Some public health associations with specific enteropathogens in childhood diarrhoea in Cape Town

H Moeng
ESVHEL001

Submitted to the University of Cape Town in partial fulfilment of the requirements for the degree Master in Public Health

Faculty of Health Sciences
UNIVERSITY OF CAPE TOWN

Supervisor: Prof G. Swingler

DECLARATION

I, *Helimamy Moeng,* herby declare that the work on this dissertation is based on my original work (except where acknowledgements are given) and has not, in whole or in part, been submitted towards another degree in this or any other university.

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ACKNOWLEDGEMENTS

I specially wish to thank Prof George Swingler for his encouragement and support during the different phases I have gone through with this study. His guidance, comments and patience during the past year were invaluable for the writing of study.

I wish to thank Victoria Pillay and those who helped her for the data that was collected, cleaned and corrected for her Ph D study. Her thorough knowledge of the data collected paved the way for this analysis. Her advice and support while starting the study were of great value to me.

I would like to thank the staff at the UCT Health Sciences Library for the patience and assistance during the various literature searches.

I wish to thank Mr. Greg Distiller who provided statistical advice during the analysis.

I would like to thank the South African Weather service for their assistance in obtaining the relevant information for this study.

I would like to thank Professor Rodney Ehrlich who granted leave of absence and supplementary years for this study.

I would also like to thank ex-fellow students and now colleagues who gave encouragement and support.

ABSTRACT

Background

Diarrhoea remains one of the leading causes of mortality in young children. After dehydration and malnutrition, electrolytes disturbances are an important complication of diarrhoea. In Cape Town, fluctuation of plasma sodium and potassium has been observed in childhood diarrhoea and were seasonal in their occurrence. A study conducted at Red Cross Children's Hospital found that seasonal fluctuations of plasma sodium and potassium were associated with specific enteropathogens but did not identify associations that suggested potential public health interventions that could target the electrolytes disturbances. It is possible that by identifying association with the enteropathogens directly, one could identify public health interventions to avoid seasonal electrolyte disturbances.

Objectives

To identify clinical, nutritional, socio-economic, socio-demographic and seasonal associations with childhood diarrhoea caused by the enteropathogens *Shigella, Salmonella*, enterotoxigenic *E. coli*, enteropathogenic *E.coli*, *Cryptosporidium*, rotavirus or *Campylobacter.*

Methods

This study was a secondary analysis of a cross-sectional study of infants less than 2 years old admitted to the Rehydration Unit of the Red Cross Children's Hospital with diarrhoea. Data were obtained through routine medical records and a questionnaire. Multiple logistic regression was performed to identify determinants of the diarrhoea-associated electrolytes disturbances.

Results

Height for age was negatively associated with enteropathogenic *E. coli* (OR: 0.82, p-value: 0.039), *Campylobacter* (OR: 0.87, p-value: 0.082) and *Salmonella* (OR: 0.79, 0-value: 0.029) in the bi-variate analysis. Weight for

height and height for age were associated respectively to rotavirus (OR: 1.24, p-value: 0.011) and enterotoxigenic *E.coli* (OR: 1.16, p-value: 0.064). Water source was positively associated with Enteropathogenic *E.* coli, *Campylobacter* and inversely associated with rotavirus. Toilet was positively associated with enteropathogenic *E.* coli and *Salmonella.* Average temperature was positively associated with Enteropathogenic *E. coli,* Enterotoxigenic *E.* coli, *Salmonella* and *Shigella.*

In the multivariate analysis breast feeding was negatively associated with *Cryptosporidium* infection (OR: 0.13; 95% CI 0.20-0.62) while average humidity was positively associated with *Cryptosporidium* infection (OR: 1.10; 95% CI 1.05-1.16). Enteropathogenic *E. coli* infection was positively associated with average ambient temperature (OR: 1.16, 95% CI 1.03-1.30).The child's age was a determinant of *Campylobacter*, (OR: 1.05; 95% CI 1.00-1.11). Formula feeding, (OR: 0.45; 95% CI 0.20-0.99) average temperature (OR: 0.87; 95% CI 0.81-0.95) and humidity (OR: 0.91; 95% CI 0.80-0.94) were all protective of rotavirus infection. None of the socio-economic variables were associated with specific enteropathogens.

Discussion

The only determinant associated with Enteropathogenic *E coli* identified in this study was average temperature. This concurs with its summer isolation. No significant determinant was identified for Enterotoxigenic *E. coli* in this analysis. For *Cryptosporidium,* in the multivariate analyses were duration of diarrhoea, breast-feeding and average humidity were identified as significant determinants. With the exception of breast-feeding, none of these determinants could be amended through public health intervention.

For *Campylobacter,* age and adequacy of drinking were identified as significant determinants during the multivariate analysis. Adequacy of drinking was the only significant determinant associated with *Salmonella.*

Formula feeding, plasma pH, average humidity and average temperature were associated with rotavirus while associations identified with *Shigella* were duration of vomiting, plasma pH and average temperature. As above, none of the identified determinants could be changed to modify the occurrence of these enteropathogenic diarrhoea.

Conclusion

Although associations with specific enteropathogens were identified, none of the associations identified suggest public health interventions to avoid seasonal electrolyte disturbances.

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1. BACKGROUND

1.1 Burden of diarrhoeal disease

Acute diarrhoeal disease is one of the commonest childhood diseases worldwide. It is estimated that 1.4 billion cases of diarrhoea occur in children less than five years of age in developing countries every year (O'Ryan et al, 2005). Worldwide, three million children die of diarrhoea every year (Booroah, 2004). The World Health Organisation reported that 21% of all deaths in children under five years were due to diarrhoea and the highest mortality rate (8.5 per 1000 per year) was observed in children less than one year old (Kosek et al, 2003, O'Ryan et al, 2005). Although the mortality associated with diarrhoea has declined, morbidity has continued to be relatively constant (O'Ryan et al, 2005).

In developed countries, diarrhoea is not a major cause of mortality but accounts for two percent of all outpatient consultations in children under five years old (Hoekstra, 2001). Thapar and Sanderson estimated in the USA that four percent of hospital admissions in children under five years are due to diarrhoea (Thapar and Sanderson, 2004). A positive trend was observed between country income and rotavirus related hospitalisation (Parashar et al, 2003) from 20% in low-income countries to 34% in high-income countries.

In South Africa, 10.6% of all specified causes of mortality in children under the age of five years in 2000 were due to diarrhoea (Bradshaw et al, 2003). The South African Demographic Household Survey in 1988 reported a 13% prevalence of diarrhoea in children under five years in the Western Cape (Department of Health, 2006).

In Cape Town, the setting of the present study, mortality due to diarrhoea accounted for 9.8% of causes of death in children younger than four years in 2001. In children under the age of one, diarrhoea represented 10.3% of the causes of mortality (Groenewald et al, 2003). In children under the age of five years, an increase in diarrhoea incidence was observed from 83.8 per 1000 in 2001 to 91.8 per 1000 in 2002 (Health Systems Trust, 2003).

1.2 Electrolytes disturbances in diarrhoea

Diarrhoea is commonly defined as the passing of three or more loose stools a day (O'Ryan et al, 2005). Different patho-physiological mechanisms cause diarrhoea from different causes but the net effect is a loss of water and electrolytes from the body. If water and electrolytes are not adequately replaced during diarrhoea, dehydration occurs. Children are more at risk of becoming dehydrated quickly compared to adults due to relatively higher fluid losses and their dependence on fluid replacement. Severe dehydration in children can result in death.

After dehydration and malnutrition, electrolytes disturbances are an important complication of diarrhoea. Hypernatremia and hypokalemia affect the function of the body, particularly of muscle or nerve cells and extreme values of plasma sodium above 158 mmol/l (Adelman and Solhung, 1996, Bruck, 1969) or potassium below 2.5 mmol/l (Travis, 1996) can result in death.

In Cape Town a striking parallel fluctuation of plasma sodium and potassium concentrations has been observed in childhood diarrhoea (Swingler and Power, 2002). The prevalence of hypokalemia in children with diarrhoea admitted to the Rehydration Unit of the Red Cross Children's Hospital in Cape Town peaked during the summer while, for hypernatremia, the highest prevalence was during winter. The association of hypernatremia with cooler season or winter has also been observed in Egypt (Fayad et al, 1992) and the USA (Bruck, 1969). No similar seasonal fluctuation of potassium has been reported elsewhere, but a subsequent prospective study in Cape Town confirmed the findings of the earlier study (Pillay, 2006).

1.3 Seasonality of diarrhoea

Diarrhoea and the pathogens causing diarrhoea have a seasonal occurrence in many settings. In Egypt (Fayad et al, 1992), Rotavirus related diarrhoea is common in winter while Escherichia coli is more prevalent in summer. On the west coast of the USA, hospitalisation due to diarrhoea in children less than two years was at its highest in winter (Parashar et al, 1998). In the Western Cape, South Africa, Househam found that diarrhoea had a marked summer peak (Househam, 1988). A study of diarrhoeal mortality (Yach et al, 1989) in South Africa found a marked seasonal summer peak in black and coloured populations. This summer peak has been observed in other developing countries. In Bangladesh (Pathela et al, 2006), in children less than two years, spring and summer peaks have been detected. While in Mexico (Villa et al, 1999), mortality due to diarrhoea in children under five years old showed a bimodal distribution with a peak in the winter months and a higher one in the summer months from 1989 until 1992.

1.4 Determinants of seasonal electrolyte variations

At Red Cross Children's Hospital, Pillay (2006) has investigated determinants of the plasma sodium and potassium fluctuations. Her study aimed to identify associations that could explain the 10-20 fold fluctuation associated with severe hypokalemia and severe hypernatremia in the study setting and by extension identify possible public health interventions that could prevent those disturbances.

That study found that the seasonal fluctuations of plasma sodium and potassium were associated with specific enteropathogens. Sodium fluctuation was associated with enteropathogenic *E. coli* and enterotoxigenic *E. coli* which were commonly isolated enteropathogens in summer and autumn in this study setting. Living in a brick house and younger age were other determinants associated with plasma sodium levels but they were not seasonal. Infection with *Cryptosporidium* was associated with lower plasma potassium concentration, with the same seasonal variation. Duration of diarrhoea was also positively associated with hypokalemia, as was lower parental education, but without seasonal links.

However, Pillay's study did not identify associations with seasonal electrolyte fluctuations that suggested potential public health interventions that could target the electrolytes disturbances. It is possible that a more direct approach of studying determinants of identified enteropathogens themselves (rather than electrolyte levels) could identify associations amenable to public health intervention. The purpose of this study is therefore to identity factors associated with the enteropathogens.

2. LITERATURE REVIEW

Review of literature on the determinants of the enteropathogens

A PubMed search was performed for studies reporting determinants of specific enteropathogens identified in the study by Pillay. The following search term was used for each individual enteropathogen: <Enteropathogen>Infections/epidemiology. The search was limited to articles in English and French, describing studies that included children younger than 23 months old i.e. older age groups that were included in the published study were included in this review. This would have resulted, if anything, in an over-inclusion of determinants. The other inclusion criteria were studies that reported, in the abstract, factors associated (seasonally or otherwise) with a specific enteropathogen. Outbreak related studies were excluded.

For **Campylobacter**, the search resulted in 276 hits. Fifty-four abstracts met the inclusion criteria. Seven studies were performed in infants and children under 59 months: three case-control, two surveys and two prospective studies. Associations are listed in Table 2.1. Commonly reported risk factors associated with *Campylobacter* infection in humans were food related: consumption of chicken prepared outside the household (Unicomb et al, 2008, Friedman et al, 2004, Ikram et al, 1994) or consumption of undercooked meat or barbecued meat (Carrique-Mas et al, 2005, Schöenberg-Norio et al, 2004, Kapperud et al, 2003, Neimann, 2003, Studhal and Andersson, 2000, Ikram et al, 1994, Kapperud et al, 1992) and unpasteurized milk consumption (Michaud et al, 2004, Neimann et al, 2003, Studhal and Andersson, 2000, Pearson and Healing, 1992). Other risk factors were pet keeping (Fullerton et al, 2007, Carrique-Mas et al, 2005, Ali et al, 2003, Tenkate and Stafford, 2001, Kapperud et al, 1992, Brieseman, 1990), contact with poultry or other farm animals (Potter et al, 2003, Tenkate and Stafford, 2001, Studhal and Andersson, 2000, Pönkä et al, 1984) where infection could occur mainly due to lack of hygiene. This is substantiated by two studies (Green et al, 2006, Potter et al, 2002) done in Canada and the USA respectively, where the highest incidence of infection was found next to farming areas (Green et al, 2006) and that the estimated risk was higher in areas with high density poultry compared to low density (Potter et al, 2002). The drinking of untreated water either from a well or a lake (Fullerton et al, 2007, Carrique-Mas et al, 2005, Schöenberg-Norio et al, 2004, Ali et al, 2003, Kapperud et al, 2003, Adak et al, 1995) is an environmental risk factor.

Protective factors were limited to treated water (Sandberg et al, 2006), eating at home (Adak et al, 1995, Ikram et al, 1994) and breast-feeding (Fullerton et al, 2007, Mégraud et al, 1990).

Campylobacter infection has been reported to be seasonal in sixteen studies. In several developed countries such as Canada, England, Finland, Norway and Sweden, the peak of infection occurs in summer (Thompson et al, 1986, Louis et al, 2005, Pönkä et al, 1984, Lassen and Kapperud, 1984, Ekdahl and

Andersson, 2004) while in Portugal and Taiwan (Cabrita et al., 1992, Lin et al, 1998), the peak occurs in winter.

Five abstracts mention that the highest isolation of *Campylobacter* was observed in children under the one year (Desheng et al, 1992, Pazzaglia et al, 1991, Hopkins and Olmsted, 1985, Rishpon et al, 1984, Richardson et al, 1983) while Gedlu (Gedlu and Aseffa, 1996) in Ethiopia and Ali (2003) in Pakistan found that the peak incidence was between four to twenty four months and 12 – 24 months respectively. Eight abstracts report that the highest rate of infection was observed in children under the age of five years (Workman, 2006, Green et al, 2006, Louis et al, 2005, Kapperud et al, 1992, Brieseman, 1990, Walckiers et al, 1989, Skirrow, 1987, Sibbald and Sharp, 1985).

