	0.0359
HasYourChildHad	1.0266	0.0894	11.7916	0.0262	1.2455	0.0211	0.9832
IfWhichPneMONIA	0.5717	0.0726	4.5013	-0.5591	1.0528	-0.5311	0.5954
otherindis	10.8305	0.2496	469.9106	2.3824	1.9236	1.2385	0.2155
DoYouUseOther	287217.4203	0.0000	>1.0E12	12.5680	277.0002	0.0454	0.9638
HasAnybody	0.5674	0.1161	2.7732	-0.5667	0.8096	-0.7000	0.4839
motherageless20cc3	0.3876	0.0311	4.8326	-0.9478	1.2874	-0.7363	0.4616
mothereduccc3	0.7054	0.1182	4.2114	-0.3490	0.9116	-0.3828	0.7019
BRELESSTHAN7CC3	3.9361	1.2538	12.3571	1.3702	0.5837	2.3474	0.0189
HasTheChild	6.8276	2.2388	20.8220	1.9210	0.5689	3.3766	0.0007
noORSUSE	12.7887	3.9193	41.7290	2.5486	0.6034	4.2237	0.0000
WasTheStool	18.9258	0.9844	363.8691	2.9405	1.5083	1.9495	0.0512
WasTheStoolBloody	52.5063	2.0396	1351.6948	3.9609	1.6573	2.3900	0.0168
CONSTANT	*	*	*	-46.5668	554.0286	-0.0841	0.9330

Convergence:	Converged
Iterations:	12
Final -2*Log-Likelihood:	98.5398

Test	Statistic	D.F.	P-Value
Score	58.2845	14	0.0000
Likelihood Ratio	72.2723	14	0.0000

LOGISTIC cc4 = HasYourChild HasYourChildEver IfWhichPneMONIA otherindis DoYouUseOther HasAnybody motherageless20cc3 mothereduccc3 BRELESSTHAN7CC3 HasTheChild noORSUSE WasTheStool WasTheStoolBloody

Previous Procedure Next Procedure Current Dataset

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
HasYourChild	0.4116	0.1169	1.4491	-0.8877	0.6422	-1.3823	0.1669
HasYourChildEver	4.9981	1.1256	22.1935	1.6091	0.7606	2.1155	0.0344
IfWhichPneMONIA	0.5652	0.0964	3.3130	-0.5705	0.9022	-0.6323	0.5272
otherindis	10.8200	0.2485	471.0660	2.3814	1.9253	1.2369	0.2161
DoYouUseOther	286980.9315	0.0000	>1.0E12	12.5672	277.0002	0.0454	0.9638
HasAnybody	0.5661	0.1174	2.7286	-0.5690	0.8025	-0.7091	0.4783
motherageless20cc3	0.3875	0.0311	4.8311	-0.9480	1.2873	-0.7364	0.4615
mothereduccc3	0.7054	0.1181	4.2114	-0.3491	0.9117	-0.3829	0.7018
BRELESSTHAN7CC3	3.9387	1.2567	12.3445	1.3709	0.5828	2.3520	0.0187
HasTheChild	6.8377	2.2607	20.6814	1.9225	0.5647	3.4044	0.0007
noORSUSE	12.7850	3.9207	41.6906	2.5483	0.6031	4.2255	0.0000
WasTheStool	18.9730	0.9959	361.4526	2.9430	1.5037	1.9572	0.0503
WasTheStoolBloody	52.6978	2.0855	1331.5907	3.9646	1.6478	2.4060	0.0161
CONSTANT	*	*	*	-46.5439	554.0276	-0.0840	0.9330

Convergence:	Converged
Iterations:	12
Final -2*Log-Likelihood:	98.5402

Test	Statistic	D.F.	P-Value
Score	58.2656	13	0.0000
Likelihood Ratio	72.2718	13	0.0000

LOGISTIC cc4 = HasYourChild HasYourChildEver IfWhichPneMONIA otherindis HasAnybody motherageless20cc3 mothereduccc3 BRELESSTHAN7CC3 HasTheChild noORSUSE WasTheStool WasTheStoolBloody

