THE BURDEN OF SEVERE ACUTE GASTROENTERITIS AND RISK FACTORS ASSOCIATED WITH POOR OUTCOME IN A COHORT OF SOWETAN CHILDREN UNDER FIVE YEARS OF AGE

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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, in partial fulfilment of the requirements for the degree of Master of Science in Medicine in the field of Epidemiology and Biostatistics

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DECLARATION

I, Michelle Jennifer Groome declare that this research report is my own work. It is being submitted for the degree of Master of Science in Medicine in the field of Epidemiology and Biostatistics in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other university.

Michelle J Groome
20 July 2010

DEDICATION

I dedicate this work to all the parents who have allowed their children to participate in research towards finding a safe and effective vaccine against rotavirus. May the introduction of the rotavirus vaccine in South Africa be a major step in the fight to decrease under five mortality in this country.

I also dedicate this report to my loving and supportive family – my husband Chris and my two wonderful daughters, Rebecca and Shannon. Thank you for allowing me the time to complete my Masters degree and for supporting me during assignments, examinations and the writing of this report.

PUBLICATIONS AND PRESENTATIONS ARISING FROM THE RESEARCH REPORT

Presentations

Poster presentation at the Federation of Infectious Diseases Societies of South Africa Congress 2009, Sun City, 20-23 August.

MJ Groome, SA Madhi. Burden of Severe Acute Gastroenteritis and Risk Factors for Poor Outcome in Sowetan Children under Five Years.

ABSTRACT

Introduction

In developing countries, diarrhoea is a major cause of morbidity and mortality among children under five years of age. This study aimed to determine the effect of age and HIV infection status on incidence of acute gastroenteritis and to identify risk factors associated with death and prolonged hospitalisation.

Methods

A secondary data analysis was performed using an existing cohort of children enrolled on a pneumococcal vaccine efficacy study performed in 1998-2005 in Soweto.

Results

The incidence rate of acute gastroenteritis requiring hospitalisation was 10.13 ($CI_{95\%}$ 9.68, 10.58) per 1000 person years. Incidence was highest in those under six months of age, decreased with increasing age, and was 5.42 times ($CI_{95\%}$ 4.89, 6.01) higher in those infected with HIV compared to that in HIV-uninfected children. HIV-infected children were more likely to be malnourished, have severe dehydration and have a concomitant diagnosis of lower respiratory tract infection (LRTI). HIV-infected children were four times more likely to die in hospital (OR 3.99 $CI_{95\%}$ 2.04, 7.81) and almost twice as likely to be hospitalized > 2 days (OR 1.81 $CI_{95\%}$ 1.38, 2.38) compared to HIV-uninfected children. Presence of malnutrition, severe dehydration and a concomitant diagnosis of LRTI were also significant risk factors for death and prolonged hospitalisation.

Conclusions

Acute gastroenteritis is an important cause of hospitalisation in children under 2 years, especially among HIV-infected children. Prevention and management of severe dehydration, malnutrition, HIV infection and concomitant LRTI need to be targeted to decrease mortality and shorten the duration of hospitalisation in children admitted with acute gastroenteritis.

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NOMENCLATURE

AIDS	Acquired immunodeficiency syndrome
ART	Antiretroviral treatment
BCG	Bacille Calmette-Guérin
CHBH	Chris Hani Baragwanath Hospital
$CI_{95\%}$	95% Confidence interval
CRP	C-reactive protein
ELISA	Enzyme-linked immunosorbent assay
EPI	Expanded programme on immunisation
GEU	Gastroenteritis Unit
GSK	Glaxo Smith Kline
HIV	Human immunodeficiency virus
ICD 9	International Classification of Diseases, 9th Revision
ICU	Intensive care unit
IQR	Interquartile range
IRR	Incidence rate ratio
LRTI	Lower respiratory tract infection
OPV	Oral polio vaccine
OR	Odds ratio
PCR	Polymerase chain reaction
PCV	Pneumococcal conjugate vaccine
RMPRU	Respiratory and Meningeal Pathogens Research Unit
RR	Relative risk
SD	Standard deviation

USA	United States of America
WCC	White cell count
WHO	World Health Organisation

1. INTRODUCTION

1.1 The Global and Local Burden of Diarrhoeal Disease

Despite a decline in child mortality, preventable and treatable diseases such as pneumonia, diarrhoea, malnutrition, malaria and measles are still the leading cause of death in children. Worldwide diarrhoea is estimated to account for 18% of the 10.6 million annual deaths in children younger than five years of age. The burden of disease is greatest in the African region, which accounts for 40% of deaths from diarrhoea despite only contributing to 18% of the world under-five population (1). Mortality from diarrhoea has decreased over the past forty years, however, the incidence has remained unchanged and morbidity remains high (2). High incidence rates are clinically significant as diarrhoea in children can have an adverse effect on growth as well as physical and cognitive function (3, 4). In South Africa, diarrhoeal disease is still a major cause of morbidity and mortality in the under-five age group, accounting for just over 10% of deaths in this age group in 2000 (5).

