Acute diarrhoea in children in rural Gambia: Knowledge, attitude and practice, aetiology, risk factors and consequences among children less than five years of age

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Abstract

Background

Diarrhoea is a leading cause of mortality and morbidity among under-five year old children worldwide and the greatest burden of the disease is in sub-Saharan Africa. Nearly half of the approximately 9 million deaths annually in children aged under five years old are in Africa and 18% of them are due to diarrhoea. A comprehensive study was conducted to understand knowledge, attitudes and practices about diarrhoea in rural Africa, identify the pathogens responsible, assess possible risk factors and document early and medium term outcome after a diarrhoeal episode.

Method

A four year study was conducted in the Upper river region, The Gambia. Repeated health care utilization and attitude surveys (7 times in three years) among 1140 randomly selected caregivers of children aged under five years old were performed. A case control study of moderate to severe diarrhoea in under-five children was conducted. Cases presenting to health facilities and age, sex and area matched controls were recruited to detect aetiological agents from their stool samples. Risk factors were ascertained through interview and home visits. Both the case and control cohorts were followed after 60-90 days and 18-24
months to assess linear growth retardation after an acute episode of moderate to severe diarrhoea.

**Results**

*Health Care and Attitudes Survey:* The period prevalence and point prevalence of diarrhoea in the community were 23.3% and 7.7% respectively. 48.4% of the caregivers brought their children to a health centre for treatment. Only 10.3% of the caregivers thought dehydration was a matter of concern. Among the children who had diarrhoea, only 17% were given oral rehydration solution (ORS) at home as a part of management of diarrhoea, before attending a health centre. Caregivers of 43% of the children did not give any treatment, 72.5% of them gave less or withheld food during the episode of diarrhoea.

*Case Control study:* There was a decline in the facility based annual incidence rate of diarrhoea during the study period (13, 8 and 6 /100 child year of observation in 2008, 2009 and 2010 respectively). Bloody diarrhoea was observed in 24.1% of the case children; the rest had watery diarrhoea. Rotavirus (OR 16.6, 95% CI: 9.8-28.2; p=<0.001), *Shigella* spp.(OR 4.7, 95% CI: 3.2-6.9; p=<0.001), *Cryptosporidium* spp. (OR 2.7, 95% CI: 1.9-3.8; p=<0.001), Enterotoxigenic Escherichia coli (ETEC) (OR 1.4, 95% CI: 1.1-1.7; p=0.009), and norovirus (OR 1.8, 95% CI: 1.3-2.3; p=<0.001) were the top five pathogens isolated from the children with acute moderate to severe diarrhoea (MSD). The overall pathogenicity indices of the top five pathogens in the children aged under five years old were 7.4, 3.8, 2.4, 2.2 and
2.1 for rotavirus, *Shigella spp.*, norovirus (G2), *Cryptosporidium spp.* and ETEC producing heat stable (ST only) toxin respectively. Primary caregivers’ illiteracy (OR 3.1, 95% CI: 2.4-4.0; p=<0.001), having a donkey/horse/mule (OR 3.3, 95% CI: (2.5-4.3); p=<0.001), giving storage water (OR 5.2, 95% CI: (3.8-7.0); p<0.001) and untreated water (OR 8.4, 95% CI: (4.2-6.6); p<0.001), and absence of toilet facilities (OR 8.5, 95% CI:1.1-4.4; p=0.038), were risk factors for diarrhoea in the children. Hand washing before food handling, cooking and use of soap were found to be protective.

*Follow-up on linear Growth Assessment:* Children in Gambia are generally stunted and on average both the cases and controls had below zero for the reference length/height for age Z score (HAZ). HAZ further declined during the two follow-up periods. More children in the case cohort died over follow-up, especially in the first few months, and the catch up growth was slower in the case cohort than the control cohort at both follow-up assessments. This was not significant in the 0-11 month age group. However, the case cohort in the 12-23 months and 24-59 months age groups grew at significantly lower rates than the controls (p=0.001 and 0.004 respectively).

**Conclusion**

Diarrhoea remains a major problem in children aged under five years old in rural Gambia, there are clear opportunities for improved management in the community, the aetiologies of MSD have been clarified in this study and risk factors have been identified. Children with MSD are more likely to die in the months after an
admission to hospital in The Gambia than community controls and their growth rates continue to track lower. A multipronged approach is necessary to face the challenge of controlling MSD in children. Now is the time to apply them in practice.
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Abbreviations

BHDSS-Basse Health and Demographic Surveillance System
CI-Confidence Interval
CRF-Case report Form
DSS-Demographic Surveillance System
EAEC- Enteroaggregative E.coli
EHEC- Enterohaemorrhagic E.coli
ELISA-Enzyme-linked Immuno Sorbent Assay
EPEC- Enteropathogenic E.coli
EPI-Expanded programme of Immunization
ETEC- Enterotoxigenic E.coli
FGD-Focus Group Discussion
GCP-Good clinical Practice
GPS-Global Positioning System
HAZ-Height for Age Z score
Hib- Haemophilus influenzae B
HIV-Human Immunodeficiency Virus
HUAS-Health Utilization and Attitudes Survey
ID-Identification Number
IMCI-Integrated Management of Childhood illness
MDG-Millennium Development Goal
MRC-Medical Research Council
MSD-Moderate to Severe Diarrhoea

MUAC-Mid Upper Arm Circumference

MUACZ-Mid Upper Arm Circumference Z score

OR-Odds Ratio

ORS-Oral rehydration solution

ORT-Oral rehydration therapy

PCR-Polymerase Chain Reaction

PCV-Pneumococcal Conjugate Vaccine

PR-Prevalence Ratio

RR-Risk Ratio/Relative Risk

SD-Standard Deviation

SCC-Scientific Coordinating Committee

SOP-Standard Operating Procedure

UK-United Kingdom

URR-Upper River Region

USD-United States Dollar

WAZ-Weight for Age Z score

WHO-World Health Organization

WHZ-Weight for Height Z score

* Children less than five years of age are described as children aged under five years old throughout the thesis.
Chapter 1- Diarrhoea in children

WE SHALL OVERCOME
1.1 Introduction

Mortality and morbidity in children less than five years of age (children aged under five years old) are important indicators of a country’s development. Childhood infection is one of the main stumbling blocks to the achievement of Millennium Development Goal (MDG) 4 set out by the United Nations in the early 1990’s. Most of the causes of childhood morbidity and mortality are well known and the measures to address them are simple, readily available and affordable. About 80% of the diarrhoeal deaths occur in Asia and Africa, 46% in Africa. Much progress has been made in Asian countries, yet the situation remains grim in much of sub-Saharan Africa. Three quarters of the diarrhoea mortalities occur in fifteen countries, and ten of these are located in the sub-Saharan Africa. Diarrhoea is the leading cause of preventable mortality in the children aged under five years old in this region.

1.2 What is diarrhoea?

Acute diarrhoea is defined as the passage of three or more abnormally loose stools in a 24 hour period. Distinct episodes of diarrhoea are defined as being separated from each other by the presence of normal stools for 48 hours. This definition requires mothers or alternative primary caregivers to identify diarrhoeal illness in their children. If a single episode of diarrhoea continues for
more than 14 days, it is defined as persistent diarrhoea. The aetiology, pathophysiology, management and consequences of acute and persistent diarrhoea are different. Acute diarrhoea, for the purpose of management, can further be subdivided into watery and bloody/mucoid types. The presence of blood in the stool is also known as dysentery. Diarrhoeal disease is mainly water borne but the route of transmission can be water, food or person to person. In human it is mainly considered to be faeco-oral.

1.3 Aetiology

Diarrhoea is caused by a diverse set of organisms including bacteria, viruses, protozoa and helminths. With the advent of molecular technology, conventional microbiology has been aided tremendously to diagnose the plethora of organism causing diarrhoea. The molecular techniques enable an apparently identical organism (e.g. E. coli) to be separated into sub-groups. These expensive diagnostic tests have been evaluated, standardized and made available in the developed world but are yet to be established in resource poor settings where the disease burden is extremely high. Application of these new tools will help in the identification of the exact diarrhoeagenic pathogens thereby facilitating the prevention and management of diarrhoeal illness in children.
1.4 Pathophysiology

The mechanism of acute diarrhoea depends on the causative organism. Watery acute diarrhoea mainly results from the disturbance of the fluid and electrolyte imbalance in the gut, specifically in the small intestine. Many viruses and bacteria have been identified as causative pathogens for watery diarrhoea. Notable among the viruses is rotavirus. Vibrio cholerae is the commonest of the bacteria causing watery diarrhoea. Among others causing watery diarrhoea are Enterotoxigenic Escherichia coli (ETEC) and Enteropathogenic Escherichia coli (EPEC). Enterohaemorrhagic Escherichia coli (EHEC) and Enteroaggregative Escherichia coli can cause both watery and bloody mucoid diarrhoea. The mechanisms through which these pathogens cause diarrhoea are different but the ultimate result is decreased absorption from the intestinal lumen and increased secretion or water loss into the lumen. Either or both these mechanisms can co-exist. Bacteria like Shigella spp., Salmonella spp., Campylobacter spp., two types of E. coli mentioned above, and parasites like Entamoeba histolytica invade the mucosal lining. There is an inflammatory response mediated by the proteins released by the pathogens, leading to ulceration and haemorrhage and ultimately causing bloody mucoid stool.
1.5 Syndromic diagnosis of diarrhoea

Empirical treatments for diarrhoea are frequently given at health facilities that lack laboratories, or where the time to get a result is not compatible with the need to get on and manage the patient. In Acute watery diarrhoea, excessive fluid loss quickly results in a severe dehydration, electrolyte imbalance and often has a fatal outcome.\textsuperscript{21} The signs of severe dehydration are easily recognized by a trained health worker.\textsuperscript{4, 22} Watery diarrhoea is often associated with severe vomiting, lethargy and coma. Bloody diarrhoea, implying intestinal damage, can also cause significant systemic abnormalities, secondary sepsis or lead to death. Bloody diarrhoea is usually associated with fever, abdominal pain and tenesmus (straining during defecation). The causative organisms for both watery and bloody diarrhoea are distinct with little overlap. With \textit{a priori} knowledge of the circulating pathogens causing either watery or bloody diarrhoea in a community it is possible to make a reasonable presumptive diagnosis of acute diarrhoea presenting at the health care facilities. Thus a syndromic diagnosis for the management of acute diarrhoea is practical and imperative.\textsuperscript{23, 24}

1.6 Management

The cornerstone of diarrhoea management is fluid replacement to replenish the on-going loss. Oral rehydration solution (ORS) has been described as the biggest discovery of the millennium, saving millions of lives from diarrhoea.\textsuperscript{25} In most
cases the diarrhoea is self-limiting but antimicrobial therapy can help to reduce duration of diarrhoea and stool frequency.\textsuperscript{26}

1.7 Nutrition

Diarrhoea is endemic and malnutrition is common in most developing countries, setting up a vicious combined cycle of co-morbidity. It has been shown that the prognosis with diarrhoea is particularly bad for malnourished children, while diarrhoea itself can cause malnutrition and developmental delay.\textsuperscript{27, 28}

1.8 Summary

Over the last few decades much has been achieved in diarrhoea research. The pathophysiology has been well established. New diagnostic tools have been developed to identify a wide range of enteropathogens causing diarrhoea. Oral rehydration solution has become established in management. New effective antimicrobial therapies have been found. A road map was established to improve water and sanitation. A link was established between diarrhoeal disease and malnutrition in children. Despite all these achievements WHO predicts that diarrhoea will continue to play an important role in the five million deaths in children aged under five years old by 2025.\textsuperscript{29, 30} The question remains why a preventable disease cannot be contained?
1.9 Rationale for Diarrhoea Study in The Gambia

A consortium of diarrhoeal disease research was formed under the auspices of the Bill and Melinda Gates Foundation, USA. MRC, The Gambia is an active partner in this consortium and the only rural site in sub-Saharan Africa. We planned to conduct a diarrhoeal disease study in The Gambia, a small West African Nation surrounded by Senegal on three sides and the Atlantic Ocean to the West. It is typical of a sub-Saharan African setting with a mixture of Sahel and Savannah eco-climatic zones (Figure 1-1).

Figure 1-1. Location of The Gambia

The Gambia has successfully implemented the Expanded Programme of Immunization (EPI) in its health structure, and was the first African country to implement vaccination against *Haemophilus influenzae* B (Hib). Eventually Hib meningitis was eliminated from the country. The Gambia is also among the few countries in Africa to introduce hepatitis B vaccination. It conducted the largest
ever Pneumococcal Conjugate Vaccine (PCV) trial following which the vaccine was also included in the EPI and is expected to bring down childhood mortality from invasive pneumococcal disease. Reports published after the PCV trial indicated that diarrhoea is the second leading cause of mortality in Gambia, like many other parts of the world. Recently the country reported reduced child mortality in one of its Demographic Surveillance Areas (DSS) to a level that suggests achievement of Millennium Development Goal (MDG) 4, seven years ahead of the target date. This reduction is mainly attributed to reduction and containment of malaria that is endemic in the country. However, the overall goal of reducing child mortality further will remain a challenge in the absence of containment of diarrhoeal disease. There was a need to conduct a comprehensive and well-designed study to understand the causes of, and barriers to, controlling diarrhoeal disease in children aged under five years old in The Gambia.

1.10 Aim

The aims of the study were to,

1. Understand the knowledge, attitude and practice regarding diarrhoeal disease in caregivers of children aged under five years old.

2. Identify facility based incidence, aetiology and risk factors for diarrhoea in children aged under five years old through a case control study.
3. Assess the short and long term consequence of diarrhoea through follow-up of the case and control cohorts.

1.11 Hypotheses

In the under-five year old children in The Gambia:

1. The facility based incidence of diarrhoeal disease is high.

2. The aetiological agents of MSD can be largely identified using currently available diagnostic tools.

3. There are modifiable personal, water, sanitation and hygiene risk factors for MSD.

4. An acute episode of MSD is associated with increased risk of failure to thrive.

1.12 My role

I was the site principal investigator for the study and a member of the epidemiology and clinical steering committee of the consortium. I was engaged from the outset of the study in the design, implementation, conduct and process development. I trained the study team for clinical assessment, anthropometric measurement, specimen collection and data collection in the clinic and field. As a part of quality assurance I made routine clinic and field visits, checked the case report forms (CRF) for consistency and performed standardization on random
samples. Even though it is a part of the multisite study, the analysis is independent; I developed the analysis plan myself with the help of my statistical supervisor. I developed, designed and implemented the long term follow-up of both the case and control cohorts, to describe the linear growth pattern after acute MSD in children.
1.13 Description of the chapters

Chapter 1: Brief description of diarrhoeal disease and aims of the study undertaken for the thesis.

Chapter 2: Review of the literature published from 1990-2010 to show the knowledge gaps in diarrhoeal disease among children aged under five years old in sub-Saharan Africa. The review sets the background demonstrating the necessity for a comprehensive research programme on diarrhoea in children.

Chapter 3: Describes the methods of the research studies in detail.

Chapter 4: Describes The Health Care Utilization and Attitude Survey (HUAS) conducted prior to the case control study and an abridged version of the HUAS conducted during the last two years of the case control study. This survey was conducted among the caregivers of children who had diarrhoea and who did not have diarrhoea in the two weeks preceding the interview. This documented community perceptions about diarrhoea, care seeking practice and home management.

Chapter 5: Aetiology of diarrhoea in rural Africa, comparing cases with age and sex matched controls and calculating pathogen-specific pathogenicity indices. The top five pathogens detected from diarrhoeal stool were evaluated in more depth. The characteristics of diarrhoea cases are also described for the diagnosis of syndromic diarrhoea.

Chapter 6: The risk factors for diarrhoea are elaborated. The risk factors discussed are socio demographic, water availability and quality, sanitation and
hygiene practice. An analysis was done to identify the independent risk factors. Finally pathogen specific risk factors are also described.

**Chapter 7**: Stunting is evaluated using follow-up data on cases and controls in both the short and long term.

**Chapter 8**: Conclusion and general recommendations from the study with discussion of future research questions.
Chapter 2- Diarrhoea in children aged under five years old in rural Africa: A review

HOW FAR TO GO
2.1 Introduction

Diarrhoea is the leading cause of mortality and morbidity among children aged under five years old in sub-Saharan Africa. Over the last three decades efforts have been made to estimate diarrhoeal disease in this population. A review of studies conducted from 1954-1979 provided a global mortality estimate of 4.6 million deaths per year. The mortality was estimated to be 3.3 million per year from studies conducted between 1980 and 1990. There was a further decline in the estimated mortality to 2.5 million deaths per year over the following decade. While the global decline in diarrhoeal disease mortality by 30-50% was reassuring, it was of concern that the decrease was relatively much less in sub-Saharan Africa than elsewhere. Only 11% of the global population lives in the region but account for half of the under-five deaths. Unfortunately diarrhoea occurring in the first few months of life contributed the highest proportion of deaths (18%). It is unclear why an easily treatable and preventable disease remains uncontainable in the region despite momentum and international commitment to address infant and child diarrhoea. It is estimated that only 20% of diarrhoea cases receive the recommended treatment comprising of low osmolarity oral rehydration solution and zinc tablet supplementation. There is a lack of qualified health care providers and access to health care facilities is often limited in remote and rural communities.
and living conditions are affected by extreme poverty, and only 42% of the population of sub-Saharan Africa have access to improved sanitation facilities.

Risk factors for diarrhoeal disease in children are multifactorial, complex and intertwining. Since the wellbeing of a child is closely linked to the primary caregiver or the mother, the caregivers educational status, social standing and overall family situation are likely to be important. It is generally perceived that educated mothers will practice good hygiene behaviour that can reduce the incidence of diarrhoea in their children. Overcrowding with poor sanitation was found to be a risk factor for diarrhoea in a number of studies and limited access to safe water worsens the situation further. It is possible to reduce incidence of childhood diarrhoea through improved water supply. Overall, availability and provision of safe potable water and sanitation themselves can reduce diarrhoeal deaths by up to 88%. However, the provision of water and availability of latrine facilities are not enough if effective hand washing practice is not followed by the children and their caregivers. Hand washing alone can reduce diarrhoea incidence by 35%. Proper hand washing with disinfectant is advocated, especially after defecation and cleaning a child. Awareness and availability of disinfectant and its use can considerably reduce the morbidity in a community. However soap use is strikingly low in the community in Africa. Overall improvements in common domestic hygiene practices are likely to reduce the burden of diarrhoea disease. Improved food safety can reduce diarrhoeal disease considerably as unhygienic
preparation of food, improper storage; inadequate reheating and improper handling of cooked food are associated with diarrhoea.\textsuperscript{65, 66, 67, 68}

Diarrhoeal disease, particularly among children, shows a seasonal pattern. Most bacterial infections are seen during wet and humid weather,\textsuperscript{69} whereas viral infections (especially rotavirus diarrhoea) are notably present during the cold and dry months of the year,\textsuperscript{70, 71} and Cryptosporidium infection is most common in the wet season.\textsuperscript{72, 73} The presence of animals in the household is associated with an increased risk for diarrhoea in children, as children in the community often play or come in close contact with them.\textsuperscript{74, 75, 76} It is thus likely that the risk factors depend on knowledge, attitude and practice regarding diarrhoea disease and its aetiology.

The vicious cycle of malnutrition in childhood and infection is equally applicable to diarrhoeal disease in children.\textsuperscript{77} Malnutrition predisposes to diarrhoea, increases its duration, alters its severity, and increases case fatality.\textsuperscript{78, 79, 80, 81, 82, 83, 84, 85} Conversely, during the acute phase of diarrhoea, the intake and absorption of nutrients decline and there is alteration of the normal metabolism contributing to malnutrition. These combined effects can impact on the function and physical growth of a child.\textsuperscript{86} However, in the absence of information on pre-existing illnesses, environmental factors and immunity, it is difficult to establish the direction of causality. In developing countries, where the diet is poor in protein and energy, nutritional supplementation may be the only way to maintain growth and provide nutritional support both during and after an acute phase of diarrhoea.\textsuperscript{87}
2.2 Aim

The aim of this chapter was to identify the gaps in knowledge of diarrhoeal disease in sub-Saharan Africa through literature review. A literature review was undertaken on:

1. Knowledge, attitude and practice about diarrhoea in sub-Saharan Africa
2. Aetiology of diarrhoea in children aged under five years old in sub-Saharan Africa
3. Nutritional consequences of diarrhoea in children aged under five years old in sub-Saharan Africa

This chapter will form the basis for the rationale for the research undertaken for the PhD thesis.

2.3 Review method

Articles published in English in peer reviewed journals from 1990-2010 were reviewed. Studies on diarrhoeal disease only were searched. Those were in relation of their knowledge, attitude and practice, aetiology and nutritional consequences. We searched the OVID search engine, Embase (containing evidence based medicine and Pubmed), Cochrane Review database, Scopus, and Web of Science. The terms used in the search were: diarrhoea (diarrhoea), knowledge, attitude, practice, aetiology (aetiology), nutrition, stunting, Africa. Studies from the references of the
searched articles were also considered for the review.

A preferred reporting item for systematic reviews and meta-analysis (PRISMA) method was followed for this review. The inclusion criteria of studies for review are described in each section below. Figure 2-1 shows the flow of search, inclusion and review process.

Figure 2-1: Review Flow Chart.

**Knowledge, Attitude and Practice:**
Articles identified through database and cross reference search: 31

- No. of articles after removal of duplicates: 29
  (6 of the studies also included information on illnesses other than diarrhoea)

- No. remained for screening: 23
  (12 studies did not meet the eligibility criteria)

- No. of articles assessed for eligibility: 11
  (One study interviewed only 29 caregivers)

- No. included in the final review: 10

**Aetiology:**
Articles identified through database and cross reference search: 54

- No. of articles after removal of duplicates: 54
  (3 studies on persistent diarrhoea)

- No. remained for screening: 51
  (36 studies did not meet the eligibility criteria)

- No. of articles assessed for eligibility: 15

- No. included in the final review: 15

**Nutritional Consequence:**
Articles identified through database and cross reference search: 41

- No. of articles after removal of duplicates: 38
  (25 included information on other factors related to malnutrition)

- No. remained for screening: 13
  (2 studies did not meet the eligibility criteria)

- No. of articles assessed for eligibility: 11
  (Two studies did not elaborate on the Z- Scoring and follow up status)

- No. included in the final review: 9
2.4 Systematic review of studies of knowledge, attitude and practice of diarrhoea in rural Africa

Like any other childhood illnesses, prevention and management of diarrhoeal disease depends heavily on the primary caregivers’ knowledge, attitude and practice. The knowledge, attitude and practice survey is a standardised tool that also can be used to estimate disease prevalence in a community. The aim was to identify studies that looked into these parameters of the caregivers of children aged under five years old in Africa.

Inclusion criteria

The reporting period in any peer reviewed journal was 1990-2010. Community and hospital based cross sectional studies that investigated one or more of knowledge, attitude, and practice among the mothers or primary caregivers of children aged under five years old towards diarrhoea only were included. The term ‘AND’ or ‘OR’ and combination of both were used to identify the studies. In the absence of valid sample size estimation for the studies, a minimum of 100 interviews conducted among the primary caregivers of children aged under five years old were required for inclusion.
Results

Initial search returned 31 full text articles. Two duplicate articles and 6 articles that reported about diarrhoea along with other childhood illnesses and where the detail of diarrhoeal disease as per our inclusion criteria was not given were removed. Twelve of the studies investigated knowledge, attitude and practice related to food, ORS, antimicrobials and other factors when the child had diarrhoea; one study only interviewed 29 mothers. Finally 10 studies were included in the analysis.
Table 2-1. Summary of knowledge, attitude and practice surveys on diarrhoeal disease among the primary caregivers of children aged under five years old conducted in different countries of sub-Saharan Africa from 1990-2010

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<th>Study locale, design and objective</th>
<th>Outcome</th>
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<td>1. A community based cross sectional study conducted at Sikasso region, Mali; During the period of March-April, 2004. The study was conducted to understand primary caregivers’ knowledge and home treatment practices for diarrhoea among children aged under five years old. Recall period for an acute episode of diarrhoea was two weeks.</td>
<td>352 primary caregivers of under-five children were interviewed. Two week period prevalence of diarrhoea in the systematically sampled survey was 64.7%. The study emphasized understanding local terminologies of diarrhoea. Based on these terminologies, 43% of the children had severe diarrhoea. Diarrhoea with fever (42.6%)</td>
<td>Treatment of diarrhoea typically begins at home followed by traditional healers. Children were taken to a health centre if the condition worsens. Caregivers’ preferred to continue both the traditional and modern medicine. Teething was considered as one of the major cause of diarrhoea and caregivers believed that certain serious</td>
<td>The study did not describe the basic demographic indicators (e.g. primary caregiver and their education, overcrowding and socioeconomic condition) of the population. Severity of diarrhoea was not assessed according to the WHO standard. In the absence of enquiring for severity signs, diarrhoea was possibly denoted as an associated</td>
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<td>weeks. Ellis, A.A. et al. 2007</td>
<td>and teething (50.3%) were the main symptoms that were considered as severe. Teething (50.3%), malaria (42.6%), and dirty water (22.4%) were considered as causative of diarrhoea by the primary caregivers. Traditional medicine was given to 21.1% of the children with diarrhoea. A child was taken to a health centre two to three days after the onset and if the condition had not improved by then. The caregivers preferred to</td>
<td>forms of diarrhoea can be cured only by traditional medicine. ORS use was low in this community as people did not consider it to be a medicine.</td>
<td>illness (of malaria and teething) rather than an independent disease. Even though a large proportion of the children in the community were malnourished, their proportions in diarrhoea illnesses were not mentioned. It would be useful to know the level of education of the primary caregivers’ to understand whether such media coverage were helpful in disseminating the role of ORS in diarrhoea. The study was conducted for a two</td>
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<td>continue both traditional and modern methods of treatment. An antibiotic was given to 30.7% of the children with diarrhoea. ORS use was promoted in the media but its use in the community was only 3.1% and use of sugar salt solution was 0.9%. The primary caregivers had an understanding of fluid replacement through ORS but they were unsure of the curative ability. Women folk specially the elders were responsible for home based care.</td>
<td>month period only and did not present the seasonal variation of the diarrhoea prevalence. While qualitative assessment of terminologies of diarrhoea, attitude of care seeking behaviour, financial implication of care, and ORS use was assessed, there is obvious absence in quantitative representation of the data. Prevalence of diarrhoea varied in different age strata even among the under-five age children and the study did not provide such data.</td>
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<td>treatment and the primary caregiver would seek permission from the male head of the household for seeking care at the health centre. 30.4% of the primary caregivers would not offer any treatment for diarrhoea in their children. The Prevalence of malnutrition among the children in the area was 47%.</td>
<td>age stratified analysis. No information regarding the knowledge on ORS use or treatment seeking pattern of the primary caregivers whose children did not have diarrhoea were available for comparison of knowledge and attitude towards the disease. It may not be prudent to attribute these findings to the population.</td>
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<tr>
<td>2. A clinic based survey in an urban locality of Nigeria. Quantitative data were collected from 250 nursing mothers of children less than one year of age and who came to the clinic</td>
<td>Food restrictions during an episode of diarrhoea were a common belief regardless of</td>
<td>Hospital based survey. Study did not follow the standard definition of diarrhoea and no</td>
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<td>collected on background and demographics and also on knowledge, attitude and practice around acute diarrhoea in infants. Emphasis was laid to the information on food withholding practice during acute diarrhoea. Data were collected during November 2003-February 2004. Only the nursing mothers of infants who presented with diarrhoea at the health care centres were interviewed. Ogunbiyi, B.O. et al. 2010</td>
<td>for the treatment of their child were enrolled in the study. Most of the mothers were from a Yoruba ethnic group (94%) and 46.8% of them had secondary education. Majority of the population (62.4%) used well water and 67.6% used public refuse dump to dispose their waste products, 56% of the respondents had access to the water toilet system. Nearly half (48%) had knowledge about acute diarrhoea and 54.8% were aware of use of ORS during diarrhoea. 71.2%</td>
<td>the primary caregivers’ educational level. A high percentage of caregivers were aware of the use ORS for the management of diarrhoea. Mothers also thought that over consumption of the breast milk could be a potential cause of diarrhoea. There was also paucity of knowledge related to the nutritional management during an episode of diarrhoea in children. Mass media was found to be the main and a well source of information on diarrhoea. But treatment was</td>
<td>information on duration and severity was available. Only descriptive results without any central tendency or dispersions were shown. Information on association between socioeconomic conditions and knowledge, attitude and practice; were not shown. The study cannot be generalized to primary caregivers of the under-five population in the community. In the absence of information on severity signs it was not clear what did drive a mother to bring their child</td>
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and 44% of the mothers reported of withdrawing food and fluid during the episode of diarrhoea. 

Guided by cultural beliefs and advises from the elders regardless of their education. 

With diarrhoea to a health centre. It is possible that mothers, who were comparatively educated, well off and living close to the health care facility brought their children for treatment; giving rise to a selection bias of the enrolled subjects. The study did not have a comparison group of mothers of children without diarrhoea. Mothers are usually pre-emptive on the care seeking when their children are sick and their response to
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<td>3. A cross sectional survey, conducted among women trading at markets; located at the urban Enugu state of Nigeria and who had children 0-24 months of age under their care. Study was conducted between September 1993 and February 1994. Comparisons</td>
<td>Two hundred sixty three women reported diarrhoea (35.6%) in their children. Teething (70.3%) was reported to be the main cause of diarrhoea followed by food (4.6%) and dirty water (3.0%). Of the total interviewed mothers 96.1% were aware of</td>
<td>Study highlighted the local belief of teething as a cause of diarrhoea. Thus diarrhoea is regarded as a normal phenomenon during the process of growth and not due to other reasons. Use of medication from the pharmacies was high during</td>
<td>knowledge, attitude and practice would differ from the mothers of children without the disease. Data were also collected for a period of three months, prohibiting any seasonal analyses. Definition of diarrhoea used in the study was unclear and it did not assess severity of diarrhoea. The recall period was also long (one month) risking misclassification and recall bias. Information on demographic, socioeconomic conditions was not available.</td>
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<td>were made between the women who brought their children to the market with those who left their children at home. Data were collected on socioeconomic condition, diarrhea incidence in the last one month, feeding and diarrhea management using a structured questionnaire. The aim of the study was to understand perception regarding the cause and management of diarrhea. Ene-Obong, H.N. et al. 2000</td>
<td>sugar salt solution but only 35.3% mentioned of their use for treatment of diarrhea. Only 10.9% of the mothers perceived of care seeking at a health centre. The study prospectively followed 216 mothers and their children for six months and 80 of them developed diarrhea during this follow up period. Among these children 28.8% mothers thought the diarrhea was due to teething and 58.8% considered it simple watery diarrhea. Only 17.5% of the</td>
<td>an episode of diarrhea possibly due to association of fever. ORS was not considered as a part the treatment of diarrhea.</td>
<td>The study population comprised a particular working group and the results could not be generalized.</td>
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<td>children were given home-made ORS, 28.8% had any form of medication from the pharmacies, herbal medicine was given to 3.8% and 7.5% received no treatment.</td>
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<td>A cross sectional study conducted in two markets (one clean and the other one unhygienic) among the market women in a semi urban Ibadan of Nigeria from September 1996 to March 1997. The objective of the study was to evaluate perceptions of</td>
<td>A sample size of 260 from each market was required. Participants (N=526) were the mothers of children aged less than five years of age. About one third of the mothers from both the market places were in the 26-30 years of age group. Mothers, trading at the clean</td>
<td>Occurrence of diarrhoea in children aged under five years old was similar in both the markets. Diarrhoea was probably related to the food handling practice at home. There was no association of diarrhoea with the educational status of the mother. Mothers</td>
<td>Severity of diarrhoea was not assessed. Interviewing working mothers’ in the market place puts in a time constraint to respond clearly. Apart from the age and education of the mother, there was no information on other demographic and socio</td>
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<td>diarrhoea in regard to knowledge and management, care seeking, feeding and ORS use at home. A pretested, structured questionnaire was used to obtain data on socioeconomic indicators, occurrence of diarrhoea among the children, assessing knowledge, practice, care seeking and management of diarrhoea Omokhodion, F.O. et al. 1998</td>
<td>market were significantly (p&lt;0.001) more educated (39% completed secondary education) compared to the mothers (23% completed secondary education) trading at the unhygienic market. 484 (92%) mothers responded to the question of occurrence of diarrhoea, 34.9% of the mothers reported one or more episode diarrhoea in their children in the preceding three months; 33% of the mothers working in the unhygienic market and 37% of the mothers</td>
<td>were less inclined to use traditional medicine for the treatment. Importantly most of the mothers were aware of the cause of diarrhoea and use of ORS for its management.</td>
<td>economic indicators to compare between the groups. Diarrhoea recall period was long risking recall bias. The severity of diarrhoea was also not assessed hence it is difficult to gauge the use of ORS at home or necessity of seeking care at the health centres. Study compared mothers working at clean and unhygienic markets but did not compare the perception of diarrhoea or use of ORS in mothers whose children did not have diarrhoea.</td>
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<td>working in the clean market. Overall 52.5% of the participant mothers thought diarrhoea was caused by dirty food while 19.0% attributed it on water and another 12.7% on teething. Majority of the mothers (78.9%) knew how to prepare ORS at home. Health centre attendances were 32.5%. 25.1% of the mothers gave ORS at home.</td>
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<td>5. As a part of Assessment of hygiene intervention, a combination of qualitative and</td>
<td>116 families having children under- five years of age were chosen, using systematic</td>
<td>Traditional beliefs guided the hygiene behaviour. Mothers were unaware of the basic signs were not assessed The</td>
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<td>quantitative approaches to understand the relation between hygiene behaviour and diarrhoeal disease was done. Two villages in north east district that were demographically similar and typical of Botswana were selected for the study purpose. Study period was July 1990-July 1991. For the qualitative part of the study, two focus groups; comprising of mothers having under-five years of age children and one in each village were asked about the random sampling. There were 208 children in those 116 families. Mothers were asked to fill up a pretested pictorial from to denote presence of diarrhoea on a daily basis to minimize the recall bias. This was monitored for 10 weeks. A detailed SES and demographic information was obtained (data not shown). Mothers attributed diarrhoea not to faeco oral transmission but to teething, improper food, cold weather and bewitching. Taking to a traditional healer transmission of diarrhoea by faeco oral route. Observation suggested that children less than one year of age and children living in poor hygienic condition were more prone to diarrhoea. Traditional healers were considered best for the prevention of diarrhoea and ORS use was very low.</td>
<td>sample size calculation was not clear. Sample size did not allow for a conclusion about the relationship between hygiene behaviours and diarrhoea. Questionnaire did not include attitude or practice towards disease and health care use. It is not possible to generalize to rural Botswana. The study had limited emphases on water, sanitation and hygiene and their relation to diarrhoea. It was not clear from the study whether information was obtained from</td>
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<td>cause and treatment of diarrhoea. Kaltenthaler, E.C. <em>et al.</em> 1996 <em>35</em></td>
<td>immediately after birth was considered as the best way of prevention. Traditional herbal tea was the main treatment given. There were considerable difficulties in preparing SSS and only 4% had ORS at home. Mean duration of diarrhoea was 3.1 days. Children under one year of age had more diarrhoea than the others. Families with poor hygienic condition had more diarrhoea.</td>
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<td>caregivers of children who did not suffer from any episode of diarrhoea. Overall demographic and socioeconomic information was not available</td>
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<td>6. A WHO standard survey was conducted in six provinces</td>
<td>Diarrhoea incidence was 3.0-6.6 episode/year in under-five</td>
<td>Continued high morbidity and mortality due to diarrhoea.</td>
<td>Sample size estimation was not given. A large sample size is</td>
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<td>of Mozambique (both rural and urban) from 1985-1987. The objective was to determine diarrhoea mortality, morbidity, treatment and household knowledge. In some parts the questionnaire included information on target diseases of the EPI, embedded in the maternal and child health evaluations. Cliff, J. et al. 1990</td>
<td>age children and mortality rates were between 4.1 to 14.7/1000/year. 89% of the mothers were aware of the ORS but only 33% of those whose children had diarrhoea used it. ORS packets were not readily available in the health centres. Instructions on ORS preparation were inadequate. Only 42% of the mothers were able to prepare the solution correctly. Study conducted a dehydration prevalence survey in one province. 413 children</td>
<td>There was continued high morbidity and mortality due to diarrhoea. However, there was distinction between knowledge vs. use and correct preparation of ORS. Study suggested rewriting of treatment norms and improve health worker training. Survey recommended KAP studies for future programme evaluation.</td>
<td>required to show effective change. Study evaluated mortality and morbidity due to other diseases along with diarrhoea in some provinces and only diarrhoea in some. Eliciting information for multiple diseases may have caused some information bias. There was no information regarding the attitude and knowledge of diarrhoea among the mothers whose children did not have diarrhoea. Study did not provide any information on</td>
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<td>(13% of the total sample) under five years of age had diarrhoea and 50% of them were dehydrated according to WHO standard. Household knowledge survey was conducted in six provinces. 93% of the mothers were aware of ORS, 67% knew correct preparation. Only 33% thought or believed that children with diarrhoea should drink more.</td>
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<td>care seeking pattern. Absence of demographic and SES information made it difficult to correlate them with diarrhoea and their practice.</td>
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<td>A sample size of 196 was determined, interviews were conducted with 161 mothers</td>
<td>High frequency of diarrhoea in under- five population, perceived as a normal</td>
<td>Definition of diarrhoea used in the study was not clear. Did not assess the severity of</td>
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<td>Temeke municipality Dar es Salaam, Tanzania from February 2007 - May 2007. Primary caregivers of under-five children were asked about occurrences of diarrhoea in their children at any time. Semi structured questionnaire was used with a face to face interview. Information was collected on socioeconomic status (SES), demographic characteristics, diarrhoea incidence and management of such episode both at home and at health centres. Assessment having an under five child. All the mothers reported diarrhoea in their children. 61.5% of them managed it at home. Children had a median age of 2 years. 20.5% of the interviewed mothers were illiterate. One third of the mothers (33%) were not aware of any risk factors for diarrhoea, 28.6% attributed diarrhoea related with growth phase of the child, 7.4% to water and 9.3% to poor sanitation. 43.5% entirely dependent on traditional</td>
<td>condition. Considerable gaps in knowledge of diarrhoea risk factors. Illiteracy was a main factor. Poor hygienic and sanitary conditions. There was reluctance to attend a health centre due to unknown reasons. Low use of ORS needs to be addressed.</td>
<td>diarrhoea. Diarrhoea recall period was long, hence everybody reported diarrhoea. Did not ask regarding the attitude towards diarrhoeal disease. Purposive sampling may lead to bias. No information on the SES condition of the population. No comparison group of mothers whose children did not have diarrhoea. No information on the seasonality of the episodes.</td>
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<td>was also made on the knowledge of diarrhoea and their management in respect to their level of education. The objective was to understand care givers perception and knowledge about diarrhoea and management Mwambete, K.D. et al. 2010</td>
<td>management. ORS was cited by only 10.5% and metronidazole by 35%. 77% of the respondents were happy with the traditional management. Only 1.2% of the primary caregivers sought immediate help from a health centre. 82% of the mothers reported not to have taken any precautionary measures while preparing food or cleanliness of the utensils they have used. 56.5% used untreated well water.81% thought diarrhoea had no influence on growth.</td>
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8. Cross sectional study conducted in six villages of two districts in Lake Victoria Basin. Multistage random sampling was done to identify the districts, wards and then villages to select under five study population. The primary caregivers were interviewed using a structured questionnaire. Information on demographic characteristics, knowledge, attitudes and practices regarding malaria/febrile illness and...
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<td>diarrhoea were obtained. Symptoms of severity of diarrhoea and food and fluid management during the episode of diarrhoea were asked. Kaatano, G.M et al. 2006</td>
<td>was 36.8% and antidiarrheal medication was given to 29.5% of the children. 13.5% of them reported going to the traditional healer and another 13.5% gave self-medication before going to the health centre. Breastfeeding varied from 53.3% to 100%. Half of the children received normal amount of fluid.35.2% received increased amount. The food habit was the same during the episode of diarrhoea. Boiling water, wash hands and avoiding contaminated food</td>
<td>especially at the hospitals (36.9%).</td>
<td>children who did not have diarrhoea.</td>
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Study locale, design and objective

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<td>9. Periurban, household survey with in the Demographic Surveillance System Bandim, Guinea-Bissau conducted during September 1991-June 1993. The population was approximately 26,000. Demographic information including nutrition, infection and vaccination were collected at every three monthly DSS round. Survey preceded a focus group discussion (FGD) with 8-12 mothers who had a child with persistent diarrhoea</td>
<td>was seen as protective</td>
<td>Education or socio economic condition did not modify the diarrhoea classification or treatment seeking behaviour. Experience possibly was more important than school education. Medical concepts of severity and dehydration were inapplicable. They were poor predictors for seeking care. Mothers recognized the severity sign poorly as individuals (except thirst by way of increased demand for breast feeding) but as a</td>
<td>Purposive sampling and not random. Only mothers with diarrhoea were interviewed. There was no information on how diarrhoea was perceived in the general population. Use of ORS and home management was not asked. Mothers with diarrhoea were likely to over report so without a comparison group the study cannot be generalized. Weighted analysis was not done so the results could not be attributed to the population.</td>
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319 mothers of under-five children having an episode of diarrhoea were interviewed. Median age of the children were 10.5 (IQR 5.9-15.5) months. Mother was the primary caregiver for 236(74.5%) of the children. 123/319 (38.6%) had no education and 149/319 (46.7%) had primary education. 201/319 (63.0) were from middle socio economic group. More than 50% of the episodes stopped at day 5/6.
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<td>or a child died because of diarrhoea. FGD was on popular concept of diarrhoea and management. Children for the household survey were identified through the weekly morbidity survey. 319 children were enrolled. Interview was conducted during the on-going period of diarrhoea and up to 14 days or till the time of hospitalization. Interview recorded signs and symptoms, overall impression of the severity and reasons for seeking and not seeking care.</td>
<td>Of the total 265(83.1%) acute watery diarrhoea, 23(7.2%) dysentery and 31 (9.7%) had persistent diarrhoea. Only 77(24%) were brought for consultation with in the first 48 hours, overall 104(32.6%) were brought for consultation at health centre or hospital, 138/319 (43.3%) received traditional treatment, 10(7%) had ORS at home. 126/215 (58.6%) mothers who did not bring their children for consultation did not think it was necessary.247/319 (77.4%) combination they were meaningful. Mothers have their own way of severity assessment and can recognize only an advanced stage. Perception of teething diarrhoea is important to note. There is an idea of normal diarrhoea that needed to be addressed. Working mothers tend not to bring their children for consultation. Good number went to traditional healer.</td>
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<td>Open ended and structured questionnaire was used. Dehydration symptoms asked were thirst, sunken eyes, depressed fontanelle, and less urine. Paediatric nurse took the interview and also assessed the severity of the child. Study assessed the caregivers’ ability to recognize the signs of dehydration, gravity of the disease and care seeking practice. Sodemann, M. et al. 1996</td>
<td>reported of at least one sign of dehydration. 120/319 (37.6%) had more than one symptom. Infants were more likely to have multiple symptoms and brought for consultation. 101/319 (31.7%) mothers thought their child’s condition was serious. Presence of any sign of dehydration, thirst and increase demand for breast milk, sunken eyes was the predictor for bringing for consultation. Bloody diarrhoea(OR 1.7;95%CI: 1.1 - 2.4), mother as a caregiver (OR</td>
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<td>10. A cross sectional study, conducted in a rural community at Wolayta district</td>
<td>707 (11.1%) of the interviewed children had diarrhoea in the previous two weeks. Highest</td>
<td>Traditional treatment was related to death or mortality. Assessment of mothers’</td>
<td>Diarrhoea definition was not clear. Severity signs were not assessed. Not possible to</td>
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<td>in southern Ethiopia during October –March (year not mentioned). The aim was to understand the caregivers’ knowledge on cause, severity, treatment practice, care seeking behaviour and consequences of diarrhoea. 764 mothers were targeted for interview and 654 were interviewed. A structured questionnaire was used. 10 questions on treatment practice including ORS, 6 questions on severity and outcome of the present rate was for 12-23 months age group (16%). Subsequent analysis was done on 619/707 children. ORS or cereal based ORT was given to 127(20.5%). Only breast milk to 140(22.6%). Breast feeding continued for 164(26.5%), stopped fluid to 53(8.6%), decreased in 262(42.3%). Stopped food to 94 (15.2%) and decreased to 337 (54.4%). No treatment sought for 313(50.6%) traditional healer 128 (20.7%), health centre 45 (7.3%) ORS/ORT to 48(15.7%), herbal remedy 30</td>
<td>perception of treatment was the main focus. Home treatment was generally poor as they stopped fluid and food. ORS use was low. They had good knowledge of safe water, use of latrine and personal hygiene. Knowledge was not associated with modern treatment but with home treatment. Traditional treatment was associated with severity (frequency of diarrhoea). Modern treatment had better outcome than traditional treatment</td>
<td>estimate why they were not taken to modern facilities. A weighted analysis was not conducted. Study was conducted in a single season. Good comparison between those who had and had not diarrhoea.</td>
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<td>episode, 21 questions about causes, consequences and treatment of diarrhoea and other questions on demographic information. They also used a scoring system for certain composite variables. 22 female interviewers from the village conducted the interview. If an under five child had no diarrhoea, it was noted an elaborate interview was not conducted. Total number of children aged under five years old visited were 6384. Olango, (9.8%), tablets 60(19.6%), injections 125 (40.8%). 91.4% had only up to grade 1 education. 92.1% thought teething was main cause of diarrhoea, 51.7% knew about ORS, 82.6% knew diarrhoea cause loss of body fluid. 59.9% of the caregivers’ did not give food or fluid if the child vomited and 69.1% gave only water when the child had diarrhoea. 20% of the children with mild diarrhoea were taken to a traditional healer; the odds of death was 8 times</td>
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<td>Health utilization is poor. Need to improve the service of community health agents.</td>
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<td>P. et al.1990 ^100</td>
<td>higher (95% CI:5.3-13.3) among these children. 18% sought modern treatment and had a high improvement rate (OR 3.4, 95% CI:2.0-5.5).</td>
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Summary

In this systematic review 10 studies met search criteria. These were either cross-sectional studies conducted in the community or household surveys. There was considerable information on diarrhoea prevalence, its management at home and health care seeking patterns. Primary caregivers of children under five years of age were interviewed through structured questionnaires. However, it was observed that none of the studies were complete in gathering information about knowledge, attitude and practice. Ascertainment of an episode of diarrhoea using a standard definition is important to avoid any mis-representation of illnesses by the caregivers. In the review some of the studies did not do this and relied on reporting by the primary caregivers only.\textsuperscript{92, 93, 95, 98} There was a notable absence of assessment of the primary caregivers’ perception regarding the common signs of dehydration, except for one study in Tanzania.\textsuperscript{98} Most often, the primary caregivers of a child who had diarrhoea were interviewed; and the recall period either was not mentioned or exceeded two weeks.\textsuperscript{92, 93, 94, 99} Studies focused on caregivers of children with diarrhoea might not reflect the general impression about the illness in the community. Desirable practices are usually over reported by respondents. Studies conducted among the caregivers trading at market places\textsuperscript{93, 94} or attending the health centres\textsuperscript{92} are not generalizable to the population. Studies obtaining information on other comorbidities or common childhood illnesses\textsuperscript{98} and hygiene behaviour\textsuperscript{95} may underestimate the importance of diarrhoeal disease. None of the studies mentioned
training of the interviewers that are important in gathering information. Shortage of trained personnel and time and cost are the major limitations in conducting a survey.