Four abstracts refer to children as the study population without specifying the age range (Pazzaglia et al, 1995, 1993, 1991, Gedlu and Aseffa, 1996) and four studies were done in children under the age of seven (Carrique-Mas et al, 2005, Saidi et al, 1997, Desheng et al, 1992, Calva et al, 1988). The case-control study done in Sweden (Carrique-Mas et al, 2005) in children less than six years old identified three risk factors from a multivariate analysis: a dog in the household, drinking water from a lake or river and the consumption of grilled meat.

Table 2.1 Summary of identified associated/protective factors for *Campylobacter*

Reference	Country	Associated factors (OR, CI or p-value of χ^2)
Unicomb 2008	Australia	Person in household with gastroenteritis (OR 3.5; CI 1.6-8.0)
		Restaurant prepared chicken (OR 2.5; CI 1.5-3.5)
		Restaurant prepared roast beef (OR 4.5; CI 1.4-14.1)
		> 2 "fast" food meals in a week (OR: 2.0; CI 1.3-3.1)
Fullerton 2007	USA	Drinking well water (OR 4.4; CI 1.4-14.2)
		Riding next to meat or poultry (OR 4.4; CI 1.2-13)
		Travel outside USA (OR 22.6; CI 3.5-144.5)
		Eating food prepared at home (OR 2.5; CI 1.2- 4.9)
		Pet with diarrhoea in the home (OR 7.6; CI 2.1-28)
		Visiting or living in a farm (OR 6.2; CI 2.2-17)
		Travel outside USA (OR 18.2; CI 1.2-284.2)
Carrique-Mas 2005	Sweden	Having a dog (OR: 8.4;CI 2.0-35.5)
		Water from lake, river (OR: 7.4; CI 1.5-36.5)
		Eating grilled meat (OR: 5.5; CI 1.7-18.1)
Michaud 2004	Canada	Eating raw, rare or undercooked poultry (OR: 4.51; CI 1.67-12.14)
		Consuming raw milk or raw milk products (OR: 3.12; CI 1.78-5.48)
		Professional exposure/ contact with animals (OR: 2.53; CI 1.44-4.13)
Schönberg-Norio 2004	Finland	Swimming in water from natural sources (OR: 2.80; CI 1.23-6.39)
		Tasting or eating raw or undercooked meat (OR: 10.79; CI 1.31-89.09)
		Drinking water from a dug well (OR: 3.36;CI 1.37-8.24)
Tenkate 2001	Australia	Pet chicken ownership (OR 11.8; CI1.37-101.75)
		Puppy ownership (OR 16.58; CI 3.73-73.65)
		Mayonnaise consumption (OR 4.13; CI 1.61-10.59)
Studahl 2000	Sweden	Drinking unpasteurized milk (OR: 3.56; CU 1.46-8.94)
		Eating chicken (OR: 2.29; CI1.29-4.23)
		Barbecuing (OR:1.98; CI 1.10-4.34)
		Daily contact with chickens or hens (OR: 11.83; CI 3.41-62.03)
Kapperud 1992	Norway	Sausage consumption at barbecue (OR: 7.64; CI 1.83-31.89)
		Daily contact with dog (OR: 4.26; CI 1.21-15.01)
		Eating poultry which was brought into the house in raw form (OR: 3.20; CI 1.17-8.76)

Reference	Country	Protective factors (OR, CI or p-value of χ^2)
Fullerton 2007	USA	Breast-feeding (OR 0.2; CI 0.1-0.6)
Mégraud 1990	Algeria	Breast-feeding (OR 0.1; CI 0.04-0.74)
Figueroa 1989	Chili	< 9 months, decreased *Campylobacter* isolation (p<0.01)
Georges-Courbot 1987	Bangui	Younger age 2-3 months compared to 4-6 months (p<0.01)

For ***Cryptosporidium***, 362 hits were obtained from the PubMed search using the terms *Cryptosporidium*/epidemiology. Forty-three met the inclusion criteria. Of these, 11 were done in Africa, 12 in western European countries and eight in the USA. Three studies done in Africa found that the highest prevalence of *Cryptosporidium* infection is seen in children under the age of two (Gatei et al, 2006, Perch et al, 2001) and in Burkina Fasso, the highest incidence of infection was seen between 6 and 23 months (Nacro et al, 1998). This peak in prevalence in the children less than two years has also been observed in other developing countries: Bangladesh and Pakistan (Khan et al, 2004, Iqbal et al, 1999) and Reinthaler in 1989 reported similar results in tropical countries although the abstract does not refer to particular countries. In developed countries, the only study reporting a similar age group was done in Spain with the highest prevalence observed in the age group one to three years, the peak prevalence in the two studies done in the USA and Korea was respectively between one to nine years, 30 to 39 and 50 to 59 years (Yoder, 2007, Chai, 2001).

Associations with *Cryptosporidium* are listed in Table 2.2. Improvement of water quality through purification or membrane filtration has been associated with a decline of *Cryptosporidium* infection in developed countries (Semenza and Nichols, 2007, Goh et al, 2005, Redlinger et al, 2002). None of the studies done in developing countries reported on this issue. One study in Guinea Bissau found breast-feeding to be protective (Mølbak et al, 1994), other factors negatively associated with *Cryptosporidium* infection were eating tomatoes and raw vegetables (Hunter et al, 2004, Roy et al, 2004).

Table 2.2 Summary of identified associated/protective factors for *Cryptosporidium*

Countries	Associated factors	References
Developed countries		
Australia	Public pool swimming(OR: 2.7; CI: 1.9-3.8)	Robertson 2002
England	Un-boiled water consumption per glass (OR: 1.13; CI 1.09-1.12)	Hunter 2004
	Travel outside UK (OR: 5.65; CI 2.86-11.16)	Hunter 2004
	Contact with cattle (OR: 3.87; CI 1.41-10.04)	Hunter 2004
USA	Fresh water swimming (OR: 1.9; CI 1.04-3.5)	Roy 2004
	Consumption of municipal water (OR: 10.1; CI 1.10-92.8)	Leach 2000
	Surface water swimming (OR: 3.7; CI: 1.02-13.5)	Gallaher 1989
	International travel (OR: 7.7; CI 2.7-22)	Roy 2004
	Travel to another country (OR: 34.7; CI 3.58-327)	Khalakdina 2003
	Contact with cattle (OR: 3.5; CI 1.8-6.8)	Roy 2004
	Peak: summer through fall	Yoder 2007
	Peak in late summer	Dietz 2000
	Age (OR: 1.29; CI: 1.05-1.58)	Leach 2000
	Lower household income (OR: 2.14; CI: 1.22-3.70)	Leach 2000
Developing countries		
Guatemala	Rainy months	Bern 2000
Guinea Bissau	Contact with dogs (OR: 2.1; CI: 1-4.2)	Mølbak 1994
	Contact with pigs (OR: 2.5; CI: 1.4-4.7)	Mølbak 1994
	Storage of cooked food for later consumption (OR: 1.8; CI: 1-3.3)	Mølbak 1994

Countries	Associated factors	References
Developing countries		
Guinea Bissau	Rainy months	Perch 2001
India	Rainy months	Nath 1999
Indonesia	Contact with cats (OR: 7.05; CI: 3.61-13.76)	Katsumata 1998
Indonesia	Rainy season (OR: 10.65; CI: 1.38-82.77)	Katsumata 1998
	Number of persons per house (OR: 1.46; CI 1.19-1.79)	Katsumata 1998
Jordan	Contact with animals (Fisher test, $p<0.0005$)	Nimri 2003
	Source of drinking water (Fisher test, $p<0.028$)	Nimri 2003
	Eating unwashed vegetables (Fisher test, $p<0.00005$)	Nimri 2003
Uganda	Wasted (OR: 1.59; CI 1.27-1.99)	Tumwine 2003
	Rainy months	Tumwine 2003

For **Enteropathogenic *E. coli***, the PubMed search resulted in 98 hits, eight abstracts met the inclusion criteria. Summer seasonality has been observed in Teheran and South Africa (Dallal et al, 2006, Robins Browne, 1984) but in Brazil the seasonal peak was during the spring (Gomes et al, 1991). Gomes found that isolation of this enteropathogen decreased with age and a subsequent analysis in Brazil ascertained that in children over two years infection with Enteropathogenic *E coli* is rare (Carbonare et al, 2003). This observation was similar to the findings of two studies done in Singapore and Nigeria (Lim et al, 1992, Antai and Anozie, 1987). Other factors associated with Enteropathogenic *E. coli* infection identified by a case-control study in Brazil (Blake et al, 1993) were: day care centre exposure, low family income, and a household member with diarrhoea. Breast-feeding and drinking boiled water were found to be protective.

Table 2.3 Identified associated/protective factors for Enteropathogenic *E. coli*

References	Country	Associated/protective risk factors
Dallal 2006	Iran	Highest isolation in summer
Long 2006	Mexico	Vitamin A supplementation (RR: 0.52; CI 0.23-0.86)
Carbonare 2003	Brazil	Over 2 years, infection rare
Blake 1993	Brazil	Hospitalisation before onset (OR: 12, p<0.01)
		Exclusive breast-feeding (OR: 0.1, p<0.01)
Lim 1992	Singapore	Infants at greater risk
Gomes 1991	Brazil	Isolation decreased with increasing age
		Peak isolation in spring
Antai 1987	*Port Harcourt*	0-18 months more susceptible than 19-36 months
Robins Browne 1984	South Africa	Summer prevalence

The PubMed search for **Enterotoxigenic *E. coli*** resulted in 117 hits, 26 abstracts met the inclusion criteria. The commonly identified associations and protective factors are summarised in Table 2.4.

Table 2.4 Identified associated/protective factors for Enterotoxigenic *E. coli*

Associated factors	Country	Reference
Summer	Australia	Gunzburg 1992
	Bangladesh	Qadri 2000
	Brazil	McAuliffe 1986
Warmer months (OR: 1.85; CI: 1.26-2.72)	Egypt	Abu-Elyazeed 1999
Hot wet month	Burma	Tin-Aye 1989
Under-nourished (WAZ < -2SD), p< 0.001	Bangladesh	Qadri 2007

Associated factors	Country	Reference
Preparation of rice, bean in the morning for serving after noon (OR:8, $p < 0.05$)	Brazil	Sobel 2004
Preparation of soup in the morning for serving after noon (OR: 9, $p<0.05$)	Brazil	Sobel 2004
Oats consumption (Hazards rate 4.01; p: 0.04)	Mexico	Long 1999
Highest incidence 6-12months (2.26/child/year)	Egypt	Rao 2003
Highest isolation in < 2 y (66%)	Burma	Tin-Aye 1989
Low quality water (Chi-square, p: 0.04)	Ecuador	Brüssow 1991
Not organised sewage system (Chi-square, p: 0.05)	Ecuador	Brüssow 1991
Supplementary food intake >100kcal (Fisher exact test, p: 0.05)	Ecuador	Brüssow 1991
Protective factors	**Country**	**Reference**
Sanitary latrine (OR: 0.49; CI: 0.32-0.72)	Egypt	Abu-Elyazeed 1999
Shorter duration of disease with vitamin A supplementation ($p < 0.02$)	Mexico	Long 1999

A study in Malaysia found that isolation of Enterotoxigenic *E. coli* was higher in females (Samuel et al, 1997) while in Nigeria the inverse was observed (Ako-Nai et al, 1990). Steinsland ascertained in a cohort study that previous Enterotoxigenic *E. coli* infection lead to 47% protection against a new infection with the same strain (Steinsland et al, 2003). An analysis of Enterotoxigenic *E. coli* antibody (Brüssow et al, 1992) in seven to ten months old infants found that antibodies were associated with poor quality drinking water, lack of sewage system and supplementary feeding.

Another factor associated with Enterotoxigenic *E. coli* infection was blood group A or AB (Qadri et al, 2007).

When a PubMed search was performed for **Rotavirus**, 864 hits were obtained of which 127 met the inclusion criteria. The peak incidence mentioned in 52 studies worldwide varies between six to twenty four months old. In temperate countries there are four distinct seasons (spring, summer, autumn and winter) while in tropical countries there are two periods: warm and cooler; a winter or cold season peak of Rotavirus diarrhoea was mentioned in 47 abstracts. In Japan, though, an analysis looking at the rotavirus peak between 1983 and 2003 found that the winter peak has shifted to spring (Suzuki et al, 2005) and in Middle East countries such as Egypt and Jordan a summer peak has been described (Naficy et al, 1999, Nirmi and Hijazi, 1996). In three settings: Turkey, Chile, Malaysia, the researchers found no seasonal pattern (Bozdayi et al, 2008, O'Ryan et al, 2007, Hung et al, 2006).

Breast-feeding was associated with lower rate of rotavirus infection in four studies (Dennehy et al, 2006, Zarnani et al, 2004, Naficy et al, 1999, Clemens et al, 1993). Other factors identified are summarised in the following table.

Table 2.5 Summary of identified associated factors for rotavirus

Associated factors	Reference	Country
Child care attendance <24 months (OR:1.5; CI: 1.047-2.2)	Dennehy 2006	USA
Child care attendance 24-59 months (OR:3; CI: 1.8-5.3	Dennehy 2006	USA
Another child<24 months in the household (OR: 1.6; CI: 1.1-2.3)	Dennehy 2006	USA
Not breast-fed <6 months (OR: 5.1; CI: 1.9-13.3)	Dennehy 2006	USA
Not breast-fed <24 months (OR: 2; CI: 1.2-3.3)	Dennehy 2006	USA
Child attending day care (OR: 2.4; CI: 1.1- 5.1)	Reves 1993	USA
Poor environmental sanitation* (OR: 3; CI: 1.03-8.9)	Menon 1990	USA

Associated factors	Reference	Country
9 -11 months (OR: 1.8; CI: 1.05-3.07)	Binka 2003	Ghana
Weight for height (wasted Z<-2: OR: 1.33;CI: 1.03-1.71)	Binka 2003	Ghana
Detection higher in males (40.8% vs 31.8%, p: 0.002)	Karadag 2005	Turkey
Patients younger than 2 years (43.7% vs 25.1%, p<0.001)	Karadag 2005	Turkey
Rented council accommodation (OR: 3.78; CI: 1.21-11.85)	Sethi 2001	UK
Contact with a person with diarrhoea (OR: 3.57; CI: 1.88- 6.81)	Sethi 2001	UK

* Poor environmental sanitation scored taking into account: animal in the yard, unprotected garbage bin, dirty diapers on the ground, standing water in the yard, small wooden house.