1.2 The Burden of Rotavirus Disease

Acute diarrhoea can be caused by a variety of viral, bacterial and parasitic enteropathogens but rotavirus is recognised as the most important cause of severe gastroenteritis in infants and young children throughout the world, and generally results in more severe gastroenteritis than that caused by other enteropathogens (6, 7). The contribution of the various pathogens to diarrhoeal illness often varies between countries depending on geographical and socio-economic conditions (8).

Rotavirus is the main cause of acute gastroenteritis worldwide, accounting for 527 000 deaths per year and 29% of all deaths due to diarrhoea in children under five years of age in 2004 (9). A review of studies published from 1986 to 1999 found that rotavirus was responsible for a median of 22% of cases of severe diarrhoea in children (10). An updated review of studies published from 2000 to 2004 showed that an increased proportion of hospitalisations were due to rotavirus compared to preceding years, and that rotavirus now accounted for 39% of severe diarrhoea cases (11). Hospital-based global sentinel surveillance data show that rotavirus infection accounts for about 40% of hospitalisations for diarrhoea in children under 5 years worldwide (12). Rotavirus is isolated more frequently in children who are hospitalised with acute gastroenteritis (severe disease) than those in the community (7), often resulting in severe dehydrating diarrhoea. More than 80% of all deaths related to rotavirus were estimated to occur in the developing countries of sub-Saharan Africa and south Asia (11).

Rotavirus infection has been shown to be most common in children aged 6 to 24 months (13), however, Steele et al reported that a sizeable proportion of infants under 6 months of age also developed rotavirus illness (14). In developed countries the majority of children with rotavirus infection are in their second year of life, whereas in developing countries just over 80% of hospitalisations for rotavirus occurred during the first year of life, with 38% before 6 months of age (7, 15). In an observational study in Greece, children with rotavirus infection were more likely to have severe gastroenteritis, be hospitalised, and have a longer duration of hospitalisation compared to gastroenteritis due to other pathogens (16). They also had a higher severity score (17).

Diarrhoea occurs year-round but there is usually a period with increased rotavirus prevalence. In a review of fifteen African countries, rotavirus infection occurred throughout the year but seasonal peaks were commoner in the dry rather than the wet seasons, although this did not apply to all countries (7). In Southern Africa there was generally a single peak of rotavirus infection during the dry season (autumn and winter). In South Africa, Steele *et al* reported a defined seasonal pattern with one peak in May (autumn) and a second smaller peak in August and September (late winter and early spring) (14).

1.3 Diarrhoea and Human Immunodeficiency Virus (HIV) Infection

HIV infection is common among children in many sub-Saharan African countries, including South Africa, with high mortality rates (18,19). The burden of paediatric HIV infection is highest among infants and young children due to the high prevalence of maternal HIV infection and subsequent mother to child transmission. HIV infected children are more likely to be malnourished, to have been previously hospitalised, to have a longer duration of hospitalisation, are less likely to respond to antibiotic therapy and more likely to die during admission compared to HIV-uninfected children (18, 19). Diarrhoeal disease has been shown to be a leading cause of morbidity and mortality in HIV-infected children (20, 21). The incidence , duration, severity and mortality from diarrhoea is greater in HIV-infected children (21, 22).

In a study performed in HIV-infected and HIV-uninfected children hospitalised for diarrhoea at Chris Hani Baragwanath Hospital (CHBH), HIV-infected children were more likely to have prolonged diarrhoea, require hospitalisation for more than 4 days (odds ratio (OR) 5.11, 95% confidence interval ($CI_{95\%}$) 1.49, 17.52) and had a higher rate of

concomitant pneumonia compared to HIV-uninfected children (22). Pavia *et al* showed that HIV-infected children had more frequent episodes of diarrhoea, were more likely to present with severe dehydration, to have persistent diarrhoea and have a fatal outcome. Diarrhoea among HIV-infected children was associated with malnutrition and advanced HIV disease in the mother (21). In Zaire HIV-infected infants had more recurrent and persistent diarrhoea, and the incidence of persistent diarrhoea in HIV-exposed uninfected infants was nearly twice that of HIV-unexposed uninfected infants (20). HIV-infected children had higher frequency of malnutrition, persistent and recurrent diarrhoea and recurrent hospital admissions in a rural South African study (23).

There is little evidence to suggest that the aetiology of diarrhoea among HIV-infected children is different to that in HIV-uninfected children. Diarrhoea in adults with Acquired Immunodeficiency Syndrome (AIDS) has been associated with opportunistic infections, for example *Isospora belli,* but this has not been observed in children (22). The frequency of excretion of enteric viruses was shown to be greater in HIV-infected children compared to HIV-uninfected children but associated illness was not more common or severe in the HIV-infected children (24). Asymptomatic and mildly symptomatic HIV infection has not been shown to influence the outcome of rotavirus infection. In a study of Malawian children, HIV-infected children shed rotavirus in their stools for a longer period but this did not lead to increased incidence or severity of diarrhoea. Rotavirus does not seem to be an opportunistic pathogen in HIV-infected children (25). No data is available on the course of rotavirus infection in severely immunocompromised children.