Only two of the reviewed studies gave a sample size whereby the validity of the study could be relied upon\textsuperscript{94,97} and one of the studies did not have adequate sample size to make an association between diarrhoea and perceived practice of hygiene.\textsuperscript{95}

In the review, none of the studies performed a weighted analysis to relate the findings to the population. The best survey would include a random sample from a population under demographic surveillance. Such a survey would provide information on the general demographic characteristics of the population, caregivers' perceptions about the signs and symptoms of the disease, impression about the existing health system, health care seeking patterns and possible barriers for health care utilization. A robust and population-based survey can give insight into home and hospital management, food and fluid replacement during an episode of illness and willingness to accept and comply with available interventions for the prevention and management of diarrhoeal illness. Such a survey would help policy makers to mobilize resources in an appropriate and pragmatic way.
2.5 Systematic review of aetiology

The aetiology of diarrhoea in children is diverse. With the advent of molecular techniques, the possibility of detecting a potential pathogen from diarrhoeal stool has increased enormously. Recently, some diarrhoeal aetiology studies have used more advanced techniques to identify different *E. coli* (enterotoxigenic, enteropathogenic, entero-aggregative, enteroadhesive), *Campylobacter* spp., a range of viruses (rotavirus, norovirus, astrovirus, sapovirus, and adenovirus), *Cryptosporidium* spp. and other organisms. However, no study has comprehensively identified all possible pathogens causing diarrhoea in children aged under five years old. Such a study is needed as management of the diarrhoea will largely depend on the aetiology. Accurate diagnosis also helps in judicious use of antibiotics and avoiding the spread of drug resistance. Importantly, short term studies without a control group are unable to show seasonality and clarify pathogenicity.

The aim was to identify published studies that investigated the aetiology of childhood diarrhoea.

**Inclusion criteria**

The time period was 1990-2010. Studies that aimed to identify and report aetiology of acute diarrhoea, either among hospitalized children or from the community were included. Studies that aimed to detect and reported a single pathogen only (e.g.
rotavirus, Cryptosporidium spp. or ETEC) were excluded. Studies restricted to children with human immunodeficiency virus (HIV) and abnormal nutritional status (both well and malnourished population) was not included. Reports of outbreak investigations, those taking place during a natural disaster (e.g. flood and draught), war and refugee conditions, and those that only looked at the antimicrobial susceptibility patterns for the purpose of screening only (e.g., as a part of deworming programme among the school children) were also excluded.

Results

Initial search and cross references from that search returned 54 studies. Three of the studies reported aetiology of persistent diarrhoea. Thirty six studies did not fulfil inclusion criteria and finally 15 studies were eligible for the review of aetiology.
Table 2-2. Summary of diarrhoeal disease aetiology study among children aged under five years old conducted sub-Saharan Africa from 1990-2010

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<td>1. A hospital based case control study designed to establish the aetiology of diarrhoea in &lt; 5 years of age children in Nigeria. Study was conducted between December 1989 and May 1990 among the children of &lt;5 years attending paediatric emergency room of Lagos University Teaching Hospital and at Gbaja health</td>
<td>Among the enrolled children, 98.4% were &lt;3 years of age and none of them were &gt;4 years old. More than half (60%) sought care within 7 days of the start of symptoms. Male: Female ratio was 2:1. A pathogen was detected from 161/215 (74.9%) cases and 28/100(28%) control specimens. Major pathogens detected</td>
<td>The study had a large detection rate and identified some new pathogens like EAEC and EHEC for the first time. Rota virus was the leading cause of diarrhoea in children and for hospital attendance. Among the bacteria, toxin (LT) producing ETEC as the leading cause of diarrhoea followed by EPEC.</td>
<td>Definition of acute diarrhoea was not clear and severity information was not available. There was no sex matching and there were fewer controls than cases. There were no children above 4 years of age. About 40% of the children had diarrhoea for more than 7 days excluding the acute nature of diarrhoea. The seasonal</td>
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<td>centre, Surule. Faecal samples were collected from 215 diarrhoea children and 100 age matched non diarrhoea children attending the hospital. None of the children received antimicrobial treatment in the previous two weeks. Specimens were processed within 2 hours of collection. Both stool microscopy and conventional standard microbiological methods were applied for the pathogen identification/detection. <em>E.coli</em> were rotavirus 22.3% vs. 9%, ETEC 14.4 vs. 6.0%, EPEC 10.7% vs. 5.0%, EAEC 9.3% vs. 4.0%, EHEC 5.1% vs. 3%, <em>Salmonella</em> spp. 3.3% vs.1.0%, <em>Shigella</em> spp. 5.1% vs. 0%, <em>G.lamblia</em> 0.9% vs. 0% in cases and controls respectively. ETEC was the common amongst cases (14.4%). <em>Shigella</em> spp. was exclusively isolated from cases. Over all 57% of the <em>Salmonella</em> were detected from children &lt; 1 year of age and 93.8% of rota virus was isolated from children &lt; 2</td>
<td>Concluded that LT producing ones are responsible for diarrhoea in urban children. Study showed a high carriage rate of ST in control group. ETEC cases were &lt; 1 year and controls were 30.1 month. Parasitic infestations were rare.</td>
<td>variation of the pathogen causing diarrhoea could not be elicited. Notable absence was detection of viruses other than rotavirus.</td>
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<td>was screened for toxin production. Ogunsanya, T.I. et al. 1994.</td>
<td>years of age.</td>
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<td>2. Hospital based study. Children with diarrhoea attending Bulpelia health centre were enrolled. Bulpelia caters 50000 population of low socio economic status from Dagomba community. This study was conducted between November 2005 and January 2006 in the dry season to assess a large spectrum of enteropathogens causing diarrhoea in urban Tamale,</td>
<td>During the study period, 445 met inclusion criteria but only 243 (54.6%) could provide stool sample and were included in the study/analysis. Of the total enrolled children enrolled in the study, 98% of the patients were under five years of age. An enteropathogen was detected from 186/243 (76.5%) and 66/124 (53.2%) of the Control children. More than half</td>
<td>Study claimed to have undertaken one of the largest diagnostic approaches in the region and concluded that acute diarrhoea was common in this population. Confirmed rota was major pathogen causing diarrhoea in dry season in Ghana with 55% prevalence in children. They also detected other important viruses causing diarrhoea, especially adenovirus and</td>
<td>Study was conducted in a dry season when the isolation of rota virus is generally high. More than half of the randomly selected controls had watery stool and about half of the controls yielding pathogen in their stool sample raise a concern of true control selection from the community. Also the number of controls per case was less. Controls were a random sample but it</td>
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<td>Ghana. Study also included clinical characteristics, risk factors and clinic epidemiological features of diarrhoea. Study enrolled children, aged less than 12 years of age and with acute diarrhoea. Children were not treated with antimicrobials in the previous 48 hours and they had no other severe condition that required referral. Controls were, 124 non diarrhoea children residing in same part of Tamale but attended routine child welfare clinic at Bulpelia</td>
<td>(65.3%) of the control stools were classified as loose or watery. Major pathogens detected were Rotavirus 54.7% vs. 12.1%, adenovirus 27.6% vs. 31.5%, norovirus 9.5% vs. 8.9%, <em>Salmonella</em> spp. 2.4% vs. 0, <em>Shigella</em> spp. 1.6% vs. 0.8%, <em>G.lamblia</em> 3.7% vs. 9.7%, <em>Cryptosporidium</em> spp. 0.4% vs. 0.8% in cases and controls respectively. <em>Aeromonas</em> and <em>Vibrio</em> were not detected. Co-infection was more common (p=0.01) in cases 64/243 (26.3%) than the controls 18/124</td>
<td>norovirus. Co infections with enteric pathogens among the diarrhoea cases in this population were high.</td>
<td>was not clear whether they were healthy or not. Approximately 2/3 control children reported/provided loose stool. Study did not make any effort to detect diarrhoegenic <em>E.coli</em>, responsible for a large proportion of diarrhoea in children.</td>
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<td>health centre. Faecal specimens were immediately processed. Standard microbiological techniques were applied for the detection/identification of pathogens. Reither, K. et al. 2007.</td>
<td>(14.5%). Rota virus was the commonest pathogen (more than half); adjusted for age and sex (OR 7.7; 95% CI: 4.2-14.2). Isolation rate of rota was 66.1% in less than 1 year and 42.2% in older age group. Other viruses did not show any difference between cases and controls. *G.<em>lamblia</em> identification was twice as frequent in controls as cases. Other parasites had sporadic identification. Bacterial pathogens were more frequented in cases (OR 4.8; 95% CI: 0.9-27.0).</td>
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<td><strong>3. This hospital based study</strong> was conducted to identify aetiology of diarrhoea in children attending Saint Camille clinic Ouagadougou at Burkina Faso. Area that serves the hospital was highly endemic for diarrhoea and malnutrition. Study was conducted during May 5, 2006 to June 22, 2008. Approximately 5% of the enrolled children were HIV seropositive. Standard conventional culture method**</td>
<td>Study enrolled 648 children aged 2-41 months. About half (45.1%) of the enrolled children were 2-10 months of age. Pathogens were detected from the stool samples of 41.2% of the cases. Major pathogens detected were rotavirus 21.1%, adenovirus 1.8%, <em>G. lamblia</em> 7.6%, <em>E. histolytica</em> 1.1%, <em>E coli</em> 4.2%, <em>Salmonella</em> spp. 3.4%, <em>Shigella</em> spp. 1.9%, <em>Yersinia</em> 1.7% of the samples.</td>
<td>Rota virus was the major pathogen causing diarrhoea among the children coming to the hospital. Bacterial isolation was 41.0% and EPEC was the leading bacterial pathogen. Parasite was not detected in children &lt;10 months of age. HIV positive patients had more co infection.</td>
<td>It was hospital based surveillance for aetiology of diarrhoea. No controls were taken. Age group limited to 2-41 months. PCR and other molecular technique or any other improved diagnostic tools were not used for identification of the pathogens.</td>
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<td>was used for detection of bacteria. Immunochromatographic technique was used for adenovirus and rotavirus detection. Microscopy was done for <em>E.histolytica</em> and <em>G. lamblia</em>. Simpore, J. <em>et al.</em> 2009.</td>
<td>66 children were enrolled in the study from January to July 2006. Seven of them were seropositive for HIV. Majority of children (46.9%) were in 2-11 months of age. Virus was detected from 24.24% of the</td>
<td>Diarrhoea was more common in children in 2-11 months of age (46.97%). Rotavirus was the common cause of diarrhoea (22.73%). Highest prevalence (38.75%) was noted in 2-11 months age group and</td>
<td>Clinic based aetiological study for viruses and parasites only. Conducted for six months in a small sample size. Since only one stool specimen was collected it probably underestimates the real</td>
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<td>4. Hospital based study to identify the viral and parasitic agents causing diarrhoea in 2-60 months age old children attending Saint Camille Medical centre at Ouagadougu, Burkina Faso.</td>
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<td>Children with diarrhoea attending the hospital during January to July 2006 were eligible for the enrolment in the study. Group A rotavirus and Adenovirus were detected using immunochromatographic technique with commercially available kits. Parasites were identified by microscopy. Djeneba, O. et al. 2007.</td>
<td>stool samples. Among them 22.73% were rota and 1.52% were adeno virus. Rotavirus was isolated from 2-24 months age group only and 38.71% were from 2-11 months age group. Parasite detection rate was 18.18%. Among them 12.12% were protozoan and 6.06% were helminths.</td>
<td>12.5% in 13-24 month age group. Parasites were found more in 2-11 months age group.</td>
<td>prevalence. No community control. Seasonal variation could not be assessed. Improved methods of diagnostic tests were not used.</td>
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<td>5. Health facility based study aimed to identify bacterial pathogens causing diarrhoea in &lt; 5 year children. Stool and Isolation rate of bacterial pathogens was 21% from cases and 3.9% from controls. Major pathogens isolated were EPEC EPEC, Salmonella and Shigella are predominant bacterial pathogens isolated from the children with diarrhoea and</td>
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<td>Both the case and control children were enrolled form the hospital. It was indicated that some patients received</td>
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<td>rectal swabs (when it was not possible to collect stool sample) were obtained from 1015 children with diarrhoea and 401 children without diarrhoea but attending different health centres located at former East Central State of Nigeria. Children were predominantly from the Ibo community. Efforts were made to collect samples from the age matched control at the same time. Specimens were collected before antimicrobial treatment (where possible). Stool/swab</td>
<td>12.0% vs. 2.7%, <em>Salmonella</em> spp. 3.1% vs. 0%, <em>Shigella</em> spp. 2.1% vs. 0%, <em>C. jejuni</em> 2.5% vs. 1.2, <em>Y. enterocolitica</em> 0.4% vs. 0%; <em>V. parahaemolyticus</em> 1.0% vs. 0% among cases and controls respectively. Among the controls only EPEC (2.7%) and <em>C. jejuni</em> (1.2%) were isolated. There was high infection rate in first two years of life and decline thereafter. <em>Shigella</em> spp. was isolated mostly from 1-2 year age, <em>Salmonella</em> spp. from 1-4 year age group and <em>Campylobacter</em> spp. from 3-4</td>
<td>attending health centres in the region</td>
<td>antimicrobials while rectal swab was taken for some of them which could influence the pathogen yield. Time period of the subject enrolment was not mentioned and hence seasonal variation could not be assessed. Number of controls was less than the cases and whether they were matched or not was not stated. Study only looked for bacteria and used only conventional microbiology. No PCR was done for identification of virulent <em>E.coli</em>.</td>
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<td>samples were processed transported and cultured using standard microbiological technique for identification of bacteria including <em>E.coli</em>. Anyanwu, B.N. <em>et al.</em> 1997.</td>
<td>years age group. EPEC isolation was also higher in 0-11 month age group. Serotyping for <em>Salmonella</em> and <em>Shigella</em> was done. <em>S. boydii</em> was the most common (47.6%) followed by <em>S. dysenterie</em> (38.1%) and <em>S.sonnei</em> (14.3%). No <em>S. flexnerii</em> was isolated.</td>
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<td>No statistical comparison of isolation between cases and controls were shown.</td>
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6. A community based study to assess prevalence, characteristics and risk factors for diarrhoea in children <5 years of age in urban Kinshasa, Zaire was assessed. Total 2644 children were screened from 1411 household. There was high point prevalence of diarrhoea in the community. Overall incidence is high in <2 years. Persistent diarrhoea was common in 2-3 years. Study was conducted over a six week period and during the rainy season. Seasonal variation could not be assessed. Isolation rate of Cryptosporidium and rota...
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<td>children with diarrhoea and 155 age matched children without diarrhoea were cluster sampled from the community and 18 children with diarrhoea who came for care seeking at hospital were enrolled in the study. Study site, Elonga health zone had a defined DSS and served by one health centre. Sampling was done for a six week period in 1990 in the rainy season. Standard definition of diarrhoea was used and severity information was obtained from the primary</td>
<td>was 8.5 days. 19.7% (34) had more than 14 days of duration. Dehydration was reported in 50.9% of the diarrhoea cases. Major pathogen isolated were EPEC 6.9% vs.5.8%, <em>Salmonella</em> spp. 3.5% vs.0%, <em>Cryptosporidium</em> spp. 15.6% vs. 12.9%, adenovirus 15.0% vs. 12.9%, rotavirus 1.2% vs. 1.3%, in cases and controls respectively. EPEC was more commonly isolated &lt;2 years of age. Rota virus was isolated from the stool samples of children &lt; 2 years of age. Rate</td>
<td>However, primary caregivers tend to treat their children with diarrhoea at home as evidenced by small number of children attending the health facility.</td>
<td>virus reflected the seasonality bias in sampling. The enrolled subjects had prolonged diarrhoea</td>
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<td>caregiver (standard signs of dehydration was used). Fresh stool sample was collected from both the cases and controls. Standard microbiological methods were used for the detection of parasite and bacteria. Henry, M.C. et al. 1995.</td>
<td>of crypto isolation among cases were high especially in children who attended the hospital. Significantly higher bacterial and viral pathogen were detected among the cases than the controls (p=0.03). Heavy load of <em>Trichuris trichuria</em> (4.0% vs. 0), <em>E.histolytica</em> and <em>G. lamblia</em> (2.8% vs. 0), and <em>Trichomonas hominis</em> (3.5% vs. 0%) was also detected in cases. These were statistically significant (p=&lt;0.05).</td>
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<td>7. Community survey through household screening was done. This study aimed to describe the main aetiological infectious agents of diarrhoea in urban Yaounde among children 6-59 months of age. Standard definition of acute diarrhoea was used. One child was randomly selected from a household that had multiple number of under-five children. Study was purposely conducted in wet season (April-June 2005) to identify</td>
<td>437 stool samples from children aged 6-59 months of age were collected. 358/437 (81.9%) were male. 72/437 (16.5%) were in 6-11 months age group, 178/437 (40.7%) in 12-23 months age group and rest 187/437 (42.8%) were in 24-59 month age group. Overall 14.4% of the children had diarrhoea. Co infection was found in 125/437 (48.1%) children. A potential infectious agent was found in 260/437 (59.5%) cases. Major</td>
<td>This was the first ever study to detect any pathogen causing diarrhoea in Cameroon at field level. At least one pathogenic organism was found from 59.5% of the cases. Males suffered from diarrhoea more than the females. <em>C. jejuni</em> isolation rate was high and <em>Salmonella</em> isolation was similar to other African sites. <em>Shigella</em> was also common. Infection with multiple pathogens was very common and study concluded that multiple</td>
<td>Study was conducted during the rainy season and for a brief period. Low isolation rate of rota could be due to seasonal sampling of the cases. Hospitalized patients with diarrhoea were not included. There was also no control group enrolled in the study.</td>
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<td>the more prevalent organisms. Stratified random sampling procedure was used for survey. 3034 household were selected from 20 neighbourhoods that contain six types of households. Structured questionnaire was used. When a diarrhoea case was reported fresh stool was collected, processed immediately and sent to laboratory for etiological studies. Standard microbiological method was applied for bacteria isolation.</td>
<td>pathogens detected from these children were rotavirus 1.1%, adenovirus 2.7%, <em>Salmonella</em> spp. 11.2%, <em>Shigella</em> spp. 8.8%, <em>E.coli</em> 7.3%, <em>C.jejuni</em> 9.6%, <em>G.lamblia</em> 13.2%, <em>E. histolytica</em> 8.4%, <em>Cryptosporidium</em> 2.6%. <em>Salmonella</em> and <em>C. jejuni</em> were more found in 12-35 months age group and rota was more in &lt;23 month age group.</td>
<td>pathogens might have acted synergistically to cause diarrhoea.</td>
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<td>ELISA was done for rota detection. Microscopy was done for parasites. Campylobacter was identified by standard method and PCR. Nguendo Yongsi, H.B. <em>et al.</em> 2008.</td>
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<td>8. An aetiological study conducted among children &lt;12 years and admitted in the Benedictine hospital during April 1997 to June 1998. Approximately 4% of the total population in Nongoma district in the northern KwaZulu Natal suffers from</td>
<td>113 cases were detected during the study period. This constituted 41% of the total children tested. 50% of the diarrhoea cases were in children below 2 years. 37% were between the ages of 5-12 years. Common organisms found were EPEC 7.1%,</td>
<td>A wide range of enteric pathogens were responsible for childhood diarrhoea in this population. Antibiotic resistance pattern was done. Most of the pathogens were resistant to conventional and common antibiotics.</td>
<td>In this hospital based study no control was enrolled. Main focus was antimicrobial resistance. Age range was wide. A method of isolation was not described.</td>
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<td>diarrhoea. No statistics on aetiology were available. Lin, J. et al. 2002.</td>
<td><em>Shigella</em> spp. 19.5% (predominantly <em>S. flexnerii</em> 45.5%), <em>Salmonella</em> 2.8%, Parasites 11.5% (including <em>E. histolytica</em>, <em>G. lamblia</em>, <em>T. hominis</em>, <em>Ascaris lumbricoides</em>). EPEC was common in children &lt; 2 years.</td>
<td>There was higher rate of isolation of pathogens in cases compared to the controls. Rota was the most common cause of diarrhoea. Majority of the rota isolation was from children &lt;24 months. <em>E. coli</em> and <em>Salmonella</em> were common.</td>
<td>Definition of acute diarrhoea was not given. Controls were also selected from the hospital and the numbers of controls were less than the cases. Study did not look for viruses other than rota and did not use PCR for confirmation. <em>E. coli</em> and</td>
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<td>80% of the hospital admissions from December to April are due to diarrhoea compared to about 10% in other months. Children aged 0-43 months with acute diarrhoea and hospitalized in the three major hospitals were enrolled. Diagnosis of acute diarrhoea was established by paediatrician. 150 children with diarrhoea and 50 age, sex and area matched controls without diarrhoea were studied. Fresh stool samples were collected from both cases and controls</td>
<td>rates were rotavirus 23.35 vs. 0%, E.coli 15.3% vs. 4.0%, <em>Salmonella</em> spp. 11.3% vs. 2.0%, <em>Shigella</em> spp. 3.3% vs. 0%, <em>C.jejuni</em> 2.7% vs. 0%, <em>G.lamblia</em> 2.7% vs. 0%, <em>E.histolytica</em> 3.3% vs. 0% among cases and controls respectively. Higher frequency of isolation was reported from infants.</td>
<td>bacterial pathogens detected. Parasite isolation rate was low.</td>
<td>their type.</td>
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<td>and they were subjected to bacterial, viral and parasitic identification. Standard parasitological, bacteriological technique and ELISA (for rotavirus) were performed. Ogbu, O. <em>et al.</em> 2008.</td>
<td>103 diarrhoea cases attending the hospital were included. 206 age and sex matched healthy children attending the MCH clinic at the hospital were enrolled as controls. Among the children with diarrhoea 10.7% had signs of dehydration. Stool samples of</td>
<td>A high percentage of pathogenic yields in diarrhoea cases. Diarrhoeagenic <em>E.coli</em> was detected from both the cases and controls and there was no difference in isolation rate between them. <em>Shigella</em> was the only pathogen associated with diarrhoea. Asymptomatic</td>
<td>Controls were selected from the hospital. Study done in the peak diarrhoea season during the rains gave more bacterial isolation than viral. Also a seasonal variation could not be elicited.</td>
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Study locale, design and objective | Outcome | Conclusion | Comments
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season (March to May) in 1997 as cases of diarrhoea increase during the rain. Standard case definition was used. Children attending the Maternal and Child Health Clinics were enrolled. Children who had antibiotics in the previous two weeks were excluded. Controls were healthy children who did not have any illness during the 2 weeks preceding the enrolment. Microscopy used for parasitological studies, Standard microbiological techniques | 96.1% cases and 52.2% controls yielded a pathogen. Major pathogens detected were *Shigella* spp. 13/103 (12.6%) vs. 8/206 (3.8%), ETEC 16/103 (15.5%) vs. 25/206 (12.0%), EPEC 4/103 (3.9%) vs. 8/206 (3.4%), rotavirus 4/103 (3.9%) vs. 0/206 (0%), *G. lamblia* 15/103 (14.6%) vs. 32/206 (15.5%), *Cryptosporidium* spp. 1/103 (1.0%) vs. 0/206 (0%) in cases and control respectively. *Shigella* spp. was associated with diarrhoea (OR 2.9, 95% infection is high in this setting |
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<td>was used for bacterial isolation including <em>E. coli</em>. PCR was used for <em>E. coli</em> pathotype. Particular emphasis was given to the identification of pathogenic <em>E. coli</em>. Gascon, J. <em>et al.</em> 2000.</td>
<td>CI: 1.1-7.7</td>
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<td>11. An aetiological prevalence study to determine the prevalence of enteropathogens causing diarrhoea among children &lt;5 years old in Ifakara, Tanzania during dry and rainy season. Study site was urban Ifakara with 40,000 population. Children admitted were collected 451 children (348 during dry and 103 in the rainy season). Acute diarrhoea was present in 70.7% and 72.81% of the patients in dry and wet seasons respectively. 11/103(10.7%) and 40/348(11.5%) had severe</td>
<td>Fresh whole stool samples were collected 451 children (348 during dry and 103 in the rainy season). Acute diarrhoea was present in 70.7% and 72.81% of the patients in dry and wet seasons respectively. 11/103(10.7%) and 40/348(11.5%) had severe</td>
<td>There was higher isolation of enteric pathogens in dry season. EAEC was incriminated as a causative agent for diarrhoea in the dry season. No seasonal difference of <em>Campylobacter spp.</em> isolation. A high frequency of rota confirmed the pathogenicity of</td>
<td>It was a hospital based aetiological study and there was no control. Inclusion of <em>E. coli</em> detection caused a higher isolation rate. Only 10% of the cases were severe. Less numbers of children were enrolled in the rainy season. Study compared pathogen</td>
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<td>to the St. Francis Hospital in Ifakara, during the period between July and September 1996 (dry season) and from February to May 1997 (rainy season) were enrolled. Standard diarrhoea definition was used. Conventional method was used for bacterial culture including <em>E.coli</em>. Microscopy was done for parasites and ELISA was done for rotavirus detection. PCR was done for identification of virulence factor for <em>E.coli</em> using specific primers.</td>
<td>diarrhoea. 240/348(71.8%) in dry season and 65/103(63.1%) in the wet season yielded at least one pathogen. Comparative isolation between dry and wet season were as follows: <em>E.coli</em> 37.4% vs. 30.1%; <em>Shigella spp.</em> 24.1% vs. 12.6%; <em>E.histolytica</em> 2.9% vs. 1.9%, <em>G.lamblia</em> 1.2% vs. 14.5% and rotavirus 23.6% vs. 3.9% in dry and wet season respectively. <em>Campylobacter spp.</em> 2.6%, <em>Salmonella spp.</em> 1.4% was found only in dry season. While 1.0% <em>Cryptosporidium</em> was found only in dry season.</td>
<td>rota in dry season.</td>
<td>isolation between dry and wet season. There was an outbreak of <em>S. flexneri</em> so result of <em>Shigella</em> in dry season should be interpreted cautiously.</td>
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<td>Vargas, M. et al. 2004.\textsuperscript{111}</td>
<td>was isolated in rainy season only. Total 130 <em>E.coli</em> was isolated. Among the <em>E.coli</em>, ETEC was mostly isolated in rainy season, EAEC in dry season and EPEC equally in both dry and wet season. <em>Shigella</em> was isolated more in dry season. <em>G.lamblia</em> more in wet season (1.2% in dry season vs. 14.5% in wet season, p&lt;0.001). Rota virus was more in dry season (23.6% in dry season vs. 3.9% in wet season, p&lt;0.001).</td>
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12. A birth cohort study was conducted in peri-urban Bandim II and Belem of Guinea Bissau to identify potential enteropathogens associated with diarrhoea, age of primary infection and to estimate the relative contribution to the incidence of diarrhoea in children. A total of 603 houses were selected and 200 children born between 15 Jan 1996 to 14 Jan 1997 were recruited within three weeks of birth. Weekly monitoring was performed on 200 children were enrolled. 18 children died during the observation period. Stool sample was collected from 88% of the cohort. 10.2 % of the samples were from children with diarrhoea on the day of collection (point prevalence). Proportion was highest in 9-10 months old children. Total of 11,987 stool, sample was collected and 2350/11,987 (20.0%) were rectal swabs (RS). Overall 37.1% of the specimen grew one potential pathogen. Rotavirus was the major pathogens isolated from this cohort contributing to the burden of the disease. Heat stable (ST) toxin producing ETEC was also strongly associated with diarrhoea. Rota was the only pathogen that conferred protection against subsequent infections. With an increase in age children got protection from overall diarrhoea but not against infection.

20% of the samples were rectal swabs that could yield better result. It is not clear whether more of RS was collected from non-diarrhoea children or not as the isolation rate was higher amongst them. It is not clear from the study about the diarrhoea free period considered and it is possible that a sample was collected from the same patient in short intervals (symptoms of diarrhoea were considered when the patient did not have...
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<td>done for 24 months and stool was collected from both children having and not having diarrhoea. Mothers reporting of diarrhoea were considered and if a microorganism was isolated the child was considered infected. Fresh stool sample was collected and if it was not available, a rectal swab was taken. Standard microbiological methods were used. PCR was done to confirm virulence factors of <em>E.coli</em>. Enteric parasites were</td>
<td>A pathogen was isolated from 56% of the children who had diarrhoea and 58% from children who did not. Pathogens associated with diarrhoea were rotavirus (OR 5.8, 95%CI: 3.8-8.9), ST ETEC (OR 1.9, 95% CI: 1.3-2.7) and <em>Shigella</em> spp. (OR 1.9, 95% CI:0.9-4.2), <em>C. parvum</em> (OR 2.1, 95%CI:1.2-3.8), <em>E.histolytica</em> (OR 1.3, 95% CI:0.1-15.1)</td>
<td></td>
<td>diarrhoea. However, stool was collected from them). Diarrhoea definition was not clear. Population attributable fraction is probably is an underestimate in an endemic setting as it could explain the pathogens in in less than 10% of the population only. Possibility of other factors in causing a disease cannot be ruled out.</td>
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<td>identified through microscopy using standard procedures. Valentiner-Branth, P. et al. 2003.</td>
<td>attributable risk but could explain only less than 10% of the population.</td>
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<td>13. An aetiological study was conducted to investigate enteric bacterial infection among under five children in Zambia. Stool samples were collected from children attending the Diarrhoea Training Unit of Zambia University Teaching Hospital in Lusaka, Zambia Between May 1992 and May 1993.</td>
<td>Total 639 cases were evaluated (220 children with dysentery and 419 with non-dysentery) for presence of bacterial infection. Major pathogens isolated were E.coli (14.9%), Shigella spp.(10.2%) and V.cholerae (3.3%), Salmonella spp. 1.4%</td>
<td>Shigella was the most common bacteria causing diarrhoea. Visible blood in stool was important in diagnosing Shigella.</td>
<td>There was no control and the study looked for aetiology in diarrhoea patients attending the hospital. Study looked for bacterial pathogens only and did not use PCR for E.coli confirmation.</td>
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<td>Standard definition of diarrhoea was used. Standard microbiological techniques were used. However, PCR was not done for virulence gene detection of the <em>E.coli</em>. Nakano, T. <em>et al</em>. 1998.</td>
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<td>14. Hospital based study to determine the spectrum of bacterial enteropathogens causing childhood diarrhoea in municipal and peri-urban area of Abuja, Nigeria. Under- five children with diarrhoea attending (July- December 2008) five hospitals in Abuja</td>
<td>404 children were enrolled in the study. Majority of the pathogens were detected from 7-24 months of age with the peak age being 2-12 month. 17.9% were from the admitted cases. A bacterial was recovered from 277/404 (68.6%) of the samples. Among</td>
<td>The study identified at least 8 bacterial species from the stool. Relatively high isolation rate from the children with diarrhoea attending a hospital. There was no variation of isolation from the municipal area and satellite towns. <em>E.coli</em> was associated with diarrhoea</td>
<td>Exclusion of children with blood and mucous in their stool possibly led to a lower isolation rate of bacteria specifically <em>Shigella spp</em>. PCR not done for <em>E.coli</em> virulence detection. Data were not presented for rota and other parasites even though tests</td>
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<td>Children with blood and mucous in their faeces were excluded from the study. Whole stool was collected from these children. Microscopy was done for parasites, helminths and ova. ELISA was done for rotavirus detection. Standard culture and biochemical test was done for bacterial isolation. Cajetan, I.C.I et al. 2009. 114</td>
<td>the isolates <em>E.coli</em> 43.1%, <em>Salmonella Typhi</em> 2.2%, <em>Proteus spp.</em> 8.4%. <em>E.coli</em> isolation was high in 23-24 month age group. More in &lt; 3 years of age</td>
<td>in children &lt;3 years of age.</td>
<td>were performed. There was no control group.</td>
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<td>15. This Facility based study was designed to describe the aetiology of diarrhoea in</td>
<td>During the study period 4590 under five children were admitted, 1029 were diagnosed</td>
<td>A pathogen was isolated from 42.2% which is lower than studies carried out in other</td>
<td>Half of the children with diarrhoea and attending the hospital did not provide stool,</td>
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<td>hospital admitted patients in southern Mozambique. Study patients were recruited from a referral hospital at Manhica District. It has a population of 130,000. Mainly a rural area under DSS. A hospital surveillance process is in place for age group of under 15 years old. Study was conducted between Sept 2000 and Sept 2001. Fresh stool sample was collected from every one of two hospitalized children younger than five years of age and</td>
<td>with diarrhoea and stool sample was collected from 529 (51.0%) of them. 54% of the children were &lt; 1 year of age. 223/529 (42.2%) were positive for at least one pathogen. Bacteria were isolated from 27.2%, parasites from 14.4% and viruses from 0.6% of cases. Helminths and EPEC were more common in children &lt; 1 year age. Rota virus was isolated from only 3 children in &lt; 24 months age group. Incidence of diarrhoea was higher in rainy season then dry</td>
<td>African countries. Diarroheogenic <em>E. coli</em> was most frequently found pathogen in these children and its role was established. Rota virus isolation was low. Parasite isolation was similar to other studies. No seasonal variation. There is age dependency in pathogen isolation. Helminths were more in older age group because of the soil contact. <em>E. coli</em> was more frequent in &lt; 1 year of age.</td>
<td>risking selection bias. It is unclear why rotavirus isolation was so low in this study. Antibiotics were given to some patients before culture, which could lower the isolation rate. A delay in processing of the samples (especially for the ones collected at night) also may have influenced the yield. Study also did not include any controls.</td>
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presenting with diarrhoea. Standard definition of diarrhoea was used. Mandomando, I.M. et al. 2007.115

season. But when all pathogens were taken into account there was no seasonal variation. Major pathogens detected were E.coli 22.6%, *Salmonella* spp. 2.5%, *G.lamblia* 2.5%, *Campylobacter* spp. 1.7%, rotavirus 0.6%. Lactose fermenting bacteria was isolated from 404(76%) samples, *E.coli* was confirmed from 335 (83%) and 120 (35.8%) were pathogenic. Pathogenic *E.coli* was EAEC (15.2%), ETEC (10.7%), and EPEC (6.9%).
Summary

Fifteen studies that met the inclusion criteria were included in the review. Six were case control studies. One of these enrolled both cases and controls from the community and relied on the caregivers’ definitions of diarrhoea. In the absence of standard definition of diarrhoea, caregivers’ responses regarding a diarrhoeal episode, especially during a routine household visit is not reliable. Identification of pathogens from stool samples from those children may be wrongly attributed to diarrhoea. For other case control studies controls that came for routine immunization could be considered as healthy. Controls that attended the health centre for other illnesses than diarrhoea may not have been representative of the population from which the cases were drawn. Comorbidities are common in children with diarrhoea. It is possible that diarrhoea was downgraded as a presenting illness in some children with co-morbidity. The studies did not clarify how children with comorbid conditions were regarded with respect to selection as cases or controls. Only three studies had enrolled one or more control per case; no good reason was given for selection of less controls in the other studies. The other studies, which did not enrol controls, failed to establish relative pathogenicity and asymptomatic infection in the children. Less than half of the studies enrolled children between 0-59 months of age. One study followed a birth cohort for until two years of age and of the prominence of rotavirus was expected. Some studies were restricted to the isolation of bacteria virus or
parasite only. Few enrolled children and analysed their stool samples over at least one year to document seasonality. Two of the studies did not mention the time period and others were conducted either for a short period or sampling was not evenly spread across all the seasons.

Aetiological studies used conventional and standard microbiological and molecular techniques. Diarrhoeagenic *E. coli* are important enteropathogens causing diarrhoea in under five children and molecular techniques using PCR and specific primers are necessary to detect them from stool samples. Some studies reported isolation of *E. coli* but did not use PCR for detection of virulent pathotypes.

Only two of the case control studies evaluated associations the rest were descriptive in nature. The Cohort study presented outcome of a multivariate analysis.

2.6 Systematic review of nutritional status and outcomes in children with diarrhoea in sub-Saharan Africa

The most important and visible nutritional risk factor for diarrhoea is growth faltering assessed through anthropometric indices. Other risk factors include micronutrient status and feeding behaviour. It is estimated that nearly one third of growth faltering in children is due to enteric infection. Nutritional consequences among children with diarrhoea are amplified by seasonal variations and food supply. There is some evidence that early childhood diarrhoea reduces linear
growth up to the age of six years and beyond but may be mitigated by nutritional supplementation. A multi-country meta-analysis showed that the risk of stunting is high among children with diarrhoea and that height tends not to ‘catch up’.

**Inclusion criteria**

The reporting period in any peer reviewed journal was 1990-2010. Both community and hospital based studies that aimed to assess any one of the anthropometric indicators of height, weight, mid upper arm circumference and the age based Z score were included. Studies that assessed the changes of these nutritional indicators due to diarrhoea were included. Studies where diarrhoea was a co-morbid condition were excluded.

**Results**

Initial search and the cross references from that search returned 41 full text articles. Three duplicates were removed from the initial search, 25 of the articles provided information on malnutrition that not only related to diarrhoea but also on other co-morbid conditions. Two studies did not mention Z score but reported severity only, another two did not report the age (reported of all paediatric children with diarrhoea) and did not elaborate on the follow-up and Z score. Summary results were from 9 selected studies.
Table 2-3. Summary of studies on effect of diarrhoea on growth (as assessed by anthropometric indices) conducted in different countries of sub-Saharan Africa from 1990-2010.

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<td>1. A retrospective case control study explored the relationship between admission weight and different diseases and their outcome in relation to the weight of the under five children. Children admitted to urban RVTH (633 children from 1993-95) and rural Sibanor health centre (188)</td>
<td>Data from 13579 children admitted with different diseases in both the hospitals were analysed. 633 from RVTH and 188 from Sibanor were enrolled due to acute diarrhoea. Mean admission weight for diarrhoea cases were lower than the controls. Mean WAZ for cases from RVTH was -2.5 and that from</td>
<td>There is overall low WAZ for cases and controls compared to NCHS standard. Cases were more affected than the controls. Even after taking dehydration (5-10%) reduction into account the cases were more malnourished than the controls. 106/813 (13.0%) children with acute diarrhoea malnutrition died.</td>
<td>Timing of measurement for hospital children and community children were different. Admission weights were obtained from the hospital record. No standardization was done. There is a typical hunger season in Gambia that could potentially influence the anthropometric indices.</td>
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### Study locale, design and objective

Children from 1994-95 were enrolled in the study. Control children were from household survey conducted during 1993-1995, Rotavirus vaccine trial in 1997 and children coming to health centre for vaccination throughout Gambia. Measurements from 7399 children were available. Children with acute diarrhoea <14 days of duration were included. Severity was not assessed. Weight for Age Z (WAZ) score was calculated using NCHS standards.

### Outcome

| Sibanoor was -2.1. Difference in WAZ between cases from RVTH and controls were 1.7 and the difference between cases from Sibanoor and controls was 1.2. These differences were significant (p<0.001). During the first five months nutritional status was comparable/better than the NCHS. It started falling afterwards from 0.04 at five months to -1.47 at 12 months. Thereafter the Z score remained constant at around -1.3. |

### Conclusion

### Comments

Definition of acute diarrhoea was not clear and it was not evident whether persistent diarrhoea cases were excluded or not. There was also possibility that malnutrition and not diarrhoea could be a potential determinant for hospitalization.
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<td>Comparison of mean and difference in WAZ score between cases and controls were done. Man, W. D. et al. 1998.</td>
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<td>2. Prospective community based study conducted between April 1987 and April 1990 to assess the interaction between nutritional status and cryptosporidiosis in a cohort of children &lt; 3 y of age in Guinea Bissau. Children born after June 1, 1994 and residing in 301 houses selected randomly were eligible. Weekly visit was</td>
<td>1064 children were followed for 1441 child years. 102 children died and 310 moved out of the area. Total 5072 weight and 4264 height measures were taken for these children. Pooled WAZ showed 0.3 at 2 month, -1.6 at 12 month, -1.6 at 24 month and -1.2 at 36 month. 236 children had cryptosporidiosis.</td>
<td>Pre-infectious nutritional status was not associated with cryptosporidiosis and duration of diarrhoea. Modelling to see this association removed the random effect of confounding of socio economic status and maternal factors. Crypto infection had a marked and lasting effect on linear growth and weight. Since it affected</td>
<td>Looked at the association of malnutrition with Cryptosporidium induced diarrhoea only. There was no control group. Diarrhoea information was obtained from mothers and it was a standard definition. Children only up to 3 years of age were included.</td>
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Diarrhoea was ascertained from the mothers and stool was collected from such children. A new episode was separated with a disease-free period of 2 or more days. Anthropometric measurements were taken every three months (both weight and height). Z score for the NCHS standard was used for assessment of the nutritional status.