Previous Procedure Next Procedure Current Dataset

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
HasYourChild	0.4338	0.1244	1.5134	-0.8351	0.6375	-1.3099	0.1902
HasYourChildEver	4.9272	1.0966	22.1398	1.5948	0.7666	2.0802	0.0375
IfWhichPneMONIA	0.5451	0.0918	3.2368	-0.6068	0.9089	-0.6677	0.5044
otherindis	9.3705	0.2292	383.1086	2.2376	1.8933	1.1818	0.2373
HasAnybody	0.6409	0.1368	3.0014	-0.4449	0.7878	-0.5648	0.5722
motherageless20cc3	0.4403	0.0354	5.4736	-0.8203	1.2859	-0.6379	0.5235
mothereduccc3	0.8684	0.1569	4.8056	-0.1411	0.8729	-0.1616	0.8716
BRELESSTHAN7CC3	3.8465	1.2377	11.9545	1.3472	0.5785	2.3285	0.0199
HasTheChild	6.6983	2.2192	20.2178	1.9019	0.5636	3.3743	0.0007
noORSUSE	12.5476	3.8508	40.8858	2.5295	0.6027	4.1971	0.0000
WasTheStool	29.2567	1.4680	583.0647	3.3761	1.5267	2.2114	0.0270
WasTheStoolBloody	122.5079	5.1913	2891.0061	4.8082	1.6129	2.9811	0.0029
CONSTANT	*	*	*	-24.0260	7.5648	-3.1760	0.0015

Convergence:	Converged
Iterations:	7
Final -2*Log-Likelihood:	99.5934

Test	Statistic	D.F.	P-Value
Score	56.7577	12	0.0000
Likelihood Ratio	71.2186	12	0.0000

LOGISTIC cc4 = HasYourChild HasYourChildEver IfWhichPneMONIA otherindis HasAnybody motherageless20cc3 BRELESSTHAN7CC3 HasTheChild noORSUSE WasTheStool WasTheStoolBloody

Previous Procedure Next Procedure Current Dataset

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
HasYourChild	0.4305	0.1238	1.4975	-0.8428	0.6360	-1.3251	0.1851
HasYourChildEver	5.0424	1.1517	22.0768	1.6179	0.7534	2.1474	0.0318
IfWhichPneMONIA	0.5476	0.0924	3.2459	-0.6023	0.9080	-0.6633	0.5071
otherindis	9.1920	0.2145	393.8708	2.2183	1.9172	1.1571	0.2472
HasAnybody	0.6395	0.1364	2.9977	-0.4470	0.7882	-0.5672	0.5706
motherageless20cc3	0.4456	0.0360	5.5214	-0.8083	1.2842	-0.6294	0.5291
BRELESSTHAN7CC3	3.8383	1.2363	11.9169	1.3450	0.5780	2.3269	0.0200
HasTheChild	6.6935	2.2152	20.2251	1.9011	0.5642	3.3697	0.0008
noORSUSE	12.6864	3.9199	41.0585	2.5405	0.5992	4.2397	0.0000
WasTheStool	28.1595	1.4773	536.7713	3.3379	1.5039	2.2194	0.0265
WasTheStoolBloody	117.3864	5.2220	2638.7467	4.7655	1.5881	3.0008	0.0027
CONSTANT	*	*	*	-24.2001	7.5199	-3.2181	0.0013

Convergence:	Converged
Iterations:	7
Final -2*Log-Likelihood:	99.6196

Test	Statistic	D.F.	P-Value
Score	56.7443	11	0.0000
Likelihood Ratio	71.1925	11	0.0000

LOGISTIC cc4 = HasYourChild HasYourChildEver IfWhichPneMONIA otherindis motherageless20cc3 BRELESSTHAN7CC3 HasTheChild noORSUSE WasTheStool WasTheStoolBloody