Contact with a person with diarrhoea (de Wit et al, 2003, Sethi et al, 2001), bottle feeding or mixed feeding (Sethi et al, 2001, Kurugöl et al, 2003) were found to be associated with rotavirus infection.

The PubMed search for ***Salmonella*** resulted in 798 hits; 44 abstracts met the inclusion criteria. For studies done in industrialised countries such as Canada, Sweden, UK, USA; travelling outside the country especially in the Indian sub-continent, in the Middle East and Central Africa has been associated with *Salmonella* infection (Jones et al, 2006, Marcus et al, 2007, Ekdahl et al, 2005b, Doré et al, 2004, Banatvala et al, 1999, McCarron, 1998). In developing countries, household contact (Brent et al, 2006, Sur et al, 2006, Vollard et al, 2004) is one of the associated factor and *Salmonella* infection tend to occur in children under five (Saha et al, 2001, Sinha et al, 1999). Frequently associated factors identified from the literature search are represented in table 2.6.

Table 2.6 Summary of identified associated factors for *Salmonella*

Associated factors	Country	References
Summer	Teheran	Dallal 2006
	Pakistan	Siddiqui 2006
	Europe	Ekdahl 2005a
	Japan	Kayaba 2002
	Bangladesh	Saha 2001
	UK	Banatvala 1999
Breast feeding (OR: 0.5; CI 0.3-0.6)	USA	Jones 2006
Meat consumption (OR: 1.7; CI: 1.1-2.7)	USA	Jones 2006
Rode in a cart next to meat or poultry (OR: 3.2; CI: 2.1-5.1)	USA	Jones 2006
Undercooked egg (OR: 2.1; CI: 1.1-3.9)	USA	Marcus 2007
Chicken strips** (OR: 6.8; CI: 1.9-38.1)	Canada	Currie 2005
Undercooked eggs** (OR: 5.5; CI: 1.2-51.1)	Canada	Currie 2005
Liquid diet (breast/formula/water) (OR: 6.67; CI: 1.07- ∞)	USA	Rowe 2004
Consumption of undercooked ground beef (OR: 3.8; CI: 1.7-8.4)	France	Delarocque-Astagneau 2000
Consumption of thoroughly cooked beef(OR: 0.4; CI: 0.2-0.8)	France	Delarocque-Astagneau 2000
Consumption of undercooked egg (OR: 1.9; p:0.003)	Germany	Kist 2000
At least some breastfeeding (OR: 0.20; CI: 0.11-0.37)	Italy	Borgnolo 1996
Formula feeding (OR: 9.15; CI: 2.71-30.9)	USA	Haddock 1991
Chicken outside the home (OR: 2.6; CI: 1.6-4.4)	USA	Marcus 2007
Live on livestock farm (OR: 4.9; CI: 1.3-18.9)	Canada	Doré 2004
Having puppies, kittens, turtles (OR: 6.8, p: 0.002)	Germany	Kist 2000
Lack of hygiene	India	Kumar 2007
No use of soap for hand washing (OR: 1.91; CI: 1.06-3.46)*	Indonesia	Vollaard 2004
No toilet in the household (OR: 2.20; CI: 1.06-4.55)*	Indonesia	Vollaard 2004
Socio-economic status	India	Kumar 2007
Low economic level (OR: 2.9; CI: 1.5-5.3)	Viet Nam	Luxemburger 2001
Father unemployed or unskilled blue collar (OR: 1.86; CI: 1.03-3.38)	Italy	Borgnolo 1996
Contact household with diarrhoea	India	Sur 2006
Household member with diarrhoea (OR: 13.16; CI: 1.77-∞)	USA	Rowe 2004
Recent typhoid in the household (OR: 2.38; CI: 1.03-5.48)*	Indonesia	Vollard 2004
Recent contact with typhoid cases (OR: 11.9; CI: 2.3-60.7)	Viet Nam	Luxemburger 2001

Associated factors	Country	References
Recent antibiotic use (<4 wks) (OR: 5.2; CI: 1.8-15.3)	Canada	Doré 2004
Recent antibiotic use (<1month) (OR: 2.3; CI: 1- 5.5)	France	Delarocque-Astagneau 2000
<60 days antimicrobial exposure (OR: 5.78; CI: 1.32-25.33)	Italy	Borgnolo 1996
Less than 3 years (OR: 64.83; CI: 3.9-1078)	Kenya	Kariuki 2006
Age 0-6 years (OR: 44.2; CI: 10.6-184)	Sweden	Ekdahl 2005a
Incidence peaked in infant < 1 year	USA	Vugia 2004
Higher proportion of cases in < 5 years	Bangladesh	Saha 2001

* Typhoid fever, community controls
** *Salmonella heldeberg*

Other identified factors were: recent hospital admission which could be the result of hospital acquired infection (Brent et al, 2006, Riley et al, 1984), day care attendance (Jones et al, 2006), eating ice-cream or ice cubes (Luby et al, 1998, Vollard et al, 2004) and raw milk consumption (Taylor et al, 1982) which is uncommon now with flash pasteurisation.

One analysis has established that breast feeding is protective for *Salmonella* infection (Haddock et al, 1991) and a study done in Italy found that breast feeding was associated with a five-fold decreased risk (Borgnolo et al, 1996).

For ***Shigella***, two search terms were used: bacillary dysentery and *Shigella*. The search for *Shigella* and bacillary dysentery resulted respectively in 528 and 344 hits. Fifty-eight references met the inclusion criteria of which 38 were common. Half of these studies, which exclude outbreaks, were done in the Indian sub-continent. Breast-feeding was found to be protective for *Shigella* infection in Bangladesh, Egypt and Mexico (Abu-Elyazeed et al, 2004, Ahmed et al, 1997, Guerrero et al, 1994, Ahmed et al, 1992, Glass and Stoll, 1989) and one analysis found that the protection associated with breast-feeding decreased with age (Ahmed et al, 1992). In various settings: Brazil, Burundi and USA, hand-washing was identified as another protective factor (Sobel et al, 2004, Birmingham et al, 1997, Gibson et al, 2002).

Table 2.7 Frequently identified associated/protective factors for *Shigella* infection

Associated factors		Country	Reference
Age group with highest isolation	< 2 y (Incidence 8.81 and 9.97 /1000 in M and F)	Bangladesh	Emch 2008
	12- 47 m (Incidence: 488.4/100000 child years)	Mozambique	Mandomando 2007
	< 1y (Incidence: 759/1000/year)	Indonesia	Agtini 2005
	From 6m, increase of *Shigella* incidence (p<0.001)	Mexico	Guerrero 1994
	1-4 highest isolation then 0-11m	Israel	Vasilev 2004
	12-23 m	Egypt	Abu-Elyazeed 2004
		Chile	Prado 1999
	5-15 y (77% of isolated enteropathogens)	Brazil	Diniz-Santos 2005
Lack of hygiene	Re-use of water	Ecuador	Sempértegui 1995
	N. of non-septic latrine (OR: 1.46; CI: 1.10-1.93)	Bangladesh	Emch 2008
	Improper sewage	Nigeria	Eko 1991
Consumption of powdered milk	OR: 2.22. p<0.05	Brazil	Sobel 2004
Handwashing of child's caretaker	OR: 0.35, p<0.05	Brazil	Sobel 2004
Use of powdered milk	p< 0.05	Brazil	Blake 1993
Summer season		Iran	Dallal 2006
		China	Wang 2006
		Egypt	Abu-Elyazeed 2004
		Israel	Vasilev 2004
		Mexico	Guerrero 1994
		India	Dutta 1992
		Bangladesh	Hossain 1990

Associated factors	Country	Reference
Winter	Senegal	Sow 2002
	UK	Shears 1995
	Saudi-Arabia	al-Eissa 1992

y: year; m:months; N.: number of non-septic latrines in neighbourhood

With regard to nutritional status, one analysis found that as nutritional status worsened, diarrhoea duration increased (Black et al, 1984) and another study established that children with diarrhoea due to *Shigella* had a lower nutritional status compared to other diarrhoeal children.

Overall, with the exception of *Campylobacter*, all the other enteropathogens were found to have marked seasonality in their occurrence, with summer predominance for Enteropathogenic *E. coli* , Enterotoxigenic *E. coli, Salmonella* and *Shigella*. The lack of hygiene in food preparation such as the consumption of undercooked meat, the quality of water as well as polluted environment in the absence of sanitary latrine or contact with animals were associated with all the enteropathogens excluding Enteropathogenic *E. coli.* Breast-feeding was identified as a statistically significant protective factor for *Cryptosporidium*, Enteropathogenic *E. coli* and *Salmonella*.

3. METHODS

The aim of this study was to identify clinical, nutritional, socio-economic socio-demographic and seasonal determinants associated with *Cryptosporidium*, enterotoxigenic *E. coli*, enteropathogenic *E.coli*, *Campylobacter*, rotavirus, *Salmonella* and *Shigella* infections.

3.1 Study design

This study is a secondary analysis of the data collected from a database compiled by Pillay over one calendar year. The primary study was of determinants of seasonal fluctuations in plasma sodium and potassium disturbances in childhood diarrhoea.

3.2 Source population

The original research study was conducted at the Rehydration Unit at Red Cross War Memorial Children's Hospital, Cape Town. This Unit, which serves large parts of the Cape Town metropolitan area, receives children with dehydrating diarrhoeal disease who use public health facilities. The children accessed the Unit via referral from primary health care sites to this combined secondary and tertiary hospital or directly when children bypassed the primary health care sites, which were open only eight hours of the day in 2002. Only very seriously ill children were admitted directly to the Intensive Care Unit of the Hospital.

3.3 Sampling

Patients were eligible for inclusion in the primary study if at the time of admission to the Rehydration Unit they were between six weeks and two years of age and had been residing in the Cape Town metropolitan area for more than one month. The enrolment in the primary study occurred during daytime working hours. Patients treated with diuretics prior to their admission were excluded form the primary study as these would lead to loss of sodium or potassium. In order to provide a sample of a similar proportion of admissions per month during the year, the target enrolment for each month in the study was determined by using the mean proportion of patients admitted each month of the year to the Unit from 1999 to 2001.

Enrolment occurred over a full year (15 April 2002 to 14 April 2003), to allow for seasonal fluctuations in the aetiology of diarrhoea. Seasons for the original study were determined by the seasonal patterns of diarrhoeal disease rather than geographical seasons and were determined thus i) November to January as summer, ii) February to April as autumn, ii) May to July as winter and iv) August to October as spring.

Since the Unit was admitting patients 24 hours a day and children needed to be enrolled on admission, systematic sampling was not feasible as it would have necessitated 24-hour data collection. A consecutive sampling technique was thus used between 10h00 to 18h00. Patients admitted to the Unit who met the inclusion criteria were approached to participate in the study. The enrolment continued each day until the required number was reached. When enrolment was behind target all eligible patients were approached on subsequent days until the shortfall was made up.

From April 2002 to July 2002, patients were recruited into the project during the above specified times. At the end of July a persisting shortfall of patients was observed because fewer patients were being admitted that met inclusion criteria in the winter months (June to July). On review of the admission records it was noted that many of the patients were admitted soon after 18h00. Therefore for the rest of the study period (October 2002 to April 2003) patients were recruited between 11h00 and 19h00.

After written consent was obtained from the caregiver, a questionnaire comprising socio-economic data, current and previous residence, diet and treatment before admission was administered by Pillay, with a translator if the caregiver spoke Xhosa. Blood and stool collection that have not been performed as part of routine care were done once written consent was obtained. Other data such as clinical presentation and patient management were collected by Pillay from the patient's folder and the clinical examination chart. The data on humidity, rainfall and temperature were later obtained for this study from the South African Weather Service by the author of this dissertation.

3.4 Independent variables and potential confounding factors

Clinical determinants such as age (measured in completed months), gender, weight, HIV status (exposed/unknown/ positive), presence of shock and co-morbidity were extracted from routine clinical records by Pillay. She calculated the percentage dehydration using the following formulae: ([{discharge weight – admission weight}/discharge weight] X100). Height was measured by her with a measuring board and was used to compute weight for height (Z-score) as a measure of wasting and height for age (Z-score) as a measure of stunting. From the questionnaire administered to the caregivers, Pillay extracted the following data: duration of diarrhoea per completed day, duration of vomiting per completed day, adequacy of drinking (not drinking at all / drinks less than normal / drinks more than normal / drinks normally), persistent diarrhoea defined by the number of previous admissions for diarrhoea in Pillay's original study, treatment before admission (oral rehydration solution and antibiotic therapy) and the patient's diet: (breast milk, formula milk, solids or semi-solids).

Socio-economic indicators covered in the questionnaire administered to the caregiver by Pillay and the translator included parental education (taken as the highest grade passed), the presence of electricity in the house as well as stove and electrical appliances. The type of house (shack or brick), the presence of a flush toilet as well as piped water formed part of the socio-economic indicators of these children.

Plasma pH was extracted by Pillay from laboratory records. If this element was absent from laboratory records it was recorded as missing, as performing a venesection merely for the study was considered to be unethical.

For this dissertation seasonal data were obtained post hoc from the South African Weather Service. The mean humidity, rainfall and temperature of the seven days preceding the admission of the child to the hospital was computed for every patient to take into account the weather pattern before arrival to the hospital.

3.5 Dependent variables

The enteropathogens associated in the Pillay study with serial sodium and potassium fluctuations were the primary dependent variables for this study: *Cryptosporidium*, enteropathogenic *E coli,* and enterotoxigenic *E. coli.*

Other enteropathogens in the primary study such as *Campylobacter*, rotavirus, *Salmonella* and *Shigella* were examined as a secondary analysis in the present dissertation.