Molbak, K. et al. 1997.124

3. A longitudinal prospective study included 209 children. Duration of diarrhoea was investigated. Mothers reporting diarrhoea...
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<td>A study in children aged 6-18 months in rural Malawi was conducted to determine the relationship between symptoms of acute infectious disease and growth. The outcome measures were HAZ, WAZ and MUACZ. Mothers’ report of diarrhoea, fever and cough were considered. Participants were from rural farming community. All the children were breast fed ad lib and were assigned to one of two interventional complementary foods.</td>
<td>the study. Duration of diarrhoea was significantly more in 6-12 months old than 12-18 months old (15 days vs. 7, p&lt;0.001). HAZ score decreased by approximately 0.5 during a 12 month observation period. Duration of diarrhoea affected HAZ, MUACZ and WAZ. Fever was associated with low MUACZ and WAZ. Cough was associated with lower WAZ only. Gender, socio economic status, food supplementations were not associated with</td>
<td>associated with reduction in growth parameters. Even with supplementation growth faltering occurred, signifying the role of infection and need for control of infection in maintaining nutritional status in children.</td>
<td>were considered, with the possibility of recall bias. Standard diarrhoea definition was used but severity was not assessed. There was no control to see the effect of supplementation on normal growth.</td>
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<td>Children were followed fortnightly. Weight and MUAC were measured monthly and height three monthly until age 18 months. Children followed for at least 280 days were analysed. Z score was calculated using WHO standard. Linear mixed model was used to determine the effect of reported symptoms on growth. Weisz, A. et al. 2011.\textsuperscript{125}</td>
<td>reduction of growth parameters.</td>
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<td>4. A clinic based surveillance of childhood infection and malnutrition. From 1979 to 1993</td>
<td>Overall 1190 children aged 0-2 years are analysed for the study. These children made</td>
<td>Diarrhoea incidence declined impressively and also the duration of diarrhoea declined</td>
<td>There was no non diarrhoea control to address whether there was generally poor</td>
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<td>children below two years attending the clinic at Keneba, Gambia from the neighbouring villages were screened for diarrhoea. It was a complete documentation of morbidity recorded in the health centre for this population. These prospectively collected data were used to see the interaction between diarrhoea and early childhood growth in Gambian children. All the children under two years of age were screened. Their diagnoses and treatment were</td>
<td>52,577 attendances. Among them 9492 were due to diarrhoea. The distribution of diarrhoea showed similar pattern in all the years. A consistent peak level was observed in rainy season and with a small peak early mid-dry season. The overall incidence of diarrhoea declined from 30.2% in 1979 to 7.6% in 1993. However, there was no improvement of WAZ score over the years. At one year mean WAZ was -1.8 both at 1979 and 1992. At two year</td>
<td>but the growth faltering continued. Thus the idea that reducing diarrhoea incidence can reduce growth faltering was not a tenable argument. Dietary intake could be a major determinant for growth acceleration.</td>
<td>nutritional status in the community. All severe infectious diseases in the community were not reported at the health centre to compare with the data suggesting that diarrhoea cases were declining.</td>
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<td>recorded. All new born were invited into the call clinic for immunization. Weight and heights were taken and compared to international standards for calculating Z score. Poskiitt, E.M.E. et al. 1999.</td>
<td>it was -2.0 in 1979 and -1.9 in 1993. For height mean HAZ at year one was -1.3 at 1979 and -1.7 in 1993. At year two it was -2.0 in 1979 and -2.1 in 1993.</td>
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<td>5. Prospective cross sectional study to determine aetiology of acute diarrhoea and their relation to nutritional status in under-five children in southern Ghana. Study enrolled all the under five children with diarrhoea</td>
<td>Total of 287 children &lt; 5 years of age were recruited. 13 excluded for insufficient data. 170 had diarrhoea and 104 no diarrhoea. Acute diarrhoea was diagnosed in 85.3% and persistent diarrhoea in 7.6% for other 7.1% duration was</td>
<td>Severe growth faltering occurred in both the diarrhoea and non-diarrhoea children.14% of the total children studied were severely malnourished and 73.7% of them had diarrhoea suggesting a link between diarrhoea and</td>
<td>Both the cases and controls were selected from the clinic and no follow up was done after the initial recruitment. Severity was not assessed. Both acute and persistent diarrhoea were included</td>
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<td>attending the Princess Marie Loiuse Childrens Hospital (PML), Accra Ghana between August 2007 and May 2008. A control group of children were recruited from the outpatient department but without diarrhoea and visiting routine child welfare care. No follow up was done as a part of the study. Standard definition of diarrhoea was used. Weight and height was measured. Z score was calculated based on the international standard reference.</td>
<td>not recorded. Weight measurements were taken from 269 children. 55.4% of the diarrhoea children and 40.6% of the non-diarrhoea children were malnourished (mild to severe, OR 1.8, 95%CI: 1.1-2.9; p=0.023) by WAZ. Height was measured for 170 children. 44.2% of the diarrhoea children and 27.4% of the non-diarrhoea children were stunted (OR 2.1, 95%CI: 1.1-4.0; p=0.026). For 161 children both height and weight were taken. 29.3% of children with</td>
<td>malnutrition.</td>
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<td>Opintan, J.A. et al. 2010.</td>
<td>diarrhoea and 16.5% without diarrhoea showed moderate to severe wasting(WHZ below -2, OR 2.1, 95% CI: 1.0-4.5; p=0.062)</td>
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<td>6. A birth cohort was prospectively followed to investigate the growth and development of severe stunting and identify factors that are independent predictors during infancy. Study was conducted in southern Malawi in a 17000 population residing in 4200 of the 729 new-borns 89 died and 27 were lost to follow up. Total 613 infants were included in the study. At the end of the follow up anthropometric data were available for 597 infants. Both WAZ and HAZ at birth were below the normal in this population and this trend</td>
<td>Moderate to severe malnutrition was a major burden in Lungwena. There was a need to improve overall nutrition than treating acute conditions. Infections were important contributors to malnutrition.</td>
<td>Followed a birth cohort for 24 months. Regular home visit may have potentiated mothers to take more care of their children. HIV infection status and their influence on growth were not assessed.</td>
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<td>households in three villages.</td>
<td>continued throughout the infancy. At one year median. WAZ was -1.7 and HAZ was -2.6. At 3 month WAZ&lt;-2 was 2% but at 12 month this was 40% and for HAZ it was 27% at 3 month and 71% at 12 month. Boys were more frequently more stunted specially after 6 month of age. Severe stunting was associated with short maternal stature, poor gestational weight gain, and pre-term birth. Total number of any illnesses and diarrhoea were also associated</td>
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<td>Study subjects were single children from 795 women recruited between June 1995 and September 1996 in a child survival study. This comprised of 95% of the total new borns in the area. For the first 12 months every child was visited monthly Anthropometric measurements were taken soon after birth and every month during the home visit. Anthropometric indices were calculated using NCHS reference. Age adjusted Z</td>
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<td>scores were used in all analyses. Espo, M. et al. 2002.</td>
<td>with severe stunting.</td>
<td>Malnutrition is common in the hospitalized children and persistent diarrhoea had worse nutritional indices than those who had acute diarrhoea.</td>
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<td>7. Facility based study described the characteristics of children with persistent diarrhoea and compared them with acute diarrhoea. From July 1993 to April 1994, children aged 6-60 months having acute diarrhoea were recruited at the outpatient of University College Hospital, Ibadan. They were followed for 15 days. Weight, height and MUAC of the children</td>
<td>307 children were enrolled during the study period, 36911 (7%) of them developed persistent diarrhoea and 271 (88.3%) had acute diarrhoea. Half of the children were in 6-12 month of age group. 43% of the children with persistent diarrhoea and 59% with acute diarrhoea were classified as normal according to Welcome classification of nutrition. Under weight (WAZ&lt;-2) was</td>
<td>Hospital based study and there was no healthy control. Severity of acute diarrhoea was not assessed. Pre-existing conditions not known so it will be difficult to presume that malnourished children get more of one form of diarrhoea than the other. Did not look at the after effect of diarrhoea.</td>
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<td>were taken. Z score was calculated for the NCHS reference population. Sodeinde, O. et al. 1997.129</td>
<td>common in the persistent diarrhoea group. Mean WHZ and WAZ for persistent diarrhoea group was significantly lower than those in acute diarrhoea group (p&lt;0.03).</td>
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<td>8. This cross sectional Study took place during a rainy season (July-August 2002, when malaria is high and food shortage is evident) of 2002 to determine the prevalence of underweight, stunting and wasting among under five children and ascertain the</td>
<td>175 children were included in the survey. Mean age in the survey was 27.6 (range 9-60) months. There was no difference between the boys and girls. Heights were obtained from 157 children. Children were below the ref population (both for WAZ and</td>
<td>There is high prevalence of malnutrition in this community. The findings are similar to elsewhere in Kenya. Children at second year of life are more affected by malnutrition.</td>
<td>This was cross sectional community study. Conducted for short time and in a particular time of the year when food shortage is evident. This could overestimate the malnutrition in the community. Diarrhoea definition and severity was</td>
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<td>predictors of childhood under nutrition. Children were from three villages in Siaya district of Western Kenya. They were from the Luo ethnic group, the third largest in Kenya (13% of the national population). All households in the villages were surveyed and children &lt;5 years were enrolled. Mothers were interviewed. 89% of the eligible children in the villages were enrolled. Height and weight was taken following CDC manual. Z score was calculated based on NCHS HAZ). Greatest deficits appearing in 23-24 months (60% stunted, 46% underweight and 10% wasted). Overall 30% of the children were underweight, 47% were stunted, and 7% were wasted. Children of mothers who had no formal education were more malnourished (OR 1.64). Weaning at six months was also associated with malnutrition (OR 2.28). 79% of the children had diarrhoea. Most of the illnesses were associated with malnutrition</td>
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<td>uncertain. Recall bias in reporting illness could not be ruled out. Study also did not include all the eligible children creating possible selection bias.</td>
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<td>reference data. Extreme values (&lt;-5/&gt;+5) of Z score were removed. Predictors including household composition, health behaviours, illness and agricultural practices were assessed both by bivariate and multivariate models. Bloss, E. <em>et al.</em> 2004.</td>
<td>(diarrhoea OR 3.19, URI OR 3.1). Overall 35.9% of the children who had diarrhoea with in the last 30 days were underweight (OR 3.2, 95% CI: 1.3-7.8) and 49.5% were stunted (OR 1.3, 95% CI: 0.7-2.7).</td>
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<td>9. A retrospective case control study to identify risk factors for fatal diarrhoea was conducted in an urban town hospital located at Yirga Alem town of southern Ethiopia. The hospital caters 21 non-bloody fatal acute diarrhoea cases were eligible for analysis. For every case 4 controls (total 84 controls) were selected. Mean age of the cases was 22.8 month and for the controls it was 12.6</td>
<td>21 non-bloody fatal acute diarrhoea cases were eligible for analysis. For every case 4 controls (total 84 controls) were selected. Mean age of the cases was 22.8 month and for the controls it was 12.6</td>
<td>Malnutrition is prevalent in the catchment area. Such children are at higher risk of getting infectious disease. Nutrition is an important risk factor for fatal outcome in diarrhoea.</td>
<td>Hospital based retrospective chart review. Controls were not properly matched. Accuracy of measurement and reliability of patient information form a chart review is a major concern in</td>
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<td>55,000 outpatients and 5000 outpatients in the hospital annually. Children represent 14.4% of the patients and 22.6% of them report with diarrhoea. Children coming to hospital between September 1985 and August 1987 were enrolled. Patient information including height and weight was obtained through chart review. Severity of diarrhoea was assessed. NCHS standard was the base for calculating nutritional indices. Lindjorn, B. <em>et al.</em> 1991.</td>
<td>months. Fever was more frequent in cases. Cases had lower HAZ, WAZ and WHZ than the controls. Cases had stunting and wasting in 55.6% and controls had stunting in 13.1% and wasting in 25.0%.</td>
<td>the study.</td>
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Summary

Nine studies were identified for the review that captured the anthropometric indices during an episode of diarrhoea. All except one study measured weight, height and calculated WAZ and HAZ scores for the enrolled children. Three of the studies were of case control design. Cases for these studies were from health facilities. The study in The Gambia had controls from the community. However, the cases and controls were not recruited at the same time. The Ethiopian study concluded that diarrhoea in a malnourished child can increase fatality compared to children who did not have diarrhoea. The Gambian and Ethiopian studies collected data from hospital records and from other surveys. It was not possible to ascertain the accuracy of anthropometric measurement from a retrospective data analysis. The prospective case control study in Ghana enrolled both cases and controls from the Children’s Hospital at Accra and the cases included both persistent and acute diarrhoea. For other studies, the objective was to establish the relation between an acute infection and their pre-existing nutritional condition. Excepting the birth cohort of Malawi, none of the studies followed the children to see the effect of an acute episode of diarrhoea on nutritional status. Other than hospital based studies in The Gambia and Ghana, it was not clear whether a standard definition of diarrhoea was used. In most of the studies diarrhoea was ascertained from the primary caregivers reporting, leading to a possible misclassification. Dehydration, in
severe forms of diarrhoea causes weight loss of 5-10% and only one study took note of this and adjusted the weight for calculation of the Z score for those children.\textsuperscript{123} The review of studies revealed that there is considerable and significant deceleration of growth in children with diarrhoea, especially due to certain pathogens like Cryptosporidium spp. \textsuperscript{124, 127} Growth can also be affected by the duration of diarrhoea.\textsuperscript{125} Long term surveillance among children showed a decline in overall diarrhoea episodes in a particular age group but the proportion of children stunted or wasted continued to increase, thus failing to establish a causal relation between malnutrition and diarrhoea only.\textsuperscript{126} Follow-up of the birth cohorts for just one year and community based studies with no follow-up is probably inadequate to show any tangible association between diarrhoea and malnutrition.\textsuperscript{128, 130} The reviewed studies provided an insight into the relation between diarrhoea and growth in young children. However studies with no follow-up, those with a single measurement of growth, and lacking background morbidity, should be interpreted with caution.\textsuperscript{127, 129, 130}

2.7 Overall summary

From the literature review of the studies conducted from 1990-2010, it was observed that published knowledge, attitude and practice surveys were unable to give a composite picture of the understanding of diarrhoeal disease from caregivers’ perspectives in sub-Saharan Africa. Some of the studies were hospital
based and interviews were conducted among the caregivers of children who had a diarrhoeal episode. The caregivers in such situations likely overemphasized the need to treat and use of different measures. It is not possible to understand the general perspective of the disease in a community from hospital based surveys. In most instances, community based surveys were focused either on knowledge, diarrhoea management, food withholding practice, or beliefs about causes and traditional measures. However, none of the studies used a single questionnaire to interrelate all these together and understand the disease as a whole. Care seeking practice is dependent of the caregivers’ recognition of the symptoms and signs of dehydration. Only a few among these studies aimed to assess caregivers’ ability to recognize these symptoms and signs.

The introduction of newer techniques has enabled the researchers to better diagnose the known pathogen and also detecting the emerging new enteric pathogen in the last few decades. It is now possible to identify the array of pathogens responsible for diarrhoeal disease. Several identified studies involved children of very limited age range, leading to a focus on pathogens that are only prominent in that age group (e.g., rotavirus isolation rate tends to be high in studies conducted on children 0-24 months of age). Some enteric infections are seasonal, and studies conducted for a short period or at a particular time of the year may not reflect year-long epidemiology. Hospital based case control studies often fail to include a community control from where the case arose.
Under nutrition is a risk factor for diarrhoea. There is evidence that repeated diarrhoea episodes interfere with both developmental and cognitive function, and also linear growth, although relevant studies have some limitations. It is important to follow children upon returning to their home environment after an acute episode of diarrhoea and to assess growth longitudinally over the next 24 months or so at least.
Chapter 3- Methods

LOOK OUT FOR STRATEGIES
3.1 Introduction

In this chapter the administrative, clinical, laboratory and field methods for the studies are described. The activities and a brief description of the study site are also given to understand the socio-demographic characteristics of the population.

3.2 Major activities planned

1. Study initiation activities.

2. Setting up of a Demographic Surveillance System (DSS) in the study area.

3. Assess health care utilization patterns in the defined population.

4. Perform a case control study to identify the aetiology, risk factors for moderate to severe diarrhoea (MSD) that requires care seeking at the health care centres located in the DSS.

5. Follow-up of the case and control cohorts to quantify the sequelae of moderate to severe diarrhoea.

3.3 Study Site

As a part of the diarrhoeal disease consortium, this study was undertaken under the auspices of the Medical Research Council in the eastern part of The Gambia.
The Gambia is one of the poorest countries of sub-Saharan Africa; the per capita income is USD 440, far below the average of sub-Saharan Africa of USD 1165. The economy is agriculture based, as the majority of the population are subsistence farmers. In addition to farming, they are involved in small trades such as selling a variety of household items, fruits and vegetables, working as artisans, and keeping livestock. The country has a population of ~1.6 million; 17% are under five years old. Malaria is endemic in the country, the HIV prevalence is low (<2%) and immunization coverage under the expanded programme of immunization (EPI) is high (over 95%). The under-five mortality is 80.7/1000 and currently The Gambia is ranked 37th from the bottom out of 192 countries in terms of under-five mortality. MRC, The Gambia Unit, is the largest research unit of MRC (UK), outside the United Kingdom and has been operating in The Gambia for the last 60 years. The unit has state of art laboratory facilities that are designated as a reference laboratory for the World Health Organization (WHO) in West Africa.

The URR has one major town, Basse, (geographical coordinates at 13°18′35”N, 14°13′47”W) where the MRC has an established field site and where the large Pneumococcal Conjugate Vaccine trial was conducted in early 2000. The study involved the population on the south bank of the river Gambia that divides the country distinctively into north and south (Figure 3-1).
The temperatures in the region vary between 15°C and >40°C. There are two distinct seasons: the dry season from November to April and the wet season (mean annual rainfall 876 mm) from May to October. There are three predominant ethnic groups: Mandinka, Fula and Sarahulleh. Most of the villages are under the public water supply system where the ground water is pumped from a bore hole to a centrally located overhead reservoir. The water gets treated with chlorination at the reservoir and is then distributed through the pipes to different locations in the villages. It is noteworthy that there is no existing quality control on this treatment. But the people rely on bore holes, deep tube and open wells due to the unreliable nature of the water supply. Collection of rainwater and use of surface water are very rare in this population.
3.4 Basse Health and Demographic Surveillance System (BHDSS)

An updated population census was conducted at the beginning (March-June 2007) and during the study period (2008 to 2010), to provide an accurate enumeration of the under-five population that was required for the incidence calculations. According to the last census conducted in 2003, the population in the south bank of URR was approximately 130,000 in an area of 1084 square kilometres with 223 villages. The area was divided into 20 smaller hamlets with an approximate population of 6500 in each hamlet. Two field workers who had prior experience in enumeration (a similar exercise was undertaken during the PCV trial), made door to door visits in each of the hamlets and counted the number of inhabitants in each household. The population of 136,793 (21,445 under five children) counted, formed the baseline study population. From July 2007 this census was converted into a live DSS with 4 monthly visits to each household to collect data on births, deaths, in and out migration, pregnancies and marriages. The BHDSS also conducts verbal autopsies using a standardized questionnaire. We also appointed a village reporter for each village to record any births and deaths on a weekly basis. The reporters are usually local elderly people, religious leaders or village chiefs who are traditionally involved in ceremonies of death and birth in the community. The BHDSS has one major health centre at Basse and five other health posts. They are mainly run by trained
nurses while the major health centre is partly supported by the expatriate physicians. The health centres are expected to provide service adhering to the government policy and following integrated management of childhood illness (IMCI) guidelines. The referral hospital is located 65 kilometres from the major health centre. The farthest health post from the referral hospital is about 100 km, while the nearest is 35 km. A few villages have pharmacies, which are mostly operated by a medically untrained person. There is no public transport system and privately owned transport lacks reliability in its service. The most common mode of transport is donkey cart.

3.5 Health-care Utilization and Attitude Survey (HUAS)

A Health Care Utilization and Attitudes Survey (HUAS) was conducted prior to the initiation of the case control study and, thereafter, an abbreviated survey was conducted coinciding with each round of DSS (designated “HUAS-lite”). Randomly selected 1140 primary caregivers of under-five children within the DSS in three different age strata (400 children in 0-11 months and 370 children each in 12-23 months and 24-59 months age group) to include at least 333 children in each age strata for the final analysis were interviewed. The purpose of starting out with a larger sample was to allow for children who are on the list but are not actually eligible because their age fell outside the age group for which the list is prepared, a child not found due to emigration from the DSS,
death of a child, and errors in the surveillance. The reason for oversampling in the 0-11 month age group was the potential difficulties locating children in this age group, e.g., as yet undetected births which occurred between DSS rounds, and higher mortality rates. Through a pre-designed questionnaire comprising 65 questions, information was solicited from the caregivers on their behaviour in seeking care when their children have diarrheal disease and their attitudes and practices concerning diarrhoea, its prevention and treatment. The questionnaire was used to confirm eligibility and obtain information on household, primary caregivers’ attitude and perception of diarrhoea and health care utilization. Both open and closed questions were asked about the perception of and attitude towards diarrhoea to understand knowledge, attitude and practice regarding the disease. This information was used to optimize the surveillance for understanding diarrhoea in the community, to guide the choice of the number of controls selected per case to maintain power of the case control study, and to calculate population-based prevalence rates. HUAS methods were adapted from the Generic protocol for a community-based survey on utilization of health care services for gastroenteritis in children under 5 years of age.¹³⁶

A trained field worker visited the household to identify the child. A household is defined as people living together and taking meals from the same cooking facility. The interviewer then described the study and its purpose and sought consent from the caregiver for their participation in the study. Written informed
consent was obtained before final enrolment. The interview was conducted if the respondent was the primary caregiver of the identified child. If a primary caregiver was not available the interviewer left a message indicating when the interviewer is likely to return. The interviewer made three attempts before considering the child as a non-responder.

For an abridged version (HUAS-lite) of the survey a questionnaire with 21 questions was administered to a random selection of 1140 children from the BHDSS population every 4 months for two years (2009-2010). Other than demographic characteristics we collected information on episodes of diarrhoea in the previous two weeks, signs of dehydration as observed by the caregivers, home management and their care seeking behaviour.

**Sample Size**

The sample size of 999 was chosen to be sufficient to estimate the proportion of children with diarrhoea who received care at a health centre. It was assumed that the diarrhoea prevalence in the community would be between 3% and 40% and about 50% -80% of those children with diarrhoea would seek care at the health centres. For this sample size, the margin of error (half width of the 95% confidence interval) will be no more than 18%.
3.6 Case Control Study

The case control study was conducted to describe the aetiology, and to identify the risk factors and consequences of diarrhoea. The cases were recruited from all the health centres within the BHDSS area. The screening of children less than five years of age, seven days a week provided the necessary information for facility based diarrhoea incidence in the population.

Definitions

Standard definitions used in the study were as follows:

**Age:** recorded in months. The primary caregivers were asked to show the infant welfare card for recording age. In the absence of such a card, an event calendar was used to estimate the age.

**Household:** a group of people who live and eat together, sharing a kitchen or cooking fire. A house or compound may contain more than one household.

**Diarrhoea:** Three or more abnormally loose stools in a 24-hour period (Standard World Health Organization definition).

**Dysentery:** Diarrhoea with visible blood in one or more stools.

**Persistent diarrhoea:** Diarrhoea lasting 14 days or longer.

**Diarrhoea episode:** Contiguous days with diarrhoea ending when diarrhoea is not present for 7 days.
Moderate-to-severe diarrhoea (MSD): We adopted the WHO definition of dehydration for the case definition of MSD in our study: diarrhoea plus any of the following:

a. Moderate to-severe dehydration, defined as the presence of one of the following: sunken eyes, more than normal and decreased skin turgor. In addition if a health care provider decided to give intravenous fluid as a measure of correction of dehydration, it was also considered as MSD.

b. Dysentery (diarrhoea with visible blood in stool according to either the caregiver or the clinician) as it is evidence of mucosal inflammation or injury.

c. Decision of the provider to hospitalize the child with diarrhoea or dysentery.

Aetiology: if a single pathogen was isolated, the episode was attributed to that agent; if multiple pathogens were identified, they were classified as multiple bacterial pathogens, multiple viral pathogens, multiple protozoan pathogens, or polymicrobial.

Pathogenicity index: The pathogenicity of an aetiological agent was defined by the ratio of isolation among cases divided by the isolation among controls for that pathogen.

Access to improved water: use of any of the following types of water supply for drinking: piped water, public tap, borehole or pump, protected well, protected spring or rainwater. Improved water sources do not include vendor-provided
waters, bottled water, tanker trucks or unprotected wells and springs, rivers or ponds.\textsuperscript{139}

**Improved sanitation facilities include:** connection to a public sewer or septic system, pour-flush latrine, simple pit latrine, or ventilated improved pit latrine. Unimproved sanitation facilities include public or shared latrine, open pit latrine, bucket latrine.\textsuperscript{139}

**Stunting:** We used the Z score for height for age to calculate stunting categories.\textsuperscript{140, 141}

- Moderate= Z score below -2 standard deviations (SDs) from the median height for age of the reference population.
- Severe= Z score below -3 standard deviations (SDs) from the median height for age of the reference population.

**Ethics**

The study protocol including the consent forms, case report forms and other supporting documents were approved by the Scientific Coordinating Committee (SCC) of MRC, The Gambia Unit and the joint ethics committee of the MRC and The Gambia Government and the institutional review board (IRB) of University of Maryland at Baltimore (UMB), USA. The study is purely an observational one and did not form any part of a clinical trial or intervention. The WHO and the existing Gambia government protocol in management of diarrhoea in all the
health centres were followed. No newer aspect of management, such as supplementation with zinc or use of low osmolar ORS was introduced. The study provided appropriate management including medicine and ORS to the subjects screened and enrolled. Routine microbiological culture reports were provided to the health care facility, from where subjects were enrolled to facilitate their treatment. There was no or very minimal risk involved in participating in the study, however a detailed informed consent was sought from the primary caregiver of the child before the child was enrolled. The parent or primary caregiver was given a copy of the consent form to read or share with confidents who are able to read and they were given ample time to understand and ask any questions. An impartial third party witnessed the consent process if the primary caregiver was illiterate.

Facilities

Basse field station of MRC, The Gambia Unit has the required infrastructure to conduct large field studies. The government health centres spread over the whole DSS area were selected for the case recruitment. Initially there were four health centres and later on during the course of the study two more health centres were established and we recruited patients from all of them. Separate screening areas for the study purposes at all the health centres were established. Appropriate management of diarrhoeal disease following standard medical
practice delivered at the local governmental facilities were provided. All children, regardless of whether they were eligible for the study, received an evaluation of their diarrheal illness and were given ORS packets free of charge. They were given prescriptions for medications and referrals for additional care as appropriate. The laboratory at Basse is equipped to conduct all microbiological assays, perform ELISA and extraction. Only molecular identification of the pathogens was done at Fajara at the coast, where the main facilities of MRC, The Gambia Unit are located.

**Staff recruitment, training and process development**

A study team was formed with a medically trained epidemiologist, a microbiologist, two clinicians, a laboratory scientific officer, a data base developer, nurses, laboratory technicians, field workers and data entry clerks. The field workers and nurses were recruited on the basis of their knowledge of at least two of the three local languages. Training was given to each staff member with respect to their responsibilities in the study that included group discussion and role playing. A decision to recruit patients round the clock seven days a week from the government health centres was made and a nurse was posted at each of the six designated health centres. However as a measure of capacity building and cooperation, all the government nurses posted in the entire south bank of URR were also trained. This was also done in anticipation that due to
any eventuality (sudden illness, emergency travel etc.) if any of our study nurses were unable to attend the clinic the government nurse could step in for them. In training, the principles of Good Clinical and Laboratory Practice and human subject research ethics were followed. The interaction between the clinical, field and laboratory team focused on the physical examination, specimen collection, transport and storage of specimens and document observation and file storage. Along with others the data team were trained to maintain the confidentiality of the study participants.

One of the major components of the study is anthropometric measurement to assess growth faltering. A two day long training session for the nurses and field workers in obtaining height, weight and mid upper arm circumference was conducted. A trained epidemiologist in anthropometry from University of Maryland assisted in conducting the training session. At the end of the session one clinician and one senior nurse was identified to supervise all the subsequent measurements in the field and standardize the procedure. They were asked to validate the measurements done by each nursing staff and field worker at least once a fortnight.

All the staff were trained as they were required to write and fill in the case report forms in a standardized English format of alphabetic and numeric data in order to reduce error rates in data recording and entry. One of the challenges was to
understand the local terminologies for the various signs and symptoms of diarrhoea and the associated features of dehydration. The people tend to use those local terms instead of the terminologies expressed in their own ethnic dialects. The training period was used to collate those terminologies and all the field workers were made aware of them. For everyday use and reference a user manual for the nurses, field workers, supervisors and data entry clerks was made available to all staff. Similar standard operating procedures (SOPs) were developed for the laboratory personnel. The training continued periodically in small groups throughout the study period.

After the completion of the training the case control study was piloted following which final case report forms (CRFs) were designed and created using Frame Maker and Microsoft Word in English. The interviewers conducted the interviews in one of three local languages, namely Mandinka, Fula and Sarahule spoken by the population. The consent forms were translated in three different languages for the perusal of the participants. The three year subject recruitment for the case control study finally started in December 2007 and continued until December 2010. The recruitment continued round the clock and seven days a week except from 4th October to 1st November 2010 due to a flood that led to the temporary closure of the field station at Basse.
Management

The case control study involved case recruitment from the government health centres and controls from the community. The regional health team was engaged before the initiation and a quarterly update was provided to them. The government nurses were involved in the training. Periodic community visits were made and the elders and leaders of the villages were met during the visit. An annual community sensitisation was done where the importance of the study was explained to the community people. The local performers also helped send the messages across through locally relevant songs and dances.

The clinical, field, laboratory and data team, led by their supervisors, met weekly for routine assessment and management of the work. The full team (except the staff on duty at the health centres) met fortnightly at the field station. Areas were identified for interdisciplinary actions, and prioritized according to need. The team at Fajara performing the molecular techniques were in regular contact with the Basse laboratory team for transport of specimens and sending results. Figure 3-2 illustrates the study flow.

Sample size

For the three year-long matched pair case control study a sample size of 600 analysable cases and an equal number of controls in each age specific stratum (0-
11, 12-23 and 24-59 months) was required. This was estimated to provide 80% power for a normal approximation test to detect a two sided significant difference (p<0.05) in isolating a pathogen causing diarrhoea when cases are compared with controls if the isolation of that pathogen was 5.8% in cases and 2.5% in controls. The sample size was based on the isolation rate of pathogens. We also allowed for 10% dropout and migration thus we planned to enrol 660 cases and an equal number of controls to achieve the desired sample size. The following table (Table 3-1) shows the sampling frame.

Table 3-1. Isolation rates that will allow 80% power to find significant differences, assuming N cases & N matched controls per stratum over 3 years (α=0.05, 2-sided)

<table>
<thead>
<tr>
<th>Pathogen-specific isolation rate in cases</th>
<th>Pathogen-specific isolation rate in controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>N = 500</td>
<td>N = 600</td>
</tr>
<tr>
<td>4.6%</td>
<td>4.2%</td>
</tr>
<tr>
<td>6.1%</td>
<td>5.8%</td>
</tr>
<tr>
<td>7.6%</td>
<td>7.2%</td>
</tr>
<tr>
<td>9.0%</td>
<td>8.5%</td>
</tr>
<tr>
<td>10.3%</td>
<td>9.8%</td>
</tr>
<tr>
<td>12.9%</td>
<td>12.4%</td>
</tr>
<tr>
<td>15.4%</td>
<td>14.8%</td>
</tr>
<tr>
<td>17.8%</td>
<td>17.2%</td>
</tr>
<tr>
<td>20.2%</td>
<td>19.5%</td>
</tr>
</tbody>
</table>

The HUAS study revealed that less than 50% of the MSD cases actually visit any of the health centres and this was also observed in the initial phase of the case
control study. The lower frequency of visits was more pronounced in the older age group and thus to keep the study adequately powered it was decided to increase the number of controls per case in a step ladder fashion. Table 3-2 shows the sampling frame for the increased case control ratio.

Table 3-2. Isolation rates that will allow 80% power to find significant differences with increased case to control ratio

<table>
<thead>
<tr>
<th>Isolation rate in cases</th>
<th>Isolation rate in controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>150 cases</td>
<td>225 cases</td>
</tr>
<tr>
<td>2 controls / case</td>
<td>3 controls / case</td>
</tr>
<tr>
<td>7.5%</td>
<td>6.8%</td>
</tr>
<tr>
<td>8.7%</td>
<td>8.0%</td>
</tr>
<tr>
<td>10.4%</td>
<td>9.7%</td>
</tr>
<tr>
<td>11.9%</td>
<td>11.3%</td>
</tr>
<tr>
<td>13.5%</td>
<td>12.8%</td>
</tr>
<tr>
<td>16.3%</td>
<td>15.6%</td>
</tr>
<tr>
<td>19.1%</td>
<td>18.3%</td>
</tr>
<tr>
<td>21.7%</td>
<td>21.0%</td>
</tr>
<tr>
<td>24.3%</td>
<td>23.5%</td>
</tr>
</tbody>
</table>

**Screening and case ascertainment**

Cases were enrolled from the health centres following the case definition and criterion set for identifying moderate to severe diarrhoea. In every health centre,
a registration log to record the total number of children younger than 60 months of age seeking medical care for any reason and the number who met the case definition of diarrhoea were maintained. The log recorded whether the child was from the catchment area or not and whether s/he belonged to the Demographic Surveillance System (DSS) population. Study personnel had access to the DSS database to confirm the status of the child to be resident of DSS.

For the children who met the definition of diarrhoea, the aims and objectives of the study were explained to the primary caregiver. A trained health care provider (in most of the cases a nurse) examined the child and recorded on a case eligibility form simple demographic data, duration of diarrhoea (in calendar days), and whether it was a new episode, administration of intravenous fluid administered, and care providers decisions regarding hospitalization. Under normal circumstances this examination would form a part of the procedure followed for any children presenting with diarrhoea in the health centre. Presence of any of these would meet the enrolment criterion (Table 3-3), subject to voluntary informed consent given by the primary caregiver and if the child had produced stool for microbiological evaluation. Recording was maintained if the child was enrolled, and if not, the reason for non-enrolment was recorded. The plan was to enrol 220 children in each age strata (0-11, 12-23 and 24-59 months) in each year making a total enrolment of 1,980 cases over a period of
three years. We also decided to limit the enrolment to 9 in each stratum/per fortnight to maintain an even sampling throughout the year.

Table 3-3. Inclusion criterion for cases and controls

<table>
<thead>
<tr>
<th>Case</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Age: 0-59 months.</td>
<td>1. Age-matched to index case (table-3.4)</td>
</tr>
<tr>
<td>2. Resident in BHDSS catchment area</td>
<td>2. Resident of the BHDSS catchment area</td>
</tr>
<tr>
<td>3. Seeking care at any of the health centre at BHDSS</td>
<td>3. Same gender as case</td>
</tr>
<tr>
<td>4. Diarrhoea as per definition</td>
<td>4. No diarrhoea with in the 7 days of enrolment</td>
</tr>
<tr>
<td>5. The onset of the diarrheal episode is within 7 days of enrolment</td>
<td>5. Concomitant : within 14 days of presentation</td>
</tr>
<tr>
<td>(new and acute) into the study, with a diarrhoea free period of ≥7</td>
<td>of the matched case</td>
</tr>
<tr>
<td>days since the last occurrence of diarrhoea</td>
<td>6. Same or nearby village or community as case</td>
</tr>
<tr>
<td>6. The diarrhoea must be “moderate-to-severe”, meaning that the child</td>
<td></td>
</tr>
<tr>
<td>must meet at least one of the following criteria:</td>
<td></td>
</tr>
<tr>
<td>a. Sunken eyes, more than normal</td>
<td></td>
</tr>
<tr>
<td>b. Loss of skin turgor</td>
<td></td>
</tr>
<tr>
<td>c. Intravenous rehydration fluid administered or prescribed</td>
<td></td>
</tr>
<tr>
<td>d. Dysentery (diarrhoea with visible</td>
<td></td>
</tr>
</tbody>
</table>
e. Hospitalized with diarrhoea or dysentery

**A case and control pair can be bilateral. If a child selected as a control subsequently develops diarrhoea at a later date, the original diarrhoea case can serve as a control for the child who was previously a control and who is now a case (inclusive design).

Demographic and epidemiological information about the primary caregiver, maternal education, household size, structure of the indwelling, household assets, and presence of domestic animals in the household, source and availability of water, method of water purification, latrine facilities, faeces disposal and hand washing was obtained. Information on breastfeeding, any other associated symptoms with diarrhoea, recognition of signs of dehydration and provision of drinks and solid food during the current episode of diarrhoea were collected. There was also interest in understanding the home management of diarrhoea and enquired about providing any oral rehydration solution and antibiotics before attending the health centre. The study also recorded both the direct (out of pocket e.g. buying medicine, paying for transport to the health centre) and indirect (income loss while attending the sick child) costs.
Clinically the care provider recorded the temperature and respiratory rate, documented the signs and symptoms of dehydration (Table 3-3), and recorded the anthropometric measurements (weight, heights twice and mid upper arm circumference twice) and obvious signs of malnutrition. The provider also recorded any prescription of rehydration fluid, antibiotics and any advice for hospitalization. If the child was admitted he/she was reassessed after four hours and again at the time of discharge recording weight and signs of dehydration. The outcome was also recorded at the time of discharge from the health centre.

Control selection

A list of six (a minimum of four) potential controls was identified by computer from the demographic surveillance database. A field worker visited the potential controls until one was identified who was eligible (Table 3-3), agreed to participate, and was able to provide an adequate (at least three grams) whole stool in a timely fashion. The age matching of the controls to the index case was as follows: ±2 months for cases 0-11 months, and ±4 months for cases 12-59 months. Thus a control for an 11 month old case had to be between the ages of 9 and 11 months and a control for a 13 month old had to be between the ages of 12 and 17 months (Table 3-4). Controls were enrolled upon obtaining written informed consent. As for the cases, reasons for non-enrolment of selected controls were also documented.
During the course of the study it was realized that it was not possible to enroll cases as anticipated and in September, 2008, the protocol was revised to increase control enrolment and maintain statistical power. During periods of slow recruitment when the required number of cases could not be enrolled, the strategy would be to enrol 2-3 controls per case. Since a matched analysis was to be performed, it was thought that confounding would not occur if the case: control ratio is altered according to high or low diarrheal incidence season. The ratio followed the following algorithm, 1:1 if 8-9 cases are enrolled, 1:2 if 4-6 cases were enrolled and 1:3 if 3 or fewer cases were enrolled.
Table 3-4. Allowable age range (in months) of controls by age of matched cases

<table>
<thead>
<tr>
<th>Matched Control</th>
<th>Matched Control</th>
<th>Matched Control</th>
<th>Matched Control</th>
<th>Matched Control</th>
<th>Matched Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case LL UL</td>
<td>Case LL UL</td>
<td>Case LL UL</td>
<td>Case LL UL</td>
<td>Case LL UL</td>
<td>Case LL UL</td>
</tr>
<tr>
<td>0 0 2 12 12 16 24 24 28 36 32 40 48 44 52</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 0 3 13 12 17 25 24 29 37 33 41 49 45 53</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 0 4 14 12 18 26 24 30 38 34 42 50 46 54</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 1 5 15 12 19 27 24 31 39 35 43 51 47 55</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 2 6 16 12 20 28 24 32 40 36 44 52 48 56</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 3 7 17 13 21 29 25 33 41 37 45 53 49 57</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 4 8 18 14 22 30 26 34 42 38 46 54 50 58</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 5 9 19 15 23 31 27 35 43 39 47 55 51 59</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 6 10 20 16 23 32 28 36 44 40 48 56 52 59</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 7 11 21 17 23 33 29 37 45 41 49 57 53 59</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 8 11 22 18 23 34 30 38 46 42 50 58 54 59</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11 9 11 23 19 23 35 31 39 47 43 51 59 55 59</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

UL=upper limit of the age for control matched to the case

LL=lower limit of the age for control matched to the case

For the analysis, it is important to determine whether the same child is enrolled in the study repeatedly as either a case or a control. All the cases and their matched controls were assigned a unique ID number which in turn was linked to the unique DSS ID of the child and was kept as a separate log file. The CRF did not contain the DSS ID number and thus from the CRF alone the child’s identity could not be established. The DSS census ID number contained the village name, census cluster number, compound number, household number.
3.7 Collection and processing of stool sample at the field and health centres

Effective communication between the clinical, field and laboratory teams was established to collect and deliver stools in a timely manner prescribed in the protocol. Both the cases and the controls’ caregivers were asked to secure at least three grams of stool for them to be enrolled in the study (For the understanding of the study team and the mothers, the stool containers were marked approximately at three gm. level and also mentioned that three gm. generally would be 3 peas). The primary caregivers were given a clean plastic container with a lid, a pair of gloves and a scoop to collect stool samples at the health centers. They were asked to scoop stool from a potty lined by a plastic sheet. The trained nurse inserted two cotton swabs in to the container dipping into the stool. It was imperative to swab the blood and mucus if it was visible. One of each swab was placed in to modified Cary Blair Medium and the other into Buffered Glycerol Saline.142, 143 Both the swabs and remaining stool in the container were placed in a polystyrene foam container or in a commercial cold box containing a frozen cold pack. The specimen was transported to the laboratory and plated within 24 hours of collection. In some cases the child was unable to pass stool in a timely manner and the medical condition warranted administration of antimicrobials. In such instances two rectal swab specimens were collected (moistening the cotton tip with the culture media mentioned above and inserting them gently into the child’s rectum, rotating 360° and
putting them back into the medium). However, all these cases needed to provide a whole stool sample within 12 hours of first antibiotic administration for final enrolment.

The stool collection from the control was arranged by the trained field workers. They usually visited the identified control children a day before the interview was performed, explained the study and the necessity for the stool collection to the primary caregiver. They left behind a plastic container, a scoop and a pair of gloves, a clean polythene sheet (asking them to try to have the child defecate on that), a polystyrene foam container with a frozen cold pack for the collection of stool samples on the following day (preferably the first stool in the morning). The field worker visited the household in the morning (it could be very early if they were called by the parents) and collected the stool specimen and processed it in the same way as described above before sending it to the laboratory for further processing. The field workers stayed in the villages and the availability of cell phones made communication easier. In some instances the primary caregiver brought the stool specimen to the nearby health center where a study nurse was posted and the field worker conducted the interview later.

Once the specimen was collected and ready for delivery to the laboratory, the laboratory personnel were informed so that it could be processed without delay. The laboratory was operational around the clock. The specimens were evaluated
for bacterial (e.g. Shigella spp., Salmonella, Aeromonas, Campylobacter, Vibriosp and E.coli), viral (rotavirus, adenovirus, sapovirus, astrovirus and norovirus), protozoal (E.histolytica, G.lamblia, Cryptosporidium spp.) pathogens using standard microbiological and molecular techniques.

3.8 Microbiological Methods

Standard and established microbiological and molecular methods in isolating and identifying the pathogens from the cases and controls were used. All the methods for isolation of pathogens from stool were piloted in both the laboratories at Basse and Fajara before the initiation of the study. A standard operating procedure (SOP) was developed to define the purpose and procedure following a set guideline. All the laboratory technicians had access to the SOP. The laboratory supervisor ensured the adherence to SOP by regular quality checks. Table 3-5 below shows the methods used in isolating the pathogens from the stool samples of cases and their matched controls.
Table 3-5. Summary laboratory method for pathogen detection and isolation

<table>
<thead>
<tr>
<th>Bacterial agents</th>
<th>Methods</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em>, <em>Salmonella</em> and <em>Shigella</em> species</td>
<td>Stool samples received in transport media (Cary-Blair – Oxoid, and Buffer Glycerol Saline - Hotline) were plated out on McConkey agar (MA) and Xylose Lysine Desoxycholate (XLD) agar media (Oxoid). The plates were incubated overnight at 37°C and then examined for lactose fermenting (LF) and non-lactose fermenting (NLF) colonies. Both the LF(s) and NLF(s) were purified and then incubated for another 24 hours. Indole test on the LF(s) using Kovac’s reagent was performed. Positive reaction was appearance of pink colour and that was indicative of <em>Escherichia coli</em>. Biochemical identification test was done on the purified NLF colonies using Analytical Profile Index (API) 20 E (BioMerieux, Catalogue no. 20 100/20 160) to identify <em>Salmonella</em> and <em>Shigella</em>. Serologic identification test was done using polyvalent and monovalent antisera from MAST-group for <em>Salmonella</em> and Reagensia for <em>Shigella</em> grouping and serotyping following the Murray, P.R. <em>et al.</em> 144</td>
<td>Murray, P.R. <em>et al.</em> 144</td>
</tr>
<tr>
<td>Methods</td>
<td>References</td>
<td></td>
</tr>
<tr>
<td>---------</td>
<td>------------</td>
<td></td>
</tr>
<tr>
<td>manufacturer’s instructions.</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Aeromonas</strong></td>
<td>Stool samples received in transport media (Cary-Blair – Oxoid) were plated out onto the Ryan agar medium containing inhibitory agents and were incubated overnight. Plates were examined for dark green colonies with darker centres indicative of <em>Aeromonas</em>. Suspected Aeromonas colonies were purified on McConkey agar for oxidase and catalase tests, NaCl (0%, 6% and 8%) concentration test and disc O129. Positive oxidase and catalase test, no growth in 0% NaCl and resistant to disc O129 confirmed the isolate as <em>Aeromonas</em>.</td>
<td>Murray, P.R. et al. 144. Bernagozzi, M. et al. 145.</td>
</tr>
<tr>
<td><strong>Vibrio cholerae</strong></td>
<td>Stool samples received in transport media (Cary-Blair – Oxoid) were plated out onto the Thiosulfate Citrate Bile Salt Sucrose (TCBS) agar and Alkaline Peptone Water (APW) media. After 24 hours incubation, cultured media were examined for suspected colonies (flat yellow and green) of <em>Vibrio</em> which are sub cultured onto the Tryptone Soy agar for further identification tests such as oxidase and NaCl (0%, 6% and 8%) concentration tests. Colonies positive to the two tests above is subjected to serologic testing using O1 and O139 antisera. Colony positive to O1 antisera is subtyped for Inaba and Ogawa antigen</td>
<td>Murray, P.R. et al. 144.</td>
</tr>
</tbody>
</table>
### Methods

| Campylobacter species | Stool samples received in transport media (Cary-Blair – Oxoid) were plated onto the Campy blood agar medium containing inhibitory agents and incubated at 42°C for 48 hrs. Following incubation, plates are examined for suspected colonies of *Campylobacter* which are oxidase and catalase positive. Modified Gram stain revealed the Gram negative curved or S shape morphology. Sodium hippurate is positive for *C. jejuni* and negative for *C. coli*. | Murray, P.R. *et al*.144 Blaser, M.J. *et al*.146 |

| Protozoal agents | The three protozoan agents targeted include *E. histolytica*, *G. lamblia* and *Cryptosporidium*. *E. histolytica*, *G. lamblia* and *Cryptosporidium* were detected using Techlab Monoclonal ELISA reagents the instructions are detailed in catalogue number PT5017, PT5012 and PT 5014 respectively. | Boone, J.H. *et al*.147 Youn, S. *et al*.14 Blessmann, J. *et al*. 148 |

<p>| Viral agents | We used IDEIA™ Rotavirus enzyme immunoassay reagent (catalogue no. K602011-2) in the 50% of the faecal test samples for the detection of Rotavirus group A antigen detection. Whilst ProSpecT™ Rota virus enzyme immunoassay reagent (catalogue no. R240396) was used in the other 50% faecal test samples. | Catalogue no. K602011-2 |</p>
<table>
<thead>
<tr>
<th>Methods</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Adenovirus IDEIA™</strong></td>
<td>Adenovirus enzyme immunoassay reagent (catalogue no. K602111-2) was used in the detection of Adenovirus hexin antigen in the 50% of the faecal test samples. Whilst ProSpecT™ Adenovirus enzyme immunoassay reagent (catalogue no. R240096) was used in the other 50% faecal test samples. Catalogue no. K602111</td>
</tr>
<tr>
<td><strong>Adenovirus serotype 40 and 41</strong></td>
<td>Adenovirus serotype 40 and 41 in human faecal samples were detected using an enzyme immunoassay reagent Premier Adenoclone – Type 40/41 (catalogue no. 696006) Catalogue no. 696006</td>
</tr>
<tr>
<td><strong>Norwalk (Norovirus GI/II)</strong></td>
<td>Viral nucleic acids were extracted from stool samples for use in Real Time – PCR to detect Norwalk (Norovirus GI/II), Astrovirus and Sapovirus. For this Vertre1TM XF (Mille Stepheson MS-782), Nuclisens Isolation Reagent (BioMerieux, catalogue no. 284160) and Lysis buffer (BioMerieux catalogue no. 284135) were used on 50% of the stool samples. On the other 50% of stool samples, extractions of viral nucleic acids were done using Vertre1™ XF (Mille Stepheson MS-782) and QIAamp(R) Viral Mini Kit (Qiagen catalogue no. 52906). Real time PCR was done on these extracts for the identification of the viral pathogens.</td>
</tr>
<tr>
<td><strong>Astrovirus</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Sapovirus</strong></td>
<td></td>
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</tbody>
</table>

**Multiplex-PCR to detect ETEC, EPEC, and EAEC strains Diarrhoeagenic E.**
<table>
<thead>
<tr>
<th>Methods</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>coli (DEC)</strong></td>
<td></td>
</tr>
<tr>
<td>Diarrhoeagenic</td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td></td>
</tr>
<tr>
<td>Three morphologically different <em>E. coli</em> colonies stored at -20°C were thawed and suspended in 0.5 ml of water. The suspension was boiled for 10 minutes and centrifuged. The supernatant were aseptically taken and regarded as the DNA template</td>
<td>Nataro J.P. *et al.*¹⁴⁹</td>
</tr>
<tr>
<td>A total volume of 20µl PCR reaction is prepared to include; 2.5µl of buffer (2mM MgCl2), 7.37µl of H₂O, 2µl dNTP (1.25mM) and 0.4µl forward and reverse primer (20 pmol/µl) for LT, ST, <em>bfpA</em>, CVD32, aaiC, 0.44µl for eae; 0.25µl Taq polymerase and 3.0µl DNA template. Amplification was performed using the Thermo cycler- Eppendorf Master cycler gradient (Preheat at 96°C for 4 minutes, denaturation at 95°C for 20 seconds, annealing at 57°C for 20 seconds, elongation at 72°C for 1 minute, run for 35 cycles. Final extension at 72°C for 7 minutes). Amplified PCR products were analysed on a 2% agarose gel containing ethidium bromide</td>
<td>Nguyen, T.V. *et al.*¹⁵⁰</td>
</tr>
</tbody>
</table>
(0.1 m ml-1 in 1 x TBE buffer and visualised on
the 2% (w/v) agarose gel under Ultral-Violet
(UV) radiation. The gel images were captured
digitally with a gel documentation system.

### 3.9 Follow-up of cases and controls

Noting that the study might miss a number of acute diarrhoea cases who subsequently develop persistent diarrhoea (a single diarrhoea continuing for more than 14 days), we introduced a memory aid card through which the primary caregivers would be able to record any abnormal stool continuing for 14 days or more. This was applicable for both the cases and controls. This memory aid card was collected at the 60-day (range 50-90 days) follow-up visit.

The nurses and the field workers collected information on the water and sanitation facilities of the households from where the cases and controls arose and a follow-up home visit allowed direct observation of these facilities and practices to evaluate to what extent growth faltering continues and in what direction (wasting, stunting etc.), we made two follow-up visits to the children: one at 60 days (range 50 to 90 days) and another at 18 months (range 18 to 24 months). The field workers recorded anthropometric measurements during both the visits following published methods and as described below. Further attacks
of diarrhoea and any other comorbidity during this period were also recorded. For logistic purpose we aimed to interview and assess the linear growth of at least one non MSD child per one MSD child. Usually, the first corresponding non MSD child was interviewed and assessed. If the first non MSD child was not found or available, the second in the list was considered. All efforts were made to follow up a child from non MSD cohort within 7 days of follow up of the corresponding child from MSD cohorts. At least three attempts were made before a follow up was abandoned due to unavailability of a caregiver.

3.10 Anthropometric measurements

Weight, height/length and mid upper arm circumference (MUAC) of the cases and controls at the time of enrolment and then during the two scheduled follow-ups were measured. The nurses at the health centres and the field workers at the community took the anthropometrical measurements.

A two day training session with the assistance of an epidemiologist as mentioned previously was conducted. At the end of the training, 10 children (5 aged 0-23 months and 5 aged 24-59 months) participated in a standardization session. Each of the nurses and field workers took two independent anthropometrical measurements of each child. The epidemiologist also took the measurements in the same away and we considered it as the “gold standard”.
Intra-rater reliability and validity were calculated using this gold standard. The measurement of height/length and MUAC taken by the nurse or field worker was unacceptable if the difference between the two measurements on the same child or when comparing with that of the gold standard were more than 0.5 cm. They were retrained for further accuracy. Length was measured for children aged 0-23 months of age or those that were older but unable to stand unassisted. Standing height was measured for children 2 years of age and older using a board with a fixed head and sliding foot piece (Shorr Productions, Olney MD). We used a 25 cm paper tri-colored single-slotted insertion tape (Shorr Productions, Olney MD) to measure MUAC. Both length/height and MUAC were taken to the nearest 0.1 cm. Length/height and MUAC were each measured three times; the average was calculated during analysis.