Previous Procedure Next Procedure Current Dataset

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
HasYourChild	0.3833	0.1146	1.2817	-0.9589	0.6159	-1.5569	0.1195
HasYourChildEver	5.7698	1.3606	24.4671	1.7526	0.7371	2.3777	0.0174
IfWhichPneMONIA	0.4952	0.0892	2.7497	-0.7027	0.8746	-0.8034	0.4217
otherindis	8.8699	0.2640	297.9928	2.1827	1.7931	1.2173	0.2235
motherageless20cc3	0.4655	0.0362	5.9827	-0.7646	1.3028	-0.5869	0.5573
BRELESSTHAN7CC3	3.9951	1.2893	12.3794	1.3851	0.5770	2.4004	0.0164
HasTheChild	6.7240	2.2342	20.2363	1.9057	0.5621	3.3900	0.0007
noORSUSE	13.5258	4.1940	43.6217	2.6046	0.5974	4.3596	0.0000
WasTheStool	26.3392	1.4095	492.1869	3.2711	1.4938	2.1898	0.0285
WasTheStoolBloody	108.4031	4.9407	2378.4448	4.6859	1.5757	2.9738	0.0029
CONSTANT	*	*	*	-24.8476	7.3796	-3.3671	0.0008

Convergence:	Converged
Iterations:	7
Final -2*Log-Likelihood:	100.3146

Test	Statistic	D.F.	P-Value
Score	57.1906	10	0.0000
Likelihood Ratio	72.2563	10	0.0000

LOGISTIC cc4 = HasYourChild HasYourChildEver IfWhichPneMONIA otherindis BRELESSTHAN7CC3 HasTheChild noORSUSE WasTheStool WasTheStoolBloody

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
HasYourChild	0.3774	0.1140	1.2497	-0.9744	0.6109	-1.5951	0.1107
HasYourChildEver	6.1192	1.4805	25.2924	1.8114	0.7240	2.5019	0.0124
IfWhichPneMONIA	0.4891	0.0895	2.6730	-0.7153	0.8666	-0.8254	0.4092
otherindis	9.4983	0.2837	317.9513	2.2511	1.7913	1.2567	0.2089
BRELESSTHAN7CC3	4.1664	1.3602	12.7620	1.4271	0.5711	2.4986	0.0125
HasTheChild	6.6053	2.2128	19.7172	1.8879	0.5580	3.3834	0.0007
noORSUSE	13.9733	4.3215	45.1818	2.6371	0.5988	4.4044	0.0000
WasTheStool	36.1997	2.4592	532.8747	3.5891	1.3721	2.6158	0.0089
WasTheStoolBloody	136.5580	6.4551	2888.8983	4.9167	1.5571	3.1576	0.0016
CONSTANT	*	*	*	-27.4108	6.5198	-4.2042	0.0000

Convergence:	Converged
Iterations:	7
Final -2*Log-Likelihood:	101.0125

Test	Statistic	D.F.	P-Value
Score	59.0362	9	0.0000
Likelihood Ratio	75.4648	9	0.0000

LOGISTIC cc4 = HasYourChild HasYourChildEver otherindis BRELESSTHAN7CC3 HasTheChild noORSUSE WasTheStool WasTheStoolBloody

Previous Procedure Next Procedure Current Dataset

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
HasYourChild	0.3375	0.1055	1.0802	-1.0861	0.5935	-1.8299	0.0673
HasYourChildEver	4.9100	1.3686	17.6147	1.5913	0.6518	2.4414	0.0146
otherindis	9.9980	0.2723	367.0986	2.3024	1.8384	1.2524	0.2104
BRELESSTHAN7CC3	4.2197	1.3832	12.8729	1.4398	0.5691	2.5300	0.0114
HasTheChild	7.0483	2.3829	20.8480	1.9528	0.5533	3.5293	0.0004
noORSUSE	13.5850	4.2121	43.8153	2.6090	0.5975	4.3667	0.0000
WasTheStool	34.6949	2.2648	531.4954	3.5466	1.3924	2.5471	0.0109
WasTheStoolBloody	140.5972	6.4176	3080.2305	4.9459	1.5750	3.1403	0.0017
CONSTANT	*	*	*	-28.3844	6.6054	-4.2972	0.0000

Convergence:	Converged
Iterations:	7
Final -2*Log-Likelihood:	101.6997

Test	Statistic	D.F.	P-Value
Score	58.1814	8	0.0000
Likelihood Ratio	74.7776	8	0.0000

LOGISTIC cc4 = HasYourChild HasYourChildEver BRELESSTHAN7CC3 HasTheChild noORSUSE WasTheStool WasTheStoolBloody