3.6 Data management

The principal investigator of the primary study (Pillay) coded information on each collected variable for the analysis. Data capture was performed by the Biostatistics Department of the Medical Research Council (MRC), Cape Town, South Africa. The Access database provided by the MRC was cleaned and transferred into STATA™ (Version 8, College Station, TX, USA). Addition of temperature, rainfall and humidity variables was done on an Excel 2003 spreadsheet and transferred back into STATA™. Before the analysis phase the STATA™ database was rechecked to identify and correct any errors that could have occurred in the transfer process.

3.7 Statistical analysis

Associations between the enteropathogens and each of the independent variables were assessed by logistic regression using STATA™. Variables associated with enteropathogens in the bivariate analyses ($p<0.10$) were included in a multiple logistic regression for each of the enteropathogens to assess association. Mean substitution was performed for variables when less than five percent of the sample had missing observations. If more than five percent of the data on a potential determinant were missing and the association was significant in the bi-variate analysis, the determinant was excluded from the multivariate analysis. For variables with less than five observations in a cell when presented in a two by two table, the Fisher's exact test ($p<0.10$) was performed.

Potential effect modifiers of associations with enteropathogens were assessed by testing for interactions in the regression models between significant variables and breastfeeding, age and nutritional status (z-scores for weight for height).

3.8 Ethical issues

Ethical approval for this secondary analysis was granted by the Research and Ethics Committee of the University of Cape Town (ref 115/2007). The research that generated the database being used in this dissertation was approved by the Research Ethics Committee of the University of Cape Town (ref 097/2000). Anonymity of participants was maintained.

4. RESULTS

4.1 Patient characteristics

Between April 1st 2002 and March 31st 2003, 2270 patients were admitted to the Rehydration Unit. Of these, 411 met the inclusion criteria and 14 refused consent, resulting in 397 enrolments. Forty-one patients were discharged before stool collection occurred and another six patients were excluded from the analysis because of missing data. The remaining 350 children were included in the analysis.

4.1.1 Clinical determinants

Of the 350 included children, 47% were female. The median age was eight months (inter-quartile range five-thirteen months). The median duration of diarrhoea and vomiting was 3.5 days (inter-quartile range two-seven days) and two days (inter-quartile range one to four days) respectively. Sixty-nine children (19%) had previously been admitted for diarrhoea. Only 20% of the patients' caregivers reported adequate drinking before admission to the hospital. More than 35% of the children were not drinking at all and 43% drank less than what they would in normal circumstances. Of the 135 patients with known HIV status, 27 (20%) were HIV positive, 61 (45%) were HIV negative and 47 (35%) had been exposed to HIV (i.e. the mother was infected but the child's infection status was not yet confirmed). Data on shock were available for only 100 patients, of whom 14 were stated to be in shock. A summary of patient characteristics is included in table 4.1.

Table 4.1: Summary of patient characteristics

	Median	Inter Quartile Range
Age (months)	8.5	5 to 13
Height for age (z- score)	-0.09	-1 to 0.93
Weight for height (z- score)	-1.2	-2.05 to -0.36
Duration of diarrhoea (days)	3.5	2 to 7
Duration of vomiting (days)	2	1 to 4
Plasma pH	7.28	7.22 to 7.48
Degree of dehydration (% of body weight)	4.21	1.66 to 4.21

4.1.2 Nutritional determinants and treatment prior to admission

Fifty two infants out of 349 were exclusively breastfed (14.8%) and 85.1% were bottle–fed. Fifty three children [15%] received mixed feeding. Fourteen percent of the patients had received antibiotic therapy before admission.

4.1.3 Socio-economic determinants

The median education level of the caregivers was grade 10 (inter-quartile range eight to twelve) and 78% of the caregivers had benefited from secondary level education. 45% of the patients lived in shacks and less than 45% had access to piped water in the house. A summary of the patients' diet and socio-economic characteristics are presented in Table 4.2.

Table 4.2: Diet and socio-economic characteristics of the patients

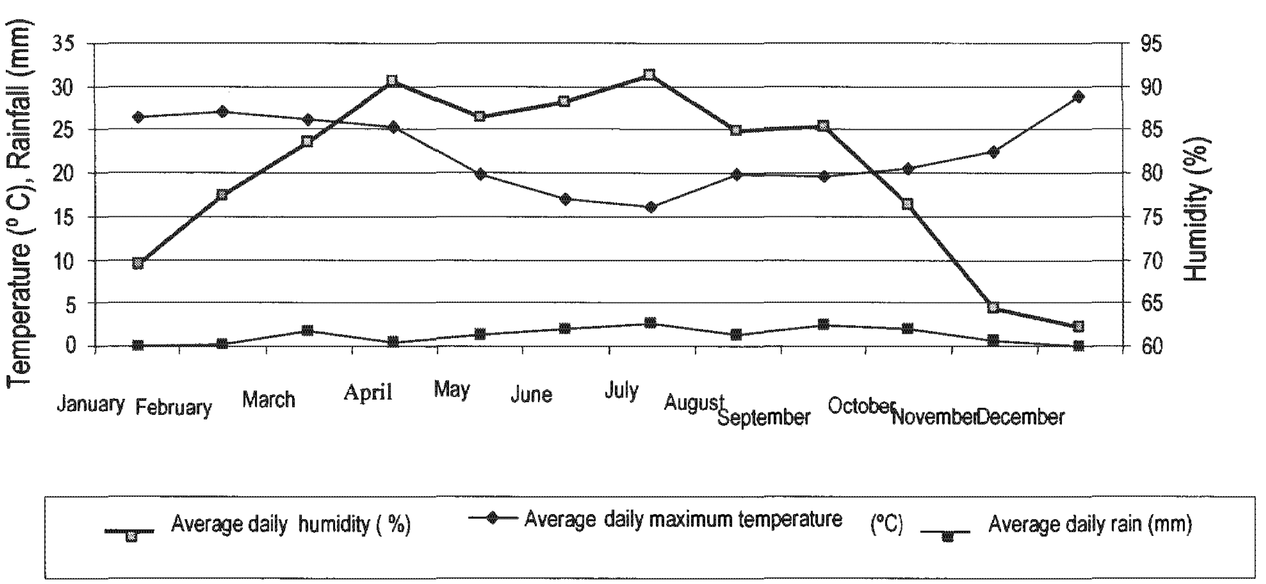
Breast-fed N=349	Bottle-fed N=350	Semi-solids N=350	ORS* use N= 350	House type N=350	Electrical appliances N=350	Access to flush toilet N=349	Access to piped water N= 349
				Brick	No electricity, fridge or deep freeze	In house	In house
				191 (54.6%)	67 (19.1%)	144 (41.3%)	156 (44.7%)
				Shack	Electricity but no functional appliance	In site	On site
104 (29.8%)	298 (85.1%)	308 (88.0%)	309 (88.2%)	159 (45.4%)	81 (23.1%)	90 (25.8%)	101 (28.9%)
					Electricity and a functional appliance	Off site	Off site
					202 (57.7%)	27 (7.7%)	92 (26.3%)
						None	
						88 (25.2%)	

*ORS: Oral Rehydration Solution
N: 349 if a data was missing for that specific determinant

4.1.4 Seasonal determinants

The highest monthly number of patients included in the analysis occurred in early February with 55 infants included in the analysis and the lowest number in June with nine infants enrolled. More than 39% of the enteropathogens identified in this analysis were isolated between December and February and the lowest in winter (June to August): eight percent. Cape Town has four seasons, as in other temperate areas, and the variation in temperature is relatively limited. Figure 4.1 represents the mean maximum temperature, humidity and the monthly rainfall for the study period.

Figure 4.1: Average seasonal determinants for the study period



4.1.5 Enteropathogens isolated

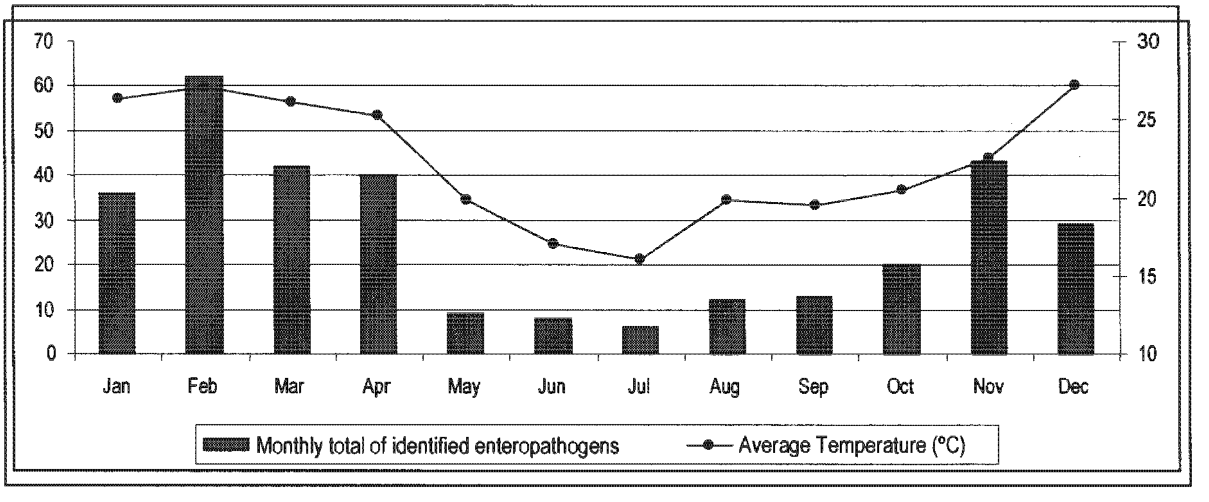
Hundred and fifteen patients did not have enteropathogens in their stool while for 235 infants, enteropathogens were isolated in heir stools and. The following enteropathogens were identified. In the case of *Cryptosporidium, Campylobacter* and rotavirus, data were missing for respectively two, eight and 6 patients.

Enteropathogens	Frequency	N
Cryptosporidium	61 (14.0%)	348
Enteropathogenic *E. coli*	37(10.5%)	350
Enterotoxigenic *E. coli*	38 (10.8%)	350
Campylobacter	61 (17.8%)	342
Rotavirus	101(29.3%)	344
Salmonella	24 (6.8%)	350
Shigella	10 (2.8%)	350

4.2 Bivariate analysis

All determinants associated with enteropathogens at the 0.1 level of significance are represented in table 4.3. Average temperature was positively associated with five enteropathogens ($p<0.1$) with the remaining pathogen (rotavirus) being negatively associated (OR = 0.94). One unit increase (^{O}C) in the average temperature of the seven days prior to admission was associated with a six percent decrease of risk of rotavirus diarrhoea. There were stronger positive associations of a unit increase in temperature with enteropathogenic *E coli*, Enterotoxigenic *E coli*, *Salmonella* and *Shigella*. Overall, the highest number of enteropathogens isolated occurred while the temperature was above 22°C (figure 4.2).

Figure 4.2: Enteropathogens isolated during the year, and the average temperature



Height for age was positively associated to Enterotoxigenic *E. coli* but the association was in the opposite direction for *Campylobacter*, Enteropathogenic *E coli* and *Salmonella*. (Table 4.3)

The lack of access to flush toilet was associated with Enteropathogenic *E coli*, rotavirus and *Salmonella* while the lack of access to piped water was associated with *Campylobacter*, Enteropathogenic *E coli* and rotavirus.

When a two by two table was constructed for the presence of shock by *Campylobacter* and Enterotoxigenic *E coli,* and *Shigella* by ORS and antibiotic, the values in one of the cells of the two by two table was less than 5. A Fisher's exact test found no significant association between *Campylobacter* and shock ($p = 0.139$), Enterotoxigenic *E coli* and shock ($p = 0.145$), *Shigella* and ORS ($p = 0.282$); *Shigella* and antibiotic ($p = 0.216$).

Table 4.3: Bivariate associations with enteropathogens (p<0.1)