During initial enrollment for cases we recorded weight (to the nearest 0.1 kg) before administering any rehydration fluids, four hours after rehydration if the child was admitted in the health centre and again at the time of discharge. Controls needed to be weighed only once during the initial phase. Respecting the local culture, this weight was taken with the child naked or in light clothing. The digital scale used for taking weight was calibrated at least weekly (model 314, Tanita Corp. of America, Arlington Heights, IL). For children 0-23 months of age, the weight of the mother alone and with the child was recorded,
and the child’s weight was computed during analysis. For the older age group weight of the child alone was recorded.

3.11 Data management

The data supervisor was responsible for quality checks and ensuring data integrity. He/she checked all the CRFs and confirmed the accuracy. The research clinician and the principal investigator also did a random check of the CRF’s. Since this was a part of a multi-centre study all the CRF’s were faxed to the data coordinating centre in the USA where they were entered into a database populated using intelligent character recognition software provided by the Data Fax system. As a security measure all the CRF’s were scanned and filed in the password protected folder on the computer of the data base developer. A separate back up file was also created as a security measure which ensured restoration capabilities.

3.12 Analysis

Analysis was performed using STATA (StataCorp, College Station, Texas 77845 USA and R (R package version 3.26) software as follows:
HUAS
Both the point and period prevalence was calculated. Point prevalence was the proportion of children having diarrhoea on the day of interview, while period prevalence referred to diarrhoea episodes during the two weeks preceding the interview. Summary proportions were weighted to the proportion of all age and sex following the standard approach to statistical analysis of stratified samples, reflecting this weighting back to the distribution of the DSS source population. Analyses were carried out using the survey modules in STATA 12.0 (StataCorp, 4905 Lakeway Drive College Station, Texas 77845 USA) and R for calculation of median (range) and censored data analysis. Weighted proportions and their 95% CIs are reported.\textsuperscript{151, 152, 153} The Chi square ($\chi^2$) test was used to compare categorical variables. Poisson regression with jack-knife standard errors were used to produce prevalence ratios and 95% confidence intervals.

Case Control study
Case characteristics were presented as proportions. Incidence rates were calculated using the mid-year population derived from the DSS as a denominator. Proportions of pathogens isolated from both the cases and controls were presented. Conditional logistic regression was used to estimate of the odds ratios (OR) and 95% confidence intervals for the association between each pathogen and diarrhoea status.
**Risk factor analysis**

The risk factors were categorized as (a) sociodemographic, (b) domestic animal, (c) water source, storage and treatment, (d) toilet facility and (e) hand washing. Conditional logistic regression was used for bivariate and multivariate analysis of risk factors for diarrhoea. Variables under each category were subjected to bivariate analysis. Tables of covariates according to case/control status were explored to see their relationship and confirm adequate numbers in each group to fit in a model. Biologically plausible and factors that were significant in the bivariate analysis at a level of p<0.05 were included in a multivariate model, and a stepwise backward regression approach was used to develop a parsimonious model including the likely predictors (both risk and protective factors) for diarrhoea. The possibilities of interactions between variables were also explored. The variables were ordered for hierarchical backward elimination, so that at each stage the least significant factor was dropped.

**Follow-up of the case and control cohorts**

The primary outcome of interest in the follow-up study was stunting, and whether or not this differed between cases and controls. Stunting was measured using the height for age Z-score (HAZ) according to WHO standards\(^{154}\). The extreme values that were biologically implausible and that were inconsistent between the time periods of follow up (Z score > or < than +6/-6) following the
same WHO standard were excluded. Mean (standard deviations) HAZ scores were presented, as were the proportions with moderate and severe stunting. The mean baseline HAZ levels in case and controls were compared using a linear regression model, with robust standard errors to account for the matching. There was substantial variation in timing of the follow-up measures (although for each matched case and control set the visits were at the same time). It was decided to use time since diagnosis as a continuous measure in order to better capture the change in HAZ score over time. Linear mixed models were used to model the relationship between disease status and change in HAZ score over time. The matching factors (age and sex) were included in the models, and retained if they explained variation in HAZ scores. The case control set was included as a random effect, as was the individual (to allow for repeated measures over time). Sampling weights were used to account for the sampling of case and controls. To account for individual heterogeneity we declared the dataset as panel data. In order to develop a model which captured a non-linear pattern of change in HAZ score over time, a first order fractional polynomial was fitted to identify the best fitting power transformations (this was done using generalized estimating equation as STATA does not allow use of fractional polynomials in xtmixed). We did a fractional polynomial for linearity assumption for the two continuous variable HAZ and time. The fractional polynomial was not used for any parameter estimation. For building the mixed models, all main effects and their interactions with time (in powers according to those found in the fractional
polynomial models) were included initially. A backward stepwise elimination approach was then used to obtain the final model.

Figure 3-2 below shows the study flow chart.
Figure 3-2. Study flow chart

Clinical Team
- Screening and eligibility ascertainment at the Health Centre
- Enrolment, Interview and stool sample collection
- Management of the case at the HC
- Quality control for all clinical CRF
- Submission of CRF to Data Centre
- 60-90 days follow up of cases at home
- 18-24 months follow up of cases at home
- Quality control for all field CRF
- Submission of CRF to Data Centre

Field Team
- Control selection at the community
- Interview and Stool collection from home
- Linkage of cases and controls to BHDSS database
- Collection, scanning and transmission of CRF to DCC
- 60-90 days follow up of controls at home
- 18-24 months follow up of controls at home

Data Team
- Confirmation of case to be a resident of BHDSS
- Identification of potential controls from BHDSS
- Linkage of cases and controls to BHDSS database
- Collection, scanning and transmission of CRF to DCC
- 60-90 days follow up of controls at home
- 18-24 months follow up of controls at home
- Quality control for all laboratory CRF

Laboratory Team
- Confirmation of stool collection
- Identification of pathogen through microbiological and molecular methods
- Reporting of result to clinical and field team
- Quality control for all laboratory CRF
- Submission of laboratory CRF to Data Centre
Chapter 4- Health Care Utilization and Knowledge, Attitude and Practice

THE JOURNEY BEGINS
4.1 Introduction

There is a lack of community-based surveillance to provide accurate diarrhoea prevalence and incidence rates in sub-Saharan Africa, particularly in rural Africa. Hospital-based data have limitations with respect to representing the true burden of disease and identifying associated risk factors. Indeed, many deaths from diarrhoeal disease likely occur in the community and are often not recorded in death registries. Verbal autopsy remains the only way to assess the cause of death and it has well described limitations.

A health care utilisation and attitudes survey (HUAS) in The Gambia in a rural population under demographic surveillance was conducted. The aim was to identify the point prevalence of diarrhoea in children under five years of age, the proportion with diarrhoea in the previous two weeks (period prevalence), and the proportion taken to health care facilities and other providers. Primary caregivers’ knowledge of diarrhoea and their perceptions and attitudes towards the illness were recorded. Detailed methods for this study are described in chapter 3 (section 3.5).
4.2 Demographic Surveillance, Population and Sample

The Basse Health and Demographic Surveillance System (BHDSS) was established in 2006 on the South bank of the River Gambia and this census was used as the sampling frame. The BHDSS population within the 1084 km² study area resides within 223 villages across two districts that are served by one major government health centre and five smaller primary health centres. The total population during the study period in year 2007 was 136,793 and the under-five population was 21,445 (15.7%), including 4,649 aged 0-11 months; 4,398 aged 12-23 months and 12,398 aged 24-59 months. For this cross-sectional survey we randomly selected 1140 children in the under-five age group, including 400 aged 0-11 months, 370 aged 12-23 months and 370 aged 24-59 months.

4.3 Results

General characteristics

Completed interviews were conducted with primary caregivers of 1,012 children; five interviewees who were not the primary caregivers of the children were excluded; the various reasons why others could not be interviewed are summarized in Figure 4.1.
Figure 4-1. Survey flow chart

Children under five in the DSS (Survey Population): N=21,445

Randomly selected for HUAS: n=1139

- Refusal: n=16
- Death: n=11
- Migration/Travel: n=65
- Misclassification by age: n=9
- Non responders: n=17

Interview conducted: n=1021

Interview not conducted: n=118

Interview given by primary caretaker: n =1012

0-11m: n= 390
12-23m: n=302
24-59 m: n=320

Had diarrhoea in the previous two weeks: n =258

No. diarrhoea in previous two weeks: n =754

0-11m: n=96
12-23m: n=98
24-59m: n=64
There were 390 children in the 0-11 month age stratum and 302, and 320, respectively, in the 12-23 and 24-59 months age strata (Table 4-1). The proportion of male children was slightly over 50% in each age stratum (Table 4-1) similar to the distribution in the DSS population. Almost all of the primary caregivers were the mother of the child (99.5%) but the proportion decreased with increasing age stratum (99.5%, 98.3% and 94.4%).

The majority (71.9%; 95% CI: 68.4-75.0) of the primary caregivers in the population had no formal education and most households had few assets (Table 4-1). The median numbers of people living in a household, number of sleeping rooms and children under-five in a household were 20 (range 3-125), 7 (range 1-47) and 4 (range 1-32), respectively.
Table 4-1. Socio demographic, economic and educational characteristics of the primary caregivers attending the survey (N=1012)

<table>
<thead>
<tr>
<th>Age strata</th>
<th>Overall *Weighted percentage, (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-11 m</td>
<td>12-23 m</td>
</tr>
<tr>
<td>n=390</td>
<td>n=302</td>
</tr>
</tbody>
</table>

| Gender, male        | 213 (54.6) 165 (54.6) 166 (51.9) 52.9 (49.2 to 56.6) |
| Primary caretaker, mother |
| Where does the mother live? |
| In the household    | 388 (99.5) 297 (98.3) 302 (94.4) 96.1 (94.2 to 97.4) |
| Died                | 1 (0.3) 1 (0.3) 1 (0.3) 0.3 (0.08 to 1.2) |
| Abroad/outside      | 1 (0.3) 3 (1.0) 13 (4.1) 2.7 (1.7 to 4.5) |

| Where does father live? |
| In the household    | 303 (77.7) 236 (78.1) 239 (74.7) 75.9 (72.6 to 79.0) |
| Died                | 6 (1.5) 2 (0.6) 11 (2.8) 2.5 (1.5 to 4.2) |
| Abroad/outside      | 81 (20.8) 64 (21.2) 70 (21.9) 21.5 (18.7 to 24.7) |

| Primary caretaker's education |
| Religious (Koranic) education only | 287 (73.6) 215 (71.2) 229 (71.6) 71.9 (68.4 to 75.1) |
| Attended primary and post primary schools | 103 (26.4) 87 (28.8) 91 (28.4) 28.1 (25.0 to 31.6) |

<p>| Floor Type |
| Finished (Vinyl) | 285 (73.1) 239 (79.1) 225 (70.3) 72.6 (69.1 to 75.8) |</p>
<table>
<thead>
<tr>
<th>Age strata</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-11 m</td>
<td>12-23 m</td>
</tr>
<tr>
<td>n=390</td>
<td>n=302</td>
</tr>
<tr>
<td>*Weighted percentage, (95% CI)</td>
<td></td>
</tr>
<tr>
<td>strips/carpet/cement etc.)</td>
<td></td>
</tr>
<tr>
<td>Natural</td>
<td>105 (26.9)</td>
</tr>
<tr>
<td>(earth/sand/dung)</td>
<td></td>
</tr>
<tr>
<td><strong>Household possessions</strong></td>
<td></td>
</tr>
<tr>
<td>Electricity</td>
<td>116 (29.7)</td>
</tr>
<tr>
<td>Television</td>
<td>95 (24.4)</td>
</tr>
<tr>
<td>Scooter/Motor cycle</td>
<td>145 (37.2)</td>
</tr>
<tr>
<td>Radio</td>
<td>355 (91.0)</td>
</tr>
<tr>
<td>Bicycle</td>
<td>341 (87.4)</td>
</tr>
<tr>
<td>Car/Truck</td>
<td>53 (13.6)</td>
</tr>
<tr>
<td>Phone</td>
<td>296 (75.9)</td>
</tr>
<tr>
<td>Refrigerator</td>
<td>41 (10.5)</td>
</tr>
<tr>
<td>Motor boat</td>
<td>0 (0)</td>
</tr>
<tr>
<td>None of the above</td>
<td>12 (3.1)</td>
</tr>
<tr>
<td><strong>Diarrhoea:</strong></td>
<td></td>
</tr>
<tr>
<td>Diarrhoea at the time of survey</td>
<td></td>
</tr>
<tr>
<td>28 (7.2)</td>
<td>36 (11.9)</td>
</tr>
<tr>
<td>Diarrhoea in the preceding 2 weeks</td>
<td></td>
</tr>
<tr>
<td>96 (24.6)</td>
<td>98 (32.5)</td>
</tr>
</tbody>
</table>

Figures are n (%) unless mentioned otherwise

*calculated using the sampling weights for age groups and sex.
The weighted point prevalence of diarrhoea among the children aged under five years old in the population was 7.7% (95% CI: 6.1-9.9). The numbers of children who had diarrhoea at the time of the survey were 28/390 (7.2 %) among the 0-11 month old children, 36/302 (11.9%) in 12-23 month and 21/320 (6.6%) in 24 to 59 month age strata. 258 of the 1,012 surveyed children had diarrhoea in the two weeks preceding the interview (weighted period prevalence of 23.3%; 95% CI: 20.5 to 26.5). The period prevalence was significantly higher in the 12-23 month age stratum (32.5%, 95% CI: 27.1-37.8) than in the other two age strata 24.6% (95% CI: 20.3- 28.9) and 20.0% (95% CI: 15.6-24.4) at 0-11 months and 24-59 months, respectively (p=0.002).

**Perception and use of health care facility**

Figure 4-2 summarizes the knowledge of the primary caregivers regarding dehydration.
Three quarters of the caregivers were aware that sunken eyes were a sign of dehydration (75.5%; 95% CI: 72.2-78.5) but less than half (46.1%; 95% CI: 42.4-49.7) of caregivers thought that a dry mouth is a sign of dehydration. Only a quarter (25.4%; 95% CI: 22.3-28.8) appreciated that decreased urination could be due to dehydration.
Primary caregivers believed that bloody diarrhoea is a danger sign (85.6%; 95% CI: 82.9-88.0). The presence of fever was considered to be of concern by only 30.2% (95% CI: 26.9-33.7), while 61.0% believed that vomiting was of importance (95% CI: 57.3-64.5). A smaller proportion held the belief that a large number of stools per day (27.1%; 95% CI: 24.0-30.5)] or presence of dehydration (10.3 %; 95% CI: 8.2-12.9) were of notable concern.

One third (33.4%, 95% CI: 30.0-36.9) of primary caregivers said they would walk to the health care facilities if their child needed care for diarrhoeal illness; the rest said they would use either commercial or private transport. A smaller proportion (5.4%; 95% CI: 4.0-7.4) expressed unwillingness to attend any of the health care centres in the DSS. Less than half (39.9 %, 95% CI: 36.4- 43.6) thought that they could reach the health centre of their choice within half an hour. Mothers of 81.1% (95% CI: 78.0-83.8) of the children were the decision makers for their child but seeking care at the health centre could be delayed for financial reasons (17.8%, 95% CI: 15.2-20.8) and lack of transport (34.4%, 95% CI: 31.0-38.1). Nevertheless, 55.9 % (95% CI: 52.2-59.5) reported there would be no difficulties in reaching a centre of their choice.
Wariness, awareness and attitudes towards diarrhoea

Overall, 57.7% (95% CI: 54.0-61.3) of primary caregivers were aware of children under-five suffering from watery diarrhoea in the community; a similar proportion (62.9%, 95% CI: 59.3-66.4) thought that prevention was available. A higher proportion of the caregivers believed that death is more common in those with bloody diarrhoea than those with watery diarrhoea (13.8%, 95% CI: 11.5-16.5% vs. 9.2%, 95% CI: 7.2-11.5 respectively). Relatively more caregivers were worried about their child suffering from watery stools (77.7%; 95% CI: 74.5-80.7) compared to their child having bloody diarrhoea 69.3%( 95% CI: 65.8-72.6).

Important preventive methods such as breast feeding 14.7% (95% CI: 12.4-17.4), adequate nutrition 16.4% (95% CI: 13.8-19.3), and proper disposal of human waste 18.5% (95% CI: 15.9-21.6) were reported as such by relatively few primary caregivers. However, clean food and water (62.1%, 95% CI: 58.5-65.6) and hand washing (37.2%, 95% CI: 33.7-40.9) were acknowledged widely as the best methods of prevention. Some of the caregivers also believed that giving any medication (15.1%; 95% CI: 12.7-17.9) could prevent their children from having diarrhoea. Primary caregivers believed that bloody diarrhoea is both dangerous (71.6%, 95% CI: 68.1-74.8) and costly to treat (69.7%, 95% CI: 66.2-73.0). Only 6.7% (95% CI: 5.0-8.9) thought that a vaccine could be a way of preventing diarrhoea. However, almost all caretakers thought that vaccines are important and would give one to their child if available. More than 90% believed that there
is medication or treatment available for both watery and bloody diarrhoea and that ORS is effective.

**Diarrhoea and health care utilisation**

Two hundred and fifty eight of the surveyed children had diarrhoea in the previous two weeks. The weighted median (range) duration of illness with diarrhoea was 5 days (1-21). According to the primary caregivers 25.9% (95% CI: 19.0-32.9) of the children had visible blood in their stool (Table 4.2) during the episode of diarrhoea; 81.3% (95% CI: 75.9-86.3) had fever and 31.5% (95% CI: 25.6-38.0) had vomiting.
Table 4-2. Reported stool characteristics, signs of dehydration and types of diarrhoea among the children who had diarrhoea in the previous two weeks (n=258)

<table>
<thead>
<tr>
<th>Age strata</th>
<th>Male</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-11 m, n=96</td>
<td>60 (62.5)</td>
<td>56.3 (48.9 to 63.5)</td>
</tr>
<tr>
<td>12-23 m, n=98</td>
<td>54 (55.1)</td>
<td>87.5 (81.2 to 91.9)</td>
</tr>
<tr>
<td>24-59 m, n=64</td>
<td>35 (54.7)</td>
<td>25.9 (19.0 to 32.9)</td>
</tr>
</tbody>
</table>

Weighted percentage & (95% CI) P value

| Reported stool characteristics: |  
| Max stool, 3-6 times/day | 93 (96.9) | 87.5 (81.2 to 91.9) |
| Blood in stool | 11 (11.5) | 25.9 (19.0 to 32.9) |
| Mucus/pus in stool | 47 (49.0) | 51.4 (44.1 to 58.7) |
| Watery stool | 36 (37.5) | 35.1 (28.5 to 42.3) |

Signs of Dehydration:

| Decreased urination | 32 (33.0) | 38.5 (31.6 to 45.8) |
| Very thirsty | 75 (78.1) | 84.9 (79.3 to 89.2) |
| Dry mouth | 45 (46.9) | 51.9 (44.6 to 59.2) |
| Sunken eyes | 68 (70.8) | 75.7 (69.0 to 81.4) |
| Wrinkled skin | 41 (42.7) | 45.5 (38.3 to 52.9) |

Diarrhoea with:

| Fever | 89 (92.7) | 81.3 (74.9 to 86.3) |
| Vomiting | 41 (42.7) | 31.5 (25.6 to 38.0) |
| Lethargy | 52 (54.2) | 48.6 (41.4 to 56.0) |
| Coma | 5 (5.2) | 3.2 (1.6 to 6.4) |

Figures are n (%) unless mentioned otherwise. *calculated using the sampling weights for the age groups and sex.
Blood was visible in stools less often in children with diarrhoea age 0-11 months (11.5%, p=0.001), than in children with diarrhoea aged 12-23 months or 24-59 months, vomiting was least common in the 24-59 months age group (20.3%, p=0.009) and fever was most common in 12-23 months age group (70.4%, p=0.007).

Two hundred and ten children (weighted 81.5%; 95% CI: 75.1-86.6) were taken out of their home for treatment whereas 48.4% (95% CI: 41.2-55.7) of them were taken to a health centre for care; the rest were taken to the other health care facilities. The median number of days before seeking of care was three (range 1-10) days. When primary caregivers sought care outside of their home, 33.6% (95% CI: 27.1-40.8) preferred to go to licensed practitioners, including pharmacists, and 28.5% (95% CI: 22.4-35.6) preferred unlicensed practitioners and friends. Only 7.1% (95% CI: 4.2-11.7) preferred a traditional healer. Among various responses about why care was not sought (n=48), 48.5% (95% CI: 32.2-65.1) thought the child did not need it, followed by the perceived high cost of treatment in 24.3% (95% CI: 12.6-41.7); 5.2% (95% CI: 1.9-13.7) expressed unhappiness about the clinical service in the health centres.
Home and hospital management

With respect to treatment at home before seeking care, 43.0% (95% CI: 35.9-50.3) of children who had diarrhoea were given no treatment at all. ORS was used by 17.0% (95% CI: 12.1-23.2) of caretakers and 19.1% (95% CI: 14.0-25.4) of children with diarrhoeal illness were given some kind of homemade fluid before being taken outside the home for care. A few caregivers (9.7%, 95% CI: 6.2-14.9) reported giving antimicrobial agents to their children at home. They were generally reluctant to give food during an episode of diarrhoea; 72.5% (95% CI: 65.4-78.5) gave their children less than usual amounts to eat. However, 63.9% (95% CI: 56.7-70.5) gave their child more than usual volumes to drink. Among the children with diarrhoea, 3.0% (95% CI: 1.3 to 6.9) were admitted to a health care facility. Among those attending a health care facility, ORS was given to 55.1% (95% CI: 44.6-65.1), 18.6.0% (95% CI: 12.0-27.9) were given antimicrobials; injectable medicines were given to 43.7% (95% CI: 33.6-54.3). Caregivers of 2.7% (95% CI: 0.7- 9.8) of children with diarrhoea reported that their child had received intravenous fluids. None of the caregivers reported receiving Zinc from the treatment facilities.

Predictors of diarrhoea and health care seeking behaviour

Regression was used to identify possible predictors of diarrhoea and of the decision to seek treatment at a health facility; we considered biologically
plausible and logically independent variables first and then constructed
multivariate models (Table 4-3).

Table 4-3. Assessment of putative risk factors for diarrhoea in 1012 study children

<table>
<thead>
<tr>
<th></th>
<th>Prevalence ratio (PR)</th>
<th>95% Confidence Interval</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age: &lt;2 Y</td>
<td>1.4</td>
<td>1.1 to 1.9</td>
<td>0.005</td>
</tr>
<tr>
<td>Gender (male)</td>
<td>1.1</td>
<td>0.9 to 1.5</td>
<td>0.311</td>
</tr>
<tr>
<td>Primary Caregivers’ education: Religious (Koranic) education</td>
<td>1.2</td>
<td>0.9 to 1.6</td>
<td>0.340</td>
</tr>
<tr>
<td>&gt; 21 people residing in the household for the last 6 months</td>
<td>1.1</td>
<td>0.9 to 1.4</td>
<td>0.426</td>
</tr>
<tr>
<td>&gt;7 sleeping rooms in the household</td>
<td>1.0</td>
<td>0.8 1.4</td>
<td>0.743</td>
</tr>
<tr>
<td>&gt; 3 under five children in the household</td>
<td>1.1</td>
<td>0.8 to 1.4</td>
<td>0.727</td>
</tr>
<tr>
<td>&gt;2 under five children under the care of the primary caregiver</td>
<td>0.9</td>
<td>0.6 to 1.5</td>
<td>0.746</td>
</tr>
<tr>
<td>Floor type non- cemented</td>
<td>1.0</td>
<td>0.7 to 1.3</td>
<td>0.953</td>
</tr>
<tr>
<td>Household has electricity</td>
<td>0.9</td>
<td>0.7 to 1.2</td>
<td>0.448</td>
</tr>
<tr>
<td>Household has television</td>
<td>1.0</td>
<td>0.7 to 1.3</td>
<td>0.902</td>
</tr>
<tr>
<td>Household has motor cycle</td>
<td>0.9</td>
<td>0.7 to 1.2</td>
<td>0.616</td>
</tr>
<tr>
<td>Household has radio</td>
<td>0.9</td>
<td>0.6 to 1.4</td>
<td>0.624</td>
</tr>
<tr>
<td>Household has bicycle</td>
<td>0.9</td>
<td>0.6 to 1.3</td>
<td>0.447</td>
</tr>
<tr>
<td>----------------------</td>
<td>-----</td>
<td>------------</td>
<td>-------</td>
</tr>
<tr>
<td>Household has car</td>
<td>1.0</td>
<td>0.7 to 1.4</td>
<td>0.871</td>
</tr>
<tr>
<td>Household has telephone</td>
<td>1.0</td>
<td>0.7 to 1.3</td>
<td>0.858</td>
</tr>
<tr>
<td>Household has refrigerator</td>
<td>0.9</td>
<td>0.5 to 1.3</td>
<td>0.510</td>
</tr>
</tbody>
</table>

The prevalence ratios estimate the prevalence of diarrhoea in the previous two weeks for children with each characteristic (compared to the prevalence in those without the characteristics).

Children under two years of age were more likely to have diarrhoea than those aged two years and over, with a prevalence ratio (PR) of 1.4 (95% CI: 1.1-1.9, p=0.005). Of the three age strata, the 12-23 months age group was most likely to suffer from diarrhoea (PR 1.3; 95% CI: 1.0-1.64, p=0.042). Gender of child, primary caregivers’ level of education, overcrowding and socioeconomic status were not significant predictors of diarrhoea in either the bivariate or multivariate models.

Children having watery diarrhoea were more likely to be taken to a health centre (PR 1.2, 95% CI: 1.0- 1.3, p=0.011) than those with other forms of diarrhoea. Among the signs of dehydration, dry mouth, lethargy and diarrhoea with fever and vomiting were found to be significant predictors of seeking treatment at a health facility. However when they were included in a multivariate analysis none of these predictors remained significant.
Table 4-4. Assessment of health care seeking behaviour of the primary caregivers when their child had Diarrhoea, expressed as prevalence ratios (PR) (n=258).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Prevalence ratio (PR)</th>
<th>95% Confidence Interval</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age: 12-23 m</td>
<td>1.0</td>
<td>0.8 to 1.2</td>
<td>0.866</td>
</tr>
<tr>
<td>Age: 24-59 m</td>
<td>1.0</td>
<td>0.9 to 1.2</td>
<td>0.554</td>
</tr>
<tr>
<td>Gender, female</td>
<td>1.0</td>
<td>0.9 to 1.2</td>
<td>0.629</td>
</tr>
<tr>
<td>Blood in stool</td>
<td>1.0</td>
<td>0.8 to 1.2</td>
<td>0.758</td>
</tr>
<tr>
<td>Mucus/Pus in stool</td>
<td>1.1</td>
<td>0.9 to 1.2</td>
<td>0.356</td>
</tr>
<tr>
<td>Watery stool</td>
<td>1.2</td>
<td>1.0 to 1.3</td>
<td>0.011</td>
</tr>
<tr>
<td>Decreased Urination</td>
<td>1.0</td>
<td>0.9 to 1.2</td>
<td>0.991</td>
</tr>
<tr>
<td>Thirst</td>
<td>1.0</td>
<td>0.8 to 1.2</td>
<td>0.806</td>
</tr>
<tr>
<td>Dry mouth</td>
<td>1.2</td>
<td>1.0 to 1.4</td>
<td>0.013</td>
</tr>
<tr>
<td>Sunken Eyes</td>
<td>1.2</td>
<td>1.0 to 1.5</td>
<td>0.054</td>
</tr>
<tr>
<td>Wrinkled skin</td>
<td>1.1</td>
<td>0.9 to 1.2</td>
<td>0.307</td>
</tr>
<tr>
<td>Fever</td>
<td>1.3</td>
<td>1.0 to 1.7</td>
<td>0.060</td>
</tr>
<tr>
<td>Vomiting</td>
<td>1.1</td>
<td>1.0 to 1.3</td>
<td>0.034</td>
</tr>
<tr>
<td>Lethargy</td>
<td>1.2</td>
<td>1.0 to 1.4</td>
<td>0.017</td>
</tr>
<tr>
<td>Coma</td>
<td>1.1</td>
<td>0.9 to 1.4</td>
<td>0.269</td>
</tr>
<tr>
<td>Mode of transport: Walk</td>
<td>1.0</td>
<td>0.9 to 1.2</td>
<td>0.553</td>
</tr>
<tr>
<td>Time to Health facilities: within 30 minutes</td>
<td>1.2</td>
<td>1.0 to 1.3</td>
<td>0.022</td>
</tr>
</tbody>
</table>

Note: The prevalence ratios estimate the proportion taken to a health care centre among children with each characteristic (compared to those without the characteristic).
Among children with diarrhoea in the previous two weeks, neither the child’s age nor gender were predictors of seeking treatment at a health facility (Table 4-4).

4.4 HUAS-lite

We analysed the information obtained from the interview of 3124 and 3180 primary caregivers of under five year old children in two consecutive years. The survey showed the period prevalence of diarrhoea to be 22.8% (712/3124), overall weighted 19.9% (95% CI: 18.4-21.4) in 2009 and 22.6% (720/3180), overall weighted 18.9% (95% CI: 17.5-20.4) in 2010. The primary caregivers of 44.8% (95% CI: 39.7-50.0) of the children who had diarrhoea in the previous two weeks took them to a health centre within the DSS in year one of the study, while 48.2% (95%CI: 42.9-53.6) did so in year two. Among those who had diarrhoea, 45.8% (95% CI: 41.8-49.9) and 41.8% (95% CI: 37.7-45.9) in two consecutive years were reported to have moderate to severe diarrhoea based on the signs perceived by the primary caregivers. Only about a quarter 25% (95% CI: 20.3-30.4) and 26.5% (95%CI: 21.1-32.7) of the children who had signs of MSD visited any of the health centres in the health and demographic surveillance area. In the health centres, ORS was given to 36.6% (95% CI: 32.8-40.6) and 37.9% (95% CI: 33.9-42.0) of the children as a treatment for diarrhoea in years 2009 and 2010 respectively.
4.5 Discussion

This study provides baseline information on diarrhoeal illness among children from a largely rural West African population. The point prevalence of diarrhoea in children under five years of age was 7.7%, signifying the magnitude of the disease. The primary caregivers in the households in the BHDSS have very low educational backgrounds and important gaps in knowledge and perception of diarrhoeal illness and its management, as has been seen in places like Ethiopia. The study found a higher prevalence of diarrhoea in children under two years of age, in particular among the 12-23 month age group compared with the 0-11 and 24-59 month age group, similar to the findings in other African settings of Nigeria, Sierra Leone and Congo.160, 161, 162

We asked caregivers about the WHO signs of dehydration.163 About one half of the caregivers reported awareness of these signs, a common observation; reported by other studies in Africa.98 This knowledge gap may delay the initiation of home management and of seeking care at a health facility. Of the signs, only “sunken eyes” was well recognized, although this can also be a sign of malnutrition. Despite distances of up to 25 km to a health facility from the farthest village, and unreliable transport, more than half of the primary caregivers did not consider transport to be a potential problem. Therefore, increasing the number of health care facilities may not be the most important solution with respect to diarrhoea in young children in this community.
Despite a rural setting and an impoverished population, a great majority (82.2%) sought care outside of their home. Less than half of the diarrhoea cases were taken to health centres, indicating that facility-based surveillance would grossly underestimate the disease burden. ORS, was given to only 55.1% of the children taken to a health centre, while the tendency to give medicine by injection shows, albeit misguided, enthusiasm to treat diarrhoea. The primary caregivers sought care at a health facility on average on day three and the median duration of diarrhoea was five days, signifying generally delayed care seeking behaviour. Boys were not more likely to be taken to the health centres than girls, unlike the findings in some Asian and African settings, and health care use was similar across all three age strata. As expected, crowding was positively related to diarrhoea, while having certain household assets (an indicator of economic wellbeing) showed an inverse relation. However, these were not statistically significant. A substantial proportion of children were taken to pharmacists/licensed practitioners and traditional healers/unlicensed practitioners. In The Gambia, registered nurses are the primary health care providers in the health care centres and are the only ones who can operate a pharmacy and be licensed to practice modern medicine. Close proximity and the availability of instant advice and medication are thought to be the main reason for seeking care at these facilities. On the other hand, traditional healers use indigenous medicine and are not bound by any law. The attendance at traditional healers and unlicensed practitioners follows similar patterns.
observed elsewhere in West Africa. Less than 5% of the children who had diarrhoea in the previous two weeks were actually admitted to a health care facility for treatment, suggesting a lack of understanding of the progression and consequences of the disease among the primary caregivers working at the health centres. The most common reason for not seeking care was the caregiver’s personal opinion (46.7%), while cost was also a prominent reason (28.9%), as seen in other similar settings.

Like other countries in Africa, ORS use was relatively low. While primary caregivers thought that ORS was beneficial, very few of them used it before seeking care when their children had diarrhoea. Women in this community are involved in many other household activities and farming and it is possible that young children are generally not receiving optimal care and attention from these busy caregivers. Those who went to non-health facility providers were less likely to be prescribed ORS, as might be expected. Caregivers did display awareness that fluid replacement is the key measure to control diarrhoea associated dehydration. It is worth considering training in the use of home-made sugar and salt solution and other readily available fluids that are appropriate for the management of dehydration, especially if ORS availability remains poor. The tendency to withhold food or introduce new items is common in Africa. Unfortunately, zinc, proven to be effective in diarrhoea management, has not yet been widely promoted in The Gambia.
A multi-pronged approach is indicated to contain diarrhoeal illness in this and similar settings.\textsuperscript{178} Primary caregivers are aware of the existence of the disease. They believe that treatment is available when a child suffers from diarrhoea. The majority understand the importance of clean food, water and washing hands.\textsuperscript{179} But very few (\(<\ 20\%\)) are aware of preventive measures like nutrition, medication, breast feeding, proper disposal of human waste, and vaccines. Maintenance of good food hygiene and preparation,\textsuperscript{180} adequate supply of potable water and treatment of turbid water may improve diarrhoeal disease control in settings such as this.\textsuperscript{181}

The interview process was standardized through repeated training of the field workers but there may have been some differences in filling of forms among interviewers. The signs of dehydration are subjective; measurement of prevalence through these signs may make that variable at risk of measurement bias. It is possible that there was some information bias with respect to health seeking, as it is difficult to know for sure whether providers are licensed or not.

Both the baseline HUAS and the HUAS-lite surveys revealed that less than half of the children were taken to the health centre when they suffer from a diarrhoea episode. ORS use remained the same over these time periods. Although the primary caretakers’ perception of signs of dehydration may often be inaccurate,
the presence of MSD based on these signs and reported by the caregivers failed to bring more than a quarter of the children to the health centres. This again underscores the need to augment awareness of the severity and consequences of diarrhoeal illness and of teaching prevention and improved home based care through a well-orchestrated health education program.

While the results of this study may well be generalizable to a broader rural Africa context, there are some potential weaknesses. The study was conducted over a four-week period and the seasonality of diarrhoeal disease may have affected our prevalence estimate (as was noticed in case control study and shown in figure 5-8). On the other hand, an abbreviated survey version (HUAS-lite) carried out on six subsequent Demographic Surveillance System (DSS) rounds showed that the prevalence of diarrhoea did not change significantly over time. I believe that the period prevalence from the HUAS-lite reflects a reliable estimate of the disease among the children less than five years of age in the community. A relatively higher prevalence noted in the initial survey could be due to the seasonal effect.

4.6 Summary

The findings of this study indicate a substantial point prevalence of diarrhoea among under-five year old children in a defined population under demographic
surveillance. Lack of knowledge in recognising the early signs of dehydration and insufficient use of health care facilities and health care providers are key factors amenable to public health intervention. The government and health policy makers should consider providing more rural dispensaries and train more village health professionals to cater to the population. ORS, along with zinc, should be scaled up. ORS could be made available both through village health professionals and pharmacies and a media campaign might accelerate its use.\textsuperscript{182} Education should include training on preventive measures and home based management of diarrhoea.\textsuperscript{183} There is also a need to continue community based surveillance of diarrhoea after effective control measures are put in place.

NB: Based on this chapter an article is already published

Chapter 5- Incidence, characteristics and aetiology of diarrhoea

THE SEARCH CONTINUES
5.1 Introduction

The facility based incidence rates of moderate to severe dehydration (MSD) in children under-five under surveillance were measured. The diarrhoea cases presented and enrolled from the health care facilities within the Basse Demographic Surveillance System (BHDSS) were assessed for signs and associated symptoms. Stool samples were collected from both the cases and controls to detect an aetiological agent and their seasonality. Hypotheses around the facility based incidences and the identification of aetiological agents causing moderate to severe diarrhoea are addressed. A detailed description is given in the method chapter 3 (section 3.8).

In this chapter the reports are on

1. Facility based cumulative and annual incidence rates of moderate to severe diarrhoea (MSD).
2. The syndrome of diarrhoea in children presenting at the health centres
3. Pathogens identified from both the cases and controls and their seasonality.
5.2 Diarrhoea incidence

Children, belonging to the population within the BHDSS represented 100,786/102,059 (98.8%) of the episodes of any illnesses presenting at the health centres during the three year (2008-2010) study period. Of the total reported illnesses to any of the health facilities, 7,688 (7.6%) episodes were due to diarrhoea. It was observed that, over the study period, the absolute number of “any illness” presentations increased, but overall diarrhoeal illness declined. Table 5-1 shows the yearly presentation of illnesses in three age strata. As a subset of all illnesses, the proportion due to diarrhoea declined from 11.4% in 2008 to 7.0% in 2009 and then 5.2% in 2010. Over the study period the proportion of illness due to diarrhoea was relatively higher in the 12-23 month old age group compared to the other age groups (p=0.002).
Table 5-1. Yearly presentation of illnesses in all the health centres of BHDSS, The Gambia (2008-2010)

<table>
<thead>
<tr>
<th>Year</th>
<th>0-11m</th>
<th>12-23m</th>
<th>24-59m</th>
<th>0-59m</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Any Illness, n</td>
<td>Diarrhoeal Illness, n (%)</td>
<td>Any Illness, n</td>
<td>Diarrhoeal Illness, n (%)</td>
</tr>
<tr>
<td>2008</td>
<td>11648</td>
<td>1311 (11)</td>
<td>9508</td>
<td>1521 (16)</td>
</tr>
<tr>
<td>2009</td>
<td>13186</td>
<td>1007 (8)</td>
<td>10912</td>
<td>1043 (10)</td>
</tr>
<tr>
<td>2010</td>
<td>14195</td>
<td>734 (5)</td>
<td>11029</td>
<td>849 (8)</td>
</tr>
</tbody>
</table>

Figures are n (%)

Diarrhoea incidence in the health centres

Using the mid-year BHDSS population as the denominator, the annual incidence rates of diarrhoeal episodes were 13, 8 and 6 per 100 child years of observation in the year of 2008, 2009 and 2010 respectively. The reporting of diarrhoea cases to the health centres varied by season. There were two distinct peaks: one in the dry and cold months from November to January, and the second from June to August (Figure 5-1).
Figure 5-1. Monthly presentation of diarrhoea cases to all the illnesses presented by children aged under five years old at the health centres of BHDSS, The Gambia during the study period (2008-2010)

5.3 Enrolment

The enrolment flowchart for MSD cases reporting to any of the six health care facilities located within the BHDSS is shown in the Figure 5-2 below.
Figure 5-2. Flow chart for the case enrolment from the health centres of BHDSS, The Gambia during the study period (2008-2010)

DSS resident and had diarrhoea in the previous 24 hours:

Current episode of diarrhoea within the last seven days: 7354

Diarrhoea free for at least 7 days prior to the present episode: 7125

Eligible for enrolment based on the presence of signs of MSD: 2059

Enrolled fulfilling the inclusion criterion: 1029
Children with acute diarrhoea (7,125 episodes) were checked for eligibility. A total of 2,059/ 7,125 (28.9%) episodes in these children had at least one of the signs of moderate to severe diarrhoea and were eligible for enrolment in the study. A higher proportion of males, 570 (55.4%), met the final inclusion criteria than females, 459 (44.6%). Sunken eyes were the predominant sign in all age strata, whereas visible blood in stool was mostly noticed in children above the age of two years (Table 5-2).

Table 5-2. Eligibility criteria of the case children enrolled from the six health centres of BHDSS, The Gambia

<table>
<thead>
<tr>
<th></th>
<th>0-11 month (N=400)</th>
<th>12-23 month (N=455)</th>
<th>24-59 month (N=174)</th>
<th>0-59 month (N=1029)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sunken eyes</td>
<td>376 (94)</td>
<td>411 (90)</td>
<td>159 (91)</td>
<td>946 (92)</td>
</tr>
<tr>
<td>Loss of skin turgor</td>
<td>128 (32)</td>
<td>144 (32)</td>
<td>38 (22)</td>
<td>310 (30)</td>
</tr>
<tr>
<td>Required intravenous fluid</td>
<td>97 (24)</td>
<td>116 (26)</td>
<td>35 (20)</td>
<td>248 (24)</td>
</tr>
<tr>
<td>Visible blood in stool</td>
<td>43 (11)</td>
<td>88 (19)</td>
<td>42 (24)</td>
<td>173 (17)</td>
</tr>
<tr>
<td>Required hospitalization</td>
<td>118 (20)</td>
<td>128 (28)</td>
<td>42 (24)</td>
<td>288 (28)</td>
</tr>
</tbody>
</table>

N=number of children in each strata, other figures are n (%)

There were various reasons for not enrolling 1030 eligible subjects in the study (Table 5-3). Three hundred and seven (29.8%) of the 1030 primary caregivers declined to give consent to participate in the study. A stool sample was required
from all the eligible MSD cases to determine the aetiology of diarrhoea. However, 466 (45.2%) children were unable to produce a stool sample during their stay in the hospital and during the process of enrolment, or the stool was insufficient for a diagnostic test to be conducted. The study protocol was designed to enrol 8-9 cases per fortnight for even sampling throughout the year and 84 (8.2%) eligible children were not enrolled due to this strategy.
Table 5-3. Reasons for non-enrolment of eligible cases from the health centres of BHDSS in different age strata

<table>
<thead>
<tr>
<th>Reason for Non-enrolment</th>
<th>0-11 months (*N=397)</th>
<th>12-23 months (N=472)</th>
<th>24-59 months (N=161)</th>
<th>Total (N=1030)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Consent could not be obtained</td>
<td>131 (33.0)</td>
<td>127 (26.9)</td>
<td>49 (30.4)</td>
<td>307 (29.8)</td>
</tr>
<tr>
<td>Not invited by the study team for following reason</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unable to produce adequate stool</td>
<td>152 (38.3)</td>
<td>231 (48.9)</td>
<td>83 (51.6)</td>
<td>466 (45.2)</td>
</tr>
<tr>
<td>14 day quota filled</td>
<td>51 (12.8)</td>
<td>32 (6.8)</td>
<td>1 (0.06)</td>
<td>84 (8.2)</td>
</tr>
<tr>
<td>Child died before invitation</td>
<td>2 (0.5)</td>
<td>2 (0.4)</td>
<td>3 (1.8)</td>
<td>7 (0.7)</td>
</tr>
<tr>
<td>Child too sick</td>
<td>2 (0.5)</td>
<td>1 (0.2)</td>
<td>0 (0)</td>
<td>3 (0.3)</td>
</tr>
<tr>
<td>Flood</td>
<td>4 (1.0)</td>
<td>5 (1.1)</td>
<td>4 (2.5)</td>
<td>13 (1.3)</td>
</tr>
<tr>
<td>Sample could not reach lab within 18 hr.</td>
<td>1 (0.3)</td>
<td>1 (0.2)</td>
<td>0 (0)</td>
<td>2 (0.2)</td>
</tr>
<tr>
<td>Stool mixed with urine</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (0.6)</td>
<td>1 (0.1)</td>
</tr>
<tr>
<td>Unable to take rectal swab</td>
<td>8 (2.0)</td>
<td>4 (0.8)</td>
<td>1 (0.6)</td>
<td>13 (1.3)</td>
</tr>
<tr>
<td>Refused by the caregiver for the following reason</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caregiver too busy</td>
<td>40 (10.1)</td>
<td>63 (13.3)</td>
<td>15 (9.3)</td>
<td>118 (11.5)</td>
</tr>
<tr>
<td>Does not like research</td>
<td>5 (1.3)</td>
<td>5 (1.1)</td>
<td>4 (2.5)</td>
<td>14 (1.4)</td>
</tr>
<tr>
<td>Needs permission from husband</td>
<td>0 (0)</td>
<td>1 (0.2)</td>
<td>0 (0)</td>
<td>1 (0.1)</td>
</tr>
<tr>
<td>Needs permission from in-laws</td>
<td>1 (0.3)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (0.1)</td>
</tr>
</tbody>
</table>

*N is the number in each age strata.
** n (%) is the number and proportion in each category of non-enrolment.
5.4 Case Characteristics

Diarrhoea duration

The median (range) duration of diarrhoea for the MSD cases amongst all the children aged under five years old, presenting to the health centres was 3 (1-7) days (Figure 5-3). The median (range) duration was lower in the older age group 2(1-7) and Kruskal Wallis test was done, this was significantly different from both of the other two age strata (p<0.05). For 889 (86.4%) of the children with MSD, the first point of seeking care was the health centre. The rest were taken first to other facilities such as the pharmacy, traditional healer, licensed and unlicensed practitioners.

Figure 5-3. Duration of diarrhoea (median and interquartile range) among the cases enrolled from the health centres of BHDSS across different age strata
Stool Characteristics

Based on the pathophysiology we broadly classified diarrhoea into watery (suggestive of secretory and non-invasive), bloody and/or sticky mucoid (suggestive of non-secretory and invasive). The primary caregivers were asked about the stool characteristics during the period of illness: for 781 children (75.9% of the MSD cases) they reported watery and for 248 (24.1%) bloody and or sticky/mucoid stool. Of the 248 children presented with bloody and/or sticky mucoid stool 33%, 28% and 21% of their stool samples yielded *Shigella* spp., EAEC and EPEC respectively. The proportion with non-watery stool increased with increasing age (Figure 5-4).

Figure 5-4. Stool characteristics of proportion of cases enrolled at the health centres of BHDSS
Syndromes associated with diarrhoea

The stool frequency in the 24 hours preceding the visit to the health centre was between 3 and 6 among the 908 (88.2%) of 1029 children as reported by their primary caregivers. The rest of the caregivers reported higher frequencies (more than six) of stool in the same period. The primary caregivers were asked about 15 symptoms and or danger signs in a child that may have developed during the current episode of acute MSD. For 746 (72.5%) of the children the caregiver reported that they had frequently demanded water when they had MSD. Almost half of them, 523 (50.8%), became lethargic, 506 (49.2%) had associated vomiting and 455 (44.2%) developed abdominal pain. Fever and cough were present in 589 (57.2%) and 541 (52.6%) of the children respectively. Other severity indicators were blood in stool in 186 (18.1%), reduced fluid intake in 123 (12.0%), inability to drink in 6 (0.6%), irritability in 144 (14.0%), loss of consciousness in 6 (0.6%), rectal prolapse in 10 (1.0%), straining in 42 (4.1%), convulsion in 7 (0.7%), and difficulty breathing in 51 (5.0%).

Severity signs of diarrhoea and care seeking pattern

Primary caregivers were asked about the signs of moderate to severe diarrhoea observed during the current episode. A sunken eye was the very obvious sign...
that more than 90% of the primary caregivers observed in their child. Other signs observed are shown in Figure 5-5.