Previous Procedure Next Procedure Current Dataset

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
HasYourChild	0.6039	0.2272	1.6050	-0.5044	0.4987	-1.0114	0.3118
HasYourChildEver	3.9811	1.1909	13.3082	1.3816	0.6157	2.2438	0.0248
BRELESSTHAN7CC3	5.0665	1.6904	15.1852	1.6227	0.5600	2.8974	0.0038
HasTheChild	6.9621	2.4727	19.6028	1.9405	0.5282	3.6740	0.0002
noORSUSE	13.2956	4.1865	42.2246	2.5874	0.5896	4.3886	0.0000
WasTheStool	35.3224	2.5758	484.3734	3.5645	1.3359	2.6682	0.0076
WasTheStoolBloody	143.8561	6.3959	3235.5843	4.9688	1.5884	3.1282	0.0018
CONSTANT	*	*	*	-24.5440	5.0920	-4.8201	0.0000

Convergence:	Converged
Iterations:	7
Final -2*Log-Likelihood:	108.5236

Test	Statistic	D.F.	P-Value
Score	62.5362	7	0.0000
Likelihood Ratio	79.7531	7	0.0000

LOGISTIC cc4 = HasYourChildEver BRELESSTHAN7CC3 HasTheChild noORSUSE WasTheStool WasTheStoolBloody

Previous Procedure Next Procedure Current Dataset

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
HasYourChildEver	3.0626	1.0427	8.9959	1.1193	0.5498	2.0360	0.0418
BRELESSTHAN7CC3	4.7760	1.6238	14.0479	1.5636	0.5505	2.8406	0.0045
HasTheChild	6.6313	2.3938	18.3700	1.8918	0.5199	3.6390	0.0003
noORSUSE	11.7859	3.9099	35.5272	2.4669	0.5630	4.3820	0.0000
WasTheStool	31.1439	2.3687	409.4849	3.4386	1.3145	2.6160	0.0089
WasTheStoolBloody	124.6206	5.3134	2922.8367	4.8253	1.6097	2.9975	0.0027
CONSTANT	*	*	*	-24.1066	5.0521	-4.7716	0.0000

Convergence:	Converged
Iterations:	7
Final -2*Log-Likelihood:	109.5606

Test	Statistic	D.F.	P-Value
Score	62.3080	6	0.0000
Likelihood Ratio	78.7161	6	0.0000

Appendix 6: Multivariate logistic regression for risk factors for subsequent diarrhoea

EpiInfo 2002

Results Library
Current View: **C:\ALLDEF~1\database\IRAN2c.MDB:IRAN2**
Select: **CaseControl = 1)) AND (SelectedFor = 1)**
Record Count: **211** *Date:* **09/07/2004 00:10:54**

LOGISTIC withorwithoutdiarrhoea = agec Diainpreviousyear Dulessthan5days fatheroc Hospreviousyear Keepanimalathome mage viAsubsequence WAZSCORE WHZSCORE zincsubseq

Next Procedure

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
agec	0.8460	0.2307	3.1019	-0.1672	0.6629	-0.2523	0.8008
Diainpreviousyear	1.6290	0.7562	3.5092	0.4879	0.3916	1.2461	0.2127
Dulessthan5days	2.4495	1.0495	5.7167	0.8959	0.4324	2.0718	0.0383
fatheroc	1.6865	0.7672	3.7076	0.5227	0.4019	1.3005	0.1934
Hospreviousyear	1.4302	0.6447	3.1730	0.3578	0.4066	0.8801	0.3788
Keepanimalathome	2.3822	1.0338	5.4894	0.8680	0.4259	2.0380	0.0415
mage	0.2352	0.0267	2.0686	-1.4472	1.1092	-1.3047	0.1920
viAsubsequence	2.3981	1.1046	5.2067	0.8747	0.3955	2.2114	0.0270
WAZSCORE	1.5773	0.6085	4.0885	0.4557	0.4860	0.9377	0.3484
WHZSCORE	1.8358	0.7285	4.6261	0.6075	0.4716	1.2882	0.1977
zincsubseq	4.3412	1.2024	15.6734	1.4681	0.6550	2.2414	0.0250
CONSTANT	*	*	*	-0.4353	1.3747	-0.3167	0.7515