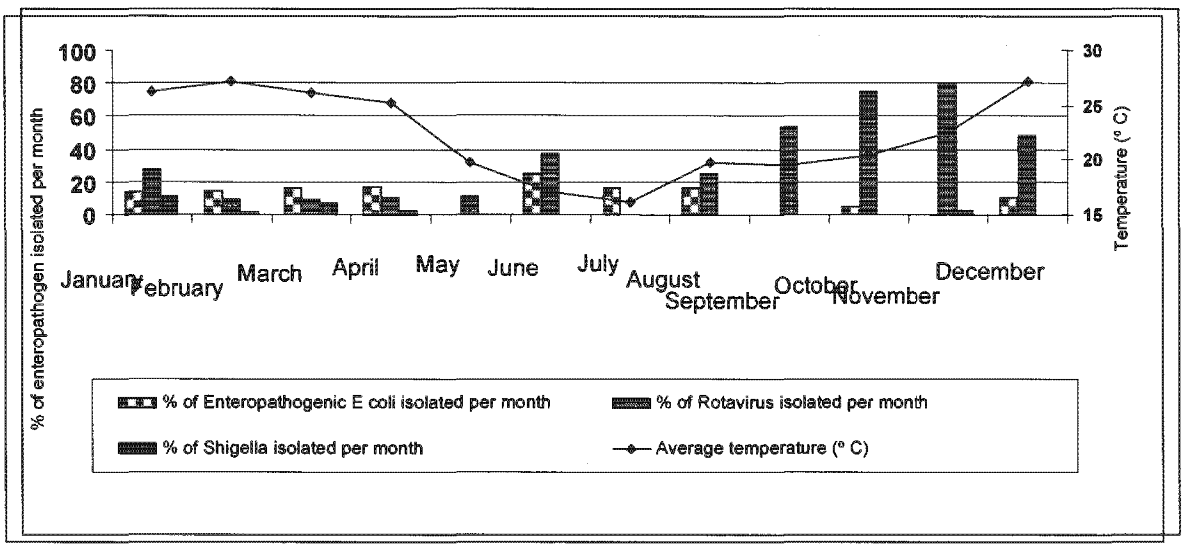
	Cryptosporidium		Enteropathogenic *E coli*		Enterotoxigenic *E coli*		*Campylobacter*		Rotavirus		*Salmonella*		*Shigella*	
	OR	p-value	OR	p-value	OR	p-value	OR	p-value	OR	p-value	OR	p-value	OR	p-value
Age (months)	1.05	0.065	-		-		1.07	0.004	-		-		-	
Gender (male/female)	-		-		-		-		-		0.43	0.074	-	
Weight for height (z-score)	-		-		-		-		1.24	0.011	-		-	
Height for age (z-score)	-		0.82	0.039	1.16	0.064	0.87	0.082	-		0.79	0.029	-	
Diarrhoea duration (days)	1.08	0	-		-		-		0.95	0.048	-		-	
Vomiting duration (days)	1.08	0.002	-		-		-		-		1.05	0.05	0.6	0.051
Adequacy of drinking (none/ less/ normal/ more than usual)	-		-		-		0.55	0.004	-		0.41	0.009	-	
Persistent diarrhoea (Number of previous admissions for diarrhoea)	-		-		-		-		-		-		-	
Shock (yes/no)	-		7.54	0.021					-		-		-	
Degree of dehydration (%)	-		-		-		-		-		-		-	
HIV (positive/negative/unknown/ exposed)	-		-		-		-		-		-		-	
Antibiotic therapy (yes/no)	-		-		0.14	0.063	-		-		-			
ORS usage (yes/no)	-		-		-		2.93	0.082	-		-			
Plasma pH	57.64	0.029	-		-		95.3	0.007	0.01	0.001	-		417971.9*	0.002
Breast milk feeding (yes/no)	0.08	0.001	-		-		-		2.4	0	-		-	
Formula milk feeding (yes/no)	4.71	0.036	-		-		-		0.4	0.003	-		-	
Solids or semi-solids diet (yes/no)	-		-		-		-		-		-			
Caregivers' highest grade	-		-		-		-		-		-		-	
House type (brick/ shack)	-		0.47	0.046	-		-		1.84	0.011	-		-	
Water source (In house/on site/off site/ none)	-		1.60	0.025	-		1.37	0.058	0.64	0.004	-		-	
Toilet (In house/ on site/off site/none)	-		1.33	0.037	-		-		0.7	0.001	1.45	0.028	-	
Electrical appliance (none/ not functional/ functional)	-		0.55	0.004	-		0.74	0.09	-		0.62	0.056	-	
Stove (electric/ or not)	-		0.41	0.01	-		-		-		0.47	0.069	-	
Average temperature (°C)	-		1.15	0.012	1.17	0.005	-		0.94	0.063	1.11	0.1	1.34	0.026
Average rain (mm)	1.18	0.066	-		0.52	0.02	-		-		-		-	
Average humidity (%)	1.1	0	-		-		-		0.92	0	-		-	

* Note the very large unadjusted OR

4.3 Multivariate analysis

In multivariate analyses, the association with average temperature persisted for Enteropathogenic *E coli,* rotavirus and *Shigella*. Figure 4.3 shows the proportion of Enteropathogenic *E coli,* rotavirus and *Shigella* per monthly total of isolated enteropathogens. Although the highest proportion of Enteropathogenic *E coli* was observed in winter: June (25%), it was isolated all year round. As the temperature increased from July, rotavirus proportion increased until it reaches its peak in November and thereafter declined, with the exception of June. In contrast, *Shigella* seemed to occur only the when temperature was above 21°C.

Figure 4.3: Proportion of monthly isolated enteropathogens



The results of the multivariate analyses for the primary dependent variables *Cryptosporidium*, Enteropathogenic *E. coli* and Enterotoxigenic *E. coli* are presented in table 4. 4.

Table 4.4: Associations with *Cryptosporidium*, Enteropathogenic *E. coli* and Enterotoxigenic *E. coli* on multiple logistic regression

	Cryptosporidium		**Enteropathogenic *E coli***		**Enterotoxigenic *E coli***	
	OR	CI	OR	CI	OR	CI
Age (months)	1.03	0.97-1.10	-		-	
Height for age	-		0.83	0.68-1.02	1.16	0.97-1.39
Diarrhoea duration (days)	**1.07**	**1.01-1.12**	-		-	
Vomiting duration (days)	1.01	0.95-1.08	-		-	
Plasma pH	19.28	0.29-1274.07	-		-	
Breast milk feeding	**0.13**	**0.02-0.62**	-		-	
Formula feeding	2.47	0.41-14.67	-		-	
Antibiotic use	-		-		0.14	0.01-1.08
House type	-		0.87	0.30-2.50	-	
Water service	-		1.23	0.54-2.82	-	
Flush toilet	-		0.80	0.45-1.43	-	
Electrical appliance	-		0.63	0.36-1.12	-	
Stove	-		1.55	0.61-3.90	-	
Shock	-		-		-	
Average temperature	-		**1.16**	**1.03-1.30**	1.07	0.94-1.22
Average rain	1.13	0.92-1.40	-		0.62	0.35-1.09
Average humidity	**1.10**	**1.05-1.16**	-		-	

Breast milk consumption was inversely related to *Cryptosporidium*, if a patient is breast-fed, he/she has an 87% decreased risk of being infected with *Cryptosporidium*. Cryptosporidum was significantly associated with average humidity defined as the average humidity in the seven days prior to hospital admission (OR= 1.11); although duration of diarrhoea is associated with

Cryptosporidium, this is presumably an effect rather than a risk factor for *Cryptosporidium* infection.

For Enteropathogenic *E. coli*, the only significant association was with average temperature determined as the average temperature for the seven days preceding hospital admission (OR =1.16). None of the independent variables were significantly associated with Enterotoxigenic *E. coli.*

None of the socio-economic variables were significantly associated with the enteropathogens at the five percent level of significance. Drinking more than normal was found to have weak association with *Campylobacter* and *Salmonella*, ORs respectively 0.58 and 0.43.

A summary of significant associations in the multivariate analysis with enteropathogens not associated with seasonal electrolytes fluctuations is represented in Table 4. 5.

Table 4.5: Significant association of enteropathogens not associated with seasonal electrolytes fluctuations (p<0.05) on multiple regression analysis

	Campylobacter		**Rotavirus**		***Salmonella***		***Shigella***	
	OR	CI	OR	CI	OR	CI	OR	CI
Age (months)	1.05	1.00-1.11						
Diarrhoea duration (days)			-					

	Campylobacter		Rotavirus		*Salmonella*		*Shigella*	
	OR	CI	OR	CI	OR	CI	OR	CI
Vomiting duration (days)							0.59	0.36-0.96
Drinks	0.58	0.38-0.87			0.43	0.21-0.86		
Plasma pH	-		0.01	0.00-0.26			**	
Breast milk feeding			-					
Formula milk feeding			0.45	0.20-0.99				
Average temperature C°			0.87	0.81-0.95			1.32	1.02-1.73
Average humidity (%)			0.91	0.89-0.94				

Average humidity of the preceding seven days hospital admission was associated with *Cryptosporidium* and rotavirus in opposite directions.

4.4 Testing for effects modifiers

Interaction terms were generated for the significant variables in the multivariate analysis with age, weight for height and breastfeeding and added to the models for each enteropathogen. With the exception of the interaction between blood pH and breastfeeding for rotavirus, none of the interactions terms had a p-value less than 0.05.

5. DISCUSSION

5.1 Summary of main findings

For enteropathogens associated with a seasonal fluctuation of plasma sodium and potassium, the significant determinants identified in the multivariate analyses were duration of diarrhoea, breast-feeding and average humidity for *Cryptosporidium*, and only average temperature for Enteropathogenic *E coli*. No significant determinant was identified for Enterotoxigenic *E coli.*

For the other enteropathogens, age and adequacy of drinking were significantly associated with *Campylobacter*, while the only significant association with *Salmonella* was adequacy of drinking. Formula feeding, plasma pH, average humidity and average temperature were associated with rotavirus while associations identified with *Shigella* were duration of vomiting, plasma pH and average temperature.

5.2 Strengths and weaknesses of the study

The lines of referral to the Rehydration Unit resulted in a study population that broadly represented children with dehydrating diarrhoea in metropolitan Cape Town, including urban and peri-urban settlements. This suggests that the

findings are broadly applicable to children in Cape Town, and perhaps in urban and peri-urban areas of other cities in low and middle income countries.
The prospective, consecutive sample (during pre-specified hours) of patients in the study took account of the seasonal patterns of the aetiological factors being assessed, with fewer patients enrolled in the quieter winter months.

Socio-economic and socio-demographic data were prospectively collected, increasing the validity of the data.

Multiple logistic regression analysis of potential determinants of plasma sodium and potassium levels helped correct for the many interrelated and potentially confounding effects. As this study is a secondary analysis of a data collected for a research with a different objective to the present analysis, the sample numbers for infrequently isolated enteropathogens might not be adequate to identify potential associations due to lack of statistical power.

The study does not address the fact that some enteropathogens could be present in the stool without the enteropathogen being responsible for diarrhoea such as the case in a healthy carrier. This is especially true when the patient had more than one enteropathogen, and would have diluted associations with actual infections.

The cross-sectional study does not allow an assessment of temporal association, limiting causal inference.

5.3 Interpretation of results

5.3.1 Enteropathogens associated with seasonal fluctuations on plasma sodium and potassium

Cryptosporidium

Breast milk was found to be protective, OR: 0.13. This is consistent with a study done in Bangladesh (Battacharya et al, 1997) where non-breast fed children under 59 months were found on multivariate analysis to be at greater risk of *Cryptosporidium* infection. This protective effect of breast-feeding differs from results obtained in Uganda (Tumwine et al, 2003) and Iran (Hamedi et al, 2005) where the difference in *Cryptosporidium* infection between breast-fed and non-breast- fed children was not statistically significant. In their analyses Tumwine and Hamedi combined infants exclusively breast-fed and those on mixed feeding and compared them with those who were not breast-fed, and none of the infants who were exclusively breast fed had *Cryptosporidium*. In this analysis, only 2 of the breast-fed patients had *Cryptosporidium*.

Diarrhoea duration was associated with *Cryptosporidium* infection in the multivariate analysis OR = 1.07, but this appears to be an effect of *Cryptosporidium* rather than a risk factor. This observation concurs with other analyses done in children up to 60 months old (Tumwine et al, 2003) as well as in older children between 6 months and 7 years (Hamedi et al, 2005). The longer duration of diarrhoea could affect loss of water and lead to electrolyte disturbances.

In contrast with the findings in the literature review, no water-related factor was identified in this study. This could be due to the consistently high quality of tap water provided to the inhabitants of the Cape Town Metropolitan area.

Cryptosporidium was found to be associated with humidity. Studies done in developing countries did not examine the percentage of humidity but examined season instead. Three studies done in developing countries found *Cryptosporidium* to have the highest prevalence during the rainy months (Tumwine et al, 2003, Bern et al, 2000, Katsumata et al, 1998). A study done at Red Cross Children's Hospital by Coltman in 1988 found that *Cryptosporidium* isolation was seasonal with a summer and an autumn peak. In this current study, more than 79% of the annually isolated *Cryptosporidium* was isolated between February and April. Therefore the seasonality of *Cryptosporidium* seems not to have changed between 1988 and 2002.

Enteropathogenic *E. coli*

On multivariate analysis the only significant association was with average temperature. Coltman (1988) also observed the summer seasonality of diarrhoeagenic E coli in Cape Town. The summer seasonality of

Enteropathogenic *E coli* has been noted in other studies, previously in South Africa (Robins Browne, 1984), in Iran (Dallal et al, 2006) and in Norway (Afset et al, 2003).

The absence of association of Enteropathogenic E coli with other potential determinants in this study could be due to the fact that of the 37 patients infected with Enteropathogenic *E. coli* only 17 were exclusively infected with Enteropathogenic *E coli*, 15 had Enteropathogenic *E. coli* and another enteropathogen, and five infants had more than three enteropathogens.

Enterotoxigenic *E. coli*

Although Enterotoxigenic *E. coli* is common in summer and autumn, no statistically significant association with average temperature was observed in this study which is different to the association with warmer season found in Egypt (Abu-Elyazeed et al, 1999).

In the multivariate analysis, none of the determinants was associated with Enterotoxigenic *E. coli*. One of the possible reasons is that more than half of the infants infected with Enterotoxigenic *E. coli* (22 out of 38) had another enteropathogen, this co-infection is common with Enterotoxigenic *E. coli* up to 40% (Qadri et al, 2005), and 27% in the same hospital as the present study in 1988 (Coltman unpublished). Another reason could be the prevalence of a carrier state of 11.7% for Enterotoxigenic *E coli* in a developing country

(Wenneras and Erling, 2004). A proportion of the isolated Enterotoxigenic *E coli* could be explained by carrier prevalence in the studied population. Another reason could be the intrinsic pathogenicity of the bacteria. It was noted in a cohort study of children less than two years old in Guinea-Bissau (Valentier-Branth et al, 2003) that only specific strains of Enterotoxigenic *E coli* were strongly associated with diarrhoea.

5.3.2 Enteropathogens not associated with seasonal plasma sodium and potassium fluctuations

Campylobacter

After adjusting for confounding, *Campylobacter* was positively associated with the patient's age and negatively associated with an adequate fluid intake by the patient. The later association is presumably an effect of the infection, not a risk factor. The median age of the study population was 8.5 months and the median age for the patients infected with *Campylobacter* was 11 months.

Although water has been identified as an associated factor for *Campylobacter* in the literature review, in this study it has not been associated with diarrhoea. More than 73% of the patients included in this study had access to high quality piped water on site, which could explain the lack of association locally.

Rotavirus

Formula feeding was associated with a 55% decreased risk of rotavirus. This differs from the conclusions of two other studies done in developing countries which found that rotavirus was less frequent in breast-fed children (Zarnani et al, 2004) and that breast-fed children have a lower incidence of rotavirus infection (Naficy et al, 1999). The observed protective effect of bottle-feeding in this study could be explained by the comprehensive advice given to mothers who choose to formula feed in the public sector. Before choosing to formula feed, the Acceptability, Feasibility, Affordability, Safety and Sustainability criteria for feeding choice are discussed with the mother. For safety, preparing the milk hygienically with clean hands, safe water, clean utensils are discussed with the mother.