Figure 5-5. Observation on the severity signs of dehydration of the primary caregivers of children with diarrhoea presenting at the BHDSS

![Signs of Dehydration](image)

**Home management of current episode of MSD**

Primary caregivers did not give any remedies to 656 (63.8%) children having MSD; 73 (7.1%) children were given oral rehydration solution at home. However, 141 (13.7%) of the caregivers gave other homemade fluids such as porridge, milk, fruit juice and plain water. Herbal medicine was given to 65 (6.3%) children and 43 (4.2%) had antimicrobial agents administered before bringing them to the health centre. The caregivers offered more drink to 588 (57.1%) children when
they had MSD, the usual quantity of drink was given to 345 (33.5%) and the
remaining 96 (9.3%) children were given less. They gave less food than usual to
435 (42.3%) children, the usual amount to 416 (40.4%) and more than usual to 178
(17.3%) children.

**Assessment by the study personnel**

The vital signs and general appearances of the enrolled children, as well as the
signs of dehydration elicited by the trained study personnel are shown below in
Table 5-4. The anthropometric indices are detailed in Chapter 3 (section 3.10).
Table 5-4. Study personnel’s assessment of general appearance and signs of MSD of the enrolled children (in different age strata) with diarrhoea from the health centres of BHDSS, The Gambia

<table>
<thead>
<tr>
<th></th>
<th>0-11 months</th>
<th>12-23 months</th>
<th>24-59 months</th>
<th>0-59 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature °C</td>
<td>37.3±0.9</td>
<td>37.3±0.9</td>
<td>37.2±1.0</td>
<td>37.3±0.9</td>
</tr>
<tr>
<td>(mean±SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiratory rate</td>
<td>40.0±9.4</td>
<td>37.7±8.3</td>
<td>34.6±6.2</td>
<td>38.1±8.6</td>
</tr>
<tr>
<td>breaths/min (mean±SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chest in-drawing, n (%)</td>
<td>21 (5.3)</td>
<td>19 (4.2)</td>
<td>2 (1.1)</td>
<td>42 (4.1)</td>
</tr>
<tr>
<td>Presence of bipedal oedema, n (%)</td>
<td>2 (0.5)</td>
<td>2 (0.4)</td>
<td>0 (0)</td>
<td>4 (0.4)</td>
</tr>
<tr>
<td>Rectal prolapse, n (%)</td>
<td>2 (0.5)</td>
<td>2 (0.4)</td>
<td>2 (1.1)</td>
<td>6 (0.6)</td>
</tr>
<tr>
<td>Abnormal hair, n (%)</td>
<td>33 (8.3)</td>
<td>41 (9.0)</td>
<td>12 (6.9)</td>
<td>86 (8.4)</td>
</tr>
<tr>
<td>Flaky paint appearance of skin, n (%)</td>
<td>10 (2.5)</td>
<td>12 (2.6)</td>
<td>2 (1.1)</td>
<td>24 (2.3)</td>
</tr>
<tr>
<td>Under-nutrition, n (%)</td>
<td>61 (15.3)</td>
<td>85 (18.7)</td>
<td>26 (14.9)</td>
<td>172 (16.8)</td>
</tr>
</tbody>
</table>

**Signs of MSD**

<table>
<thead>
<tr>
<th></th>
<th>0-11 months</th>
<th>12-23 months</th>
<th>24-59 months</th>
<th>0-59 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sunken eyes, n (%)</td>
<td>373 (93.3)</td>
<td>407 (89.5)</td>
<td>160 (92.0)</td>
<td>940 (91.4)</td>
</tr>
<tr>
<td>Dry mouth, n (%)</td>
<td>257 (64.3)</td>
<td>297 (65.3)</td>
<td>115 (66.1)</td>
<td>669 (65.0)</td>
</tr>
<tr>
<td>Loss of skin turgor, n (%)</td>
<td>124 (31.0)</td>
<td>154 (33.8)</td>
<td>40 (23.0)</td>
<td>318 (30.9)</td>
</tr>
<tr>
<td>Restless/irritable, n (%)</td>
<td>21 (5.3)</td>
<td>26 (5.7)</td>
<td>5 (2.9)</td>
<td>52 (5.1)</td>
</tr>
<tr>
<td>Lethargic/unconscious, n (%)</td>
<td>14 (3.5)</td>
<td>15 (3.3)</td>
<td>4 (2.3)</td>
<td>33 (3.2)</td>
</tr>
<tr>
<td>Blood in stool, n (%)</td>
<td>21 (5.3)</td>
<td>55 (12.1)</td>
<td>25 (14.4)</td>
<td>101 (9.8)</td>
</tr>
</tbody>
</table>
The study personnel assessed that 172 (16.8%) of the children enrolled were undernourished by their appearance. Loss of skin turgor was more evident in the less than two year old age group while the presence of blood in the stool was noticed more in the two older age strata. The study personnel also elicited the signs and symptoms of MSD in each child (Figure 5-6). These signs were similar to the observations made by the primary caregivers. The Kappa test was performed to assess agreement between the primary caregiver and the study personnel with respect to recognizable signs of dehydration. The greatest agreement was obtained for sunken eyes ($\kappa=0.86$) followed by dry mouth ($\kappa=0.76$). Reasonable agreement was observed for loss of skin turgor while there was very low agreement for lethargy ($\kappa=0.11$).

Figure 5-6. Assessment of signs of dehydration in the enrolled children with diarrhoea by study personnel at the health centres of BHDSS
Management of MSD cases and their outcome

Every child enrolled in the study was evaluated for the need and type of rehydration (oral or intravenous). Among the children enrolled, 965/1029 (93.8%) of them required rehydration. Among the children requiring rehydration 713/965 (73.9%) could be managed with oral rehydration solution (ORS) and the rest needed an intravenous solution. Study personnel advised admission for 300/1029 (29.2%) of these MSD children for correction of dehydration and other management at the health centres. The rest were prescribed ORS and sent home. Admission frequencies in each of the age strata were similar. There were 122/400 (30.1%), 133/455 (29.2%) and 45/174 (25.9%) children admitted into the health care facilities in the 0-11 months, 12-23 months and 24-59 months age strata respectively. The median (range) duration of hospital stay for all the children admitted was 3 (1-11) days (Figure 5-7), and was not significantly different by age group (p=0.2)
Outcome of acute MSD

An outcome assessment was possible at the time of discharge from the hospital. Of the 300 children admitted into a health centre, 84 (28.0%) children recovered fully and 198 (66.0%) children had improved by the time of discharge from the hospital. The condition of one child did not change and 17 (5.7%) children died during their admission. Stool samples of five of these 17 children yielded no pathogen. Single pathogens were detected from the stool samples of children in following order: EPEC=2, EAEC=2, Norovirus=1, S.flexneri=1. Stool samples from the rest of the children grew multiple pathogens; they were EPEC and EAEC=2,
EAEC and ETEC(ST)=1, *S. flexneri*, EPEC and EAEC=1, *S. flexneri* and *S. sonnei*=1, *S. flexneri* and Norovirus=1.

5.5 Aetiology of MSD and pathogens detected from stool samples of asymptomatic controls

None of the stools from cases or controls grew *V. cholerae* or *Salmonella* Typhi. *Aeromonas* spp. were isolated from only two controls and *Entamoeba histolytica* from only two cases and four controls. We did not include *Aeromonas* spp. and *Entamoeba histolytica* in the matched pair analysis given the small numbers. The standardised diagnostic tools enabled us to isolate at least one organism of interest from 85.3% of the cases and 74.1% of the controls. The isolation of one of the protozoa *G. lamblia* was higher in controls than the cases; the overall pathogen isolation excluding *G. lamblia* was 81.4% and 62.0% in cases and controls respectively. Table 5-5 below shows the isolation rate of pathogens in different age strata.
Table 5-5. Proportion of single and multiple pathogens isolated from case and control children from BHDSS, The Gambia

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>0-11 months</th>
<th>12-23 months</th>
<th>24-59 months</th>
<th>0-59 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case (N=400)</td>
<td>Control (N=585)</td>
<td>Case (N=455)</td>
<td>Control (N=639)</td>
</tr>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>No</td>
<td>45 (11.2)</td>
<td>174 (29.7)</td>
<td>75 (16.5)</td>
<td>155 (24.3)</td>
</tr>
<tr>
<td>Overall</td>
<td>355 (88.8)</td>
<td>411 (70.3)</td>
<td>383 (83.5)</td>
<td>484 (75.7)</td>
</tr>
<tr>
<td>Overall without Giardia</td>
<td>345 (86.3)</td>
<td>380 (65.0)</td>
<td>363 (79.8)</td>
<td>399 (62.4)</td>
</tr>
<tr>
<td>Single</td>
<td>182 (45.5)</td>
<td>250 (42.7)</td>
<td>169 (37.1)</td>
<td>263 (41.2)</td>
</tr>
<tr>
<td>Multiple</td>
<td>173 (43.3)</td>
<td>161 (27.5)</td>
<td>211 (46.4)</td>
<td>221 (34.6)</td>
</tr>
</tbody>
</table>

N=Number of children enrolled, figures are n(%) unless mentioned otherwise

Overall in the under-five age group of children, rotavirus proved to be the major pathogen associated with diarrhoea, being present in 19.9% of the cases compared to only 2.7% controls with an OR of 16.6 (95% CI:9.8-28.2 and p<0.001) followed by *Shigella* spp.: 11.1% in cases and 2.9% in controls (OR 4.7;95% CI 3.2-6.9 and p<0.001). The detailed isolation rate is shown in the Tables 5-6 to 5-9.
Table 5-6. Pathogens associated with moderate to severe diarrhoea in children aged under five years old in rural Gambia

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>Case (N=1029) n (%)</th>
<th>Control (N=1569) n (%)</th>
<th>OR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacterial pathogens</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Shigella</em> spp.</td>
<td>114 (11.1)</td>
<td>46 (2.9)</td>
<td>4.7</td>
<td>3.2-6.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ETEC all</td>
<td>181 (17.6)</td>
<td>201 (12.8)</td>
<td>1.4</td>
<td>1.1-1.7</td>
<td>0.009</td>
</tr>
<tr>
<td>ETEC any ST</td>
<td>114 (11.1)</td>
<td>83 (5.3)</td>
<td>2.1</td>
<td>1.5-2.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ETEC LT only</td>
<td>67 (6.5)</td>
<td>118 (7.5)</td>
<td>0.8</td>
<td>0.6-1.1</td>
<td>0.210</td>
</tr>
<tr>
<td><em>Campylobacter</em> spp.</td>
<td>41 (4.0)</td>
<td>65 (4.1)</td>
<td>1.1</td>
<td>0.7-1.6</td>
<td>0.773</td>
</tr>
<tr>
<td><em>Salmonella enterica</em> non-Typhi*</td>
<td>17 (1.7)</td>
<td>25 (1.6)</td>
<td>1.1</td>
<td>0.6-2.1</td>
<td>0.785</td>
</tr>
<tr>
<td><strong>Viral Pathogens</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rotavirus</td>
<td>205 (19.9)</td>
<td>42 (2.7)</td>
<td>16.6</td>
<td>9.8-28.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Norovirus</td>
<td>148 (14.4)</td>
<td>133 (8.5)</td>
<td>1.8</td>
<td>1.3-2.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Noro G1</td>
<td>37 (3.6)</td>
<td>61 (3.9)</td>
<td>0.8</td>
<td>0.5-1.3</td>
<td>0.465</td>
</tr>
<tr>
<td>Noro G2</td>
<td>120 (11.7)</td>
<td>75 (4.8)</td>
<td>2.5</td>
<td>1.8-3.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>35 (3.4)</td>
<td>41 (2.6)</td>
<td>1.7</td>
<td>1.1-2.8</td>
<td>0.031</td>
</tr>
<tr>
<td>Adeno 40/41</td>
<td>23 (2.2)</td>
<td>11 (0.7)</td>
<td>3.6</td>
<td>1.7-7.7</td>
<td>0.001</td>
</tr>
<tr>
<td>Adeno non 40/41</td>
<td>12 (1.2)</td>
<td>30 (1.9)</td>
<td>0.9</td>
<td>0.4-1.8</td>
<td>0.711</td>
</tr>
<tr>
<td>Sapovirus</td>
<td>46 (4.5)</td>
<td>68 (4.3)</td>
<td>0.9</td>
<td>0.6-1.4</td>
<td>0.789</td>
</tr>
<tr>
<td>Astrovirus</td>
<td>51 (5.0)</td>
<td>45 (2.9)</td>
<td>1.7</td>
<td>1.1-2.6</td>
<td>0.016</td>
</tr>
<tr>
<td><strong>Protozoal pathogens</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Cryptosporidium</em> spp.</td>
<td>114 (11.1)</td>
<td>79 (5.0)</td>
<td>2.7</td>
<td>1.9-3.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><em>G. lamblia</em></td>
<td>175 (17.0)</td>
<td>465 (29.6)</td>
<td>0.5</td>
<td>0.4-0.6</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Note: N=Number of children enrolled; figures are n(%) unless mentioned otherwise
ETEC=Enterotoxigenic *E.coli*; EAEC: Enteroaggregative *E.coli*; EPEC=Enteropathogenic *E.coli*; ST=Heat stable toxin; LT=Heat Labile toxin; NTS=Non typhoidal salmonella
S.flexneri and S.sonnei were the predominant sero-species within the Shigella spp. Seventy eight (7.6%) isolates of S.flexneri were from the MSD cases compared to 22(1.4%) from the controls (OR 7.1, 95% CI: 4.2-12.0; p<0.001). S.sonnei was isolated from 24 (2.3%) cases and 18(1.1%) controls (OR 2.1, 95% CI: 1.1-4.0; p=0.035). S.boydii was isolated from 5 cases and 4 controls while two S.dysenteriae were isolated, one case and one control. The serotyping of S.flexneri showed 2a (case: 36, control: 9), 1b (case: 14, control: 2), 6 (case:11, control:6) , X (case:5, control:1), 3a (case:5, control:1), 2b (case:4, control:2), 1a (case:1, control:0), non-typable (case:2, control:0) and 3b(case:0, control:1). Among the bacterial pathogens other than Shigella spp. Enterotoxigenic E. coli (ETEC) was responsible for acute MSD in the children. Only ST (heat stable toxin producing) containing Enterotoxigenic E. coli were pathogenic. Norovirus (G2 serotype), Adenovirus (Adeno40/41 serotype) and Astrovirus were the other viral pathogens that significantly contributed to MSD. Cryptosporidium spp. was the only protozoon which was shown to be diarrhoegenic in this population. G. lamblia was present significantly more in healthy controls (29.6%) than the cases (17.0%) (OR 0.5; 95% CI: 0.4-0.6, p <0.001)

Similar analyses across the different age strata to show association between the different diarrhoeagenic pathogens and MSD by age was done. Table 5.7 shows the distribution in the 0-11 month age group
Table 5-7. Pathogens associated with moderate to severe diarrhoea in 0-11 month age old children in rural Gambia

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>Case (N=400) n (%)</th>
<th>Control (N=585) n (%)</th>
<th>OR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacterial pathogens</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shigella spp.</td>
<td>16 (4.0)</td>
<td>4 (0.7)</td>
<td>8.0</td>
<td>2.3-27.9</td>
<td>0.001</td>
</tr>
<tr>
<td>ETEC all</td>
<td>64 (16.0)</td>
<td>66 (11.3)</td>
<td>1.4</td>
<td>0.97-2.1</td>
<td>0.066</td>
</tr>
<tr>
<td>ETEC any ST</td>
<td>38 (9.5)</td>
<td>30 (5.1)</td>
<td>2.0</td>
<td>1.1-3.4</td>
<td>0.014</td>
</tr>
<tr>
<td>ETEC LT only</td>
<td>26 (6.5)</td>
<td>36 (6.2)</td>
<td>1.0</td>
<td>0.6-1.7</td>
<td>0.982</td>
</tr>
<tr>
<td>Campylobacter spp.</td>
<td>20 (5.0)</td>
<td>30 (5.1)</td>
<td>1.2</td>
<td>0.6-2.2</td>
<td>0.599</td>
</tr>
<tr>
<td>Salmonella enterica non-Typhi</td>
<td>5 (1.3)</td>
<td>14 (2.4)</td>
<td>0.5</td>
<td>0.2-1.5</td>
<td>0.221</td>
</tr>
<tr>
<td><strong>Viral Pathogens</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rotavirus</td>
<td>100 (25.0)</td>
<td>25 (4.3)</td>
<td>12.4</td>
<td>6.2-24.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Norovirus</td>
<td>54 (13.5)</td>
<td>44 (7.5)</td>
<td>1.7</td>
<td>1.1-2.8</td>
<td>0.023</td>
</tr>
<tr>
<td>Noro G1</td>
<td>8 (2.0)</td>
<td>10 (1.7)</td>
<td>1.1</td>
<td>0.4-3.0</td>
<td>0.802</td>
</tr>
<tr>
<td>Noro G2</td>
<td>48 (12.0)</td>
<td>34 (5.8)</td>
<td>2.0</td>
<td>1.2-3.5</td>
<td>0.009</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>18 (4.5)</td>
<td>12 (2.1)</td>
<td>2.8</td>
<td>1.3-6.2</td>
<td>0.011</td>
</tr>
<tr>
<td>Adeno 40/41</td>
<td>11 (2.8)</td>
<td>5 (0.9)</td>
<td>3.4</td>
<td>1.2-9.9</td>
<td>0.027</td>
</tr>
<tr>
<td>Adeno non 40/41</td>
<td>7 (1.8)</td>
<td>7 (1.2)</td>
<td>2.2</td>
<td>0.6-7.4</td>
<td>0.208</td>
</tr>
<tr>
<td>Sapovirus</td>
<td>11 (2.8)</td>
<td>33 (5.6)</td>
<td>0.4</td>
<td>0.2-0.9</td>
<td>0.024</td>
</tr>
<tr>
<td>Astrovirus</td>
<td>18 (4.5)</td>
<td>16 (2.7)</td>
<td>1.6</td>
<td>0.8-3.3</td>
<td>0.210</td>
</tr>
<tr>
<td><strong>Protozoal pathogens</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cryptosporidium spp.</td>
<td>61 (15.3)</td>
<td>38 (6.5)</td>
<td>3.3</td>
<td>2.0-5.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>G. lamblia</td>
<td>41 (10.3)</td>
<td>81 (13.9)</td>
<td>0.8</td>
<td>0.5-1.2</td>
<td>0.274</td>
</tr>
</tbody>
</table>

Note: N= number of children enrolled; Figures are N (%) unless mentioned otherwise
ETEC=Enterotoxigenic E.coli; EAEC: Enteraggregative E.coli; EPEC=Enteropathogenic E.coli; ST=Heat stable toxin; LT=Heat Labile toxin; NTS=Non typhoidal salmonella
Viral (rotavirus, adenovirus 40/41 and norovirus G2) and protozoan (Cryptosporidium spp.) infections are a common cause of MSD in infancy with rotavirus making the biggest contribution. Among the bacterial pathogens ETEC (ST) was the main organism. In infancy G.lamblia was present more in controls than MSD cases.
Table 5-8. Pathogens associated with moderate to severe diarrhoea in 12-23 months age old children in rural Gambia

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>Case (N=455) n (%)</th>
<th>Control (N=639) n (%)</th>
<th>OR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacterial pathogens</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Shigella</em> spp.</td>
<td>70 (15.4)</td>
<td>28 (4.9)</td>
<td>4.2</td>
<td>2.6-6.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ETEC all</td>
<td>89 (19.6)</td>
<td>99 (15.5)</td>
<td>1.2</td>
<td>0.9-1.6</td>
<td>0.306</td>
</tr>
<tr>
<td>ETEC any ST</td>
<td>57 (12.5)</td>
<td>44 (6.9)</td>
<td>1.8</td>
<td>1.1-2.70</td>
<td>0.010</td>
</tr>
<tr>
<td>ETEC LT only</td>
<td>32 (7.0)</td>
<td>55 (8.6)</td>
<td>0.7</td>
<td>0.5-1.2</td>
<td>0.187</td>
</tr>
<tr>
<td><em>Campylobacter</em> spp.</td>
<td>19 (4.2)</td>
<td>26 (4.1)</td>
<td>1.2</td>
<td>0.6-2.2</td>
<td>0.637</td>
</tr>
<tr>
<td>*Salmonella enterica non-<em>Typhi</em></td>
<td>8 (1.8)</td>
<td>11 (1.7)</td>
<td>1.2</td>
<td>0.5-3.1</td>
<td>0.713</td>
</tr>
<tr>
<td><strong>Viral Pathogens</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rotavirus</td>
<td>80 (17.6)</td>
<td>10 (1.6)</td>
<td>47.3</td>
<td>11.6-193.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Norovirus</td>
<td>63 (13.9)</td>
<td>55 (8.6)</td>
<td>1.8</td>
<td>1.2-2.8</td>
<td>0.005</td>
</tr>
<tr>
<td>Noro G1</td>
<td>16 (3.5)</td>
<td>27 (4.2)</td>
<td>0.8</td>
<td>0.4-1.6</td>
<td>0.564</td>
</tr>
<tr>
<td>Noro G2</td>
<td>53 (11.7)</td>
<td>30 (4.7)</td>
<td>2.7</td>
<td>1.6-4.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>16 (3.5)</td>
<td>15 (2.4)</td>
<td>2.1</td>
<td>0.96-4.5</td>
<td>0.063</td>
</tr>
<tr>
<td>Adeno 40/41</td>
<td>12 (2.6)</td>
<td>3 (0.5)</td>
<td>8.5</td>
<td>1.8-38.7</td>
<td>0.006</td>
</tr>
<tr>
<td>Adeno non 40/41</td>
<td>4 (0.9)</td>
<td>12 (1.9)</td>
<td>0.7</td>
<td>0.2-2.3</td>
<td>0.543</td>
</tr>
<tr>
<td>Sapovirus</td>
<td>25 (5.5)</td>
<td>22 (3.4)</td>
<td>1.5</td>
<td>0.8-2.8</td>
<td>0.161</td>
</tr>
<tr>
<td>Astrovirus</td>
<td>24 (5.3)</td>
<td>15 (2.4)</td>
<td>2.2</td>
<td>1.1-4.3</td>
<td>0.023</td>
</tr>
<tr>
<td><strong>Protozoal pathogens</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Cryptosporidium</em> spp.</td>
<td>50 (11.0)</td>
<td>34 (5.3)</td>
<td>2.5</td>
<td>1.5-4.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><em>G. lamblia</em></td>
<td>76 (16.7)</td>
<td>227 (35.5)</td>
<td>0.4</td>
<td>0.3-0.5</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Note: N= total number of children enrolled; Figures are n(%) unless mentioned otherwise
ETEC=Enterotoxigenic *E. coli*; EAEC: Enteroaggregative *E. coli*; EPEC=Enteropathogenic *E. coli*; ST=Heat stable toxin; LT=Heat Labile toxin; NTS=Non typhoidal salmonella
In the second age group (12-23 months) Table 5-8, bacterial pathogens were more frequently isolated from the MSD cases than the controls. The isolation patterns of viral agents were fairly similar (with addition of astrovirus) to the youngest age group. *G. lamblia* was more frequently isolated from the healthy controls.
Table 5-9. Pathogens associated with moderate to severe diarrhoea in 24-59 months age old children in rural Gambia

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>Case (n=174)</th>
<th>Control (n=345)</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacterial pathogens</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Shigella</em> spp.</td>
<td>28 (16.1)</td>
<td>14 (4.1)</td>
<td>5.0</td>
<td>2.4-10.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ETEC all</td>
<td>28 (16.1)</td>
<td>36 (10.4)</td>
<td>1.8</td>
<td>1.0-3.3</td>
<td>0.041</td>
</tr>
<tr>
<td>ETEC any ST</td>
<td>19 (10.9)</td>
<td>9 (2.6)</td>
<td>4.5</td>
<td>1.9-10.5</td>
<td>0.001</td>
</tr>
<tr>
<td>ETEC LT only</td>
<td>9 (5.2)</td>
<td>27 (7.8)</td>
<td>0.7</td>
<td>0.3-1.6</td>
<td>0.373</td>
</tr>
<tr>
<td><em>Campylobacter</em> spp.</td>
<td>2 (1.2)</td>
<td>9 (2.6)</td>
<td>0.4</td>
<td>0.1-2.0</td>
<td>0.268</td>
</tr>
<tr>
<td><em>Salmonella enterica</em> non-Typhi*</td>
<td>4(2.3)</td>
<td>0(0)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Viral Pathogens</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rotavirus</td>
<td>25 (14.4)</td>
<td>7 (2.0)</td>
<td>10.5</td>
<td>3.6-30.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Norovirus</td>
<td>31 (17.8)</td>
<td>34 (9.9)</td>
<td>1.6</td>
<td>0.9-2.9</td>
<td>0.102</td>
</tr>
<tr>
<td>Noro G1</td>
<td>13 (7.5)</td>
<td>24 (7.0)</td>
<td>0.7</td>
<td>0.3-1.6</td>
<td>0.440</td>
</tr>
<tr>
<td>Noro G2</td>
<td>19 (10.9)</td>
<td>11 (3.2)</td>
<td>3.5</td>
<td>1.5-7.9</td>
<td>0.003</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>1 (0.6)</td>
<td>14 (4.1)</td>
<td>0.2</td>
<td>0.03-1.6</td>
<td>0.137</td>
</tr>
<tr>
<td>Adeno 40/41</td>
<td>0 (0)</td>
<td>3 (0.9)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Adeno non 40/41</td>
<td>1 (0.6)</td>
<td>11 (3.2)</td>
<td>0.3</td>
<td>0.04-2.2</td>
<td>0.221</td>
</tr>
<tr>
<td>Sapovirus</td>
<td>10 (5.8)</td>
<td>13 (3.8)</td>
<td>1.3</td>
<td>0.5-3.2</td>
<td>0.565</td>
</tr>
<tr>
<td>Astrovirus</td>
<td>9 (5.2)</td>
<td>14 (4.1)</td>
<td>1.2</td>
<td>0.5-2.9</td>
<td>0.749</td>
</tr>
<tr>
<td><strong>Protozoal pathogens</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Cryptosporidium</em> spp.</td>
<td>3 (1.7)</td>
<td>7 (2.0)</td>
<td>1.1</td>
<td>0.3-4.3</td>
<td>0.906</td>
</tr>
<tr>
<td><em>G. lamblia</em></td>
<td>58 (33.3)</td>
<td>157 (45.5)</td>
<td>0.6</td>
<td>0.4-0.9</td>
<td>0.015</td>
</tr>
</tbody>
</table>

Note: N=number of children enrolled; Figures are n(%) unless mentioned otherwise
ETEC=Enterotoxigenic *E. coli*; EAEC: Enteroaggregative *E. coli*; EPEC=Enteropathogenic *E. coli*;
ST=Heat stable toxin; LT=Heat Labile toxin; NTS=Non typhoidal salmonella
There was a similar pattern of bacterial isolation in the older age group as seen in the 12-23 months age group. *Shigella* spp. was predominant (Table 5-9). The detection of viral pathogens was low but significantly different in MSD cases and controls (only Rotavirus and Noro G2). *G. lamblia* continued to be excreted more in the stools of controls than that of the cases. In contrast to the younger age groups, *Cryptosporidium* spp. showed no significant evidence of pathogenicity in 24-59 month old children.

### 5.6 Top five pathogens

The overall pathogen isolation results for the MSD cases suggest that there are several pathogens causing acute diarrhoea ETEC, rotavirus, norovirus, adenovirus, astrovirus and *Cryptosporidium* spp.) and *Shigella* spp.

The notable and top five pathogen causing diarrhoea among the under five children in Gambia are rotavirus, ETEC (ST only), *Shigella* spp., *Cryptosporidium* spp. and norovirus (Phenotype G2). The overall pathogenicity indices (ratio of isolation among cases divided by the isolation rate of that pathogen among controls) of the top five pathogen in the under five years of age children were 7.4, 3.8, 2.4, 2.2 and 2.1 for rotavirus, *Shigella* spp., norovirus (G2), *Cryptosporidium* spp. and ETEC (ST only) respectively.
5.7 Seasonality

It was observed that certain pathogen-specific diarrhoeal illnesses in children were highly seasonal (Figure 5-8). Rotavirus is mainly isolated in the dry and cold season (January and February) while *Shigella* spp., *Cryptosporidium* spp., ETEC and norovirus are usually seen during the wet and hot months (May-September). However norovirus also had its peak in the winter.

Figure 5-8. Seasonal pattern of top five pathogens detected from the stool specimens of children aged under five years old in rural Gambia
5.8 Discussion

The pathogens causing MSD in this rural endemic African setting include viruses, bacteria and protozoa and tend to be seasonal. Epidemics and outbreaks are common in these areas but the containment of the disease remains far from reach. A great majority of children remain asymptomatic in the endemic area and without having a matched control the relative pathogenicity of organisms causing diarrhoea in the population cannot be measured. 184, 185

In this case-control study, facility based passive surveillance was conducted for case recruitment in all the health centres serving the population of BHDSS for three consecutive years. An earlier study indicated that the 88% of the primary caregivers in The Gambia could give an accurate history related to the severity of any illnesses.186 This study showed that primary caregivers observation of the signs of dehydration were very close to the ones elicited and confirmed by the trained study personnel at the time of enrolment. Given the nature and characteristics, it was expected that the primary caregivers of the children who had MSD would seek care at the facilities. HUAS study (Chapter 4) revealed that nearly half of the children with diarrhoea did not seek care at the health facilities, and thus it is possible to assume that the true incidence of diarrhoea episodes in the population may be twice that which was observed at the health facilities. It was found that the facility based incidence rate of diarrhoea episodes among children aged under five years old in rural Gambia declined over the
study period, consistent with a similar trend in other low and middle income countries, the decline was from an estimated 3.4 episodes/child year in 1990 to 2.9 episodes/child year in 2010.187

A syndromic approach to diarrhoea in children with empiric treatment has been widely accepted in public health practice and can be applied in developing settings.188, 189, 190 In this study, stool characteristics in children with diarrhoea are classified as non-invasive (watery stool) or invasive (bloody/mucoid stool). Potential danger signs during an episode of moderate to severe diarrhoea were also identified. These danger signs together with the stool characteristics make a realistic syndromic approach to diagnosing moderate to severe diarrhoea.191 This is potentially helpful for those making decisions regarding prescribing antimicrobial agents in the absence of on-going laboratory based evidence.

Primary caregivers of 63.6% of the children did not give any remedies before seeking care out of their home and only 7.1% of the children were given ORS at home. The ORS use at home was similar to other countries in the region.91, 183 This shows the lack of knowledge of disease progression and a failure to understand the gravity of the illness. However, the rationale of ORS use was well orchestrated in the health facilities. The use of ORS, as a part of the management tool was much higher (73.9%) compared to 64% overall use in sub Saharan countries.192, 193 The majority of the caregivers provided the children with
more fluids during the episode of diarrhoea but restricted administration of solid food. The use of appropriate fluid and food is important for diarrhoea management. While offering more fluids can be seen as a measure of fluid replacement, the content of fluid other than ORS can cause electrolyte imbalance in the children. The restriction of solid food may compromise nutritional status.

Nearly one third of the children with MSD were admitted to the health facilities. Both the median duration of diarrhoea and duration of the hospital stay during the current episode was three days. This was lower than the mean duration described in other low and middle income countries (reported to be 4.3 days for mild episodes and 8.4 days for severe dehydration) but still reveals a considerable burden on the health system in a resource poor setting. In an overburdened health care facility the patient turnover needs to be quick to accommodate the most serious patients. Two thirds (66.0%) of the admitted children could not be kept at the facility for full recovery. However, it was not possible to clearly establish the actual hospital stay required for an episode of acute diarrhoea or what proportion may develop persistent diarrhoea.

Most of the studies on diarrhoeal disease aetiology conducted in sub-Saharan Africa were focused on a single or only a few pathogens of interest, overlooking the diverse aetiology of childhood diarrhoea. In this study,
apart from characterising and estimating the magnitude of the disease presenting in the health facilities; the pathogens responsible for causing diarrhoea were identified through a case-control approach. At least one diarrhoeagenic pathogen from 85.3% (81.4% without the *G. lamblia*) of the cases and 74.1% (62.0% without *G. lamblia*) among the controls were identified. To our knowledge, this is the highest rate of identification in any study conducted in sub-Saharan Africa.\textsuperscript{9} \textsuperscript{201} *G. lamblia* was isolated more from the control or asymptomatic children than from the cases as seen in other studies.\textsuperscript{202, 203} The symptomatology of diarrhoea due to *G. lamblia* is genotype dependent.\textsuperscript{204} The parasite colonises in the healthy gut wall of healthy children and its presence as a co-pathogen does not alter the clinical presentation of acute diarrhoea.\textsuperscript{205} It is possible that the parasite protects children from diarrhoeal disease caused by other pathogens.\textsuperscript{206}

The top five pathogens isolated from the children aged under five years old in Gambia were rotavirus, *Shigella* spp., ETEC, *Cryptosporidium* spp. and norovirus. The pathogenicity indices of more than two show the propensity of these pathogens to produce MSD in children.\textsuperscript{101, 207, 208} All five pathogens were associated with MSD in all the age strata except *Cryptosporidium* spp., which was only seen in children in less than two years of age. Other studies in the region have shown associations of *Cryptosporidium* spp. with malnutrition and persistent diarrhoea, in immuno compromised children and children with HIV.
infection.209, 210, 211, 212, 213 The identification rate of Cryptosporidium spp. in this study, in acute MSD and in a low HIV setting seems an unexpected finding and suggests for the first time that it is an important enteric pathogen in young children in rural Africa. The overwhelming malnutrition in the under-five population in this study setting (chapter 7) could possibly explain the diarrhoea caused by Cryptosporidium spp.

Rotavirus was the leading pathogen detected in the stool samples of children. This is consistent with the other findings in Africa and elsewhere globally.214, 215 In this study we have observed that norovirus, specifically of the GII genotype, was also associated significantly with acute MSD in children.217 The emergence of norovirus as an enteric pathogen in children in rural Africa underpins its importance.218, 219 The newer and improved diagnostic techniques applied in this study facilitated the detection of other viruses such as adeno 40/41, sapo virus and astro virus in varying frequencies in different age groups and confirms their presence in the region.220, 221

Among the bacteria Shigella was the leading pathogen in all age groups. This result indicates that the clinical syndrome of dysentery could still be a problem in this population.222, 223 S. flexneri was the most frequently isolated sero-group in this population. ST producing ETEC stood out to be an important diarrhoeagenic
E. coli in this population. The overall isolation rate of ETEC remained higher than that of the Shigella spp, as seen in other countries across Africa.\textsuperscript{7, 9, 102, 115, 200}

The seasonality of detection of the top five pathogens typically reflects the interaction between the pathogen and the environment. Rotavirus was more common in the dry and cold months of the year, consistent with the findings of a large scale meta-analysis of 26 studies conducted in the tropics.\textsuperscript{224} We did not detect the presence of rotavirus diarrhoea throughout the year as some of the studies reported earlier.\textsuperscript{70, 225} Seasonality for the other four pathogens was consistent with earlier reports. Cryptosporidium spp. predominated in the rainy season, consistent with other studies conducted in Africa.\textsuperscript{209, 211, 213, 226} The humid and hot months favoured ETEC and Shigella, although these organisms were present throughout the year.\textsuperscript{227, 228} We noticed a peak of norovirus infection during the rainy season, as has been seen in Malawi \textsuperscript{219} and Madagascar.\textsuperscript{229} As an emerging enteric pathogen, the potential seasonality of norovirus diarrhoea needs further study.\textsuperscript{230} The seasonality of pathogen specific diarrhoea prevalence in a population helps in determining the policy to undertake treatment measure and inform the health care provider for emergency response to outbreak situation.

One of the limitations of the study is that a large number of children presenting with MSD could not be enrolled for various reasons. One issue was collecting
stool for analysis. It appeared that the frequency of stool reduces with time. Also primary caregivers, who were needed for enrolment of their children, often declined due to time commitments and household responsibilities.

This case-control study conducted over a period of three years described the incidence of diarrhoea presenting to health facilities, the incidence of MSD diarrhoea in these individuals and its aetiology. The study was conducted in a DSS that covers one tenth of the Gambian national population. The homogeneity, lifestyle and environment of the population in Gambia and in West Africa make this study generalizable to the greater rural Africa. The syndromes evaluated in the study will help in empirical treatment of diarrhoea in the facility. The study enabled to identify key pathogens and their seasonality in causing diarrhoea among the children aged under five years old. While Rotavirus, *Shigella* spp., ETEC and *Cryptosporidium* spp. are well recognized as pathogens, the role of norovirus needs substantial evaluation for both clinical and epidemiological outcomes. These findings are important for policy makers to rationalise treatment policy, mobilise resources and give impetus to vaccine introduction into the country.
Chapter 6- Risk factors for diarrhoea in children aged under five years old in rural Gambia
6.1 Introduction

Though risk factors for diarrhoea are known, their respective roles need to be understood in the context of the geographic location, socio-demographic strata, environment and personal hygiene. In this chapter, the assessment of various risk factors for MSD in children aged under five years old in rural Gambia are presented and the reasons behind and possible ways to mitigate the identified risk factors are discussed. The risk factors assessed in this study are based on biological plausibility and their presence both in the direct and intermediate pathways. The risk factors have been explored, and identified in studies elsewhere; in different settings and with different study designs. The risk factors were categorized into the following groups;

1. Demographic and epidemiological information that included information about the primary caregiver and their education, father, and overcrowding. Socioeconomic status that included the information on household structure, possession of certain household items and type of cooking fuel. A wealth index was calculated based on possession of all or some of these items.

2. Dwelling of certain domestic animals in the household.
3. Water, sanitation and hygiene information that included the source of water, availability, storage and treatment practice. The caregivers were also asked about the presence of type of toilet facilities in the household, and faeces disposal practice.


Possible risk factors were evaluated through a prospectively conducted case control study design. The cases were children attending the health centres in the DSS area with moderate to severe diarrhoea while controls were recruited from the community and met the inclusion criteria (chapter 3, section 3.3)

Conditional logistic regression for the bivariate and multivariate analyses of the risk factors was performed. Factors that were significant at a level of $p=0.05$ were included in a stepwise backward regression for a multivariate analysis as detailed in the method chapter (chapter 3, section 3.12).

6.2 Socio-demographics

Mothers were usually the primary caregivers of children aged under five years old in The Gambian population under study, and their level of formal schooling was poor. In table 6-1, we show the socio-demographic characteristics of the cases and controls. We observed that the number of people sleeping in a household was between 2 and 229. Because of this wide variation and since
people live in an extended family, the household densities (the number of people per sleeping room present in the household); as a marker of crowding are presented. A wealth index based on the possession of certain household items including television, radio, electricity, phone, bicycle, agricultural land, type of fuel used for cooking (details in the questionnaire CRF04A, attached as appendix-1) was calculated. Households were categorized to the lower 40%, middle 40% and upper 20% on the basis of their wealth index percentiles for the purposes of the study. However, this classification should not be considered as a marker for absolute poverty or richness.
Table 6-1. Demographic and household risk factors for moderate to severe diarrhoea in under-five children in rural Gambia

<table>
<thead>
<tr>
<th>Variables</th>
<th>Case N=1029</th>
<th>Control N=1569</th>
<th>Bivariate OR (95% CI)</th>
<th>P</th>
<th>Multivariate OR (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary caregiver other than mother</td>
<td>36 (3.5)</td>
<td>22 (1.4)</td>
<td>2.8 (1.6-5.0)</td>
<td>&lt;0.001</td>
<td>2.7 (1.5-4.8)</td>
<td>0.001</td>
</tr>
<tr>
<td>Mother died or lives out of the household</td>
<td>24 (2.3)</td>
<td>12 (0.8)</td>
<td>3.5 (1.7-7.4)</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No formal schooling of primary caregiver</td>
<td>668 (64.9)</td>
<td>699 (44.6)</td>
<td>2.6 (2.2-3.2)</td>
<td>&lt;0.001</td>
<td>2.5 (2.1-3.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Father died or lives out of the household</td>
<td>304 (29.5)</td>
<td>376 (24.0)</td>
<td>1.3 (1.1-1.6)</td>
<td>0.002</td>
<td>1.3 (1.1-1.6)</td>
<td>0.008</td>
</tr>
<tr>
<td>Household density-No. of people/rooms in the household</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-4 person/sleeping room</td>
<td>898 (87.3)</td>
<td>1367 (87.1)</td>
<td>ref</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-10 person/sleeping room</td>
<td>128 (12.4)</td>
<td>198 (12.6)</td>
<td>1.1 (0.8-1.4)</td>
<td>0.667</td>
<td>1.01 (0.8-1.3)</td>
<td>0.933</td>
</tr>
<tr>
<td>&gt;10 person/sleeping room</td>
<td>3 (0.3)</td>
<td>3(0.2)</td>
<td>1.2 (0.2-6.2)</td>
<td>0.819</td>
<td>1.02(0.2-5.7)</td>
<td>0.976</td>
</tr>
<tr>
<td>No. of under five children in the household</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-4</td>
<td>498 (48.4)</td>
<td>665(42.4)</td>
<td>0.8 (0.2-6.2)</td>
<td>0.016</td>
<td>0.8 (0.2-6.2)</td>
<td>0.065</td>
</tr>
<tr>
<td>5-10</td>
<td>449 (43.6)</td>
<td>711(45.3)</td>
<td>0.8 (0.2-6.2)</td>
<td>0.016</td>
<td>0.8 (0.2-6.2)</td>
<td>0.065</td>
</tr>
</tbody>
</table>
### Crude associations (odds ratio) between risk factors and diarrhoea on a complete set of 1029 cases and 1569 matched controls using conditional logistic regression were estimated. The strongest relationships were found for primary caregiver other than mother (OR 2.8, 95% CI: 1.6-5.0; p=<0.001), mother being out of household/dead (OR 3.5, 95% CI: 1.7-7.4; p=0.001), no formal schooling of the primary caregiver [OR 2.6 (95% CI: 2.2-3.2), and father being out of household/dead (OR 1.3, 95% CI: 1.1-1.6; p=0.002). Having more children less than five years of age in the same household was associated with decreased risk of diarrhoea in this population. Neither overcrowding nor wealth index were

<table>
<thead>
<tr>
<th>Variables</th>
<th>Case N=1029</th>
<th>Control N=1569</th>
<th>Bivariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>OR (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>&gt;10</td>
<td>82 (8.0)</td>
<td>193(12.3)</td>
<td>0.5 (0.7-1.0)</td>
<td>&lt;0.00</td>
</tr>
<tr>
<td>Wealth Index</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower 40%</td>
<td>420 (40.8)</td>
<td>619 39.5)</td>
<td>ref</td>
<td></td>
</tr>
<tr>
<td>Middle 40%</td>
<td>412 (40.0)</td>
<td>629 (40.1)</td>
<td>0.9 (0.8-1.1)</td>
<td>0.377</td>
</tr>
<tr>
<td>Upper 20%</td>
<td>197 (19.1)</td>
<td>321 (20.5)</td>
<td>0.9 (0.7-1.2)</td>
<td>0.441</td>
</tr>
</tbody>
</table>
found to be associated with moderate to severe diarrhoea in this population (Table 6-1).

It is understandable that if the mother is dead or lives out of the household, the primary caregiver for the child would be somebody else; hence we did not include the “mother lives out of household or dead” in the multivariate analysis. The multivariate analysis showed that the primary caregiver other than the mother, no formal schooling of the primary caregiver, and the father being out of the household were independently associated with MSD while an increased number of children aged under five years old remained associated with decreased risk (table 6-1).

### 6.3 Animals’ dwelling in the household

Certain animals, rodents and livestock are known to be reservoirs of some of the pathogens causing diarrhoea, notably *E.coli*, *Campylobacter* spp., *Cryptosporidium* spp. and non typhoidal *Salmonella*. Table 6-2 shows the presence of different animals in the households and their association with MSD in infants and young children.
Table 6-2. Animals in the household as risk factor for moderate to severe diarrhoea in under-five children in rural Gambia

<table>
<thead>
<tr>
<th>Variables</th>
<th>Case</th>
<th>Control</th>
<th>Bivariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=1029</td>
<td>n (%)</td>
<td>OR (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>Donkey/Horse/ Mule</td>
<td>641 (62.3)</td>
<td>695 (44.3)</td>
<td>2.8 (2.3-3.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cow</td>
<td>374 (36.3)</td>
<td>296 (18.9)</td>
<td>2.7 (2.2-3.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cat</td>
<td>276 (26.8)</td>
<td>194 (12.4)</td>
<td>2.7 (2.2-3.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Rodents</td>
<td>775 (75.3)</td>
<td>1024 (65.3)</td>
<td>1.9 (1.6-2.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fowl</td>
<td>915 (88.9)</td>
<td>1358 (86.6)</td>
<td>1.3 (1.0-1.7)</td>
<td>0.029</td>
</tr>
<tr>
<td>Dog</td>
<td>288 (28.0)</td>
<td>389 (24.8)</td>
<td>1.2 (1.0-1.5)</td>
<td>0.041</td>
</tr>
<tr>
<td>Goat</td>
<td>782 (76.0)</td>
<td>1185 (75.5)</td>
<td>1.1 (0.9-1.3)</td>
<td>0.513</td>
</tr>
<tr>
<td>Sheep</td>
<td>768 (74.6)</td>
<td>1186 (75.6)</td>
<td>0.9 (0.8-1.2)</td>
<td>0.598</td>
</tr>
</tbody>
</table>

In the bivariate analysis, animals identified as risk factors were indwelling of donkey/horse/mule (OR 2.8, 95% CI: 2.3-3.4; p=<0.001), cow (OR 2.7, 95% CI: 2.2-3.3; p=<0.001), cat (OR 2.7, 95% CI: 2.2-3.4; p=<0.001), rodents (OR 1.9, 95% CI: 1.6-2.4; p=<0.001) fowl (OR 1.3, 95% CI: 1.0-1.7; p=0.029), and dog (OR 1.2 95% CI:
The presence of a goat or sheep was not significantly associated with MSD. We also looked at the interaction between the presence of any animals that were significantly associated with MSD, with either goat or sheep and found no significant interaction. In the multivariate analysis donkey/horse/mule, sheep, cow, cat and rodents were significantly more commonly present in households with children having MSD compared to their matched controls.

### 6.4 Water source, storage and treatment

A great majority of the Basse health and demographic surveillance system area has an established system of piped water through the public distribution system. Unfortunately the system is unable to provide water throughout the day. Hence people also rely on other sources. For study purpose water source is broadly classified as improved and unimproved categories. The improved sources included piped water and water from deep/shallow tube wells and boreholes. The unimproved sources included water from covered/uncovered wells and other open sources. The primary caregivers were asked about the storage and treatment modalities of water. These were observed by the field workers during a follow-up visit between 60-90 days after enrolment. The primary caregivers were also asked whether the enrolled child was given any untreated water
during the last seven days. Both the bivariate and multivariate analysis results are presented in Table 6-3.