Convergence: Converged
Iterations: 6
Final -2*Log-Likelihood: 177.4505

Test	Statistic	D.F.	P-Value
Score	34.1937	11	0.0003
Likelihood Ratio	37.1362	11	0.0001

LOGISTIC withorwithoutdiarrhoea = Diainpreviousyear Dulessthan5days fatheroc Hospreviousyear Keepanimalathome mage viAsubsequence WAZSCORE WHZSCORE zincsubseq

Previous Procedure Next Procedure Current Dataset

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
Diainpreviousyear	1.6479	0.7691	3.5310	0.4995	0.3888	1.2846	0.1989
Dulessthan5days	2.4497	1.0508	5.7110	0.8960	0.4319	2.0746	0.0380
fatheroc	1.6838	0.7667	3.6975	0.5210	0.4014	1.2982	0.1942
Hospreviousyear	1.4239	0.6424	3.1560	0.3534	0.4061	0.8702	0.3842
Keepanimalathome	2.4026	1.0447	5.5257	0.8766	0.4249	2.0629	0.0391
mage	0.2297	0.0262	2.0092	-1.4712	1.1066	-1.3294	0.1837
viAsubsequence	2.4027	1.1069	5.2156	0.8766	0.3954	2.2168	0.0266
WAZSCORE	1.5599	0.6048	4.0235	0.4446	0.4834	0.9198	0.3577
WHZSCORE	1.8818	0.7617	4.6487	0.6322	0.4614	1.3701	0.1707
zincsubseq	4.3367	1.2028	15.6363	1.4671	0.6543	2.2421	0.0250
CONSTANT	*	*	*	-0.5813	1.2465	-0.4663	0.6410

Convergence:	Converged
Iterations:	6
Final -2*Log-Likelihood:	177.5151

Test	Statistic	D.F.	P-Value
Score	34.1848	10	0.0002
Likelihood Ratio	37.0715	10	0.0001

LOGISTIC withorwithoutdiarrhoea = Diainpreviousyear Dulessthan5days fatheroc Keepanimalathome mage viAsubsequence WAZSCORE WHZSCORE zincsubseq

Previous Procedure Next Procedure Current Dataset

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
Diainpreviousyear	1.7200	0.8192	3.6111	0.5423	0.3784	1.4330	0.1519
Dulessthan5days	2.4839	1.0676	5.7795	0.9098	0.4309	2.1117	0.0347
fatheroc	1.5596	0.7199	3.3788	0.4444	0.3945	1.1266	0.2599
Keepanimalathome	2.3813	1.0393	5.4563	0.8677	0.4230	2.0511	0.0403
mage	0.2132	0.0245	1.8580	-1.5453	1.1045	-1.3991	0.1618
viAsubsequence	2.5760	1.1960	5.5483	0.9462	0.3915	2.4172	0.0156
WAZSCORE	1.6800	0.6602	4.2754	0.5188	0.4766	1.0886	0.2763
WHZSCORE	1.9884	0.8127	4.8646	0.6873	0.4565	1.5057	0.1321
zincsubseq	4.4558	1.2458	15.9360	1.4942	0.6502	2.2980	0.0216
CONSTANT	*	*	*	-0.3851	1.2112	-0.3179	0.7505

Convergence: Converged
Iterations: 6
Final -2*Log-Likelihood: 179.6348

Test	Statistic	D.F.	P-Value
Score	34.2745	9	0.0001
Likelihood Ratio	37.4601	9	0.0000

LOGISTIC withorwithoutdiarrhoea = Diainpreviousyear Dulessthan5days Keepanimalathome mage viAsubsequence WAZSCORE WHZSCORE zincsubseq

Previous Procedure Next Procedure Current Dataset

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
Diainpreviousyear	1.8307	0.8803	3.8070	0.6047	0.3736	1.6187	0.1055
Dulessthan5days	2.3613	1.0249	5.4400	0.8592	0.4258	2.0178	0.0436
Keepanimalathome	2.7486	1.2449	6.0686	1.0111	0.4041	2.5021	0.0123
mage	0.2075	0.0239	1.7991	-1.5725	1.1020	-1.4270	0.1536
viAsubsequence	2.5006	1.1694	5.3473	0.9165	0.3878	2.3635	0.0181
WAZSCORE	1.5684	0.6254	3.9333	0.4501	0.4691	0.9595	0.3373
WHZSCORE	2.1714	0.9011	5.2328	0.7754	0.4488	1.7279	0.0840
zincsubseq	4.5545	1.3004	15.9523	1.5161	0.6395	2.3706	0.0178
CONSTANT	*	*	*	-0.2567	1.1975	-0.2143	0.8303