Average temperature was related to a 13% decrease in rotavirus diarrhoea per unit increase in temperature. Rotavirus is known to occur during the cooler months of the year in industrialised and non-industrialised countries (Tanaka et al, 2007, O'Ryan et al, 2005, Binka et al, 2003). Average humidity was the other seasonal determinant significantly associated with rotavirus. One percent increase in the average humidity was linked with a nine per cent decrease in risk for rotavirus. This negative association is consistent with laboratory experience where high humidity affects rotavirus survival (Hashizume et al, 2008)

Salmonella

Although more than 66% of the *Salmonella* identified during the study occurred in summer (December to February), average temperature was not associated with *Salmonella* in the multivariate analysis which could be due to lack of statistical power. The only determinant that was significantly associated with *Salmonella* was the adequacy of drinking and is likely to be an effect of the infection and not a determinant.

Shigella

Duration of vomiting and average temperature were the only factors associated with *Shigella*. One unit increase in temperature was linked to 32% increase in risk for *Shigella*. A high summer isolation was also observed in Egypt (Abu-Elyazeed et al, 2004) and in Brazil (Diniz-Santos et al, 2005). The summer peak observed in this population could be due to increased exposure to contaminants in the environment such as flies which can contaminate food.

Duration of vomiting was inversely related to *Shigella*. Vomiting (Abu-Elyazeed et al, 2004, Hiranrattana et al, 2005) is one of the symptoms associated with *Shigella* infection rather than a determinant.

5.4 Relationship of the study findings to existing knowledge

With the exception of studies done in Brazil (Magalhães et al, 2007, Sobel et al, 2004, Lima et al, 2000), in Egypt (Wierzba et al, 2006) in Tunisia (Sdiri-Loulizi et al, 2008) the identified studies from the literature review were cohort or case control studies with a focus on one enteropathogen. Studies of more than a single enteropathogen aimed to identify risk factors for diarrhoea rather than for a specific enteropathogen (Dallal et al, 2006, Colomba et al, 2006, Diniz-Santos et al, 2005, Haque et al, 2003).

With the exception of age; the associations found in this study do not differ from findings in other settings. Coltman in his study done at the same hospital in 1988 found that the prevalence of Enteropathogenic *E. coli* infection decreased with age as of the 17 patients with Enteropathogenic *E coli* in their stools, 47% were infants less than two months and 12% in infants between 9-11 months. This downward trend was not observed in this study.

A cohort study done in Egypt ascertained that the incidence of diarrhoea related to Enterotoxigenic *E. coli* declined with increasing age (Abu-Elyazeed et al, 1999). In this study, no association with age was observed. The mean age of infants with Enterotoxigenic *E. coli* was 9.5 months, ranging from one month to 22 months.

5.5 Implications for public health

This study did not identify determinants that suggest public health measures that could target the electrolytes disturbances associated with childhood diarrhoea. Although it confirmed the seasonality of certain enteropathogenic diarrhoea in this setting, it did not identify protective measures for specific enteropathogens associated with seasonal electrolyte fluctuations. Therefore general diarrhoea prevention messages such as clean water, hand-washing, safe garbage and safe sewage disposal should remain unchanged.

In order to replace fluid lost during diarrhoea, Oral Rehydration Solution has been made widely available for children attending public health facilities in the Western Cape especially in summer. The ingestion of Oral Rehydration Solution in diarrhoeal cases aims to replace water as well as electrolytes therefore ensuring adequate hydration and maintain normal range for plasma electrolytes.

Due to the burden of rotavirus in this study population, the decision of the National Department of Health to include rotavirus vaccine in the Expanded Immunisation Program is an appropriate public health response.

5.6 Implications for future research

While seasonal hypernatremia related to diarrhoea has been confirmed in other settings, seasonal fluctuations of potassium have not been documented elsewhere. Studies in other geographical areas could be undertaken to establish whether seasonal potassium disturbances occur elsewhere and identify the related enteropathogens.

Studies looking at other determinants such as contact with animals, cooking, weaning practices and the impact of hand-washing in preventing these electrolytes disturbances could be considered.

6. CONCLUSION

This study has identified factors associated with enteropathogens previously associated with seasonal electrolytes disturbances (such as *Cryptosporidium* and Enteropathogenic *E. coli*). It has not however suggested interventions that could prevent seasonal plasma potassium and sodium disturbances.

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APPENDICES

Appendix 1: English questionnaire of the study providing the data for this thesis

Date of admission: ___/___/___ Time:__________

Folder No ____________________

Name ____________________

DOB ___/___/___ Sex ________

Eligibility

Ma'am, how old is Baby?__________months

Were you sent here from another hospital or ward? **Y / N**

Has baby been in a research study with me before? **Y / N**

Is baby on diuretics? **Y / N**

History

Can you please tell me the following things about baby's illness

How long has baby been sick with diarrhoea?__________Day(s)

Has baby been vomiting? **Y / N**

If yes

For how long:__________Day(s)

Is baby drinking as well as s/he usually does: **Y / N**

If no

Less than usual ☐

Not at all ☐

How many times has baby had diarrhoea in the past three months where baby needed to be taken to a doctor?__________

Has baby had fits in the last 24 hours? **Y / N**

Has baby been crying more than usual? **Y / N**

If yes

Crying but you can calm baby ☐

Crying but cannot calm baby ☐

Diet

Ma'am, can you please tell me the following things about baby's feeding habits.

Is baby on the breast only? **Y / N**

If no

Is baby bottle-fed? **Y / N**

Which bottle feed? __________

Is baby on solids? ______________________________ Y / N

Does baby drink any thing else? Y / N

If yes could you tell me what

____

Treatment before admission

Ma'am I am now going to ask a few questions about how you have been treating the diarrhoea before you came here today.

Have you needed to visit anybody for treatment of baby's diarrhoea before coming the to RCCH? Y / N

'Traditional' /Pharmacist /Clinic nurse / Clinic doctor / GP

Have you been giving baby anything to drink as treatment for the diarrhoea ? Y / N

If yes, what did you give baby?

- ☐ Sachet: ______________________________
- ☐ Salt and sugar solution: ______________________________
- ☐ Other: ______________________________

How did you make it?

____

____

What liquid did you use and how much? ____________________ ml

No. spoons salt: ____________________

No. spoons sugar: ____________________

Ma'am could you please show me from all the spoons in the box which size spoon you used.

A B C

Other treatments

Have you been giving baby anything else to treat the diarrhoea? Y / N

If yes describe

Ma'am I am now going to ask a few questions about yourself and where you live.

What is the highest education level you have reached?

- No education ☐
- School (Std / Grade):____________________

Tertiary education passed

- Training college (no of years):____________________ ☐
- Technikon (no of years):____________________ ☐
- University (no of years):____________________ ☐
- Other:____________________ ☐

In what type of house do you live in?

- House on a separate site ☐
- Hut/traditional house ☐
- Flat in a block of flats ☐
- Semi-detached/cluster/town house ☐
- House or a flat in the backyard ☐
- Shack in the backyard ☐
- Shack not in the backyard ☐
- Room in a hostel or compound provided for you by your boss ☐
- Other:____________________ ☐

Please tell me whether you and your family have access to the following

- Chemical toilet ☐
- Pit latrine ☐
- Bucket toilet ☐
- None ☐

	In house	On site	Off site
Flush toilet	☐	☐	☐

Family income

Please tell me which of the following things you have in the house and whether they are working or not.

	Y	N	Yes but not working (explain)
Electricity	1	2	3 ___________
Stove (electric,gas, coal, wood, paraffin [primus])	1	2	3___________
Other:___________________________			
Refrigerator/ deepfreeze	1	2	3___________

Recent urbanisation

Where do you live? ____

When did you move into this area?_____________________________________

Has baby always lived here? **Y/ N**

During the past year has baby lived somewhere else for any period? **Y/ N**

With whom and for how long (> than 1 month):

Appendix 2: Search strategy for literature review

Cryptosporidium search done on 5th April 2008

	Most Recent Queries	Time	Result
#15	Select **60** document(s)	05:52:15	60
#13	Search **cryptosporidium/epidemiology** Limits: **Humans, English, French, All Infant: birth-23 months**	05:21:04	362

Enteropathogenic *E.coli* search done on 28th April 2008

Search	Most Recent Queries	Time	Result
#4	Search **Enteropathogenic Escherichia coli/epidemiology** Limits: **Humans, English, French, All Infant: birth-23 months**	03:52:11	98

Enterotoxigenic *E. coli* search done on 28th April 2008

Search	Most Recent Queries	Time	Result
#7	Search **Enterotoxigenic Escherichia coli/ epidemiology** Limits: **Humans, English, French, All Infant: birth-23 months**	04:06:15	117

Campylobacter search done on 5th April 2008

Search	Most Recent Queries	Time	Result
#4	Search **"Campylobacter Infections/epidemiology"[Mesh]** Limits: **Humans, All Infant: birth-23 months**	03:54:41	339
#3	Search **"Campylobacter Infections/epidemiology"[Mesh]**	03:47:37	1213

Rotavirus, Salmonella search done on 4th May 2008

Search	Most Recent Queries	Time	Result
#7	Search **"Rotavirus Infections/epidemiology"[Mesh]** Limits: **Humans, English, French, All Infant: birth-23 months**	06:58:52	864
#4	Search **"Salmonella Infections/epidemiology"[Mesh]** Limits: **Humans, English, French, All Infant: birth-23 months**	03:59:50	798

Dysentery search done on 3th May 2008

Search	Most Recent Queries	Time	Result
#4	Search **"Dysentery, Bacillary/epidemiology"[Mesh])** Limits: **Humans, English, French, All Infant: birth-23 months**	03:23:32	344

Shigella search done on 28th April 2008

Search	Most Recent Queries	Time	Result
#20	Search **"Shigella"/epidemiology** Limits: **Humans, English, French, All Infant: birth-23 months**	04:59:16	528

Appendix 3: STATA output

```
. summarize  agef, detail

                            AGEF
-------------------------------------------------------------
      Percentiles      Smallest
 1%            1              1
 5%            2              1
10%            2              1       Obs                 350
25%            5              1       Sum of Wgt.         350

50%          8.5                      Mean           9.325714
                        Largest       Std. Dev.      5.650486
75%           13             22
90%           18             22       Variance       31.92799
95%           20             22       Skewness       .4452134
99%           22             22       Kurtosis       2.180317
```

```
. summarize  diarrdur, detail

                          DIARRDUR
-------------------------------------------------------------
      Percentiles      Smallest
 1%            0              0
 5%            1              0
10%            1              0       Obs                 350
25%            2              0       Sum of Wgt.         350

50%          3.5                      Mean           5.571429
                        Largest       Std. Dev.      7.183975
75%            7             36
90%           11             49       Variance        51.6095
95%           17             60       Skewness       4.144079
99%           36             60       Kurtosis       26.25283
```

```
. summarize  vomtdur, detail

                          VOMTDUR
-------------------------------------------------------------
      Percentiles      Smallest
 1%            0              0
 5%            0              0
10%            0              0       Obs                 350
25%            1              0       Sum of Wgt.         350

50%            2                      Mean           3.545486
                        Largest       Std. Dev.      5.354264
75%            4             30
90%            7             30       Variance       28.66814
95%           11             30       Skewness       5.355015
99%           30             60       Kurtosis       44.29343
```

```
. summarize  whz, detail

                            WHZ
-------------------------------------------------------------
      Percentiles      Smallest
 1%           -4          -4.53
 5%        -3.13          -4.18
10%       -2.765          -4.18       Obs                 350
25%        -2.05             -4       Sum of Wgt.         350

50%         -1.2                      Mean             -1.133
                        Largest       Std. Dev.      1.370684
75%         -.36           2.41
90%         .585           2.87       Variance       1.878774
95%          1.3           2.88       Skewness        .701467
99%         2.41           6.43       Kurtosis       5.184461

. summarize  haz, detail

                            HAZ
-------------------------------------------------------------
      Percentiles      Smallest
 1%        -5.24          -9.27
 5%        -3.33          -7.04
10%        -2.27          -5.93       Obs                 350
25%           -1          -5.24       Sum of Wgt.         350

50%         -.09                      Mean           -.1464857
                        Largest       Std. Dev.        2.30837
75%          .93           3.02
90%         1.77           3.42       Variance       5.328571
95%         2.27           3.44       Skewness       5.241448
99%         3.02          28.93       Kurtosis       74.14197

. tab  bottle

     BOTTLE |      Freq.     Percent        Cum.
------------+-----------------------------------
          0 |         52       14.86       14.86
          1 |        298       85.14      100.00
------------+-----------------------------------
      Total |        350      100.00

. tab  breast

     BREAST |      Freq.     Percent        Cum.
------------+-----------------------------------
          0 |        245       70.20       70.20
          1 |        104       29.80      100.00
------------+-----------------------------------
      Total |        349      100.00
```

. tab gender

GENDER	Freq.	Percent	Cum.
1	185	52.86	52.86
2	165	47.14	100.00
Total	350	100.00	

. tab solsemis

SOLSEMIS	Freq.	Percent	Cum.
0	42	12.00	12.00
1	308	88.00	100.00
Total	350	100.00	

. tab ors

ORS	Freq.	Percent	Cum.
0	41	11.71	11.71
1	309	88.29	100.00
Total	350	100.00	

. tab ab

AB	Freq.	Percent	Cum.
0	301	86.00	86.00
1	49	14.00	100.00
Total	350	100.00	

. tab toiletf

TOILETF	Freq.	Percent	Cum.
0	144	41.26	41.26
1	90	25.79	67.05
2	27	7.74	74.79
3	88	25.21	100.00
Total	349	100.00	