Table 6-3. Water source, availability and storage as risk factor for moderate to severe diarrhoea in under-five children in rural Gambia

<table>
<thead>
<tr>
<th>Variables</th>
<th>Case</th>
<th>Control</th>
<th>OR (95% CI)</th>
<th>p</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type of water</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Improved water</td>
<td>839</td>
<td>1334</td>
<td>Ref</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unimproved water</td>
<td>190</td>
<td>235</td>
<td>1.5 (1.1-1.9)</td>
<td>0.010</td>
<td>1.2 (0.8-1.7)</td>
<td>0.328</td>
</tr>
<tr>
<td><strong>Time to fetch water</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within 30 mins</td>
<td>924</td>
<td>1450</td>
<td>Ref</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;30 mins</td>
<td>105</td>
<td>119</td>
<td>1.5 (1.1-2.0)</td>
<td>0.008</td>
<td>1.3 (0.9-1.7)</td>
<td>0.167</td>
</tr>
<tr>
<td><strong>Availability</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All the time</td>
<td>579</td>
<td>829</td>
<td>ref</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Occasionally</td>
<td>450</td>
<td>740</td>
<td>0.9 (0.7-1.1)</td>
<td>0.199</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gave stored water to the child</td>
<td>814</td>
<td>766</td>
<td>6.1 (4.8-7.7)</td>
<td>&lt;0.001</td>
<td>5.7 (4.5-7.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Treat drinking water at home</td>
<td>369</td>
<td>368</td>
<td>2.5 (2.0-3.1)</td>
<td>&lt;0.001</td>
<td>2.0 (1.6-2.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gave untreated water to child</td>
<td>788</td>
<td>1218</td>
<td>0.9 (0.7-1.1)</td>
<td>0.227</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
It was observed that water from unimproved source was a risk factor for MSD (OR 1.5, 95% CI: 1.1-1.9; p=0.010). Availability of water was not a risk factor for MSD but needing more than 30 minutes to fetch water was significantly associated (OR 1.5, 95% CI: 1.1-2.0; p=0.008). A higher percentage of MSD cases (79.1%) than controls (48.8%) were given stored water by their caregivers: giving stored water was strongly associated with MSD in this population (OR 6.1, 95% CI: 4.8-7.7; p=<0.001). Treatment of water was not common, however: caregivers of the case children were more likely to treat water (35.9%) than the caregivers of the controls (23.5%). Caregivers of both cases (99.5%) and controls (99.7%) used cloth as a method of filtration. It was found that treating water was a risk factor for MSD (OR 2.5, 95% CI: 2.0-3.1; p=<0.001). However, giving untreated water was not an independent risk factor [OR 0.9 (95% CI: 0.7-1.1), p=0.227].

“Time to fetch water” appeared to be more of a major issue than “water availability” among the caregivers. Caregivers of 90% of the case children and 91% of the control children fetched water from a distance that took less than 30 minutes, irrespective of the water availability. It was observed that 325/1029 (31.6%) of the case children compared to 185/1569(11.8%) of controls were given water that was both treated and stored (OR 5.8, 95% CI: 4.4-7.7; p= <0.001). More case children were given untreated and stored water than the controls [615/1029(59.8%) cases vs. 588/1569 (37.5%) controls, OR 3.1, 95% CI: 2.6-3.8;
p=0.001. Finally, in the multivariate analysis, giving the child both stored and untreated water and treating water were significantly associated with MSD.

911/1029 cases and 1456/1569 controls between 60-90 days after enrolment in the study were followed up. During that period the field workers confirmed the water source and observed the water containers, whether they were covered and water dispensing methods. Field workers were able to identify a water container in 97.5% of the case and 99% of the control households (OR 0.3, 95%CI: 0.1-0.7; p=0.009). We found that 95.6% of the control households used a covered container compared to the 94% of the case households (OR 0.6, 95% CI: 0.4-0.9; p=0.024). However, the dispensing method either by pouring [39.3% among cases vs.38.6% among controls, OR 1.0 (95% CI: 0.6-1.5), p=0.876] or scooping with a cup [63.2% in cases vs. 63.8% in controls OR 1.1 95%CI: 0.7-1.1), p=0.640) did not vary between the cases and controls.

6.5 Toilet facility

The common type of toilet used in rural Gambia is the traditional pit latrine. There were also other types of toilets that did not have proper flushing mechanisms or water seals and we considered them as improvised toilets. Also enquired in the study were existences of toilet facilities in the household and faeces disposal practice of the caregivers.
Having an improvised toilet in the household was a risk factor for MSD in the children (OR 4.8, 95% CI: 2.4-9.7; p=<0.001). The presence of any toilet was likely to enable the primary caregiver to dispose the faeces in the toilet and it was observed that about 95% of caregivers of both cases and controls disposed of faeces in the toilets. However scattering the faeces in the yard was strongly associated with MSD (OR 8.2, 95% CI: 3.1-21.7; p=<0.001). On the other hand either burying or disposing the faeces out of the yard decreased the risk (OR 0.4, 95% CI: 0.2-0.7; p=0.001).
Table 6-4. Toilet facilities and disposal of faeces as risk factor for moderate to severe diarrhoea in under-five children in rural Gambia

<table>
<thead>
<tr>
<th>Variables</th>
<th>Case (N=1029)</th>
<th>Control (N=1569)</th>
<th>Bivariate OR (95% CI)</th>
<th>p</th>
<th>Multivariate OR (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Toilet facility</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Traditional pit toilet</td>
<td>989 (96.1)</td>
<td>1551 (98.9)</td>
<td>Ref</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Improvised toilet</td>
<td>32 (3.1)</td>
<td>14 (0.9)</td>
<td>4.4 (2.2-8.6)</td>
<td>&lt;0.001</td>
<td>4.8 (2.4-9.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No facilities</td>
<td>8 (0.8)</td>
<td>4 (0.3)</td>
<td>3.5 (0.9-13.9)</td>
<td>0.078</td>
<td>4.7 (1.0-23.4)</td>
<td>0.057</td>
</tr>
<tr>
<td><strong>Faeces disposal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toilet</td>
<td>979 (95.1)</td>
<td>1498 (95.5)</td>
<td>Ref</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scatter in yard</td>
<td>31 (3.0)</td>
<td>6 (0.4)</td>
<td>8.2 (3.1-21.7)</td>
<td>&lt;0.001</td>
<td>7.3 (2.8-19.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Disposal out of the yard and bury</td>
<td>19 (1.8)</td>
<td>65 (4.1)</td>
<td>0.4 (0.2-0.7)</td>
<td>0.001</td>
<td>0.3 (0.2-0.6)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

In the multivariate analysis, use of an improvised toilet and disposal of faeces in the yard were significantly associated with increased risk of MSD while disposal or burying the faeces outside of yard was associated with decreased risk.

### 6.6 Hand washing

Enquires about the hand washing practices of the caregivers of the study children before and after certain day to day activities and whether they used any
disinfectants like soap or mud while hand washing were made. Table 6-5 shows the detailed results.

Table 6-5. Hand washing as a risk factor for moderate to severe diarrhoea in under five children in rural Gambia

<table>
<thead>
<tr>
<th>Variables</th>
<th>Bivariate</th>
<th></th>
<th></th>
<th></th>
<th>Multivariate</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case</td>
<td>Control</td>
<td>OR</td>
<td>p</td>
<td>OR</td>
<td>p</td>
</tr>
<tr>
<td></td>
<td>N=1029</td>
<td>N=1569</td>
<td>(95% CI)</td>
<td>p</td>
<td>(95% CI)</td>
<td>p</td>
</tr>
<tr>
<td>Before eating</td>
<td>988 (96.0)</td>
<td>1501 (95.7)</td>
<td>1.0</td>
<td>0.840</td>
<td>0.9</td>
<td>0.481</td>
</tr>
<tr>
<td></td>
<td>(0.6-1.5)</td>
<td>(0.5-1.3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before cooking</td>
<td>531 (51.6)</td>
<td>998 (63.6)</td>
<td>0.6</td>
<td>&lt;0.001</td>
<td>0.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(0.5-0.7)</td>
<td>(0.5-0.7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before nursing or preparing baby food</td>
<td>423 (41.1)</td>
<td>616 (39.3)</td>
<td>1.1</td>
<td>0.582</td>
<td>1.4 (1.1-1.7)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>(0.9-1.3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>After defecation</td>
<td>795 (77.3)</td>
<td>1186 (75.6)</td>
<td>1.1</td>
<td>0.606</td>
<td>1.4 (1.1-1.7)</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>(0.9-1.3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>After handling domestic animals</td>
<td>87 (8.5)</td>
<td>185 (11.7)</td>
<td>0.7</td>
<td>0.004</td>
<td>0.7</td>
<td>0.025</td>
</tr>
<tr>
<td></td>
<td>(0.5-0.9)</td>
<td>(0.5-1.0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>After cleaning the child who defecated</td>
<td>795 (77.3)</td>
<td>1307 (83.3)</td>
<td>0.6</td>
<td>&lt;0.001</td>
<td>0.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(0.5-0.8)</td>
<td>(0.5-0.8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Use of any disinfect

<table>
<thead>
<tr>
<th>Use of any disinfect</th>
<th>Bivariate</th>
<th></th>
<th></th>
<th></th>
<th>Multivariate</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case</td>
<td>Control</td>
<td>OR</td>
<td>p</td>
<td>OR</td>
<td>p</td>
</tr>
<tr>
<td></td>
<td>N=1029</td>
<td>N=1569</td>
<td>(95% CI)</td>
<td>p</td>
<td>(95% CI)</td>
<td>p</td>
</tr>
<tr>
<td>Water only</td>
<td>268 (26.0)</td>
<td>321 (20.5)</td>
<td>ref</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water and any disinfectant</td>
<td>761 (74.0)</td>
<td>1248 (79.5)</td>
<td>0.7</td>
<td>0.001</td>
<td>0.8</td>
<td>0.098</td>
</tr>
<tr>
<td></td>
<td>(0.6-0.9)</td>
<td>(0.7-1.0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
In the bivariate analysis hand washing was found to be associated with reduced odds of MSD when done before cooking (OR 0.6, 95% CI: 0.5-0.7; p=<0.001), after cleaning a child who defecated (OR 0.6, 95% CI: 0.5-0.8; p=<0.001) and after handling any domestic animals (OR 0.7, 95% CI: 0.5-0.9; p=0.004). Hand washing after defecation, before eating, before preparing food for baby or nursing was not associated with reduced odds of MSD. Use of any disinfectant while washing hands was also associated with reduced risk of MSD (OR 0.7, 95% CI: 0.6-0.9; p=0.001). It was noted that 99% of the caregivers of both cases and controls used soap as disinfectant. The role of disinfectant in hand washing was explored. Caregivers of 57.4% (591/1029) of the case children washed hands with disinfectant after defecation compared to 62.8% (985/1569) of the control children (OR 0.8, 95% CI: 0.6-0.9; p=0.001). More of the control caregivers (1207/1569; 76.9%) also used disinfectant before eating as opposed to case caregivers (726/1029; 70.6%) giving an OR of 0.7, (95% CI: 0.6-0.8; p<0.001). However, there were no notable differences in disinfectant use observed for other practices such as washing hands before cooking or preparing baby food.

In the multivariate analysis washing hands before cooking and after cleaning a child after defecation was significantly associated with reduced risk of MSD.

In order to evaluate the overall impact of the different putative risk factors a multivariate model including all the risk factors identified in the previous
sections were fitted. The processes of inclusion of independent risk factors in the multivariate model are described in the method chapter (chapter 3, section 3.12). This analysis is presented in Table 6-6.
Table 6-6. Risk and protective factors for moderate to severe diarrhoea in children aged under five years old in rural Gambia

<table>
<thead>
<tr>
<th></th>
<th>Bivariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>N=1029</td>
<td>N=1569</td>
</tr>
<tr>
<td>n (%)</td>
<td>n(%)</td>
<td></td>
</tr>
<tr>
<td>Primary caregiver other than mother</td>
<td>36 (3.5)</td>
<td>22 (1.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No formal schooling of primary caregiver</td>
<td>668 (64.9)</td>
<td>699 (44.6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Father died or lives out of the household</td>
<td>304 (29.5)</td>
<td>376 (24.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No of under five children in the household</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-4</td>
<td>498 (48.4)</td>
<td>665 (42.4)</td>
</tr>
<tr>
<td>5-10</td>
<td>449 (43.6)</td>
<td>711 (45.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;10</td>
<td>82 (8.0)</td>
<td>193 (12.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wealth Index</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower 40%</td>
<td>420 (40.8)</td>
<td>619 (39.5)</td>
</tr>
<tr>
<td>Middle 40%</td>
<td>412 (40.0)</td>
<td>629 (40.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper 20%</td>
<td>197 (19.1)</td>
<td>321 (20.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Animals in the household</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Donkey/Horse/</td>
<td>641 (62.3)</td>
<td>695 (44.3)</td>
</tr>
</tbody>
</table>

231
<table>
<thead>
<tr>
<th></th>
<th>Bivariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=1029</td>
<td>N=1569</td>
</tr>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td></td>
<td>OR</td>
<td>(95% CI)</td>
</tr>
<tr>
<td>Mule</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(2.3-3.4)</td>
<td>(2.5-4.1)</td>
</tr>
<tr>
<td>Cow</td>
<td>374 (36.3)</td>
<td>296 (18.9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cat</td>
<td>276 (26.8)</td>
<td>194 (12.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rodents</td>
<td>775 (75.3)</td>
<td>1024</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Water source, availability, storage and treatment**

|                   |           |               |
|                   |           |               |
| Improved water    | 839 (81.5)| 1334(85.0)    | Ref           |
|                   |           |               |
| Unimproved water  | 190 (18.5)| 235 (15.0)    | 1.5           | 0.010        | 1.0           | 0.863        |
|                   |           |              | (1.1-1.9)     |              | (0.6-1.5)     |
| Source reachable  | 924 (89.8)| 1450(92.4)    | Ref           |
|                   |           |               |
| Within 30 mins    | 105 (10.2)| 119 (7.6)     | 1.5           | 0.008        | 1.0           | 0.979        |
|                   |           |              | (1.1-2.0)     |              | (0.7-1.5)     |
| Gave stored water | 814 (79.1)| 766 (48.8)    | 6.1           | <0.001       | 5.3           | <0.001       |
| to the child      |           |              | (4.8-7.7)     |              | (4.0-7.2)     |
| Treat drinking    | 369 (35.9)| 368 (23.5)    | 2.5           | <0.001       | 2.1           | <0.001       |
| water at home     |           |              | (2.0-3.1)     |              | (1.5-2.9)     |

**Toilet facility and disposal of faeces**

<p>| | | |
|                   |           |               |
|                   |           |               |
| Traditional pit   | 989 (96.1)| 1551(98.9)    | Ref           |
| toilet            |           |               |
| Improvised toilet | 32 (3.1)  | 14 (0.9)      | 4.4           | &lt;0.001       | 9.4           | &lt;0.00        |
|                   |           |              | (2.2-8.6)     |              | (3.9-2.9)     | 1            |</p>
<table>
<thead>
<tr>
<th>No facilities</th>
<th>Case</th>
<th>Control</th>
<th>OR  (95% CI)</th>
<th>P</th>
<th>OR  (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8 (0.8)</td>
<td>4 (0.3)</td>
<td>3.5 (0.9-3.9)</td>
<td>0.078</td>
<td>8.6 (3.6-21.2)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Disposal of faeces in the Toilet</th>
<th>979(95.1)</th>
<th>1498(95.5)</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scatter faeces in yard</td>
<td>31 (3.0)</td>
<td>6 (0.4)</td>
<td>8.2 (3.1-1.7)</td>
</tr>
<tr>
<td>Disposal faeces out of the yard and bury</td>
<td>19 (1.8)</td>
<td>65 (4.1)</td>
<td>0.4 (0.2-0.7)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hand washing practice</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Before cooking</td>
<td>531 (51.6)</td>
<td>998 (63.6)</td>
<td>0.6 (0.5-0.7)</td>
<td>&lt;0.001</td>
<td>0.6 (0.5-0.8)</td>
<td>0.001</td>
</tr>
<tr>
<td>After handling domestic animals</td>
<td>87 (8.5)</td>
<td>185 (11.7)</td>
<td>0.7 (0.5-0.9)</td>
<td>0.004</td>
<td>1.7 (1.1-2.5)</td>
<td>0.013</td>
</tr>
<tr>
<td>After cleaning the child who defecated</td>
<td>795 (77.3)</td>
<td>1307 (83.3)</td>
<td>0.6 (0.5-0.8)</td>
<td>&lt;0.001</td>
<td>0.6 (0.5-0.9)</td>
<td>0.006</td>
</tr>
<tr>
<td>Water only</td>
<td>268 (26.0)</td>
<td>321 (20.5)</td>
<td>ref</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water and any disinfectant</td>
<td>761 (74.0)</td>
<td>1248 (79.5)</td>
<td>0.7 (0.6-0.9)</td>
<td>0.001</td>
<td>1.2 (0.9-1.6)</td>
<td>0.327</td>
</tr>
</tbody>
</table>

The overall risk factors for MSD in a multivariate analysis were primary caregiver other than the mother (OR 2.8, 95% CI: 1.3-6.5; p=0.011), father being
out of the house or dead (OR 1.3, 95% CI: 1.0-1.8; p=0.025) and no formal schooling of the primary caregiver (OR 3.1, 95% CI: 2.4-4.0; p=<0.001). Presence of animals like horse/donkey, cow, cat and rodents in the same household were also significantly associated with MSD. If the children were given stored water (OR 5.3, 95% CI: 4.0-7.2; p=<0.001) and treated (OR 2.1, 1.5-2.9; p=<0.001) they had an increased risk of MSD. Toilets other than traditional pit latrine, non-existence of any toilet facilities and faeces disposal practice in the yard were also associated with MSD. If a household had more young children, and the caregivers disposed the faeces either by throwing out of yard or burying them for the risk of MSD was reduced. Hand washing in general, and specially before cooking and before cleaning a child who defecated was protective.

6.7 Risk factors for the top five pathogens

The top five pathogens causing MSD in children aged under five years old in Gambia were Enterotoxigenic E.coli, Shigella spp, rotavirus, Cryptosporidium spp. and norovirus. Apart from identifying the risk factors for acute MSD irrespective of their causative organism, we also identified the pathogen specific risk factors for MSD in this population. In a multivariate analysis, no formal schooling of the primary caregiver, giving stored water and non-treatment of water and presence of horse/donkey or mule or cow were associated with MSD caused by all these five pathogens (Table 6-7)
Table 6-7. Pathogen specific risk factors (multivariate analysis) for moderate to severe diarrhoea in children aged under five years old in rural Gambia

<table>
<thead>
<tr>
<th></th>
<th>ETEC OR (95% CI); p</th>
<th>Shigella spp. OR (95% CI); p</th>
<th>Rotavirus OR (95% CI); p</th>
<th>Cryptosporidium spp. OR (95% CI); p</th>
<th>Norovirus OR (95% CI); p</th>
</tr>
</thead>
<tbody>
<tr>
<td>No formal schooling of primary caregiver</td>
<td>3.1 (1.5-6.3); 0.002</td>
<td>9.4 (2.4-37.0); 0.001</td>
<td>3.6 (1.9-6.6); &lt;0.001</td>
<td>3.2 (1.4-7.6); 0.006</td>
<td>3.4 (1.4-8.5); 0.008</td>
</tr>
<tr>
<td>Presence of horse/donkey or mule in the household</td>
<td>5.5 (2.7-11.3); &lt;0.001</td>
<td>18.4 (2.8-122.3); 0.003</td>
<td>2.7 (1.5-4.9); 0.001</td>
<td>4.1 (1.5-11.0); 0.005</td>
<td>5.5 (2.3-13.0); &lt;0.001</td>
</tr>
<tr>
<td>Presence of cow in the household</td>
<td>3.1 (1.6-6.3); 0.001</td>
<td>13.9 (2.0-94.6); 0.007</td>
<td>1.8 (0.9-3.4); 0.078</td>
<td>1.7 (0.7-3.8); 0.222</td>
<td>8.1 (2.4-27.4); 0.001</td>
</tr>
<tr>
<td>Gave stored water to the child</td>
<td>7.1 (2.8-14.1); &lt;0.001</td>
<td>21.2 (3.9-115.5); &lt;0.001</td>
<td>3.7 (1.9-7.2); &lt;0.001</td>
<td>5.2 (2.0-13.5); 0.001</td>
<td>9.3 (3.5-24.8); 0.001</td>
</tr>
<tr>
<td>Treat drinking water at home</td>
<td>2.1 (0.9-4.6); 0.071</td>
<td>4.4 (1.6-25.9); 0.028</td>
<td>2.4 (1.1-5.2); 0.028</td>
<td>2.6 (1.0-7.0); 0.059</td>
<td>3.9 (1.1-13.5); 0.029</td>
</tr>
</tbody>
</table>
Having a cow in the household was associated with MSD caused by ETEC (OR 2.8, 95% CI: 1.3-5.9; p=0.006), *Shigella* spp. (OR 15.7, 95% CI: 1.9-127.2; p=0.010) and norovirus (OR 8.8, 95% CI: 2.4-33.0; p=0.001). Presence of a cat in the household was associated with increased risk of MSD due to *Shigella* spp. (OR 11.6, 95% CI: 1.6-85.6; p=0.016), rotavirus (OR 2.3, 95% CI: 1.1-4.8; p=0.025) and *Cryptosporidium* spp. (OR 4.0, 95% CI: 1.5-10.8; p=0.006) The presence of a rodent in the house was significantly associated with MSD due to *Shigella* spp. (OR 4.4, 95% CI: 1.0-18.4; p=0.043) and rotavirus (OR 2.1, 95% CI: 1.1-4.2; p=0.033).

Absence of toilet facilities in the household was associated with MSD due to ETEC (OR 12.4, 95% CI: 1.1-136.6; p=0.039) and *Cryptosporidium* spp. (OR 33.1, 95% CI: 1.2-897.3; p=0.038).

Washing hands before cooking was associated with decreased risk of MSD due to *Shigella* spp. (OR 0.1, 95% CI: 0.0-0.4; p=0.008). A higher number of under five children in the household also was associated with decreased risk of MSD due to ETEC (OR 0.2, 95% CI: 0.1-0.5; p=0.002) and *Shigella* spp. (OR 0.02, 95% CI: 0.0-0.4; p=0.008)

### 6.8 Discussion

The study identified and established the socio-demographic, environmental and personal risk factors for MSD in children aged under five years old in rural
Gambia. The risk factors for diarrhoea are well explored and they are similar in both the developed and developing world. However, while developed countries have been successful in controlling these risk factors through appropriate corrective and preventive measures, developing nations are yet to formulate sufficient strategies for reducing diarrhoeal disease mortality and morbidity. In this study we identified risk factors not only for acute MSD but also for pathogen specific MSD in this population.

It is likely that in the absence of the mother, children are unattended and exposed to the conditions that are truly detrimental to their health. The absence of a father in the household and non-formal schooling of the primary caregiver was also associated with MSD. A low literacy rate among the caregivers may lead to lack of understanding of the gravity of the disease and need for care seeking, supporting the hypothesis of a positive impact of education on child health. Women in the African community are not empowered to make decisions on the well-being of their children such as seeking care when they are ill. In a strongly bonded society with extended families it is apparently a collective decision from various members of the family for the management of the child’s care. One study showed such decisions delay facility-based treatment and favour home based management. Thus in the absence of the father, the role of the mother as a decision maker is further relegated. In The Gambia, people live in an extended family with a relatively
large household size that has several rooms for sleeping. The sleeping rooms are well separated from each other. It is likely that people sharing one sleeping room have the most person to person spread of diarrhoea. However, we calculated the number of people per sleeping room to assess the role of overcrowding and found that there was no association of MSD with overcrowding, contrary to the findings of some other studies.\textsuperscript{238, 239} We postulate that in rural site like ours there is more available physical spaces per person than in the urban areas. Such availability of space may protect against person to person spread to a certain extent. On the other hand more people in a household means more help to take care of the children in the family, so any particular child may receive attention not only from the primary caregiver but from other adults as well. Economic solvency as ascertained by the wealth index was also not associated with MSD. The absence of association between diarrhoea and overcrowding and wealth index, to some extent, justifies why the Global Burden Study did not consider them as one of the potential risk factors.\textsuperscript{240}

In Basse, people keep animals, especially donkeys, horses and mules to be used on agricultural land for ploughing. Fowls are raised for eggs and meat. Livestock animals are kept for milk and they are also considered as assets in this population. Dogs and cats are stray animals in the community but very often the children play with them. Horses, donkeys and mules are also used for transport. Chicken and other birds are commonly seen in the households, often entering
the sleeping rooms. Animals, chickens and rodents dwelling in the household were found to be associated with MSD in our study. The ability of a pathogen to infect both the animals and humans and their transmission between each other is of a notable concern. Some of the animals act as a vector for transmission of diarrhoeal disease. Flies can carry the pathogens from scattered animal and human faeces. Thus enteropathogens have direct, indirect and vector borne routes of transmission to humans. Domestic animals have been incriminated as a risk factor for diarrhoea in children from studies in Guinea Bissau, Malawi and Liberia.

Since diarrhoea is a water borne disease the source of water, its quality and treatment play a major role in disease transmission. This study was not designed to evaluate water quality. The observation of improved water usage by more than 80% of both the cases and controls showed significantly better water supply in this rural community compared to the coverage of 64% in Africa overall. In the bivariate analysis, use of unsafe water and time to fetch water of more than 30 minutes, were significant. Use of unsafe water is an established risk factor for diarrhoea and studies have shown that improvement in water quality reduces diarrhoea morbidity between 22%-26%. If more time is taken to fetch water there is general tendency to store water and use it for prolonged periods. Even though people have access to improved water, the availability of such water was for less than half of the time of a day. Giving stored water, treated or untreated,
was significantly associated with MSD. These findings are suggestive of microbiological contamination of drinking water in particular types of vessels, transfer of water to storage containers at home and subsequent dipping of cups or ladles instead of pouring or using taps.\textsuperscript{248, 249, 250} Uses of un-covered containers in our study indicate contamination of water in such containers. Earlier studies conducted in West Africa observed similar practices in the population.\textsuperscript{251} One may view our findings of treatment of water as a risk factor as counter intuitive. It is possible that households with more obviously contaminated water feel that they should treat it in some way, but they do so ineffectively. Effective treatment against organisms such as Cryptosporidium spp. and G.lamblia has proved to be costly and involves multiple steps that are unlikely to be applied in a setting like The Gambia.\textsuperscript{252} Even absence or breakthrough of a simple chlorination technique can give rise to emergence of resistant organism in the water and spread to the community causing diarrhoea.\textsuperscript{253} The treatment practice used mainly in this rural African setting was through a cloth. A cloth folded four to eight times has been shown to be effective in removing zooplankton, phytoplankton and all V.cholerae attached to it and other particulates of \~20 µm size.\textsuperscript{254} Removal of other pathogens from water through cloth filtration has not yet been established. It was not possible to confirm from the primary caregivers that they use the right procedure of filtration through a cloth (e.g. folding of the cloth). It was felt that cloth could be a potential source of contamination if not washed properly after each filtration and if they were simultaneously used for other purposes like
wiping hands, mouths etc. It is likely that caregivers of case children used a contaminated cloth for the purpose of filtration.

Pit latrines, as used by the majority of the population, may not be considered as a proper sanitation facility but at least having a pit latrine was more protective than having any other toilets that do not have proper drainage or washout systems. Even though there were few households without a latrine, its association with MSD is noteworthy as it has been seen elsewhere in Africa.\textsuperscript{46,255}

We also observed that disposing the child faeces by scattering in the yard is a potential risk factor for diarrhoea consistent with similar previous findings in the region.\textsuperscript{256} The children and other domestic animals come in close contact with faeces and can get contaminated. Also during the rainy season these excreta are washed away and may seep into the drinking water systems in the community causing widespread contamination of water and outbreaks of disease.\textsuperscript{257}

The risk factors related to water and sanitation observed in our study also established the role of both the ‘domestic domain’ controlled by the household and the ‘public domain’ that includes the streets and fields. It has been postulated that the route of transmission from the domestic domain is through stored water, improper faeces disposal and absence of latrine facilities. The transmission route from the public domain is from the contaminated soil brought into the household by the domestic animals.\textsuperscript{258}
Hand washing before eating was a common practice in the population and there was no difference between the cases and controls. It was found that washing hands with disinfectant was protective, consistent with other studies.\textsuperscript{57, 259, 260} Hand washing before cooking, after handling domestic animals and after cleaning a child who defecated was also protective. This signifies the role of personal hygiene in controlling diarrhoea in children, especially the ones that are caused by ETEC as they have an animal reservoir, and \textit{Shigella} spp. due to their requirement of low inoculum to infect a person. Even though more case caregivers washed their hands, they were less likely to use disinfectant. Reviews suggest promoting hand washing causes reduction in diarrhoeal disease. But emphasis should be laid out to effectively promote the appropriate technique of hand washing.\textsuperscript{261}

One of the important aetiological findings of our study was the detection of norovirus and \textit{Cryptosporidium} spp. and their association with MSD. The risk factors of MSD due to norovirus were similar to other pathogens. But our study also showed having a cow in the household was a risk factor for MSD due to norovirus. Hence potential zoonotic transmission of norovirus should be considered for further epidemiological research.\textsuperscript{262, 263} Infected people with norovirus infection continue to shed the virus, even after recovery from the acute state and can spread the disease through contaminated food and water.\textsuperscript{264}
Several studies have isolated the same species of *Cryptosporidium* from both humans and domestic animals. They established a strong link between domestic animals with human infection. The transmission of the pathogen is from human to human via contaminated drinking water with the domestic animals acting as the reservoir.

Like any other case control study this study can be criticised for over reporting and recall biases. But a visit to the household of each enrolled children and observation made by the field worker on water storage, filtration technique, and existence of sanitation facilities and presence of any faeces in the compound during the follow-up periods likely minimized such biases to a large extent. While the pathogen specific risk factors were similar to the risk factors for diarrhoea in general, smaller numbers in those analyses led to reduced precision around the estimates.

This study was able to make a sensible assessment of the overall risk factors for MSD and establish the role of socio demographic, environmental, water sanitation and hygiene factors in children aged under five years old. There were a number of transmission routes from hygiene behaviour at home, hand washing and treatment of water for MSD in this rural population. The results clearly highlight that, intervening in a single route of transmission may not show any dramatic reduction in MSD.
Chapter 7- Linear growth of children over time:

Follow-up of the case and control cohort

TIME TO ACT
7.1 Introduction

Diarrhoea is endemic and malnutrition is common in most developing countries, setting up a vicious combined cycle of co-morbidity. Diarrhoea is responsible for the deaths of approximately 800,000 children under five years of age annually; second only to pneumonia. Malnutrition is thought to be responsible for approximately one third of the 6.9 million annual deaths in under-five year old children. Of the 165 million stunted children worldwide; more than 90% live in Asia and Africa. Diarrhoea and other illnesses act synergistically with malnutrition in early childhood to cause stunting, developmental delay and death. A single episode of moderate to severe diarrhoea causes mucosal injury and loss of intestinal permeability, growth faltering and reduced catch-up growth through lack of essential nutrients. Growth after an episode of diarrhoea depends on nutritional status, the pathogen causing diarrhoea, the duration of illness, and the presence of other co-morbidities.

A better understanding of the relation between diarrhoea and stunting is necessary, both for management of diarrhoea and also for nutritional supplementation. Both the case (MSD) and control (non MSD) cohorts at two time periods during the study period were followed. The first follow up was done 60-90 days after enrolment and the second 18-24 months after enrolment. The cohorts were followed up for anthropometric assessments and other co
morbidities. Here we describe the linear growth of cases in the context of a diarrhoeal episode and compare them with the controls, in the context of the occurrence of other illnesses during follow-up.

7.2 Methods

The detailed methods of participant selection, anthropometric measurements and statistical analyses are described in chapter 3 (section 3.10 and 3.12). We calculated the mean length/height for age Z score (HAZ) and their differences between the two cohorts of cases of MSD and the controls. The Z score allowed measurement of the dispersion of data and enabled expression of the difference between an individual subject’s height and the average height of comparable subjects in the reference population. We used both the cut off based prevalence, <-2 and >+2 Z scores and summary statistics of the Z scores (mean, SD, frequency). The cut off prevalence implies that 2.3% of the reference population are stunted at the baseline. The prevalence rates are used to assess the trigger level (medium: 20-29%, high: 30-39% and very high: >=40%) of stunting in the population and for prioritizing intervention needs. On the other hand summary statistics describe the nutritional status of the entire population. Hence, a mean Z score below zero indicates a general reduced nutritional status. Due to the large variation in timing of the follow-up, time from diagnosis (of the case) was treated as a continuous variable in the analysis. Linear mixed models were fitted
to describe the relative growth curves and to test for difference in relative growth patterns between case and controls. The MSD/non MSD set and the child were included as random effects to account for the matching and the repeated measures over time. The matching variables (age and sex) were included as covariates. The mean baseline HAZ level in MSD and non MSD cohorts were compared from the model, with robust standard errors to account for the matching. The means and standard deviations at baseline and the two follow-up points are presented. The model co-efficient and their 95% confidence intervals for the initial and the final model and their p values for each group are also presented.

7.3 Results:

Enrolment

There were 1029 eligible MSD and 1569 non MSD children enrolled initially, 1024 (399 in 0-11 month, 453 in 12-23 month and 172 in 24-59 month age group) MSD and 1562 (583 in 0-11 month, 635 in 12-23 month and 344 in 24-59 month age group) non MSD children were eligible for analysis. The baseline characteristics of MSD and non MSD children are described in chapter 6 (Table 6.6). Primary caregivers of the MSD children were less educated compared to the primary caregivers of the non MSD children. We also noticed that children with MSD were more likely to have a primary caregiver other than the mother and for the father to live outside of home or be dead. However, due to small numbers of
primary caregivers who were other than the mother in both the MSD and non MSD cohort, this should be interpreted cautiously. Wealth index was not significantly different between the MSD cases and non MSD controls. We considered primary caregivers’ education could be a possible confounder for linear growth for this population as there was difference in educational status of primary caregivers of MSD and non MSD cohort (chapter 6, Table 6.6).

Table 7-1 below shows the baseline HAZ scores and proportion stunting of the children. It can be seen that children belonging to both the MSD and non MSD cohort across all the age strata had a mean Z score below zero and also the mean Z scores lower in MSD compared to the non MSD in all age strata.
Table 7-1. Baseline HAZ (mean± SD and their 95% confidence interval) and proportion of stunting (HAZ<-2) among the MSD and non MSD children

<table>
<thead>
<tr>
<th>Age group</th>
<th>MSD</th>
<th>Non MSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean± SD</td>
<td>95% CI</td>
</tr>
<tr>
<td>0-11 month</td>
<td>-0.86±1.24</td>
<td>-0.98 to -0.74</td>
</tr>
<tr>
<td>12-23 month</td>
<td>-1.36±1.23</td>
<td>-1.48 to 1.25</td>
</tr>
<tr>
<td>24-59 month</td>
<td>-1.84±1.21</td>
<td>-2.02 to 1.66</td>
</tr>
</tbody>
</table>

From the time of enrolment to the first follow-up, 39 (3.8%) MSD children and 7 (0.4%) non MSD children died (RR 8.5; 95% CI: 3.8-18.9). Thus 990 MSD children and 1562 non MSD children were eligible for follow-up. We were not able to conduct interviews for 114 MSD children and 113 non MSD children for various reasons (Figure 7-1). Interviews and measurements were conducted for 876 MSD (85% of the original MSD cohort) and 1449 (92% of the original non MSD cohort). One of the MSD children and three of the non MSD children had biologically implausible values (HAZ <-6/>+6 SD from the median). After excluding these children with abnormal HAZ scores, 875(344 in 0-11 months, 385 in 12-23 months and 146 in 24-59 months age group) MSD and 1446 (536 in 0-11 months, 589 in
12-23 months and 321 in 24-59 months age group) non MSD children were included for analysis.
Figure 7-1: Flow chart for first follow up

Total children enrolled for case control study: 2598

MSD: 1029
- Died: 39
- Refused: 89
- Caregiver unavailable for interview: 04
- Not found/emigrated from DSS: 21
- Interview conducted: 876
- Implausible HAZ score: 01
- Analysed: 875
  - 0-11 months: 344
  - 12-23 months: 385
  - 24-59 months: 321

Non MSD: 1569
- Died: 07
- Refused: 94
- Caregiver unavailable for interview: 04
- Not found/emigrated from DSS: 15
- Interview conducted: 1449
- Implausible HAZ score: 03
- Analysed: 1466
  - 0-11 months: 536
  - 12-23 months: 589
  - 24-59 months: 321
During the period between the first and second follow-up 20 (2.0%) MSD children and 13 (0.8%) non MSD children died (RR 2.4, 95% CI: 1.2-4.9) (Figure 7-2). We were not able to conduct interviews in 136 MSD children and 124 non MSD children, for various reasons. Thus, 587 non MSD children were not selected for interview. Interviews and measurements were conducted for 834 MSD and 846 non MSD children. After exclusion for biologically implausible values, growth measurements were available for 786 MSD children (306 of the 0-11 months, 373 of the 12-23 months and 107 of the 24-59 months age group) and 803 non MSD children (317 of the 0-1 months, 372 of the 12-23 months and 114 of the 24-59 months age group).
Total children eligible for second follow up: 2552

MSD: 990
- Died during the follow up interval: 20
- Refused: 105
- Caregiver unavailable for interview: 17
- Not found/emigrated from DSS: 14
- Second Follow up conducted: 834
- Im plausible HAZ score: 07
- Age > 59 months: 41
- Analysed: 786
  - 0-11 months: 306
  - 12-23 months: 373
  - 24-59 months: 107

Non MSD: 1562
- Died during follow up interval: 13
- Refused: 87
- Caregiver unavailable for interview: 14
- Not found/emigrated from DSS: 10
- Second follow up conducted: 846
- Im plausible HAZ score: 02
  - Age > 59 months: 36
  - More than one control/case: 05
- Analysed: 803
  - 0-11 months: 317
  - 12-23 months: 372
  - 24-59 months: 114
Figure-7-3 below shows the timing of assessments for the cohorts by age strata. Because of the variation in timing of follow-up visits, particularly at the second follow-up we took the opportunity to provide a fuller picture of the growth patterns of children in this population than just at the three time points, through construction of a parsimonious model.

Figure 7-3: HAZ assessment time points for both MSD and non MSD cohort by age strata

There was considerable variability between children in the pattern of change in HAZ score over time. This variability was present in all age strata and in both
MSD and non MSD children. Figure 7-4 below shows this variability in a random selection of children from the cohort.

Figure 7-4: Linear growth pattern (Z score) at defined interval (0-3 months and 18-24 months after enrolment) for randomly selected children in different age strata.
Individual children showed differences in linear growth in different age strata. Considering mean HAZ scores at baseline and the two follow-up periods, it was observed that they were below zero in all age groups, indicating that there was general stunting in the study population (Figure 7-5).

Figure 7-5: HAZ of cases and control cohort at three different time points of follow up by age Strata

Figure 2:

HAZ: Height for age Z score

Values in X axis: 1=enrolment; 2=first follow-up; 3=second follow-up
General Health

Table 7-2 shows the co morbidities, other than death, reported during both the follow ups, as obtained from the mothers and through checking of the infant welfare card.

Table 7-2. Illnesses and health facility visits n (%) reported by the MSD and non MSD children during the two follow up times.

<table>
<thead>
<tr>
<th></th>
<th>First follow up</th>
<th>Second follow up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case (N=875)</td>
<td>Control (N=1446)</td>
</tr>
<tr>
<td></td>
<td>Control (N=786)</td>
<td>Control (N=803)</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>349 (39.9)</td>
<td>431 (29.8)</td>
</tr>
<tr>
<td></td>
<td>452 (57.5)</td>
<td>431 (53.7)</td>
</tr>
<tr>
<td>Visited Health Centre for diarrhoea</td>
<td>208 (59.6)</td>
<td>232 (53.8)</td>
</tr>
<tr>
<td></td>
<td>384 (85.0)</td>
<td>334 (77.5)</td>
</tr>
<tr>
<td>Dysentery</td>
<td>23 (2.6)</td>
<td>20 (1.4)</td>
</tr>
<tr>
<td></td>
<td>18 (2.3)</td>
<td>25 (3.1)</td>
</tr>
<tr>
<td>Visited Health Centre for dysentery</td>
<td>13 (56.5)</td>
<td>8 (40.0)</td>
</tr>
<tr>
<td></td>
<td>12 (66.7)</td>
<td>17 (68.0)</td>
</tr>
<tr>
<td>Cough</td>
<td>51 (5.8)</td>
<td>58 (4.0)</td>
</tr>
<tr>
<td></td>
<td>78 (9.9)</td>
<td>86 (10.7)</td>
</tr>
<tr>
<td>Visited Health Centre for cough</td>
<td>29 (56.9)</td>
<td>25 (43.1)</td>
</tr>
<tr>
<td></td>
<td>58 (74.4)</td>
<td>66 (76.7)</td>
</tr>
<tr>
<td>Fever</td>
<td>234 (26.7)</td>
<td>352 (24.3)</td>
</tr>
<tr>
<td></td>
<td>421 (53.6)</td>
<td>429 (53.4)</td>
</tr>
<tr>
<td>Visited Health Centre for fever</td>
<td>154 (65.8)</td>
<td>211 (59.9)</td>
</tr>
<tr>
<td></td>
<td>340 (80.8)</td>
<td>343 (80.0)</td>
</tr>
<tr>
<td>Any illness</td>
<td>461</td>
<td>608</td>
</tr>
<tr>
<td></td>
<td>599</td>
<td>591</td>
</tr>
</tbody>
</table>
During the first follow-up a higher proportion of MSD children had an episode of diarrhoea, fever or cough compared to their non MSD controls (461/875 (52.7%) of MSD vs. and 608/1466 (42.0) of non MSD; p<0.001). Of note, 283/461 (61.4%) MSD and 357/608 (58.7%) non MSD children visited a health centre for one or more of these symptoms. A higher proportion of MSD children experienced each of these symptoms, and a similar proportion of MSD and non MSD children had episodes considered severe enough to be taken to the health centre by their caregivers (table7-2 and 7-3). 101/875(11.5%) MSD children and 145/1446(10.0%) non MSD children were diagnosed with malaria at the health centre. Pneumonia was diagnosed among 17/875(1.9%) and 18/1446(1.2%) of the MSD and non MSD cohorts respectively.
Table 7-3. Illnesses and health facility visits, n (%) reported by the MSD and non MSD children by age strata during the time between enrolments and first follow up at 60-90 days

<table>
<thead>
<tr>
<th></th>
<th>0-11 months</th>
<th>12-23 months</th>
<th>24-59 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case (N=344)</td>
<td>Control (N=536)</td>
<td>Case (N=385)</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>150 (43.6)</td>
<td>173 (32.3)</td>
<td>147 (38.2)</td>
</tr>
<tr>
<td>Visited Health Centre for diarrhoea</td>
<td>84 (56.0)</td>
<td>109 (63.0)</td>
<td>91 (61.9)</td>
</tr>
<tr>
<td>Dysentery</td>
<td>8 (2.3)</td>
<td>8 (1.5)</td>
<td>12 (3.1)</td>
</tr>
<tr>
<td>Visited Health Centre for dysentery</td>
<td>4 (50.0)</td>
<td>4 (50.0)</td>
<td>7 (58.3)</td>
</tr>
<tr>
<td>Cough</td>
<td>24 (7.0)</td>
<td>23 (4.3)</td>
<td>21 (5.5)</td>
</tr>
<tr>
<td>Visited Health Centre for cough</td>
<td>13 (54.2)</td>
<td>11 (47.8)</td>
<td>12 (57.1)</td>
</tr>
<tr>
<td>Fever</td>
<td>84 (24.4)</td>
<td>140 (26.1)</td>
<td>114 (29.6)</td>
</tr>
<tr>
<td>Visited Health Centre for fever</td>
<td>60 (71.4)</td>
<td>96 (68.6)</td>
<td>71 (62.3)</td>
</tr>
<tr>
<td>Any illness</td>
<td>189 (54.9)</td>
<td>232 (43.3)</td>
<td>204 (53.0)</td>
</tr>
<tr>
<td>Visited Health Centre for any illness</td>
<td>116 (61.4)</td>
<td>156 (67.2)</td>
<td>125 (61.3)</td>
</tr>
</tbody>
</table>

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During the second follow-up period 599/786 of MSD and 591/803 non MSD children had an episode of diarrhoea, fever or cough (76.2\% of MSD vs. 73.6\% of non MSD p=0.151) and 519/599 (86.6\%) MSD and 497/591(84.1\%) healthy children visited a health centre for one or more of these symptoms. A similar proportion of MSD children experienced each of these symptoms, as were the proportions considered severe enough to be taken to the health centre by their caregivers (table 4). Of note, 65.1\% (306/470) of MSD and 57.2\% (261/456) of non MSD cohort had multiple episodes of diarrhoea.
Table 7-4. Illnesses and health facility visit n (%) reported by the MSD and non MSD children by age strata during the time between first follow up (at 60-90 days) and second follow up (18-24 months after the enrolment)

<table>
<thead>
<tr>
<th>Illness</th>
<th>0-11 months</th>
<th>12-23 months</th>
<th>24-59 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case (N=306)</td>
<td>Control (N=317)</td>
<td>Case (N=373)</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>175 (57.2%)</td>
<td>177 (55.8%)</td>
<td>215 (57.6%)</td>
</tr>
<tr>
<td>Visited Health Centre for diarrhoea</td>
<td>150 (85.7%)</td>
<td>137 (77.4%)</td>
<td>183 (85.1%)</td>
</tr>
<tr>
<td>Dysentery</td>
<td>7 (2.3%)</td>
<td>11 (3.5%)</td>
<td>8 (2.1%)</td>
</tr>
<tr>
<td>Visited Health Centre for dysentery</td>
<td>3 (42.9%)</td>
<td>7 (63.6%)</td>
<td>6 (75.0%)</td>
</tr>
<tr>
<td>Cough</td>
<td>30 (9.8%)</td>
<td>32 (10.1%)</td>
<td>37 (9.9%)</td>
</tr>
<tr>
<td>Visited Health Centre for cough</td>
<td>24 (80.0%)</td>
<td>25 (78.1%)</td>
<td>27 (73.0%)</td>
</tr>
<tr>
<td>Fever</td>
<td>172 (56.2%)</td>
<td>164 (44.0%)</td>
<td>194 (52.0%)</td>
</tr>
<tr>
<td>Visited Health Centre for fever</td>
<td>136 (79.1%)</td>
<td>137 (83.5%)</td>
<td>156 (80.4%)</td>
</tr>
<tr>
<td>Any illness</td>
<td>228 (74.5%)</td>
<td>233 (73.5%)</td>
<td>294 (78.8%)</td>
</tr>
<tr>
<td>Visited Health Centre for any illness</td>
<td>202 (88.6%)</td>
<td>200 (85.8%)</td>
<td>249 (84.7%)</td>
</tr>
</tbody>
</table>
Fractional polynomials and mixed model

As stated earlier (section 3.10 and 3.12 of chapter 3), a mixed model was built for each age group, to model a best relationship between the disease status and HAZ score over time. The case (MSD) control (non MSD) set was included as a random effect, as was the individual (to allow for repeated measures over time). Sampling weights were used to account for the sampling of MSD and non MSD. Age and sex were included in the models. Time variants (linear, squared, cubic, square root, reciprocal of square root and logarithmic) since diagnosis were used as a continuous variable. The polynomial terms included in the model were chosen by fitting a fractional polynomial model in a generalised estimating equation setting. Due to limitations of STATA in combining fractional polynomials and use of sample weights we chose to use the identified polynomials in a linear mixed model. For building the mixed models, all main effects (case control status and gender) and their interactions with time (in powers according to those found in the fractional polynomial models) were included initially. A backward stepwise elimination approach was then used to obtain the final parsimonious models, eliminating the non-significant (p>0.05) terms.
Model estimation and parameter estimates for 0-11 month age group

The matching factors age and sex, interaction with gender and time and interaction with disease status with time was put in the model. However, the model with main effects and the interaction terms of time variants with both gender and case control status showed no significant differences (table 7-5). When the interaction with disease status (MSD and non MSD) and time was dropped, the difference for gender with time was significant, with the boys being comparatively more stunted across both MSD and non MSD cohorts than the girls (p=0.049).
Table 7-5. Parameter estimates from the model with the main effects (age, gender and case control status) and the interaction of time with gender and case control status for 0-11month age group children

<table>
<thead>
<tr>
<th>Variables</th>
<th>Coefficients</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.001</td>
<td>-0.004 to 0.002</td>
<td>0.494</td>
</tr>
<tr>
<td>Gender</td>
<td>128.815</td>
<td>-303.352 to 560.981</td>
<td>0.559</td>
</tr>
<tr>
<td>Type</td>
<td>-23.250</td>
<td>-179.401 to 132.901</td>
<td>0.770</td>
</tr>
<tr>
<td>Time (linear)</td>
<td>0.886</td>
<td>-2.603 to 4.375</td>
<td>0.619</td>
</tr>
<tr>
<td>ln(√time)</td>
<td>-24.288</td>
<td>-88.657 to 40.081</td>
<td>0.460</td>
</tr>
<tr>
<td>ln(time)</td>
<td>-179.631</td>
<td>-519.604 to 160.342</td>
<td>0.300</td>
</tr>
<tr>
<td>1/√time</td>
<td>-97.999</td>
<td>-228.831 to 32.833</td>
<td>0.142</td>
</tr>
<tr>
<td>√time</td>
<td>177.316</td>
<td>-247.737 to 602.368</td>
<td>0.414</td>
</tr>
<tr>
<td>time²</td>
<td>-0.0004</td>
<td>-0.0006 to 0.0005</td>
<td>0.907</td>
</tr>
<tr>
<td>time³</td>
<td>-0.000000007</td>
<td>-0.0000000001 to 0.000000001</td>
<td>0.896</td>
</tr>
<tr>
<td>Type*time</td>
<td>-0.593</td>
<td>-5.245 to 4.060</td>
<td>0.803</td>
</tr>
<tr>
<td>Type* ln(√time)</td>
<td>13.967</td>
<td>-75.931 to 103.864</td>
<td>0.761</td>
</tr>
<tr>
<td>Type* ln(time)</td>
<td>90.238</td>
<td>-412.752 to 593.227</td>
<td>0.725</td>
</tr>
<tr>
<td>Type*1/√time</td>
<td>23.180</td>
<td>-132.988 to 179.347</td>
<td>0.771</td>
</tr>
<tr>
<td>Type*√time</td>
<td>-98.140</td>
<td>-700.900 to 504.620</td>
<td>0.750</td>
</tr>
<tr>
<td>Type* time²</td>
<td>0.0000409</td>
<td>-0.00067 to 0.00075</td>
<td>0.910</td>
</tr>
<tr>
<td>Type* time³</td>
<td>0.000000003</td>
<td>-0.0000001 to 0.965</td>
<td>0.965</td>
</tr>
<tr>
<td>Variables</td>
<td>Coefficients</td>
<td>95% CI</td>
<td>P</td>
</tr>
<tr>
<td>---------------------------</td>
<td>--------------</td>
<td>-----------------</td>
<td>-----------</td>
</tr>
<tr>
<td>Gender*time</td>
<td>-3.689</td>
<td>-12.572 to 5.194</td>
<td>0.416</td>
</tr>
<tr>
<td>Gender* ln(√time)</td>
<td>63.666</td>
<td>-84.223 to 211.558</td>
<td>0.399</td>
</tr>
<tr>
<td>Gender*ln(time)</td>
<td>306.898</td>
<td>-375.565 to 989.36</td>
<td>0.378</td>
</tr>
<tr>
<td>Gender*1/√time</td>
<td>-128.565</td>
<td>-560.748 to 303.618</td>
<td>0.560</td>
</tr>
<tr>
<td>Gender*/time</td>
<td>-410.820</td>
<td>-1353.98 to 532.341</td>
<td>0.393</td>
</tr>
<tr>
<td>Gender*time^2</td>
<td>0.0007</td>
<td>-0.001 to 0.002</td>
<td>0.439</td>
</tr>
<tr>
<td>Gender*time^3</td>
<td>-0.00000015</td>
<td>-0.0000005 to 0.0000005</td>
<td>0.452</td>
</tr>
</tbody>
</table>

After dropping the polynomial terms and their interaction in a backward stepwise elimination, gender and the time remained significant (table 7-8). Finally, tests to see the difference in growth for MSD and non MSD over time variants were not significant (p=0.703) indicating no influence of growth due to MSD over time in 0-11 month age group children. Figure 7-6 below shows the fitted curve for boys and girls in 0-11 month age group. Boys and girls, irrespective of their disease status at the baseline were below zero for their HAZ score. There was initial drop in their HAZ score followed by a catch up growth but that too remained below zero of the HAZ score for their respective age.
Figure 7-6 Growth curve based on the HAZ score for both boys and girls (0-11 month age group) from the final model showing the linear growth over time.