Convergence:	Converged
Iterations:	6
Final -2*Log-Likelihood:	180.9685

Test	Statistic	D.F.	P-Value
Score	33.6060	8	0.0000
Likelihood Ratio	36.8016	8	0.0000

LOGISTIC withorwithoutdiarrhoea = Dulessthan5days Keepanimalathome mage viAsubsequence WAZSCORE WHZSCORE zincsubseq

Previous Procedure Next Procedure Current Dataset

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
Dulessthan5days	2.4087	1.0513	5.5190	0.8791	0.4230	2.0782	0.0377
Keepanimalathome	2.6730	1.2237	5.8389	0.9832	0.3987	2.4663	0.0137
mage	0.2154	0.0246	1.8858	-1.5353	1.1070	-1.3869	0.1655
viAsubsequence	2.6005	1.2247	5.5215	0.9557	0.3842	2.4877	0.0129
WAZSCORE	1.6336	0.6579	4.0564	0.4908	0.4641	1.0576	0.2903
WHZSCORE	2.0564	0.8644	4.8923	0.7210	0.4422	1.6304	0.1030
zincsubseq	4.1036	1.1993	14.0413	1.4119	0.6276	2.2495	0.0245
CONSTANT	*	*	*	0.0622	1.1837	0.0526	0.9581

Convergence:	Converged
Iterations:	6
Final -2*Log-Likelihood:	183.6274

Test	Statistic	D.F.	P-Value
Score	31.8279	7	0.0000
Likelihood Ratio	34.8135	7	0.0000

LOGISTIC withorwithoutdiarrhoea = Dulessthan5days Keepanimalathome viAsubsequence WAZSCORE WHZSCORE zincsubseq

Previous Procedure Next Procedure Current Dataset

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
Dulessthan5days	2.5875	1.1461	5.8415	0.9507	0.4155	2.2882	0.0221
Keepanimalathome	2.4909	1.1596	5.3503	0.9126	0.3901	2.3397	0.0193
viAsubsequence	2.9012	1.3788	6.1043	1.0651	0.3795	2.8064	0.0050
WAZSCORE	1.5868	0.6382	3.9456	0.4617	0.4647	0.9935	0.3205
WHZSCORE	2.1311	0.8967	5.0648	0.7566	0.4417	1.7131	0.0867
zincsubseq	4.0315	1.1870	13.6921	1.3941	0.6238	2.2348	0.0254
CONSTANT	*	*	*	-1.4418	0.4913	-2.9347	0.0033

Convergence:	Converged
Iterations:	5
Final -2*Log-Likelihood:	186.6977

Test	Statistic	D.F.	P-Value
Score	30.1360	6	0.0000
Likelihood Ratio	32.4096	6	0.0000

LOGISTIC withorwithoutdiarrhoea = Dulessthan5days Keepanimalathome viAsubsequence WAZSCORE zincsubseq

Previous Procedure Next Procedure Current Dataset

Unconditional Logistic Regression

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
Dulessthan5days	2.7087	1.2089	6.0694	0.9965	0.4116	2.4208	0.0155
Keepanimalathome	2.5188	1.1850	5.3537	0.9238	0.3847	2.4013	0.0163
viAsubsequence	2.7939	1.3406	5.8228	1.0274	0.3747	2.7422	0.0061
WAZSCORE	2.4816	1.1728	5.2510	0.9089	0.3824	2.3768	0.0175
zincsubseq	3.8623	1.1695	12.7557	1.3513	0.6096	2.2168	0.0266
CONSTANT	*	*	*	-1.2122	0.4649	-2.6073	0.0091

Convergence:	Converged
Iterations:	5
Final -2*Log-Likelihood:	189.6987

Test	Statistic	D.F.	P-Value
Score	27.4807	5	0.0000
Likelihood Ratio	29.4086	5	0.0000