. tab watersrcef

WATERSRCEF	Freq.	Percent	Cum.
0	156	44.70	44.70
1	101	28.94	73.64
2	92	26.36	100.00
Total	349	100.00	

```
. tab  hsetypef

   HSETYPEF |      Freq.     Percent        Cum.
------------+-----------------------------------
          0 |        191       54.57       54.57
          1 |        159       45.43      100.00
------------+-----------------------------------
      Total |        350      100.00

. tab  grade

      GRADE |      Freq.     Percent        Cum.
------------+-----------------------------------
          0 |          4        1.15        1.15
          2 |          1        0.29        1.43
          3 |          1        0.29        1.72
          4 |          3        0.86        2.58
          5 |         14        4.01        6.59
          6 |         11        3.15        9.74
          7 |         20        5.73       15.47
          8 |         37       10.60       26.07
          9 |         47       13.47       39.54
         10 |         65       18.62       58.17
         11 |         57       16.33       74.50
         12 |         67       19.20       93.70
         13 |          6        1.72       95.42
         14 |          7        2.01       97.42
         15 |          5        1.43       98.85
         16 |          2        0.57       99.43
         17 |          2        0.57      100.00
------------+-----------------------------------
      Total |        349      100.00


. logistic crytospori agef diarrdur vomtdur ph breast bottle averain avehumid

Logistic regression                               Number of obs   =        348
                                                  LR chi2(8)      =      72.18
                                                  Prob > chi2     =     0.0000
Log likelihood = -105.34582                       Pseudo R2       =     0.2552

------------------------------------------------------------------------------
  crytospori | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
        agef |   1.037581   .0325924     1.17   0.240     .9756278    1.103468
    diarrdur |   1.071306   .0290628     2.54   0.011     1.015832     1.12981
     vomtdur |   1.016743   .0340114     0.50   0.620     .9522204    1.085638
          ph |   19.28999   41.24183     1.38   0.166     .2920591     1274.07
      breast |   .1349868   .1055187    -2.56   0.010     .0291682    .6247018
      bottle |   2.470605   2.245579     1.00   0.320     .4160356    14.67156
     averain |   1.138628   .1224662     1.21   0.227      .922211    1.405833
    avehumid |   1.107806   .0269738     4.20   0.000      1.05618    1.161955
------------------------------------------------------------------------------
```

```
. logistic    epathb  haz hsetypef watersrcef toiletf elecapp stovefin avetemp

Logistic regression                               Number of obs   =        350
                                                  LR chi2(7)      =      20.64
                                                  Prob > chi2     =     0.0043
Log likelihood = -107.79124                       Pseudo R2       =     0.0874

------------------------------------------------------------------------------
      epathb | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
         haz |   .8375239   .0843281    -1.76   0.078     .6875305     1.02024
    hsetypef |   .8780903   .4692007    -0.24   0.808     .3081103    2.502488
  watersrcef |   1.238242   .5206373     0.51   0.611      .543132    2.822968
     toiletf |   .8084021   .2356458    -0.73   0.466     .4565683    1.431361
     elecapp |   .6395041   .1841209    -1.55   0.120     .3637241    1.124384
    stovefin |   1.552294   .7300198     0.94   0.350     .6175426    3.901943
     avetemp |   1.165866   .0685835     2.61   0.009     1.038905    1.308343
------------------------------------------------------------------------------


. logistic etoxb haz ab avetemp averain

Logistic regression                               Number of obs   =        350
                                                  LR chi2(4)      =      23.88
                                                  Prob > chi2     =     0.0001
Log likelihood = -108.29262                       Pseudo R2       =     0.0993

------------------------------------------------------------------------------
       etoxb | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
         haz |   1.167059   .1075087     1.68   0.094      .974273    1.397993
          ab |   .1444398   .1485928    -1.88   0.060     .0192314    1.084832
     avetemp |   1.079479   .0708686     1.16   0.244      .949144    1.227711
     averain |   .6269738   .1784313    -1.64   0.101     .3589258    1.095201
------------------------------------------------------------------------------


. logistic    campylobac agef haz drinks ors watersrcef elecapp ph

Logistic regression                               Number of obs   =        342
                                                  LR chi2(7)      =      30.37
                                                  Prob > chi2     =     0.0001
Log likelihood = -145.17865                       Pseudo R2       =     0.0947

------------------------------------------------------------------------------
  campylobac | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
        agef |   1.057597    .028993     2.04   0.041     1.002271    1.115976
         haz |   .8759095   .0761947    -1.52   0.128     .7386077    1.038735
      drinks |   .5820841   .1193625    -2.64   0.008     .3894375    .8700288
         ors |   2.738917   1.735386     1.59   0.112     .7911436    9.482058
  watersrcef |   1.085454   .2451767     0.36   0.717     .6971824     1.68996
     elecapp |   .7668818   .1734683    -1.17   0.241     .4922516     1.19473
          ph |   34.23065   62.94053     1.92   0.055      .931741    1257.579
------------------------------------------------------------------------------
```

. logistic rotavirus whz diarrdur ph breast bottle hsetypef toiletf watersrcef avetemp avehumid

Logistic regression Number of obs = 344
LR chi2(10) = 78.11
Prob > chi2 = 0.0000
Log likelihood = -169.18276 Pseudo R2 = 0.1876

rotavirus	Odds Ratio	Std. Err.	z	P>|z|	[95% Conf.	Interval]
whz	1.091568	.1082948	0.88	0.377	.8986757	1.325864
diarrdur	.9962868	.02379	-0.16	0.876	.9507335	1.044023
ph	.0109321	.0177899	-2.78	0.006	.0004503	.2653808
breast	1.294755	.4221798	0.79	0.428	.6833402	2.45323
bottle	.4512096	.181682	-1.98	0.048	.2049451	.9933886
hsetypef	1.323236	.5303292	0.70	0.485	.6032416	2.902576
toiletf	.7062803	.1624647	-1.51	0.131	.4499625	1.108607
watersrcef	1.334855	.4579993	0.84	0.400	.6813654	2.615099
avetemp	.8795184	.0363561	-3.11	0.002	.8110719	.9537411
avehumid	.9198017	.013679	-5.62	0.000	.8933784	.9470065

. logistic salmonela gender haz vomtdur drinks toiletf elecapp stovefin avetemp

Logistic regression Number of obs = 350
LR chi2(8) = 23.63
Prob > chi2 = 0.0026
Log likelihood = -75.660797 Pseudo R2 = 0.1351

salmonela	Odds Ratio	Std. Err.	z	P>|z|	[95% Conf.	Interval]
gender	.4400157	.2163701	-1.67	0.095	.1678435	1.153537
haz	.8317426	.1028936	-1.49	0.136	.6526616	1.059961
vomtdur	1.044316	.0285755	1.58	0.113	.9897841	1.101852
drinks	.4302837	.1530755	-2.37	0.018	.2142575	.8641197
toiletf	1.230107	.2828045	0.90	0.368	.7838796	1.93035
elecapp	.8552316	.289634	-0.46	0.644	.4403668	1.660936
stovefin	1.264621	.7393389	0.40	0.688	.4020873	3.977413
avetemp	1.05502	.0772013	0.73	0.464	.9140586	1.21772

. logistic shigella vomtdur ph avetemp

Logistic regression Number of obs = 350
LR chi2(3) = 24.76
Prob > chi2 = 0.0000
Log likelihood = -33.028848 Pseudo R2 = 0.2726

shigella	Odds Ratio	Std. Err.	z	P>|z|	[95% Conf.	Interval]
vomtdur	.5909247	.1474687	-2.11	0.035	.3623347	.9637278
ph	417543.7	1812044	2.98	0.003	84.46622	2.06e+09
avetemp	1.329681	.1787725	2.12	0.034	1.021658	1.730571

Appendix 4: Literature review – tables

Campylobacter _ studies identified

Reference		Country	Study	Risk factors	Protective
Unicomb	2008	Australia	Case-control age specific RF	household with diarrhoea consumption restaurant chicken, beef eating two or more fast food restaurant-prepared red meet swim in older group >5y	
Fullerton	2007	USA	Case-control	0-6 m not breast feeding drinking well water riding in cart next poultry meat 7-11m visiting a farm, living pet with diarrhoea eating fruits, vegetable at home all ages: travel outside USA	Breast feeding
Sandberg	2006	Norway	Modelling		treated water
Workman	2006	Barbados	cohort	highest isolation 1-4 y number cases higher march, june-august	
Green	2006	Canada	modelling	Higher in pop living next farm animals higher incidence in 0- 4 / Winnipeg	
Tam	2006	England	time-series	Linear relationship temp/disease up to 14 degrees	
Ethelberg	2005	Denmark	Case-control	rural area low population density	
Carrique-Mas	2005	Sweden	Case-control	well in household drinking water from lake having a dog eating grilled meat	
Louis	2005	England Wales	Review	Seasonal effect - children <5 correlation with temperature	

Reference		Country	Study	Risk factors	Protective
Ekdahl	2004	Sweden		Sesonal pattern in all temperatures, peak in summer No seasonal from tropical	
Michaud	2004	Canada		eating raw poultry, raw milk seasonal variation	
Schönberg-Norio	2004	Finland	Case-control	Swimming in natural sources eating undercooked meat drinking dug-well water	
Friedman	2004	USA	Case-control	consumption of chicken in restaurant	
Ali	2003	Rawalpindi	Cross-sectional	pet keeping chicken meat consumption untreated drinking water peak incidence 12 - 21 m	
Potter	2003	USA	Case-control	poultry husbandry	
Kapperud	2003	Norway	Case-control	Un-disinfected water eating at barbecues poultry bought ray occupational exposure to animals eating undercooked pork	eating mutton eating fruits berries swimming
Neimann	2003	Denmark	Case-control	undercooked poultry red meat at barbecue grapes, un-pasteurized milk foreign travel	
Potter	2002	USA	log-linear model	high poultry density - occupational exposure	
Sopwith	2003	England	questionnaire	seasonal: peak in march	
Tenkate	2001	Australia	Case-control	ownership pet puppies pet chickens mayonnaise	

Reference		Country	Study	Risk factors	Protective
Studahl	2000	Sweden	Case-control	drinking un-pasteurized milk eating chicken eating pork with bones barbecuing living working on a farm daily contact with chickens or hens	
Lin	1998	Taiwan	Cross sectional	younger higher incidence incidence higher in winter	
Saidi	1997	Kenya	Cross sectional	difference in age distribution	
Gedlu	1996	Ethiopia	prospective	highest isolation 4 - 24 m peak was September- november	
Adak	1995	England	Case-control	occupational exposure to raw meat pet with diarrhoea untreated drinking water	eating dish cooked at home
Pazzaglia	1995	Egypt	cross sectional	isolation higher during rainy season	
Ikram	1994	Nzealand	Case-control	eating poultry at friend's house eating poultry at barbecue drinking water from non urban supply	eating at home
Pazzaglia	1993	Egypt	Case-control	isolation prevalent during rainy season keeping fowl in the home outdoor source of drinking water	
Kapperud	1992	Norway	Case-control	Sausage consumption at barbecue daily contact with dog eating poultry	
Pearson	1992	UK	? Review	infection peak in may	
Kapperud	1992	Norway	review	highest isolation first 5 y peak in warm months	
Desheng	1992	China	cross -sectional	isolation rising with age up to 12 months	infants<12m lower rates
Cabrita	1992	portugal	cross-sec	age group analysis shows higher isolation under one peak incidence in winter	

Reference		Country	Study	Risk factors	Protective
Pazzaglia	1991	Egypt	Case-control	children less than 1 greater risk	
Mégraud	1990	Algeria	Case-control		BF
Brieseman	1990	New Zealand	Review	peak incidence 0 - 4y high incidence in males, rural area peak incidences spring/summer household contact with dog	
Figueroa	1989	Chili	Prospective	lowest prevalence 9 m, highest 15 m	
Walckiers	1989	Belgium	Surveillance	isolation 54% in males 50% cases < 5years isolation rises in May high until October-November	
Mølbak	1988	Liberia	Survey	excretor increased with age correlation with supplementary feeding	inversely correlated to quality of water supply
Calva	1988	Mexico	Cohort		incidence inversely related to age
Skirrow	1987	England	survey	peak incidence 1 -4 y male predominance in summer	
Tauxe	1987		Review	highest incidence in infants peak in November	
Georges-Courbot	1987	Bangui	Survey	highest isolation in Brest Fed infants highest infection in 3 - 6 months than 4 - 6 months	
Thompson	1986	Canada	Review	peak in summer more infection in males	
Harris	1986		Case-control	consumption of raw chicken processed meat shell fish	
Sibbald	1985	Scotland	Review	rates highest in < 5 y	
Hopkins	1985	USA	Review	predominance in males predominance in < 1 y	
Ho	1985	Hong Kong	Survey	isolation in second year of life	
Rishpon	1984	Israel		incidence higher in children < 1	No association with animals at home

Reference		Country	Study	Risk factors	Protective
Pönkä	1984	Finland		abroad summer animal contacts	
Lassen	1984	Norway		highest distribution < 10 y returning travelers increase in warmer months	
Richardson	1983	Safrica	prospective	isolation higher in less 9 months	BF not protective
Gordon	1983	USA	retrospective	age, sex race not different	
Norkrans	1982	Sweden		fresh chicken	

Cryptosporidium _studies identified

Reference		Country	Study	Associated factors	Protective factors
Semenza	2007	EU	surveillance	peak in autumn Ireland, Spain : spring peak	improvement in drinking water: decline in incidence
Sanad	2007	Saudi		Infection males higher	least infected < 2 y
Yoder	2007	USA		higher in 1 -9 y and adult 30-39 peak onset in early summer through fall	
Llorente	2007	Spain	Cross sectional	More frequent in children from rural area	
Wongstitwilairoong	2007	Thailand		rainy season June -October	
Gatei	2006	Keny		highest in children 13- 24 m, November to February	
Simango	2004	Zimbabwe	Cross sectional	highest detection in children < 5 years	
Abdel-Messih	2005	Egypt	Cross sectional	younger age	
Goh	2005	England	Modelling		membrane filtration of water
Hamedi	2005	Iran	Cross sectional	underweight children	
Khan 2004	2004	Bangladesh	Case-control	most common in less than 2	
Hunter	2004	England	Case-control	travel outside UK contact with person with diarrhoea touching a cattle helping a child <5 to use toilet number of glasses of tap water	eating ice cream - strongly negatively associated eating tomatoes
Roy	2004	USA	Case-control	International travel contact with cattle contact with person > 2 to 11 years fresh water swimming	eating raw vegetables
Tumwine	2003	Uganda	Case-control	prevalence highest during rainy months malnutrition, stunting	
Nimri	2003	Jordan		higher in spring drinking water contact with animals eating unwashed vegetables	
khalakdina	2003	USA	Case-control	travel to another country	
Redlinger	2002	USA			purified water
Robertson	2002	Australia	Case-control	swimming in public pools contact with a person with diarrhoea	