Model estimation and parameter estimates for 12-23 month age group:

For the 12-23 month age group, the mixed model began with the matching factors, interaction of time with disease status and gender. In the model, the main effects and the interaction of different time variants (linear, squared, cubic, square root, reciprocal of square root and logarithmic) with case control status and gender were not significant (table7-6). When these interaction terms were dropped case control status over time was marginally significant (p=0.055).
Table 7-6. Parameter estimates from the model with the main effects (age, gender and case control status) and the interaction of time variants with gender and case control status for 12-23 month age group children

<table>
<thead>
<tr>
<th>Variables</th>
<th>Coefficients</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.0008</td>
<td>-0.003 to 0.001</td>
<td>0.471</td>
</tr>
<tr>
<td>Gender</td>
<td>-15.261</td>
<td>-182.924 to 152.402</td>
<td>0.858</td>
</tr>
<tr>
<td>Type</td>
<td>-125.337</td>
<td>-288.475 to 37.802</td>
<td>0.132</td>
</tr>
<tr>
<td>Time (linear)</td>
<td>-0.140</td>
<td>-0.552 to 0.272</td>
<td>0.507</td>
</tr>
<tr>
<td>ln(time)</td>
<td>-12.697</td>
<td>-59.447 to 34.053</td>
<td>0.594</td>
</tr>
<tr>
<td>1/√time</td>
<td>4.806</td>
<td>-11.034 to 20.646</td>
<td>0.552</td>
</tr>
<tr>
<td>√time</td>
<td>-26.296</td>
<td>-133.806 to 81.213</td>
<td>0.632</td>
</tr>
<tr>
<td>time²</td>
<td>0.00008</td>
<td>-0.0001 to 0.0003</td>
<td>0.417</td>
</tr>
<tr>
<td>time³</td>
<td>-0.00000003</td>
<td>-0.00000009 to 0.00000003</td>
<td>0.353</td>
</tr>
<tr>
<td>Type*time</td>
<td>0.452</td>
<td>-0.125 to 1.029</td>
<td>0.125</td>
</tr>
<tr>
<td>Type*ln(time)</td>
<td>53.592</td>
<td>-15.796 to 122.980</td>
<td>0.130</td>
</tr>
<tr>
<td>Type*1/√time</td>
<td>125.325</td>
<td>-37.818 to 288.468</td>
<td>0.132</td>
</tr>
<tr>
<td>Type*√time</td>
<td>-17.800</td>
<td>-40.670 to 5.070</td>
<td>0.127</td>
</tr>
<tr>
<td>Type*time²</td>
<td>-0.0002</td>
<td>-0.000044 to 0.00005</td>
<td>0.125</td>
</tr>
<tr>
<td>Type*time³</td>
<td>0.00000006</td>
<td>-0.00000002 to 0.0000001</td>
<td>0.134</td>
</tr>
<tr>
<td>Gender*time</td>
<td>0.171</td>
<td>-0.471 to 0.813</td>
<td>0.602</td>
</tr>
<tr>
<td>Gender*ln(time)</td>
<td>10.289</td>
<td>-62.637 to 83.216</td>
<td>0.782</td>
</tr>
<tr>
<td>Variables</td>
<td>Coefficients</td>
<td>95% CI</td>
<td>P</td>
</tr>
<tr>
<td>-------------------</td>
<td>--------------</td>
<td>------------------</td>
<td>--------</td>
</tr>
<tr>
<td>Gender*1/√time</td>
<td>15.396</td>
<td>-152.247 to 183.039</td>
<td>0.857</td>
</tr>
<tr>
<td>Gender*time</td>
<td>-4.963</td>
<td>-29.679 to 19.752</td>
<td>0.694</td>
</tr>
<tr>
<td>Gender*time²</td>
<td>-0.0001</td>
<td>-.0004 to .0002</td>
<td>0.423</td>
</tr>
<tr>
<td>Gender*time³</td>
<td>0.00000005</td>
<td>-0.00000004 to 0.0000001</td>
<td>0.280</td>
</tr>
</tbody>
</table>

After dropping the polynomial terms in a stepwise fashion the interaction terms for case/control status with the follow-up time became significant (Table 7-8). Also, there were significant differences (p<0.001) in linear growth between MSD and non MSD children; indicating the influence of MSD on growth over time in the 12-23 months age group. Figure 6 shows the fitted curve, which indicate that both the cases and controls were stunted at the start of the follow-up, that both experienced some recovery, but that the controls recovered more than the cases.
Figure 7-7. Growth curve based on the HAZ score for MSD and non MSD children from the final model showing the linear growth over time for 12-23 month age group children

Model estimation and parameter estimates for 24-59 month age group:

For the age cohort of the 24-59 month age group, a full model with the interaction terms with time and disease status or type were not significant (table-7). A significant difference between the MSD and non MSD cohort over time (p<0.005) was observed.
Table 7-7. Parameter estimates from the model with the main effects (age, gender and case control status) and the interaction of time variants with gender and case control status for 24-59 month age group children

<table>
<thead>
<tr>
<th>Variables</th>
<th>Coefficients</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.001</td>
<td>-0.001 to 0.003</td>
<td>0.286</td>
</tr>
<tr>
<td>Gender</td>
<td>111.800</td>
<td>-110.378 to 333.979</td>
<td>0.324</td>
</tr>
<tr>
<td>Type</td>
<td>5.668</td>
<td>-202.675 to 214.012</td>
<td>0.957</td>
</tr>
<tr>
<td>Time (linear)</td>
<td>0.3667</td>
<td>-0.218 to 0.951</td>
<td>0.219</td>
</tr>
<tr>
<td>Ln(time)</td>
<td>32.728</td>
<td>-32.902 to 98.359</td>
<td>0.328</td>
</tr>
<tr>
<td>$1/\sqrt{time}$</td>
<td>69.613</td>
<td>-80.858 to 220.085</td>
<td>0.365</td>
</tr>
<tr>
<td>$\sqrt{time}$</td>
<td>-12.277</td>
<td>-34.603 to 10.048</td>
<td>0.281</td>
</tr>
<tr>
<td>Time$^2$</td>
<td>-0.00022</td>
<td>-0.0005 to 0.00005</td>
<td>0.112</td>
</tr>
<tr>
<td>Time$^3$</td>
<td>0.00000009</td>
<td>-0.00000001 to 0.000000001</td>
<td>0.053</td>
</tr>
<tr>
<td>Type*time</td>
<td>-0.142</td>
<td>-0.934 to 0.649</td>
<td>0.725</td>
</tr>
<tr>
<td>Type*ln(time)</td>
<td>-5.891</td>
<td>-96.285 to 84.503</td>
<td>0.898</td>
</tr>
<tr>
<td>Type*$1/\sqrt{time}$</td>
<td>-6.069</td>
<td>-214.365 to 202.227</td>
<td>0.954</td>
</tr>
<tr>
<td>Type*$\sqrt{time}$</td>
<td>3.468</td>
<td>-27.073 to 34.009</td>
<td>0.824</td>
</tr>
<tr>
<td>Type*time$^2$</td>
<td>0.000124</td>
<td>-0.00024 to 0.00049</td>
<td>0.503</td>
</tr>
<tr>
<td>Type*time$^3$</td>
<td>-0.0000006</td>
<td>-0.0000002 to 0.00000006</td>
<td>0.322</td>
</tr>
<tr>
<td>Gender*time</td>
<td>-0.477</td>
<td>-1.329 to 0.374</td>
<td>0.272</td>
</tr>
<tr>
<td>Gender*ln(time)</td>
<td>-49.711</td>
<td>-146.402 to 46.979</td>
<td>0.314</td>
</tr>
</tbody>
</table>
In the final model disease status (MSD and non MSD) and the linear time variable were significant (Table 7-8). The difference between the MSD and non MSD was also significant (p=0.007); indicating the influence of MSD on linear growth over time.
Figure 7-8: Growth curve based on the HAZ score for MSD and non MSD children from the final model showing the linear growth over time for 24-59 month age group children.

Figure 7-8 shows that children belonging to both MSD and non MSD cohort were stunted at the outset, the MSD children more so than the non MSD children that both appeared to recover height during follow-up but the cases remained persistently disadvantaged.

In the final model, parameter estimates that were significant for each age group are shown in table-8.
Table 7-8. Parameter estimates from the final model of HAZ score growth curves for each of the age cohorts

<table>
<thead>
<tr>
<th>Age group</th>
<th>Difference (coefficient)</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-11 month</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>0.233</td>
<td>0.013 to 0.453</td>
<td>0.038</td>
</tr>
<tr>
<td>time</td>
<td>-0.003</td>
<td>-0.004 to -0.002</td>
<td>0.000</td>
</tr>
<tr>
<td>time²</td>
<td>0.000003</td>
<td>0.000002 to 0.000004</td>
<td>0.000</td>
</tr>
<tr>
<td>12-23 month</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>time²</td>
<td>0.000002</td>
<td>0.000001 to 0.000003</td>
<td>0.037</td>
</tr>
<tr>
<td>Type* time</td>
<td>0.0025</td>
<td>0.0005 to 0.0045</td>
<td>0.016</td>
</tr>
<tr>
<td>Type* √time</td>
<td>-0.0458</td>
<td>-0.0695 to -0.0222</td>
<td>0.000</td>
</tr>
<tr>
<td>Type*time²</td>
<td>-0.000001</td>
<td>-0.000003 to 0.000002</td>
<td>0.092</td>
</tr>
<tr>
<td>24-59 month</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case control status (Type)</td>
<td>-0.4938</td>
<td>-0.72 to -0.27</td>
<td>0.000</td>
</tr>
<tr>
<td>time</td>
<td>0.001</td>
<td>-1.621 to -1.288</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Mean HAZ estimated from the best fitted model for MSD and non MSD cohort at different time points of assessment.
Mean HAZ was estimated from the final model. The MSD children in all age strata had a lower mean HAZ; but the difference (ΔHAZ) was statistically significant only in the oldest age group (p<0.001) where MSD children showed a linear growth faltering over time.

For the 0-11 month age group there was no difference between the MSD and non MSD cohort (Table 7-9) but there was difference among the boys and girls irrespective of their disease status over time. Boys, with MSD or non MSD in 0-11 month age group had a significantly lower (p=0.04) lower mean HAZ compared to the girls with MSD or non MSD.

Table 7-9. Mean HAZ for MSD and non MSD children in the age group of 0-11 month at three time point of assessment

<table>
<thead>
<tr>
<th></th>
<th>MSD</th>
<th>Non MSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean± SD 95% CI</td>
<td>Mean± SD 95% CI</td>
</tr>
<tr>
<td>Baseline</td>
<td>-0.85± 0.06 -0.97 to -0.73</td>
<td>-0.78± 0.06 -0.91 to -0.66</td>
</tr>
<tr>
<td>3 months</td>
<td>-1.14± 0.06 -1.26 to -1.03</td>
<td>-1.07±0.07 -1.20 to -0.94</td>
</tr>
<tr>
<td>24 months</td>
<td>-1.68± 0.07 -1.81 to -1.56</td>
<td>-1.61± 0.06 -1.74 to -1.49</td>
</tr>
</tbody>
</table>

*There was no interaction with time and case control status in the model. Even though there was change in the point estimates, the difference and the p values remained same.

In the 12-23 month age group, both MSD and non MSD children were below zero for the HAZ score for the age at enrolment. On follow-up MSD children
deteriorated further compared to non MSD children; who also failed to maintain linear growth. Both the groups had a catch up growth, but MSD children remained at a significantly lower level than the controls (Table 7-10).

Table 7-10. Mean HAZ for MSD and non MSD children in the age group of 12-23 month at three time point of assessment

<table>
<thead>
<tr>
<th>Time</th>
<th>MSD Mean±SD</th>
<th>95% CI</th>
<th>Non MSD Mean±SD</th>
<th>95% CI</th>
<th>Difference (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>-1.36±0.06</td>
<td>-1.47 to -1.25</td>
<td>-1.36±0.06</td>
<td>-1.47 to -1.24</td>
<td>-0.004 (-0.17 to 0.16)</td>
<td>0.966</td>
</tr>
<tr>
<td>3 months</td>
<td>-1.67±0.06</td>
<td>-1.79 to -1.55</td>
<td>-1.44±0.06</td>
<td>-1.56 to -1.33</td>
<td>-0.23 (-0.39 to -0.07)</td>
<td>0.006</td>
</tr>
<tr>
<td>24 months</td>
<td>-1.35±0.06</td>
<td>-1.47 to -1.22</td>
<td>-1.15±0.06</td>
<td>-1.26 to -1.03</td>
<td>-0.20 (-0.36 to -0.04)</td>
<td>0.016</td>
</tr>
</tbody>
</table>

The oldest age group showed a clear difference between the MSD and non MSD cohorts. The model showed a linear relationship of linear growth over time for both the MSD and non MSD cohort. Both the cohorts were below zero of HAZ score for the age. They followed the growth trajectory, and continued to grow; but at all the time points of assessments the difference between the MSD and non MSD remained significant (Table 7-11).
Table 7-11. Mean HAZ for MSD and non MSD children in the age group of 12-23 month at three time point of assessment

<table>
<thead>
<tr>
<th>Time</th>
<th>MSD</th>
<th>Non MSD</th>
<th>Difference (95% CI)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>-1.95±0.09</td>
<td>-1.45±0.08</td>
<td>-0.49 (-0.72 to -0.27)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>3 months</td>
<td>-1.86±0.09</td>
<td>-1.37±0.08</td>
<td>-0.49 (-0.72 to -0.27)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>24 months</td>
<td>-1.25±0.11</td>
<td>-0.76±0.09</td>
<td>-0.49 (-0.72 to -0.27)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

There was no interaction with time and case control status in the model. Even though there was change in the point estimates, the difference and the p values remained same.

Primary caregivers’ education was found to be a risk factor for a child to suffer from MSD (chapter 6, table 6.6). In the final model for all these three age groups, interaction of education with disease status was considered. The primary caregivers’ education level did not significantly influence the linear growth of the child in any of the age groups (p=0.361 for 0-11month, p=0.885 for 12-23 month and p= 0.107 for 24-59 month old age group).

Overall Stunting in both cohorts:

At enrolment, 264/1024 (25.8%) of MSD and 393/1562 (25.2%) of non MSD cohort were stunted (Figure 3) and this difference was not statistically significant (p=0.747). At the first follow-up, 276/875 (31.5%) of MSD and 395/1446 (27.3%) of non MSD cohort were stunted (p=0.03) and 230/786 (29.3%) of MSD and 224/803 (28.0%) of non MSD cohort were stunted during the second follow up.
(p=0.579). For 0-11 months age cohorts the proportion of stunting gradually increased, and the trigger level accelerated from low to medium. But the stunting for two older age groups showed improvement over time, though the trigger level was still at medium to high level (figure-9).

Figure 7-9: Age stratified proportion of stunting for MSD and non MSD cohort at baseline, first and second follow up time
7.4 Discussion

In this study, the MSD cohort was more likely to die, especially by the first follow-up, and they tended to have worse general health over the follow-up period than non MSD group, although the majority of both groups had multiple episodes of diarrhoea. More than 25% of MSD and non MSD cohorts were stunted at the time of enrolment and during 18-24 months of follow-up. While the proportions that were stunted only differed significantly in the 12-23 month age group, between MSD and non MSD cohorts at any of the three time-points; more differences in mean HAZ scores by age group and gender were identified. In all age groups and at all-time points, mean HAZ scores were lower for MSD children than non MSD children. The pattern of linear growth was different in different age groups. For those 0-11 months old at recruitment, there was continued growth faltering in both the MSD and non MSD cohort, evident by decreased mean HAZ scores over follow-up. In the older two age groups, the MSD cohort showed an initial fall and then a catch up, and the non MSD cohort showed continued catch up growth. Continued breast feeding was positively associated with linear growth. It is possible that the comparatively better HAZ observed in the 0-11 month age group was due to the protection offered by the continued breastfeeding.\textsuperscript{277, 278, 279} The differences in mean HAZ scores between MSD and non MSD cohorts widened in the older age groups, reaching
significance at the first follow-up in the 12-23 month age group and for both older age groups at the second follow-up.

The overall prevalence of stunting in our study is consistent with those in other developing countries. It is estimated that 32% of children aged under five years old, in developing countries are stunted. And among 40 countries where 40% of under five years old children are stunted, 23 are in Africa.\textsuperscript{280} The basic pattern of growth observed in our study is also consistent with similar settings. Children in developing countries such as The Gambia tend to lose ground in the first 2 years of life and undergo varying degrees of catch-up. The growth pattern also depends on various socioeconomic and environmental factors but the probability of catching up is similar across all age groups in under five year olds.\textsuperscript{281, 282, 283}

Till date there are no studies that have shown the differences in stunting and mean HAZ scores over time in relation to a severe diarrhoeal episode. A study in Brazil showed a strong association between diarrhoea and subsequent malnutrition. In this study hospital admitted children with diarrhoea were followed for 36-52 months. The association was strongest in the first two years of life but it remained significant until the fourth year. Children with diarrhoea had a lower mean HAZ (-1.11) compared to the children hospitalized without diarrhoea (mean HAZ-0.64). Children in this study were not from a particularly low socioeconomic group.\textsuperscript{80} Our study findings, in a population from a lower
socioeconomic background and with moderate to severe diarrhoea, showed similar growth patterns.

A study in rural Bangladesh by Black et al showed that diarrhoea caused by *Shigella* had a negative association with linear growth within a follow up period of both at 60 days and one year. Later, Briend et al. showed that children with dysentery, had delayed linear growth up to six months following the episode. In both studies no differences were noted for diarrhoea due to other causes.  

Recently, a study in semi urban Peru by Checkley et al. revealed that height catch up did not manifest until two months following an acute episode of diarrhoea and 2.3% of diarrhoea prevalence was responsible for 2-27% of the growth deficit observed in the first 24 months of life. Diarrhoea before the age of six months resulted in long term height deficit as opposed to the transient height loss for the older age group. Studies in neighbouring Guniea-Bissau showed a significant difference in linear growth of children with *Cryptosporidium* infection (p<0.02) with the main effect in less than one year age group of children at a follow up 180 days after detected infection. This study did not show the relation of growth to pathogen specific diarrhoea, noting that it is unlikely that pathogen specific information will be routinely available in settings such as Gambia.
Of interest, there is some evidence that diarrhoea has a greater effect on growth than other significant illnesses. A study in a Guatemalan village showed a 6.3% greater increase in length in a low frequency diarrhoea group compared to high frequency group. Such a difference was not noticed in relation to fever or respiratory tract illnesses. A similar observation was made in rural Gambia in the late '70s where children under three years of age were assessed for both height and weight at one month intervals. In the study, relationships between nine categories of childhood diseases with the nutritional status were evaluated. None of those diseases except diarrhoea had a negative relation with nutritional status. A regression coefficient of 4 mm per month in height gain against diarrhoea prevalence was noted in children between the ages of 6-36 months. More than half of both the cohorts in our study reported to have multiple episodes of diarrhoea during the follow-up period. Overall, diarrhoea and other illnesses over the follow-up period were more commonly reported in cases than the controls. The large number of illnesses experienced during follow-up in both case and control groups is worthy of note. Such continuous insults compromise nutritional status and may hinder growth. In a study children under five years of age and who had recurrent diarrhoea had a height deficit compared to those who did not have diarrhoea. Widening of the difference in ΔHAZ between the MSD and non MSD cohorts, especially in the two older cohorts may well be due to the relatively higher on going morbidity in the case cohort. It seems likely that the MSD cases represent a segment of the community with more
comorbidity prior to the episode that led to recruitment, and into the future. The large number of illnesses in the non MSD cohort, especially due to diarrhoea, despite a robust method for their selection, also suggests that it is difficult in this community to select a comparator group that is very different from cases that are recruited.

It is possible that environmental enteropathy was an issue in our study. Environmental enteropathy, causes growth faltering due to mal-absorption of nutrients and is thought to be associated with poor sanitation in general. Studies suggest that 43% of the children in Gambia and about 87% of children in African settings like Malawi have environmental enteropathy and that has strong association with linear growth in children.

Other studies have shown that boys tend to be stunted more than the girls. A similar pattern is seen in the younger age group during the enrolment and first follow up but there was no difference in the long term. Also girls are more responsive to nutritional stress and can catch up growth at a relatively faster pace as seen in other studies.

Apart from the difficulties with the differences between cases and control, there were other limitations to this study. Repeated follow up with short intervals would have provided more information on the pattern of change over time.
However, it may also have led to repeated intervention such that the results would not reflect reality. The follow-up was not done on exactly the same date for each child, likely contributing to the variability in HAZ that was observed. A mixed model was built that took this variability into account. I did not account for seasonal variation of growth as countries in West Africa have a distinct ‘hungry’ season. However, recruitment was evenly spread over time for cases and controls and they were effectively matched on season. Finally the rural nature of the study site, illiteracy and low socioeconomic condition are deterrents to unconstrained growth in sub Saharan Africa. Like any other cohort study, loss to follow up was also a concern in our study. However 89% of the eligible cases and 93% of the eligible controls were interviewed during the first follow up. More than 80% of cases and an equal number of controls were interviewed during the second follow up.

This study had several strengths. MSD cases were ascertained through a well-defined method and rigorous screening. The findings are consistent with the “vicious cycle of malnutrition and infection (MSD). The management of diarrhoea does not end with the treatment given at home or hospital but needs to be continued with nutritional supplementation at home for the recovery of losses. There is evidence that the negative effect of diarrhoea on linear growth can be abated with adequate nutritional supplementation soon after the after recovery from an acute episode. It is likely that post diarrhoea management
practices were widely absent in our study locale, rendering the children with diarrhoea vulnerable to growth retardation.\textsuperscript{301}

Children under 5 years of age with MSD have lower mean HAZ scores than controls and this tends to persist over at least next 18-24 months with some widening of the gap in mean HAZ scores in the older age groups. They also are more likely to die and tend to have more illnesses over follow-up. This suggests that children with MSD themselves are both an acute and a chronic high risk group and nutritional rehabilitation should be integrated into their management. Favourable conditions need to be restored for the children with MSD to facilitate the growth acceleration.\textsuperscript{302} In the children with MSD in this population, diarrhoeal-specific community based interventions may well be worthwhile, including initiation of home based management with oral rehydration solutions and Zinc supplementation.
Chapter 8- Conclusion

HOPE ON THE HORIZON
8.1 Introduction

Diarrhoea is a very common illness and especially affects children. Diagnosis of diarrhoea is symptom based and does not require any special diagnostics test. Fluid replacement is the cornerstone of diarrhoeal management and a key intervention to avert death. Yet the disease remains the second leading cause of mortality and morbidity among children aged under five years old globally, with the greatest burden being in sub-Saharan Africa. If diarrhoeal illnesses and deaths cannot be prevented, Millennium Development Goal 4 will remain far from reach.

After decades of research on diarrhoea morbidity and mortality, there remain large gaps in our understanding of the disease. Major gaps include a lack of comprehensive data on symptoms, lack of characterization of diarrhoea in relation to symptoms, lack of clarity regarding the main aetiological agents, seasonal variations and limited known risk factors and data on the consequences of diarrhoeal illness. In the studies presented here, we were able to address all of these issues to some extent and to understand diarrhoeal disease as a whole. The source population for our study was a population of 160,000 people in rural Gambia under demographic surveillance and constituted one tenth of the total
population of the country, representing all the major ethnic groups. The study population is representative of the nation and typical of rural Africa. Thus the study has broader generalizability.

In this concluding chapter the findings and their relevance for policy and future research are highlighted.

8.2 Health Utilization and Attitude Survey

Major findings

An established generic protocol was used to understand the knowledge, attitude and practice regarding diarrhoea in a rural community in Gambia. Caregivers of children were asked about the symptoms of diarrhoea and signs of severity, the need for and place of care, and treatment. The caregivers showed poor knowledge and perception regarding diarrhoeal disease. Half did not take their child to a health facility when they had diarrhoea, ORS use was low, and zinc supplementation was not used either in the community or by the health care facilities. The period prevalence of diarrhoea in the community during the study period was estimated.
Recommendations for practice and policy

1. A media campaign to raise awareness of the diarrhoeal disease and to promote use of home-made ORS.

2. Traditional Birth Attendants and Community Health Workers should be provided with ORS sachets as the first point of care. They should be adequately trained to assess the level of dehydration and make referrals to the health centres.

3. Zinc use needs to be scaled up and practiced for the treatment of diarrhoea at health centres.

Future research

1. Continued cross sectional surveys (at least two per year to address the seasonality) across the DSS to monitor diarrhoeal disease prevalence among children aged under five years old in the community. It is possible to incorporate the abridged version of the survey with regular DSS activity.

2. Public health evaluation of an intervention using focus group discussion, nutritional and hygiene education to reduce the prevalence of diarrhoea in the community.
8.3 Case characteristics and aetiology of diarrhoea in children aged under five years old in rural Gambia

Major findings

Surveillance for MSD among children aged under five years old in the health centres provided cases for this study. The facility based incidence of MSD during the study period showed a steady decline. Cases were age, sex and area matched to healthy children in the community. The aetiological agents were identified using both conventional and advanced microbiological and molecular techniques. Of note, the top five pathogens detected causing diarrhoea in children aged under five years old in rural Gambia were rotavirus, ETEC, *Shigella spp.*, *Cryptosporidium Spp.* and norovirus. The case control design of the study allowed us to detect the pathogenicity index. The associated symptoms of MSD were identified and seasonality of diarrhoea in this population was described.

Recommendations for practice and policy

1. Emphasis should be given on ORS use by the health centres. Functional ORT (Oral rehydration therapy) corners at the triage at each of the health centre and operated by an IMCI (integrated management of childhood illness) trained nurse is essential. ORS sachets should be made available
and the caregivers need to be educated on the preparation and use of ORS.

2. Vaccination against rotavirus needs to be implemented in the EPI.

3. Proper practice guidelines need to be in place for the management of diarrhoea, especially when prescribing antimicrobial agents (Among the top five pathogens isolated from the study and apart from *Shigella* spp., none would be susceptible to antimicrobial therapy and benefit from its use)

4. Resource mobilization is necessary during the two peak seasons of diarrhoea to combat the disease.

5. Systematic surveillance of diarrhoeal disease in the major health in the major health centres to observe the trend in diarrhoeal disease in under-five population.

**Future research**

1. Rotavirus vaccine effectiveness study with an embedded case control study.

2. Trials of new vaccines in different stages of development. E.g. Phase III trial of a candidate *Shigella* vaccine; Safety and immunogenicity of ETEC vaccine.
3. Randomized controlled trial to evaluate the efficacy of Nitazoxanide (an antiprotozoal agent) against diarrhoea due to Cryptosporidium spp.

4. Case contact study to show the prevalence of asymptomatic carriers in households.

8.4 Risk factors for diarrhoea

Major findings

The socio demographic, environmental, water, sanitation and hygiene risk factors were identified for MSD in children aged under five years old under demographic surveillance. Illiteracy and the absence of any of the parents were the major social and demographic factors related to MSD. Close proximity with livestock and domestic animals were found to be environmental risk factors. Our study also revealed that storage of water, and no or improper treatment of drinking water were risk factors. Caregivers’ practice of faeces disposal, improper hand washing and absence of a toilet in the household were also associated with MSD. The pathogen specific risk factors were also similar to the risk factors for diarrhoea in general. Other independent pathogen specific risk factors are as follows: MSD due to Shigella spp. was associated with having a cow or presence of a rodent in the household and absence of toilet facilities. Children were more likely to have MSD due to Cryptosporidium spp. if there were cats in
the house and household had no toilet facilities. MSD due to ETEC was more common in children if there were cows in the household or there was no toilet facility in the household. Having cows and cats were risk factors for norovirus and rotavirus MSD respectively.

**Recommendations for practice and policy**

1. This study supports initiatives to improve female literacy and empowerment in Gambian society.

2. Availability of safe water to the community and household: existence of distribution system and access without availability is clearly suboptimal.

3. Regular treatment of water from the point of distribution should be considered for implementation.

4. Low cost filtration techniques should be considered at the community level.

5. Ventilated pit latrines should be encouraged.

6. Animal rearing in the household or compound should be discouraged.

**Future research**

1. Cluster randomized trial to evaluate the effectiveness of multiple interventions (e.g. education, filtration technique, hand washing by soap) to reduce the incidence of diarrhoea in the community.
2. Acceptability and use of ventilated pit latrine in rural community.

3. Assessment of water quality at different points of distribution (source, store) and consumption.

4. Comparison of low cost filtration technique vs. cloth filtration in reducing the microbial contamination of water.

5. Study of zoonotic transmission of enteric pathogens (isolation or detection of species from animal and human.)

8.5 Stunting in children with diarrhoea

Major findings

Both the case and control cohorts were followed to observe the association of stunting with an episode MSD. Stunting was prevalent in the population irrespective of case or control status. Children suffered from multiple episodes of diarrhoea and other childhood illnesses during the follow-up periods, which made them more vulnerable to stunting. Children with an acute episode of MSD had relatively lower growth potential than the controls. An episode of MSD maintained an association with stunting even at 18 months of follow-up.
**Recommendations and policy decision**

1. The overall nutritional status of the under-five population needs to be addressed.
2. Introduction of nutritional practices to the caregivers of the children in the health centre.
3. Nutritional rehabilitation in the hospital and in the community following discharge from a health centre.
4. Supplementation with micronutrients and other interventions are required for the active management of MSD and they should be incorporated in practice guidelines.

**Future research**

1. Scaling up zinc use in young children in rural Africa.
2. Intervention with low cost, easily available and acceptable nutrition supplements (sweet potatoes, ground nut, lentils, spinach etc.) and their long term effect on growth in children.
3. Observational studies of cognitive development and growth in children in rural Africa.
**Recommendations for immediate policy decision**

1. Media campaign to raise awareness of diarrhoeal disease, to promote use of home-made ORS and zinc for the management of diarrhoea.

2. Introduce rotavirus vaccination in the EPI.

3. Establish systematic surveillance of diarrhoeal disease in the major health centres to observe trends in diarrhoeal disease in the under-five population.

4. Encourage improvement of water sanitation and hygiene behaviour through public and private initiatives.

5. Initiate implementation of nutritional rehabilitation in the hospital and following discharge from a health centre.

These recommendations are based on the major findings from this study. They are focused on low cost and, for some, international assistance is available (e.g. vaccine against rotavirus). Some of the risk factors (hand washing, water supply) are also associated with the reduction of other childhood illnesses.\(^{247, 303, 304}\) Hence addressing them may have more widespread benefits. There is need for national commitment towards containing diarrhoeal disease.
Immediate research agenda

1. Public health evaluation of an intervention using nutritional and hygiene education to reduce the prevalence of diarrhoea in the community.

2. Rotavirus vaccine effectiveness study with an embedded case control study.

3. Randomized controlled trail to evaluate the efficacy of Nitazoxanide (an antiprotozoal agent) against diarrhoea due to Cryptosporidium spp.

4. Comparison of low cost filtration technique vs. cloth filtration in reducing the microbial contamination of water.

5. Evaluation of an intervention with low cost, easily available and acceptable nutrition supplements (sweet potatoes, ground nut, lentils, spinach etc.) and their long term effect on growth in children.

Future research needs to be given priority by donors and funding agencies, and should be focused on preventing diarrhoeal disease and improving outcomes from diarrhoeal illness. The studies presented here provide a comprehensive understanding of diarrhoeal illness in the community, of MSD presenting to health facilities, and of the outcome of the disease. There is now a need to focus on interventions to combat MSD, so that global mortality due to diarrhoea will decline.
References


adenovirus and enteric parasites among pediatric patients attending Saint Camille Medical Centre in Ouagadougou. Pak J Biol Sci 10: 4266-70.


160. Huttly SR, Blum D, Kirkwood BR, Emeh RN, Feachem RG, 1987. The epidemiology of acute diarrhoea in a rural community in Imo State,
Nigeria. Transactions of the Royal Society of Tropical Medicine and Hygiene 81: 865-70.


on 20th November 2012 at 1430 hrs


Reduction of cholera in Bangladeshi villages by simple filtration. Proc Natl Acad Sci U S A 100: 1051-5.


Appendix 1: Case Report Forms (CRFs)

<table>
<thead>
<tr>
<th>CRF 02 – REGISTRATION LOG FOR CASES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study #013</td>
</tr>
<tr>
<td>Site Center</td>
</tr>
</tbody>
</table>

Directions: Complete the following information for each child younger than 5 years old who is seeking medical care at the health facility.

<table>
<thead>
<tr>
<th>Number</th>
<th>Time</th>
<th>Cluster Unit</th>
<th>Age In Months</th>
<th>Gender</th>
<th>Hospitalized?</th>
</tr>
</thead>
<tbody>
<tr>
<td>000016</td>
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</table>

Qualifies for DSS? Yes No

Three or more abnormally loose or watery stools in the previous 24 hour period? Yes No

<table>
<thead>
<tr>
<th>Number</th>
<th>Time</th>
<th>Cluster Unit</th>
<th>Age In Months</th>
<th>Gender</th>
<th>Hospitalized?</th>
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<tr>
<td>000017</td>
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</table>

Qualifies for DSS? Yes No

Three or more abnormally loose or watery stools in the previous 24 hour period? Yes No

<table>
<thead>
<tr>
<th>Number</th>
<th>Time</th>
<th>Cluster Unit</th>
<th>Age In Months</th>
<th>Gender</th>
<th>Hospitalized?</th>
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<tbody>
<tr>
<td>000018</td>
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</table>

Qualifies for DSS? Yes No

Three or more abnormally loose or watery stools in the previous 24 hour period? Yes No

<table>
<thead>
<tr>
<th>Number</th>
<th>Time</th>
<th>Cluster Unit</th>
<th>Age In Months</th>
<th>Gender</th>
<th>Hospitalized?</th>
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</tbody>
</table>

Qualifies for DSS? Yes No

Three or more abnormally loose or watery stools in the previous 24 hour period? Yes No

<table>
<thead>
<tr>
<th>Number</th>
<th>Time</th>
<th>Cluster Unit</th>
<th>Age In Months</th>
<th>Gender</th>
<th>Hospitalized?</th>
</tr>
</thead>
<tbody>
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</tbody>
</table>

Qualifies for DSS? Yes No

Three or more abnormally loose or watery stools in the previous 24 hour period? Yes No

Interviewer’s Code: Quality Control’s Code: Date: Day Month Year
CRF 03 – ELIGIBILITY FOR CASES

1. Child's birthdate: [ ] [ ] [ ] [ ] [ ] [ ] Age: [ ] [ ] Months

2. Child's gender: [ ] Boy [ ] Girl

Eligibility Checklist

3. Does the child qualify as a DSS resident? [ ] No [ ] Yes [ ] DK

4. Do you believe that this child is not currently enrolled in GEMS as a case? [ ] No [ ] Yes [ ] DK

5. Is this child 0-59 months of age? [ ] No [ ] Yes [ ] DK

6. Did this child pass 3 or more abnormally loose stools during the previous 24 hours? [ ] No [ ] Yes [ ] DK

7. Did current diarrhea episode begin within the previous 7 days? [ ] No [ ] Yes [ ] DK

8. Before this episode began, did the child have at least 7 days without diarrhea? [ ] No [ ] Yes [ ] DK

9. Does the child have ANY ONE of the following indicating moderate/severe diarrhea?
   a. Sunken eyes, more than normal [ ] No [ ] Yes [ ] DK
   b. Loss of skin turgor [ ] No [ ] Yes [ ] DK
   c. Intravenous rehydration administered or prescribed [ ] No [ ] Yes [ ] DK
   d. Dysentery (diarrhea with visible blood in stool observed or reported) [ ] No [ ] Yes [ ] DK
   e. Hospitalized with diarrhea or dysentery [ ] No [ ] Yes [ ] DK

10. Is the child eligible for enrollment? (The child is eligible only if the answers to the Questions 3 through 8, and at least one of the Questions 9a to 9e are "Yes"). [ ] No [ ] Yes [ ] DK

10a. If any response to Questions 3 – 8 or 9a – 9e are DK, check the option that best describes why you were not able to determine eligibility.
   [ ] Caretaker not available
   [ ] Clinician not available
   [ ] Both caretaker & clinician not available
   [ ] Other, specify

(If response to Q10 is "No", STOP, and end the interview by thanking the caretaker/parent for his/her participation. Write down the name and staff code and submit the form to the DCC. If child is eligible, continue to Question 11.)

Interviewer's Name: ____________________________

Quality Control's Name: ____________________________

Staff code: [ ] [ ] [ ] [ ]

Date: [ ] [ ] [ ]

CRF03 Revised 18Nov2004

Page 1 of 2
CRF 03 – ELIGIBILITY FOR CASES

11. Was consent obtained?  No  Yes

12. Was child given antibiotic before whole stool sample could be collected?  No  Yes

13. If ‘No’ to Question 12, was a stool sample collected from the child within 12 hours of registration?  No  Yes

14. If ‘Yes’ to Question 12, were rectal swabs taken before antibiotics AND was a whole stool collected within 12 hours of registration?  No  Yes

15. Was the child enrolled?  No  Yes

16. If eligible but not enrolled, what was the reason? (Check one of the two main reasons.)

- Not invited by health center for one of the following reasons:
  - After hours presentation
  - Unable to collect a rectal swab before the child received antibiotics
  - Unable to produce adequate stool sample (10 grams with a minimum of 1 gram) within 12 hours of registration
  - 14 day quota filled
  - Child died before invitation
  - Child too sick
  - Other, specify

- Refused by parent/caretaker for one of the following reasons:
  - Parent/caretaker too busy
  - Does not like research
  - Child too sick
  - Other, specify

Notes or comments [Initial and date notes.]

Interviewer's Name

Quality Control's Name

337
Section 1: Demographic and Epidemiological Information

1. Who is [Child's Name]'s primary caretaker?
   - [ ] Mother
   - [ ] Father
   - [ ] Sister
   - [ ] Brother
   - [ ] Grandmother
   - [ ] Grandfather
   - [ ] Aunt
   - [ ] Uncle
   - [ ] No relation
   - [ ] Other relation by blood or marriage, specify____________________

2. What is your relationship to [Child's Name]?
   - [ ] Mother
   - [ ] Father
   - [ ] Sister
   - [ ] Brother
   - [ ] Grandmother
   - [ ] Grandfather
   - [ ] Aunt
   - [ ] Uncle
   - [ ] No relation
   - [ ] Other relation by blood or marriage, specify____________________

3. Where does [Child's Name]'s mother live?
   - [ ] Living in household
   - [ ] Abroad
   - [ ] Died
   - [ ] Lives outside of household
   - [ ] Whereabouts unknown

4. Where does [Child's Name]'s father live?
   - [ ] Living in household
   - [ ] Abroad
   - [ ] Died
   - [ ] Lives outside of household
   - [ ] Whereabouts unknown

5. How far did the child’s primary caretaker go in school?
   - [ ] No formal schooling
   - [ ] Less than primary
   - [ ] Completed primary
   - [ ] Completed secondary
   - [ ] Post-secondary
   - [ ] Religious education only
   - [ ] Don’t know

6. How many people have been living regularly in your household for the past 6 months? __________

7. How many people have been sleeping regularly in your household for the past 6 months? __________

8. How many children younger than 60 months live in the household? __________
9. How many rooms in your household are used for sleeping?  

10. What is the predominant floor in the house of [Child’s Name]?  

- Natural Floor  
  - □ Earth/Sand  
  - □ Dung  

- Rudimentary Floor  
  - □ Wood planks  
  - □ Palm/bamboo  

- Finished Floor  
  - □ Parquet or polished wood  
  - □ Vinyl or asphalt strips  
  - □ Ceramic Tile  
  - □ Cement  
  - □ Carpet  

□ Other, specify ______________________

11. Does your household have the following? [Must be functioning: "X" all that apply.]  

- □ Electricity  
- □ Television  
- □ Motorcycle/scooter  
- □ Radio  
- □ Bicycle/rickshaw  
- □ Car/truck  
- □ Refrigerator  
- □ Boat with a motor  
- □ Telephone (mobile or non-mobile)  
- □ Animal-drawn cart  
- □ Agricultural land  
- □ None of the above

12. What type of cooking fuel does your household use? ["X" all that apply.]  

- □ Electricity  
- □ Liquid Propane Gas  
- □ Natural Gas  
- □ Kerosene  
- □ Biogas  
- □ Coal/lignite  
- □ Charcoal  
- □ Wood  
- □ Straw/straw/grass  
- □ Animal dung  
- □ Agricultural crop residue  
- □ Other, specify ______________________

13. Do the following animals live in the compound where [Child’s Name] lives? ["X" all that apply.]  

- □ Goat  
- □ Sheep  
- □ Dog  
- □ Cat  
- □ Cow  
- □ Rodents  
- □ Fowl (chicken, duck or other birds)  
- □ No Animals  
- □ Other, specify ______________________
14. During the last two weeks, has your household ever obtained drinking water from any of the following sources? [Check all that apply.]

- [ ] Piped into house
- [ ] Piped into yard
- [ ] Public tap
- [ ] Open well in house or yard
- [ ] Open public well
- [ ] Pond or lake
- [ ] Deep tube well
- [ ] Shallow tube well
- [ ] Other, specify

- [ ] Covered well in house or yard
- [ ] Covered public well
- [ ] Protected spring
- [ ] Unprotected spring
- [ ] River or stream
- [ ] Dam or earth pan
- [ ] Rainwater
- [ ] Bought (tank, bottles, etc)
- [ ] Bore hole

15. During the last two weeks, what was the main source of drinking water for the members of your household? [Mark only one response that relates to the main source of drinking water.]

- [ ] Piped into house
- [ ] Piped into yard
- [ ] Public tap
- [ ] Open well in house or yard
- [ ] Open public well
- [ ] Pond or lake
- [ ] Deep tube well
- [ ] Shallow tube well
- [ ] Other, specify

[Use your response from Question 15 to answer Questions 16 and 17. If the response to Question 15 is “piped into house/yard”, “open or covered well in house/yard” or “rainwater”, then go to Question 18. Otherwise continue.]
16. How long does it take to go there, get water, and come back?

- [ ] Less than 15 minutes
- [ ] 15 to 29 minutes
- [ ] 30 to 59 minutes
- [ ] 1 to 3 hours
- [ ] More than 3 hours

17. Do you or other members from your household go and fetch drinking water for the household every day?

[If ‘Yes’, go to Question 17a. If ‘No’, go to Question 17b.]

17a. On average, how many trips do you and members from your household make to fetch water each day?

- [ ] No
- [ ] Yes

17b. On average, how many trips do you and members from your household make to fetch water each week?