Reference		Country	Study	Associated factors	Protective factors
Bern	2002	Peru		frequent in warm season houses without latrine - infection more frequent	
Perch	2001	Guinea Bissau		highest prevalence in children 6 -11 m marked seasonal variation just before rainy seasons	
Chai	2001	Korea	Cross sectional	Higher in 50-59 y, higher rates in spring than summer and winter weak correlation with rainfall	
Iqbal	2001	Kuwait	Cross sectional	Prevalence highest in children> 2 vs < 2 seasonal variation January to April	
Bern	2000	Guatemala		rainy season affected children at younger age	
Leach	2000	USA		consumption of municipal water instead of bottled older age lower household income	
Dietz	2000			increase in reporting in late summer	
Nath	1991	India		highest prevalence in 16 -45 y rainy months	
Newman	1999	Brazil	cohort	low birth weight living in densely crowded subdivisions	
Iqbal	1999	Pakistan		highest in children 19- 24 months	
Katsumata	1998	Indonesia		rainy season susceptible age less than 2 contacts with cats, rain , flood, crowded living conditions	
Nchito	1998	Zambia		more common in BF infants	
Nacro	1998	Burkina-Faso		highest incidence 6 - 23 months Higher April - June (rainy season) malnutrition	
Battacharya	1997	Bangladesh	case-control	stunting Not – Breast Fed	
Clavel	1996	Spain		prevalence highest 1 - 3 y autumn- winter period	
Miller	1994	Mexico		no consistent correlation between water source and other environmental data and presence of Crypto in stools	

Reference		Country	Study	Associated factors	Protective factors
Adegbola	1994	Gambia	Cross sectional	seasonal peak in association with rains and high relative humidity	
Mølbak	1994	Guinea Bissau	cohort	keeping pigs and dogs storage of cooked food male	Breast Feeding protective
Weinstein	1993	Australia	Case-control	spring water	
Dagan	1991	Israel	prospective study	summer month higher detection	
Moodley	1991	SA		high rainfall season	
Jongwutiwes	1990	Thailand		March-June: temperature +rainfall high	
Steele	1989	SA		seasonal :late summer and early autumn	
Gallaher	1989	USA		drinking surface water attending day care where other children were ill	
Reinthaler	1989			highest rate < 2, rainy season	

Rotavirus _ studies

References	Country	Study patients	Associated factors	Protective factors
Bozdayi 2008	Turkey	Children	Prevalent throughout the year	
			affected mainly 6 -23 months	
Sdiri-Loulizi 2008	Tunisia	children	winter peak, unusual peak May to September	
Kheyami 2008	Saudi Arabia	children + adults	highest detection 48- 59 months	
Tanak 2007	Bangladesh	children < 5	most RV cases 3- 24 months	
			peak during cool and dry months December - February	
Putnam 2007	Indonesia	children < 6	children significantly younger	
			associated symptoms: vomiting, fever , nausea ,dehydration	
Rodrigues 2007	Portugal		peak in February, March	
Reither 2007	Ghana	children < 5	young age	
			fever, watery stool	
Magalhães 2007	Brazil	children < 5	90% cases in young children	
Amarilla 2007	Paraguay	children +adults	incidence higher in coolest and driest months	
Misra 2007	USA	infants	no difference in incidence between exclusively BF and partially	
Van Damme 2007	EU	children < 5	highest incidence 6 - 23 m	
Van Damme 2007	EU	children < 5	fever, lethargy, vomiting, dehydration	
D'Souza 2008	Australia	children < 5	peak in winter and spring, lower in summer	
Bucher 2006	Switzerland	children < 5	peak incidence 13- 24 months	
			urban lower incidence vs rural	
O'Ryan 2007	Chile	< 3	peak 6 - 24 months	
			no clear seasonal predominance	
Dennehy 2006	USA	< 59 m	Low birth weight	Breast feeding protective in < 6 months
			children in child care	
			having another child in the house < 24 m	
			maternal age < 25	
			mother with less than high school ed	
Kiulia 2006	Kenya	children	most patients 3 - 60 months	
Hung 2006	Malaysia	< 5	highest rate 6 - 17 m	
			no seasonality	
Newall 2006	Australia		highest rate 6 - 12 months	
Lee 2006	Malaysia	< 14 y	peak 12 - 35 m	

References	Country	Study patients	Associated factors	Protective factors
Colomba 2006	Italy		mean age lower than in other virus vomiting frequent	
Kheyami 2006	Saudi Arabia		seasonality, winter in some cities, summer in others	
Rendi-Wagner 2006	Austria		peak in February, March	
			peak incidence 8- 14 m	
Charles 2006	USA	chidlren	winter peak	
			peak 3 - 24 months	
Díez-Domingo 2006	Spain	< 5	highest in < 12	
			80% in winter _ January - March	
Grimwood 2006	New Zeland	< 3	detection varied by age increasing	
			winter vs spring	
Wierzba 2006	Egypt	< 6	peak late summer, early winter	
			vomiting, dehydration	
Samarbafzadeh 2005	Iran	< 24 m	highest detection 7 - 1 2m	
Chen 2005	Taiwan	< 5	no seasonal pattern	
			most common in 7 - 23 months	
Bahl 2005	India		peak 9 -11 m	
			year round predominant in winter	
Moe 2005	Myanmar	< 5 y	peak 6 - 17 m, boys	
			peak cool, dry season: November - February	
Wang 2005	China	< 5 y	highest in 1- 2 y	
Veeravigrom 2004	Thailand		peak in winter	
Ghazi 2005	Saudi Arabia		frequent in < 2	
Karadag 2005	Turkey		boys vs girls	
			younger than 2	
			winter higher November April	
Suzuki 2005	Japan		Shift from winter to early spring	
Kane 2004	Latin amerca		winter seasonal peak	
Nguyen 2004	Vietnam		highest prevalence 13- 24	
			males vs females	
			watery diarrhoea, vomiting, fever, dehydration	
Salinas 2004	Venezuela	< 5 y	peak in dry cold months	
			3-23 months old	
Medici 2004	Italy		peak March, Jan	
Khalili 2004	Iran	< 5	seasonal variation: November to February mostly in children under 2 years	

References	Country	Study patients	Associated factors	Protective factors
Sánchez-Fauquier 2004	Spain	< 5y	cooler months November – February < 2 y 90%	
Phukan 2003	India	< 5 y	high 11- 20 m upper middle socio-economic status peak incidence in winter, inverse relation with temperature, humidity and rainfall	
Zamani 2004	Iran	< 5 y	6 -12 m most affected detection highest in spring, lowest summer	Rotavirus less frequent in Breast fed babies
Kurugöl 2003	Turkey		most younger than 2 years peak January - March not exclusively Breast fed had 2 fold risk of rotavirus diarrhoea	
de Wit 2003	Netherlands		person with gastro outside household food handling hygiene	
Binka 2003	Ghana		old age wasting dry season vomiting, dehydration	
Harrington 2003	Ireland		peak 6 - 12 months	
Cardoso 2003	Brazil	< 10 y	dry season April - August	
Urbina 2003	Colombia		frequent in less 12 m vomiting, dehydration	
Doan 2003	Vietnam	< 15 y	peak 1 -2 y slight seasonal pattern	
Steele 2003	SA		less than 12 m cooler, drier months, then spring	
Fischer 2002	Guinea-Bissau		primary infection resulted in 52% protection	
Grimprel 2001	France		peak in winter months	
da Rosa 2001	Brazil		highest 6 - 24 m March – September peak in June coldest- driest month	
Jain 2001	India		most prevalent in 7 12 m	
Cunliffe 2001	Malawi	< 5 y		HIV no difference in rotavirus severity
O'Ryan 2001	South america		seasonality, peak in November - May	
Sethi 2001	UK	< 16	living rented council housing accommodation more than 5 rooms contact with someone with diarrhea Bottle feeding in infants	

References	Country	Study patients	Associated factors	Protective factors
Cilla 2000	Spain	< 15 y	peak in winter	
O'Mahony 2000	Ireland	<, 7	spring peak < 2 most infected	
Rytlewska 2000	Poland	1 - 11y	7 - 36 m boys vs girls cold months march- may fever, vomiting, dehydration	
Bittencourt 2000	Brazil		718 m common infected higher in winter season, inversely related to average temperature	
Effler 2000	Hawaii	< 4 y	higher incidence in < 1y	
Chiu 2000	Taiwan		seasonal peak in late winter, early spring the late spring and summer in 1997 vomiting, dehydration	
Waters 2000	Canada		higher in 12 - 23 m and 24 - 35 m	
Zhou 2000	Japan		peak late winter to spring predominant in 1 - 2 y	
Nishio 2000	Vietnam		winter, autumn < 2 y	
Maneekarn 2000	Thailand		peak: dry cool season October -February most prevalent 6- 11 m	
Seo 2000	Korea		prevalent in 6 - 24 months late winter to early spring	
da Silva Domingues 2000	Brazil	infants children	peak in May - September	
Phetsouvanh 1999	Laos		dry season in January max prevalence	
Nacify 1999	Egypt	< 3	other socio-demographic, environmental factors associated July- November occurrence: warmer months	Breast Feeding associated with lower incidence
Ruggeri 1999	Italy		peak in winter predominant in 6- 24 m	
Szücs 1999	Hungary	< 14	most < 4 years peak December -February	
Koopmans 1999	Netherlands		winter, early spring	
Cunliffe 1998	Africa	infants children	Rotavirus seasonal peak dry months	
Carlin 1998	Australia		seasonal patterns like temperate regions: peak in mid to late winter	
Parashar 1998	USA		winter seasonality	
Unicomb 1997	Bangladesh		dehydration, vomiting, watery stools	

References	Country	Study patients	Associated factors	Protective factors
Ferson 1996	Australia	< 5	incidence August- September	
Nimri 1996	Jordan		prevalent in summer: June- August	
Aithala 1996	Oman		increased incidence in cooler months of the year	
Milaat 1995	Saudi Arabia		males vs females peak 6 to 11 m warmer months in Al-taif, cooler in Jeddah	
Mpabalwani 1995	Zambia	children	dry season	
Patwari 1994	India		no correlation between Rotavirus antigen temperature , rainfall, humidity	
Yachha 1994	India		no temporal, seasonal pattern observed , occurred more in winter	
Armah 1994	Ghana		High in cool dry month, low wet season	
Clemens 1993	Bangladesh		second year risk higher in Breast Fed	exclusively Breat fed protection against rotavirus RR 0.10
Gomwalk 1993	Nigeria		higher prevalence in dry season October- April infection correlated inversely with relative humidity	
Reves 1993	USA		Child day care	
Raúl Velázquez 1993	Mexico	< 2y	frequent in autumn	
Nath 1992	India	< 5 y	max in 19- 24 m girls more susceptible cooler months November - February	
el Assouli 1992	Saudi Arabia		increase in frequency in cooler months children less than 2, max 13- 15 m old	
Fun 1991	Bangladesh		peak dry winter months	
Ruuska 1991	Finland	24- 32 m	those at home had a low rate of rotavirus diarrhoea	
Henry 1990	West Indies		peak 6 to 23 m peak during dry season crowding at home associated with repeated infection	BF had lower infection
Kim 1990	Korea		no apparent seasonal trend	
Tswana 1990	Zimbabwe	< 24 m	dry cool season max 4 - 24 m	
Menon 1990	USA	< 2 y	exposure to other children with diarrhoea living homes with septic tanks poor environmental sanitation	
Mendis 1990	Sri Lanka		prevalence during rainy season watery diarrhoea, vomiting commoner in RV diarrhoea	

References	Country	Study patients	Associated factors	Protective factors
Dutta 1990	Bahrain		age group 6 - 11 m higher incidence in males up to 11 m mother with university education higher level or RV infection higher income no seasonal trend	
Dagan 1990	Israel		rate decreased as age increased hospitalisation in summer frequently	
Gomwalk 1990	Nigeria	< 5 y	virus more prevalent in 0 - 6 m decreased with an increase age dry season male and breast fed children more predisposed to infection with rotavirus	
Mertens 1990	Sri Lanka		peak 6 - 11m	
Cook 1990			winter disease in temperate zones winter peak in Americas autumn spring in other parts of the world	
Grinstein 1989	Argentina	< 2 y	peak in < 2 y	
Singh 1989	India		two peaks: early summer months , early winter	
Hjelt1987	Denmark		Highest rates January to April absent above 4	
Fayran 1987	USA		highest incidence October- December	
Steele 1986	SA		increase in autumn	
Duffy 1986			attack similar in BF and bottle fed	
Kidd 1986	SA		peak in autumn	
Ravaoarinoro 1986	Madagascar		prevailing agent during dry cool season	
Oishi 1985	Japan		0- 2 very susceptible distinctive seasonal variation: cooler months	
Sitbon 1985	Gabon		prevalent dry season	
Mutanda 1984	kenya		peak 6 - 12 m January - March hot, dry weather low humidity	
Panon 1984	New Caledonie	children	male and 6m - 24 m most affected fresh season : August - November	
Guerrant 1983	Brazil		drier months June October	
Coiro 1983	Brazil		seasonal variation January	

References	Country	Study patients	Associated factors	Protective factors
Brandt 1983			RV in November – July, younger patients	
Gurwith 1983	Canada		no protective effect of BF more often in first six months of life	
Konno 1983	Japan		infection related to temperature but not to humidity peak when less than 5°c November - December, peak in January	
Mata 1983			August through December	
Linhares 1983	Brazil		no seasonal pattern	
Jayavasu 1982	Thailand		seasonal distribution, peak January	
			highest in 6 - 11 m	
Muchinik 1981	Argentina		peak cooler months May to August	