- [ ] Number of trips/day
- [ ] Number of trips/week

18. In the last two weeks, how often has water been available from this main source?

- [ ] All the time
- [ ] A few times per week
- [ ] Several hours every day
- [ ] Less frequent than a few times per week
- [ ] No
- [ ] Yes

19. In the last two weeks, did you give [Child’s Name] stored water for drinking?

- [ ] No
- [ ] Yes

20. Do you usually treat drinking water at home?

[If ‘No’, go to Question 23.]

21. Which method do you use the most to treat drinking water at home? [‘X’ only one response.]

- [ ] Leave water in sun to disinfect
- [ ] Boil
- [ ] Filter through cloth
- [ ] Filter through ceramic or other filter
- [ ] Chlorine liquid, powder, or tablets
- [ ] Alum
- [ ] Other chemical or additive, specify

[If chlorine is not used, skip to Question 22.]

21a. If you use chlorine liquid, powder or tablets, which type do you most commonly use? [‘X’ only one response.]

- [ ] Certeza
- [ ] Watermaker
- [ ] Aquatabs
- [ ] PuR
- [ ] AquaGuard
- [ ] Unknown
- [ ] Other, specify
22. In the last two weeks did you give [Child’s Name] water which was not treated?  No  Yes

23. How do you usually dispose of [Child’s Name]’s feces?  [“X” only one response.]
   □ Scatter in yard
   □ Bury
   □ Toilet, latrine
   □ Bush/Field/Ground/Stream/Open sewer
   □ Do nothing
   □ Other, specify_____________________

24. What kind of facility does your household most commonly use to dispose of human fecal waste?  [Show pictures to confirm the identity of the facility used.  “X” only one response.]
   □ Flush toilet
   □ Ventilated improved pit (VIP) latrine
   □ Traditional pit toilet
   □ Pour flush toilet
   □ No facility: Bush/Field/Ground/Stream/Open sewer
      [If “No facility” selected, go to Question 26]
   □ Other, specify_____________________

25. How many households (other than your own) share this facility?  □□
   [Respond with a number, code “00” for none.]

26. When do you usually wash your hands?  [“X” all that apply. Do not probe.]
   □ Before eating
   □ Before cooking
   □ Before you nurse or prepare baby’s food
   □ After you defecate
   □ After handling domestic animals
   □ After cleaning child who defecated
   □ Never
   □ Other, specify_____________________

27. When you wash your hands, what do you usually use?  [“X” only one.]
   □ Water only
   □ Water and soap
   □ Water and ashes
   □ Water and mud or clay

Section 2: Clinical Information

28. Is [Child’s Name] currently breastfed?
   □ No
   □ Partial breastfeeding
   □ Exclusive breastfeeding

29. How many days including today has this episode of diarrhea lasted?  □□
30. Since [Child's Name] became ill with diarrhea, how would you best describe the stool? [
"X" the most common.]
☐ Simple watery  ☐ Rice watery stool  ☐ Sticky/mucoid  ☐ Bloody

31. During the illness, what was the maximum number of loose stools that [Child's Name] passed in a day (24-hour period)? ["X" only one response.]
☐ ≤ 6 per day  ☐ 7 to 10 times per day  ☐ More than 10 times per day

32. Did [Child's Name] have any of the following since this illness began?  
   a. Blood in stools ☐ ☐ ☐  
   b. Vomiting 3 or more times per day ☐ ☐ ☐  
   c. Very thirsty ☐ ☐ ☐  
   d. Drank much less than usual ☐ ☐ ☐  
   e. Unable to drink ☐ ☐ ☐  
   f. Belly pain ☐ ☐ ☐  
   g. Fever measured at least 38°C or parental perception ☐ ☐ ☐  
   h. Irritable or restless ☐ ☐ ☐  
   i. Decreased activity or lethargy ☐ ☐ ☐  
   j. Loss of consciousness ☐ ☐ ☐  
   k. Rectal straining ☐ ☐ ☐  
   l. Rectal prolapse ☐ ☐ ☐  
   m. Cough ☐ ☐ ☐  
   n. Difficulty breathing ☐ ☐ ☐  
   o. Convulsion ☐ ☐ ☐
33. Right now, does your child have any of the following?

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
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<tbody>
<tr>
<td>a. Very thirsty</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>b. Drinks poorly or not able to drink</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c. Sunken eyes</td>
<td></td>
<td></td>
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<tr>
<td>d. Wrinkled skin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>e. Irritable or restless</td>
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<td></td>
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<tr>
<td>f. Lethargy or loss of consciousness</td>
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<tr>
<td>g. Dry mouth</td>
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<tr>
<td>h. Fast breathing</td>
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</tbody>
</table>

34. Before coming to this hospital/health center, was [Child’s Name] given any of the following to treat his/her diarrhea? ("X" all that apply. Start with open-ended question; then probe options if not mentioned by the caretaker.)

- A fluid made from a special packet called ORALITE or ORS?
- Homemade fluid (e.g., Thin watery porridge made from maize, rice or wheat, soup, sugar salt water solution, Yogurt based drink)
- Special milk or infant formula
- Home remedy/Herbal medication
- Zinc (tablet/syrup)
- No special remedies given

If yes: Any other liquids, specify

If yes: Antibiotics, specify

If yes: Other (1), specify

If yes: Other (2), specify

35. Since [Child’s Name] developed diarrhea, how much have you been offering [Child’s Name] to drink?

- More than usual
- Usual
- Somewhat less than usual
- Much less than usual
- Nothing to drink

36. Since [Child’s Name] developed diarrhea, how much have you been offering [Child’s Name] to eat?

- More than usual
- Usual
- Somewhat less than usual
- Much less than usual
- Nothing to eat
Section 3: Health care utilization and expenses made before this visit to this hospital/health center

37. Before coming to this hospital/health center, did you seek care for [Child’s Name] outside your household for this illness?  
[If “No”, go to Question 41.]  
☐ No ☐ Yes

38. If you previously sought care for [Child’s Name] for this illness, where did you go? [Use the Health Facility Coding List to code the center(s) of choice. Do not include this center. “X” all that apply.]

☐ Pharmacy  ☐ Friend/relative  ☐ Traditional healer  ☐ Unlicensed practitioner/village doctor/bush doctor/village health worker  
☐ Licensed practitioner/private doctor (not at hospital)  ☐ Bought a remedy/medicine at the shop/market, specify remedy/drug:

☐ Hospital/Center of first choice
☐ Hospital/Center of second choice
☐ Hospital/Center of third choice
☐ Other Hospital/Center, specify

39. What were your or your household estimated out-of-pocket expenses for the following: [Have respondent answer for only those facilities (not friends or relatives) that were used in Question 38 and provide the expense in the local currency.]

<table>
<thead>
<tr>
<th>Total Medical Expenses</th>
<th>Transportation</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Pharmacy</td>
<td></td>
</tr>
<tr>
<td>b. Traditional healer</td>
<td></td>
</tr>
<tr>
<td>c. Unlicensed practitioner/village doctor/bush doctor</td>
<td></td>
</tr>
<tr>
<td>d. Licensed practitioner/private doctor</td>
<td></td>
</tr>
<tr>
<td>e. Bought remedy/medicine at the shop/market</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Total Medical Expenses</th>
<th>Transportation</th>
</tr>
</thead>
<tbody>
<tr>
<td>f. Hospital/Center of 1st choice</td>
<td></td>
</tr>
<tr>
<td>g. Hospital/Center of 2nd choice</td>
<td></td>
</tr>
<tr>
<td>h. Hospital/Center of 3rd choice</td>
<td></td>
</tr>
<tr>
<td>i. Other, specify</td>
<td></td>
</tr>
</tbody>
</table>

CRF 04A 09/01/2007
40. Where did the money come from? ["X" all that apply. Start with open-ended question, then probe options if not mentioned by the caretaker.]

☐ Cutting down expenses from meal
☐ Cutting down from other expenses
☐ Using savings
☐ Borrowing
☐ Selling assets
☐ Asking for donations outside the household
☐ Relative or friend pays on your behalf
☐ Others, specify

Section 4: Health Care Expenses when leaving the hospital/health center

[Complete this section when the child leaves the hospital after an outpatient visit or at discharge after admission.]

41. How long did it take to get here from your home (including the journey time and any time waiting for transport)?

☐ Less than 15 minutes
☐ 15 minutes to 29 minutes
☐ 30 to 59 minutes
☐ 1 to 4 hours
☐ More than 4 hours
☐ Don’t know

42. If you paid for transportation to bring the child to the hospital or clinic, how much did you pay?

□ □ □ □ □ □ Local currency

43. Other than the first trip to bring the child to the health center, how much did you pay for transport to or from this facility during the child’s stay in the facility?

□ □ □ □ □ □ Local currency

44. How much have other members of your household paid for transport to or from this facility as a result of the child’s stay in the facility?

□ □ □ □ □ □ Local currency
45. What are your estimated out-of-pocket expenses for the following? (This information applies to the period of hospitalization or visit to this center. Use the local currency):

Consultation: 

Drugs: 

Diagnostics: 

Food: 

Other, specify: 

[Only if the respondent cannot break down the expenses, use the “Total” row.
DO NOT CALCULATE THE “TOTAL” FROM ALL THE ROWS.]

Total: 

46. Where did the money that you spent during this visit or hospitalization come from? ( “X” all that apply.
Start with open-ended question; then probe options if not mentioned by the caretaker.)

☐ Cutting down expenses from meals
☐ Cutting down from other expenses
☐ Using savings
☐ Borrowing
☐ Selling assets
☐ Asking for donations outside the household
☐ Relative or friend pays on your behalf
☐ Other, specify: ____________________________

[Answer Questions 47 to 50 for the time period starting from the beginning of the illness until today.]

47. Did you lose some earnings due to seeking or providing care during [Child's Name] illness?

☐ No  ☐ Yes  If yes, how much? [Use local currency.]

48. Did other caregivers lose some earnings due to seeking or providing care during [Child's Name] illness?

☐ No  ☐ Yes  ☐ DK  If yes, how much? [Use local currency.]

49. How much time have you spent taking care of [Child’s name] when otherwise you would have been doing income generating activities (farming, selling in the market, working in a private business, etc.)? [Half a morning or afternoon = 0.25 days, a morning or afternoon = 0.50 days, a morning and afternoon = 1.00 day, anything less than half a morning or afternoon = 0 days.]

☐  ,  Day(s)
50. How much time have other caregivers spent taking care of [Child's name] when otherwise they would have been doing income generating activities (farming, selling in the market, working in a private business, etc.)? [Half a morning or afternoon = 0.25 days, a morning or afternoon = 0.50 days, a morning and afternoon = 1.00 day, anything less than half a morning or afternoon = 0 days]

Day(s)

END OF THE INTERVIEW.
THANK THE RESPONDENT(S) FOR THEIR COOPERATION.

Place sticker of Specimen ID here.

51. Specimen ID:

Notes or comments [Initial and date notes]

Interviewer's Name

Staff code

Quality Control's Name

Staff code

Day

Month

Year

CRF04A 09OCT2007

Page 11 of 11
Section 1: Physical Findings

1. Physical findings:
   a. Weight
      - 0-23 months old: (Weight of caretaker with and without child): __________. ___ kg  __________. ___ kg
      - 24-59 months old: (Weight of child alone) __________. ___ kg
   b. Height
      - 1st: __________. ___ cm  2nd: __________. ___ cm  3rd: __________. ___ cm
   c. MUAC
      - 1st: __________. ___ cm  2nd: __________. ___ cm  3rd: __________. ___ cm
   d. Axillary temperature __________. ___ °C
   e. Respiratory rate per minute
      - 1st: __________  2nd: __________
   f. Chest indrawing  □ No  □ Yes

   g. Eyes  □ Normal  □ Sunken [Confirm with the mother that the eyes are more sunken than usual.]
   h. Mouth  □ Normal  □ Somewhat dry  □ Very dry
   i. Skin pinch  □ Normal  □ Slow return [≤ 2 sec.]  □ Very slow (> 2 sec.)
   j. Mental status  □ Normal  □ Restless, irritable  □ Lethargic/unconscious

   k. Rectal prolapse  □ Absent  □ Present
   l. Bipedal edema [Both feet]  □ Absent  □ Present
   m. Abnormal hair: sparse, loose, straight  □ Absent  □ Present
   n. Undernutrition: wasted/very thin  □ Absent  □ Present
   o. Skin has ‘flaky paint’ appearance  □ Absent  □ Present

2. Did either the interviewer or the study staff observe a stool sample from this child?  □ No  □ Yes
   [If “Yes”, go to Question 3, if “No” go to Question 4.]

3. If yes, what was the nature of the stool? ['X' only one.]
   □ Loose/liquid stool without blood  □ Loose/liquid stool with blood  □ Normal stool
4. Does the child require rehydration?
   □ No  □ Yes, Oral rehydration  □ Yes, IV rehydration
   [If ‘No’, go to Section 3]

5. Will [Child’s Name] receive recommended rehydration at this hospital/health center?
   □ Yes  □ No, referred to another center  □ No, parents refused  □ Prescribed ORS for administration at home

Section 2: Outcome after rehydration
[Complete this Section if the child received rehydration therapy (oral or intravenous) in the health facility.]

Outcome 4 hours after starting rehydration
[obtain the following information 4 hours after starting rehydration therapy (oral or intravenous). If the child leaves the facility before 4 hours have passed, skip this Section and proceed to Section 3.]

6. Was the child evaluated after 4 hours?  □ No  □ Yes
   a. If “No”, what was the reason? ____________
      [If you were not able to do the evaluation after 4 hours, complete the reason and proceed to Section 3 below.]

7. Findings after 4 hours of rehydration:
   a. Weight
      0-23 months old: (Weight of caretaker with and without child) □ caretaker + child □ caretaker alone □ child □ caretaker alone
      24-59 months old: (Weight of child alone): □ □ kg
   b. Mouth □ Normal □ Somewhat dry □ Very dry
   c. Skin pinch □ Normal □ Slow return ≤ 2 sec. □ Very slow >2 sec.

8. Does the child continue to purge large volumes of watery stool?  □ No  □ Yes

9. Was the total stool output within the last four hours measured?  □ No  □ Yes
   a. If “Yes”, what was the volume? □ □ ml

10. Does the child require additional oral/IV fluid for rehydration?  □ No [skip to section 3] □ Yes
**Outcome if additional rehydration needed after 1st 4 hours**

[Complete the following if "Yes" to question 10]

10a. Was the child completely rehydrated in the hospital?  
☐ No [skip to section 3]  
☐ Yes

10b. Date of rehydration:  
<table>
<thead>
<tr>
<th>Day</th>
<th>Month</th>
<th>Year</th>
</tr>
</thead>
</table>

10c. If “Yes” to Q10a, weigh the child again after the child is completely rehydrated

| 0-23 months old: (Weight of caretaker with and without child): |
|-----------------|-----------------|-----------------|
| Caretaker + child | ☐ kg | ☐ kg |
| Caretaker alone | ☐ kg | ☐ kg |

| 24-59 months old: (Weight of child alone): |
|-----------------|-----------------|
| ☐ kg | ☐ kg |

**Section 3: Outcome when leaving the hospital/health center**

(This Section should be completed when the child leaves the health center, either after an outpatient visit or hospital admission.)

11. Weight

(Measure weight at discharge from the hospital or from health center outpatient visit if the child received rehydration therapy and at least 4 hours have passed since the child was last weighed. Check "NA" otherwise.)

| 0-23 months old: (Weight of caretaker with and without child): |
|-----------------|-----------------|-----------------|
| Caretaker + child | ☐ kg | ☐ kg |
| Caretaker alone | ☐ kg | ☐ kg |

| 24-59 months old: (Weight of child alone): |
|-----------------|-----------------|
| ☐ kg | ☐ kg |

12. Was the child admitted to the hospital?  
☐ No  
☐ Yes

[If "No", go to Question 14]

13. If admitted to the hospital, for how many days?  
| ☐ | ☐ |

13a. Is the child still in hospital > 60 days?  
☐ No  
☐ Yes

14. Child’s diagnosis upon leaving the hospital/health center. ["X" all that apply.]  
☐ Diarrhea  
☐ Dysentery  
☐ Pneumonia/lower respiratory infection  
☐ Meningitis  
☐ Other invasive bacterial infection  
☐ Malaria  
☐ Malnutrition  
☐ Other, specify ____________________________
CRF 04B – ENROLLMENT QUESTIONNAIRE FOR CASES - MEDICAL

15. A child may receive medication in the hospital and/or receive a prescription for treatment at home. For each of the following medications, cross ["X"] the appropriate boxes. ["X" all that apply.]

<table>
<thead>
<tr>
<th>Given prescription for treatment at home</th>
<th>Treatment given in health center</th>
<th>Given prescription for treatment at home</th>
<th>Treatment given in health center</th>
</tr>
</thead>
<tbody>
<tr>
<td>☐ ORS</td>
<td>☐ Anceillin</td>
<td>☐ Cotrimoxazole</td>
<td>☐ Naftidix acid</td>
</tr>
<tr>
<td>☐ Intravenous fluids</td>
<td>☐ Ciprofloxacin/Norfloxac/other fluoroquinolone</td>
<td>☐ Normal food withheld for ≥ 1 day</td>
<td>☐ Selvenir/Pvmdemicilinam</td>
</tr>
<tr>
<td>☐ Gentamycin</td>
<td>☐ Other antibiotic, specify</td>
<td>☐ Chloramphenicol/Thiamphenicol</td>
<td>☐ Zinc</td>
</tr>
<tr>
<td>☐ Erythromycin</td>
<td>☐ A (government recommended) homemade fluid</td>
<td>☐ Azithromycin</td>
<td>☐ An antimalarial drug</td>
</tr>
<tr>
<td>☐ Other antibiotics</td>
<td>☐ Other medicine, specify</td>
<td>☐ Penicillin</td>
<td>☐ Other medicine, specify</td>
</tr>
<tr>
<td>☐ Amoxicillin</td>
<td>☐ Other medicine, specify</td>
<td>☐ None prescribed/taken</td>
<td></td>
</tr>
</tbody>
</table>

16. Outcome when leaving hospital/health center. ["X" only one response.]

☐ Resolved or healthy
☐ Improved
☐ No better
☐ Worse
☐ Died in hospital/health center
☐ Unknown/lost to follow up

[If the child died, complete Question 16a and make sure a verbal autopsy will be completed according to local guidelines. Medical information will be collected using CRF10.]

16a. If the child died, what was the date of death: ☐ ☐ ☐ Day ☐ ☐ Month 20 ☐ Year

Notes or comments [Initial and date notes]

Interviewer's Name ___________________________ Staff code ☐ ☐ ☐

Quality Control's Name ___________________________ Staff code ☐ ☐ ☐ Day ☐ ☐ Month ☐ ☐ Year

CRF04B 09OCT2007
CRF 05 – 60 DAY FOLLOW-UP QUESTIONNAIRE FOR CASES & CONTROLS

Choose one:  □ Case-child    □ Control-child

**Interview Outcome**

1. What was the outcome of the follow-up interview?
   - □ Conducted
   - □ Not conducted
   - If “Not conducted”, what was the reason?
     - □ Child cannot be found
     - □ Caretaker refused
     - □ Caretaker not available after 3 visits
     - □ Other, specify __________________________

   [If the interview was not conducted, complete the above part, sign, date, and submit this page to the DCC.]

**Notes or comments [Initial and date notes]**

---

**Interviewer’s Name** __________________________    □ □ □ □  Staff code

**Quality Control’s Name** __________________________    □ □ □ □  Staff code  □ □ □ □  Year
Section 1: Clinical Information

1. What is your relationship to [Child's Name]?  
   - [ ] Mother  
   - [ ] Father  
   - [ ] Sister  
   - [ ] Brother  
   - [ ] Grandmother  
   - [ ] Grandfather  
   - [ ] Aunt  
   - [ ] Uncle  
   - [ ] No relation  
   - [ ] Other relation by blood or marriage, specify__________

3. How is [Child's Name]'s health since the last study visit? [Explain to caretaker what is meant by "the last study visit"][
   - [ ] Appears healthy
   - [ ] Health has deteriorated
   - [ ] Health improved but not back to normal
   - [ ] Died
   - [ ] No better/unchanged
   [If died, complete "a" to "c" below.]
   a. If [Child's Name] died, what was the date of death?  
      Day ____________  Month ____________  Year ____________
   b. If [Child's Name] died, what was the place of death?  
      - [ ] Health facility  
      - [ ] Home or elsewhere  
   c. If the child died in a health facility, what was the name of the health facility?  
      [Use the Health Facility Coding List to code the facility; if the health facility is not coded, use '999' and insert the name below; if health facility unknown, use '999'.]
      ________
   [If the child died, make sure a verbal autopsy will be completed (and medical information will be collected if the child died in a health facility) according to the local guidelines. For children who died, the remainder of the questionnaire needs to be completed except Section 2.]
4. Since the last study visit, has [Child's Name] experienced any of the following illnesses?
[If "Yes" to any illness, indicate if child visited a health care facility for that illness.]

<table>
<thead>
<tr>
<th>Illness</th>
<th>Visited a health facility?</th>
<th>Illness</th>
<th>Visited a health facility?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea</td>
<td>No</td>
<td>Fever with unknown origin</td>
<td>No</td>
</tr>
<tr>
<td>Dysentery</td>
<td>No</td>
<td>Other, specify</td>
<td>No</td>
</tr>
<tr>
<td>Cough with difficult breathing</td>
<td>No</td>
<td>Other, specify</td>
<td>No</td>
</tr>
</tbody>
</table>

5. To your knowledge, was the child diagnosed with any of the following at a health care facility?

<table>
<thead>
<tr>
<th>No Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typhoid</td>
</tr>
<tr>
<td>Malaria</td>
</tr>
<tr>
<td>Pneumonia</td>
</tr>
<tr>
<td>Meningitis</td>
</tr>
<tr>
<td>Other, specify</td>
</tr>
</tbody>
</table>

6. Since the last study visit, has [Child's Name] experienced any of the following:

a. Rectal prolapse [some pink tissue appears outside of the child's anus] No Yes
b. Convulsions

c. Arthritis [swollen, painful joints]

Section 2: Physical Examination

7. Physical findings

a. Weight
   0-23 months old: (Weight of caretaker with and without child): kg  kg

   24-59 months old: (Weight of child alone): kg

b. Height
   1st: cm 2nd: cm 3rd: cm

c. MUAC
   1st: cm 2nd: cm 3rd: cm

d. Axillary temperature °C

e. Respiratory rate per minute: 1st 2nd
CRF 05 - 60 DAY FOLLOW-UP QUESTIONNAIRE FOR CASES & CONTROLS

<table>
<thead>
<tr>
<th>Study # 604</th>
<th>Plate # 054</th>
<th>Visit # 002</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Site</th>
<th>Center</th>
<th>Child ID</th>
<th>Absent</th>
<th>Present</th>
</tr>
</thead>
</table>

f. Rectal prolapse

- 

g. Bipedal edema [Both feet]

- 

h. Abnormal hair: sparse, loose, straight

- 

i. Undernutrition: wasted/very thin

- 

j. Skin has 'flaky paint' appearance

- 

Section 3: Water-Sanitation-Environment

8. During the last two weeks, what was the main source of drinking water for the members of your household? ["X" only one response that relates to the main source of drinking water.]

- Piped into house
- Piped into yard
- Public tap
- Open well in house or yard
- Open public well
- Pond or lake
- Deep tube well
- Shallow tube well
- Other, specify

- Covered well in house or yard
- Covered public well
- Protected spring
- Unprotected spring
- River or stream
- Dam or earth pan
- Rainwater
- Bought (tank, bottles, etc)
- Bore hole

[Interviewer should ask to see the containers where drinking water is usually stored; based on your observations, complete parts "a" to "d" below.]

8a. Observed container(s) in use in the house?

[If "No", go to Question 9.]

- a. Yes
- b. No

8b. Type of container observed. ["X" only one response]

- Wide-mouthed container(s) - 6 cm or more across the opening
- Narrow-mouthed container(s) - less than 6 cm across the opening
- Mixture of wide and narrow-mouthed containers
- Other, specify:

8c. Are containers covered?

- a. Yes
- b. No
- Mixed (covered and uncovered)
8d. How is water removed from container? ['X' all that apply.]
- Pour (spigot or spout)
- Scoop with cup
- Scoop with ladle

9. Do you usually treat your drinking water at home?
   [If "No", go to Question 11.]
   - No
   - Yes

10. Which method do you use the most to treat drinking water at home? ['X' only one response.]
Method reported
- Leave water in sun
- Boiled
- Filter through a cloth
- Ceramic/other filter
- Chlorine
- Alum
- Other chemical

Specify

Materials observed for method reported
- 10-20 clear 1-2 L bottles on roof in sun
- By observation
- Cloth observed
- Filter observed
- Tablet/liquid/powder observed
- Alum observed
- Chemical observed

Specify

[If chlorine is not used, go to Question 11]

10a. If chlorine is the method of water treatment in Q10, record the chlorine test result.
- Positive (yellow)
- Refused test
- Negative (clear)
- No water in the container

10b. If chlorine is the method of water treatment in Q10, check the brands that you observed.
   ['X' all that apply.]
- Certeza
- Aquatabs
- AquaGuard
- WaterGuard
- Watermaker
- PuR
- Unknown
- Not applicable (none observed)
- Other, specify

11. Where do you usually wash your hands?
- In or near dwelling/yard
- Another place

[If "Another place", go to Question 13.]
12. If hands are washed in or near dwelling/yard, ask to see the place and record whether the following items are present:

<table>
<thead>
<tr>
<th>No</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Piped water source</td>
</tr>
<tr>
<td></td>
<td>Non-piped water source without tap</td>
</tr>
<tr>
<td></td>
<td>Non-piped water source with tap</td>
</tr>
</tbody>
</table>

13. Please show me where you usually dispose of the feces of your child. ("X" one only.)

<table>
<thead>
<tr>
<th>No</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flush toilet</td>
</tr>
<tr>
<td></td>
<td>Ventilated improved pit (VIP) latrine</td>
</tr>
<tr>
<td></td>
<td>Traditional pit toilet</td>
</tr>
</tbody>
</table>

(*The option "Bush/Field/Ground/Stream/Open sewer" includes dumping anywhere in the environment outside the compound)

14. [Interviewer, record whether feces observed]:

<table>
<thead>
<tr>
<th>No</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>14a. Visible feces observed in defecation area</td>
<td></td>
</tr>
<tr>
<td>14b. Visible feces observed elsewhere in house or yard</td>
<td></td>
</tr>
</tbody>
</table>

15. Please show me the facility your household most commonly use to dispose of human fecal waste. ("X" one only.)

<table>
<thead>
<tr>
<th>No</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flush toilet</td>
</tr>
<tr>
<td></td>
<td>Ventilated improved pit (VIP) latrine</td>
</tr>
<tr>
<td></td>
<td>Traditional pit toilet</td>
</tr>
</tbody>
</table>

END OF INTERVIEW.

THANK RESPONDENT(S) FOR THEIR COOPERATION.

Notes or comments [Initial and date notes]
CRF 06 ELIGIBILITY FOR CONTROLS

Index Case's Information
1. Birthday of index case: Day  Month  Year  Age:  in months
2. Gender of index case: □ Boy  □ Girl
3. Date of enrollment of index case: Day  Month  Year
4. Child ID Number of index case: 

Control's Information
5. Child's birthdate: Day  Month  Year  Age:  in months
6. Child's gender: □ Boy  □ Girl  No  Yes  Died  [If "Yes", continue; otherwise stop, write your name and staff code, date the form and send to DCC.]
7. Have you been able to identify the child? □  □  □

Eligibility Checklist
8. Is this child appropriately age-matched to the index case? □  □  □
9. Is this child the same gender as the index case? □  □  □
10. Does this child live in the same or nearby village or community as case? □  □  □
11. Was the index case enrolled within the past 14 days? □  □  □
12. Has this child been free of diarrhea for the past 7 days? □  □  □
13. Is the child eligible for enrollment? □  □  □

(The child is eligible only if the answers to the Questions 8 through 12 are "Yes".)

13a. If either Questions 8 or 12 are "DK", check the option that best describes why you were not able to determine eligibility.

□ Caretaker not available.  □ Other, specify________________________

(If not eligible, STOP, end the interview by thanking the caretaker/parent for their participation. Write down name and staff code, date the form and submit to DCC. If the child is eligible, continue to Question 14.)

Interviewer's Name ______________________  Staff code ________________

Quality Control's Name ______________________  Staff code ________________  Day  Month  Year 2 0 ______

CRF 06 Rev A 31/10/2008  Page 1 of 2
CRF 06 ELIGIBILITY FOR CONTROLS

Study # 004  Plate # 002  Visit # 001

Site  Center  Child ID (Control)

No  Yes

14. Was consent obtained?  

15. Was a stool sample collected from the child?  

16. Was the child enrolled?  

17. If eligible but not enrolled, what was the reason? ["X" one of the two main reasons.]

☐ Not invited for one of the following reasons:  
☐ Unable to produce adequate stool sample [10 grams with a minimum of 3 grams]
☐ Other, specify

☐ Refused by parent/caretaker for one of the following reasons:  
☐ Parent/caretaker too busy
☐ Does not like research
☐ Other, specify

18. If child is enrolled into the study, enter the date of enrollment:  

   Day  Month  Year

Notes or comments [Initial and date notes]

Interviewer's Name  

Quality Control's Name

Staff code

2008
Section 1: Demographic and Epidemiological Information

1. Who is [Child’s Name]’s primary caretaker?
   - [ ] Mother
   - [ ] Father
   - [ ] Sister
   - [ ] Brother
   - [ ] Grandmother
   - [ ] Grandfather
   - [ ] Aunt
   - [ ] Uncle
   - [ ] No relation
   - [ ] Other relation by blood or marriage, specify______________________________

2. What is your relationship to [Child’s Name]?
   - [ ] Mother
   - [ ] Father
   - [ ] Sister
   - [ ] Brother
   - [ ] Grandmother
   - [ ] Grandfather
   - [ ] Aunt
   - [ ] Uncle
   - [ ] No relation
   - [ ] Other relation by blood or marriage, specify______________________________

3. Where does [Child’s Name]’s mother live?
   - [ ] Living in household
   - [ ] Abroad
   - [ ] Died
   - [ ] Lives outside of household
   - [ ] Whereabouts unknown

4. Where does [Child’s Name]’s father live?
   - [ ] Living in household
   - [ ] Abroad
   - [ ] Died
   - [ ] Lives outside of household
   - [ ] Whereabouts unknown

5. How far did the child’s primary caretaker go in school?
   - [ ] No formal schooling
   - [ ] Completed secondary
   - [ ] Less than primary
   - [ ] Post-secondary
   - [ ] Completed primary
   - [ ] Religious education only
   - [ ] Don’t know

6. How many people have been living regularly in your household for the past 6 months? [ ]

7. How many people have been sleeping regularly in your household for the past 6 months? [ ]
8. How many children younger than 60 months live in the household? 

9. How many rooms in your household are used for sleeping? 

10. What is the predominant floor in the house of [Child's Name]?

   - **Natural Floor**
     - [ ] Earth/Sand
     - [ ] Dung

   - **Rudimentary Floor**
     - [ ] Wood planks
     - [ ] Palm/bamboo

   - **Finished Floor**
     - [ ] Parquet or polished wood
     - [ ] Vinyl or asphalt strips
     - [ ] Ceramic Tile
     - [ ] Cement
     - [ ] Carpet

     - [ ] Other, specify ____________________________

11. Does your household have the following? [Must be functioning; "X" all that apply.]

   - [ ] Electricity
   - [ ] Television
   - [ ] Motorcycle/scooter
   - [ ] Radio

   - [ ] Bicycle/rickshaw
   - [ ] Car/truck
   - [ ] Refrigerator
   - [ ] Boat with a motor

   - [ ] Telephone (mobile or non mobile)
   - [ ] Animal-drawn cart
   - [ ] Agricultural land
   - [ ] None of the above

12. What type of cooking fuel does your household use? ["X" all that apply.]

   - [ ] Electricity
   - [ ] Liquid Propane Gas
   - [ ] Natural Gas
   - [ ] Kerosene

   - [ ] Biogas
   - [ ] Coal/lignite
   - [ ] Charcoal
   - [ ] Wood

   - [ ] Straw/shrubs/grass
   - [ ] Animal dung
   - [ ] Agricultural crop residue

     - [ ] Other, specify ____________________________
13. Do the following animals live in the compound where [Child’s Name] lives? [“X” all that apply.]

- [ ] Goat
- [ ] Sheep
- [ ] Dog
- [ ] Cat
- [ ] Cow
- [ ] Rodents
- [ ] Fowl (chicken, duck or other birds)
- [ ] Other, specify ___________________

14. During the last two weeks, has your household ever obtained drinking water from any of the following sources? [“X” all that apply.]

- [ ] Piped into house
- [ ] Piped into yard
- [ ] Public tap
- [ ] Open well in house or yard
- [ ] Open public well
- [ ] Pond or lake
- [ ] Deep tube well
- [ ] Shallow tube well
- [ ] Other, specify ___________________
- [ ] Covered well in house or yard
- [ ] Covered public well
- [ ] Protected spring
- [ ] Unprotected spring
- [ ] River or stream
- [ ] Dam or earth pan
- [ ] Rainwater
- [ ] Bought (tank, bottles, etc)
- [ ] Bore hole

15. During the last two weeks, what was the main source of drinking water for the members of your household? [“X” only one response that relates to the main source of drinking water.]

- [ ] Piped into house
- [ ] Piped into yard
- [ ] Public tap
- [ ] Open well in house or yard
- [ ] Open public well
- [ ] Pond or lake
- [ ] Deep tube well
- [ ] Shallow tube well
- [ ] Other, specify ___________________
- [ ] Covered well in house or yard
- [ ] Covered public well
- [ ] Protected spring
- [ ] Unprotected spring
- [ ] River or stream
- [ ] Dam or earth pan
- [ ] Rainwater
- [ ] Bought (tank, bottles, etc)
- [ ] Bore hole

[Use your response from Question 15 to answer Questions 16 and 17. If the response to Question 15 is “piped into house/yard”, “open or covered well in house/yard” or “rainwater”, then go to Question 18. Otherwise continue.]
16. How long does it take to go there, get water, and come back?

☐ Less than 15 minutes  ☐ 1 to 3 hours
☐ 15 to 29 minutes  ☐ More than 3 hours
☐ 30 to 59 minutes

17. Do you or other members from your household go and fetch drinking water for the household every day? [If "Yes", go to Question 17a, if "No" go to Question 17b.]

☐ No  ☐ Yes

17a. On average, how many trips do you and members from your household make to fetch water each day? [Number of trips/day]

17b. On average, how many trips do you and members from your household make to fetch water each week? [If no trips are made, complete as "00"]

18. In the last two weeks, how often has water been available from this main source?

☐ All the time  ☐ A few times per week
☐ Several hours everyday  ☐ Less frequent than a few times per week

19. In the last two weeks, did you give [Child’s Name] stored water for drinking?

☐ No  ☐ Yes

20. Do you usually treat drinking water at home? [If "No", go to Question 23.]

☐ No  ☐ Yes

21. Which method do you use the most to treat drinking water at home? ["X" only one response.]

☐ Leave water in sun to disinfect  ☐ Boil
☐ Filter through a cloth  ☐ Filter through ceramic or other filter
☐ Chlorine liquid, powder, or tablets  ☐ Alum
☐ Other chemical or additive, specify __________________________

[If chlorine is not used, go to Question 22]

21a. If you use chlorine liquid, powder or tablets, which type do you most commonly use? ["X" only one response.]

☐ Certezza  ☐ Watermaker
☐ Aquatabs  ☐ PurR
☐ AquaGuard  ☐ Don’t know
☐ WaterGuard  ☐ Other, specify __________________________
22. In the last two weeks did you give [Child’s Name] water which was not treated?  
☐ No  ☐ Yes

23. How do you usually dispose of [Child’s Name]’s feces? [“X” only one response.]
☐ Scatter in yard  ☐ Bush/Field/ Ground/Stream/Open sewer
☐ Bury  ☐ Do nothing
☐ Toilet, latrine  ☐ Other, specify

24. What kind of facility does your household most commonly use to dispose of human fecal waste?  
[Show pictures to confirm the identity of the facility used. “X” only one response.]
☐ Flush toilet  ☐ Pour flush toilet
☐ Ventilated improved pit (VIP) latrine  ☐ No facility: Bush/Field/ Ground/Stream/Open sewer
☐ Traditional pit toilet  
[If “No facility” selected, go to Question 26.]
☐ Other, specify

25. How many households (other than your own) share this facility?  
[Respond with a number; code “99” for none.]

26. When do you usually wash your hands? [“X” all that apply. Do not probe.]
☐ Before eating  ☐ After handling domestic animals
☐ Before cooking  ☐ After cleaning child who defecated
☐ Before you nurse or prepare baby’s food  ☐ Never
☐ After you defeate  ☐ Other, specify

27. When you wash your hands, what do you usually use? [“X” only one.]
☐ Water only  ☐ Water and soap  ☐ Water and ashes  ☐ Water and mud or clay

Section 2: Clinical Information

28. Is [Child’s Name] currently breastfed?
☐ No  ☐ Partial breastfeeding  ☐ Exclusive breastfeeding
29. During the last 7 days, did [Child’s Name] have any of the following?
   a. Blood in stools
   b. Fever measured at least 38 °C or parental perception
   c. Vomiting 3 or more times per day

30. Is the child currently receiving any medicine?
   [If “No”, go to Question 31.]
   No  Yes

30a. If ‘Yes’ to Question 30, is a bottle or tablet strip or prescription available for ongoing treatment?
   [If “Yes”, go to Question 30b.]
   No  Yes

30b. What are the medicines that the child is currently receiving? (*X* all that apply.)

- ORS
- Intravenous fluids
- Cotrimoxazole
- Normal food withheld for ≥1 day
- Gentamycin
- Chloramphenicol/Thiamphenicol
- Erythromycin
- Azithromycin
- Other macrolides
- Penicillin
- Amoxicillin

- Ampicillin
- Nalidixic acid
- Ciprofloxacin/Norfloxacin/other fluoroquinolone
- Selexid/Pvmecillinam
- Other antibiotic, specify____________________
- Zinc
- A (government recommended) homemade fluid
- An antimalarial drug
- Other medicine, specify____________________
- Other medicine, specify____________________
- Other medicine, specify____________________
- Nothing

31. The last time [Child’s Name] had diarrhea, did you seek care for him/her outside your household?
   [If “No”, go to Question 33.]
   No  Yes

   If the child never had diarrhea, go to Question 35.
   Never had diarrhea
32. If you sought care for [Child’s Name]’s last episode of diarrhea where did you go? [Use the Health Facility Coding List to code the center(s) of choice. ‘X’ all that apply.]

- [ ] Pharmacy
- [ ] Friend/relative
- [ ] Traditional healer
- [ ] Unlicensed practitioner/village doctor/bush doctor/village health worker
- [ ] Licensed practitioner/private doctor (not at hospital)
- [ ] Bought a remedy/medicine at the shop/market, specify remedy/drug ______________________
- [ ] Hospital/Center of first choice [ ]
- [ ] Hospital/Center of second choice [ ]
- [ ] Hospital/Center of third choice [ ]
- [ ] Other Hospital/Center, specify ______________________

33. The last time [Child’s name] had diarrhea, how much did you offer [Child’s name] to drink?

- [ ] More than usual
- [ ] Usual
- [ ] Somewhat less than usual
- [ ] Much less than usual
- [ ] Nothing to drink

34. The last time [Child’s Name] had diarrhea, how much did you offer [Child’s Name] to eat?

- [ ] More than usual
- [ ] Usual
- [ ] Somewhat less than usual
- [ ] Much less than usual
- [ ] Nothing to eat

Section 3: Physical Findings

35. Physical findings:

a. Weight

0-23 months old: (Weight of caretaker with and without child): [ ] kg [ ] kg

Caretaker + child

Caretaker alone

24-59 months old: (Weight of child alone): [ ] kg
CRF 07 – ENROLLMENT QUESTIONNAIRE FOR CONTROLS

Study # 004  Plate # 076  Visit # 001

Site  Center  Control ID

b. Height  1st  cm  2nd  cm  3rd  cm

c. MUAC  1st  cm  2nd  cm  3rd  cm

d. Axillary temperature  °C

e. Respiratory rate per minute  1st  2nd

f. Bipedal edema (Both feet)  Absent  Present

g. Abnormal hair: sparse, loose, straight  

h. Undernutrition: wasted/very thin  

i. Skin has ‘flaky paint’ appearance  

END OF INTERVIEW
THANK RESPONDENT(S) FOR THEIR COOPERATION

36. Specimen ID:  Place sticker of Specimen ID here.

Notes or comments  [Initial and date notes]

Interviewer’s Name

Quality Control’s Name

Page 8 of 8
1. Was the Memory Aid completed? 
   No ☐ ☐ Yes ☐ ☐ Partial ☐ ☐
   [If “No”, “X” and sign the form and hand over to supervisor.]

2. If “Yes” or “Partial”, what was the first and last day of diarrhea according to the Memory Aid?

   First day of Diarrhea: ____
   Last day of Diarrhea: ____
   [Code 1 to 14 from Memory Aid, Column 1]

Notes or comments [Add date and initials or staff code]

Interviewer’s Name ___________________________ Staff code ________

Quality Control’s Name: ________________________ Staff code ________
   Day ________ Month ________ Year ________
Memory Aid to Record the Presence of Diarrhea

Site  Center  Child ID  Day  Month  Year

Please complete this form every day for each of the next 14 days.

1. Each morning when you wake up, decide whether your child had diarrhea during the previous day. Diarrhea means that your child passed 3 or more loose or watery stools that were not normal for him or her on that day.

2. Go to the correct day. "O" means today, "O O" means tomorrow, and so on. A day begins when you wake up in the morning and ends when you wake up the next morning.

3. If your child had diarrhea that day, mark "X" in the dark box for that day. If your child did not have diarrhea, mark "X" in the white box for that day. Each day, make only one "X".

4. If you forget for a few days, try to start again on the correct day.

5. Keep this form in a safe place. We will come to your house to collect it in 60 days.

DIARRHEA

NORMAL

(1) O (today)

(2)

(3)

(4)

(5)

(6)

(7)

(8)

(9)

(10)

(11)

(12)

(13)

(14)
CRF 10 – INFORMATION FROM HEALTH CENTERS ON CHILD DEATHS

Study #016  Plate #101  Visit #001

Subject ID  Day  Month  Year

1. DSS ID number:

1a. Status of the child in the study at the time of death:

☐ Case  ☐ Control  ☐ Not enrolled in GEMS Study

2. Code of the health facility where the child died:

[Use Health Facility Coding List to code. If a facility is not coded, use “090” and specify the name of health facility below.]

3. Date of visit/ hospital admission of the child:

Day  Month  Year

4. Date of death of the child:

Day  Month  Year

5. Was any medical information about the cause of death of the child obtained from the health facility?

☐ No  ☐ Yes

If no, reason:

[If no information was obtained, write down your name, staff code, date the form and submit this page to DCC.]

Interviewer's Name  Staff code

Quality Control's Name  Staff code  Day  Month  Year

CRF10  06/01/2008  Page 1 of 3
6. Were you able to see the medical chart of the child?  
   [If "No", go to Question 8. If "Yes", answer Question 6a and record the causes of death in Questions 7a-7e.  
   Record the notes from the medical chart in the space provided on Page 3.]  
   a) Date of last note in the chart: \[ \square \square \square 20 \square \]  

**Cause of death according to medical chart:**  

7a. Immediate cause of death: ____________________________  
7b. First underlying cause of death: ________________________  
7c. Second underlying cause of death: ______________________  
7d. Third underlying cause of death: ________________________  
7e. Contributing cause(s) of death: ________________________  

8. Were you able to interview a doctor/nurse who attended the child before death?  
   [If "No", go to Question 9. If "Yes" record the causes of death in the spaces provided for Questions 8a-8e.]  
   a) Immediate cause of death: ____________________________  
   b) First underlying cause of death: ________________________  
   c) Second underlying cause of death: ______________________  
   d) Third underlying cause of death: ________________________  
   e) Contributing cause(s) of death: ________________________
9. Were you able to see the death certificate?  
No □ Yes □

If “No”, write down your name, staff code, date the form and submit to DCC.  
If “Yes” record the causes of death in the spaces provided for Questions 9a-9e.

9a. Immediate cause of death: ________________________________

9b. First underlying cause of death: ________________________________

9c. Second underlying cause of death: ________________________________

9d. Third underlying cause of death: ________________________________

9e. Contributing cause(s) of death: ________________________________

Transcription of notes from the medical chart:

Notes or Comments [Initial and date notes.]

Interviewer's Name ___________________________ Staff code ____________

Quality Control's Name ___________________________ Staff code ____________ Day ____________ Month ____________ Year 20____
CRF 11 – STOOL COLLECTION

1. (Estimated) time of whole stool passed/excreted: [ ] [ ] [ ] (24 hour clock) VERSION # 2

2. Consistency of whole stool sample: (select one)
   - [ ] grade 1 (formed)
   - [ ] grade 2 (soft)
   - [ ] grade 3 (thick liquid)
   - [ ] grade 4 (opaque watery)
   - [ ] grade 5 (rice water-clear watery)

3. Characterization of whole stool sample:
   - [ ] Blood No [ ] Yes
   - [ ] Pus No [ ] Yes
   - [ ] Mucus No [ ] Yes

4. If the child is a case, did s/he receive antibiotics after arriving at the health center but before producing the whole stool specimen? If the child is a control, did s/he receive antibiotic during the 4 hours prior to stool collection?
   - No [ ] Yes [ ] DK
   [If 'Yes', check the appropriate boxes (*"X" all that apply). If 'No', go to Question 7.]
   - [ ] Ampicillin
   - [ ] Cotrimoxazole
   - [ ] Selexid/Prvnccillinam
   - [ ] Chloramphenicol/Thiamphenicol
   - [ ] Azithromycin
   - [ ] Penicillin
   - [ ] Amoxicillin
   - [ ] Nalidixic acid
   - [ ] Ciproflaxacin/Norfloxacin/other fluoroquinodone
   - [ ] Gentamycin
   - [ ] Erythromycin
   - [ ] Other macrolides
   - [ ] Other antibiotic, specify

5. If antibiotic was given:
   a. Date of first antibiotic: [ ] [ ] [ ]
   b. Time of first antibiotic: [ ] [ ] [ ] (24 hour clock)

6. If the child is a case and was given antibiotics at the health center before the child produced a whole stool specimen, were rectal swabs collected from the child before the child received antibiotics?
   - No [ ] Yes [ ]
   [If 'Yes', continue. If 'No', go to Question 7.]
   a. (Estimated) time when rectal swabs obtained: [ ] [ ] [ ] (24 hour clock)

7. Time when whole stool/rectal swab placed in transport media: [ ] [ ] [ ] (24 hour clock)

8. Swab (rectal swab/whole stool) in Cary Blair: [ ] No [ ] Yes

9. Swab (rectal swab/whole stool) in Buffered Glycerol Saline: [ ] No [ ] Yes

10. Specimen ID: [ ] [ ] [ ]

11. Time when sample received by lab personnel: [ ] [ ] [ ] (24 hour clock)

Place sticker of Specimen ID here.

Interviewer's Name

Quality Control's Name

Staff code

Day [ ] [ ] [ ] Month [ ] [ ] [ ] Year [ ] [ ] [ ]