1.4 Prevention of Diarrhoeal Disease

Prevention remains an important means to decrease both morbidity and mortality from acute gastroenteritis. Previous efforts focused mainly on early treatment of diarrhoea with oral rehydration, but the availability of an effective rotavirus vaccine provides a new way to address this problem. Preventative interventions for diarrhoeal disease and its complications include improvement of water quality and sanitation, promotion of breastfeeding, higher rates of measles immunization, and introduction of treatment programs focused on oral rehydration. These interventions have decreased mortality associated with bacterial and parasitic infections, but have not reduced the incidence of rotavirus diarrhoea (26).

Rotavirus infections occur universally in children with a similar incidence in developing and developed countries suggesting that rotavirus infection cannot be prevented by improvements in water and sanitation (27). Bacterial and parasitic agents are transmitted mainly through contaminated food and water, whereas rotavirus is often spread from person to person (11). Prevention of bacterial and parasitic diarrhoea by improvement in hygiene and sanitation most likely account for the increasing proportion of severe diarrhoea cases attributable to rotavirus over recent years. Children are more likely to die in developing countries due to poor access to oral rehydration therapy and the high prevalence of malnutrition (10).

Immunization is recognized as the most cost-effective public health intervention in developing countries (13). The introduction of a rotavirus vaccine will provide a public health tool to address mortality and morbidity from rotavirus disease, and thus from acute

gastroenteritis in general. An estimated 16% of all diarrhoeal deaths in children under five could be prevented by introduction of an effective vaccine (27). The first effective rotavirus vaccine was introduced into the routine immunization programme in the United States of America (USA) in 1998 but was withdrawn after it was associated with an increased risk of intussusception (28). Following this, two oral live-attenuated rotavirus vaccines have been evaluated in large clinical trials and were found to be safe and efficacious for the prevention of rotavirus gastroenteritis (29, 30). These are Rotarix™ (Glaxo-SmithKline Biologicals (GSK), Rixensart, Belgium), a monovalent human rotavirus vaccine and RotaTeq™ (Merck & Co, New Jersey, USA), a pentavalent human-bovine reassortment rotavirus vaccine. These are both licenced in South Africa and Rotarix™ was included in the Expanded Programme on Immunization (EPI) in South Africa since August 2009.

Of special note is that the rotavirus vaccine gave 42% protection against hospitalisation for gastroenteritis of any cause (29), which leads to a decrease in the overall burden of acute gastroenteritis. The vaccine has been shown not to interfere with other childhood vaccinations, including oral polio vaccine (OPV) (31). A study in HIV-infected children in South Africa showed that Rotarix was well tolerated and immunogenic in this group of children, when co-administered with the routine vaccines in the EPI schedule (32). An efficacy study conducted in South Africa using Rotarix™ showed vaccine efficacy in children under one year of age to be 76.9% ($CI_{95\%}$ 56.9, 88.4) and although vaccine efficacy may be lower than that in developed countries, the public health impact is expected to be greater due to the higher burden of disease (33).

Rotavirus diarrhoea occurs at an earlier age in developing countries compared to developed countries so children in developing countries will require complete vaccination earlier (13). The recommendation is to vaccinate all infants, without targeting only those with risk factors, with a mass immunization program starting at the age of six weeks (34). Rotarix™ will be given to South African infants at 6 and 14 weeks in conjunction with the existing vaccines in the vaccine schedule.

1.5 Severity of Diarrhoea

A measure of the severity of an episode of acute gastroenteritis is important as severity may vary by age, HIV infection status or other risk factors. A widely used scoring system is the Vesikari scale, which assigns a score from 0 to 20 based on duration and intensity of diarrhoea and vomiting, intensity of fever, presence of dehydration and hospitalisation (35). This scoring system has been used in the clinical trials investigating safety and efficacy of candidate rotavirus vaccines, as well as some of the observational trials where a modified score was used. Other observational studies have relied on level of dehydration, duration of diarrhoea prior to hospitalisation, duration of hospitalisation and death as indicators of severity. Most frequently clinical grading of severity is done by grading the degree of dehydration according to signs and symptoms that reflect the amount of fluid lost.

1.6 Risk Factors for Poor Outcome

The identification of risk factors associated with severe acute gastroenteritis as well as identifying predictors of poor outcome, can aid in designing prevention strategies. With

high HIV prevalence rates in South African children, the impact of HIV infection on the incidence of and outcomes from acute gastroenteritis is important when determining and tailoring interventions. Sex, age, non breastfeeding, low birth weight, HIV infection, malnutrition and co-morbidities have been associated with poor outcome in children with diarrhoea.

Sex has been associated with incidence of acute gastroenteritis, with a relative risk of males versus females equal to 1.08 ($CI_{95\%}$1.04, 1.11). Age has been identified as an important risk factor for acute gastroenteritis, with incidence decreasing with increasing age (36). About two-thirds of all rotavirus gastroenteritis cases in the REVEAL study in Europe occurred in the 6 to 23 month age group (37). In a review of the epidemiology of rotavirus infection in hospitalised South African children, it was found that more than 95% of rotavirus cases occurred in children under 18 months of age (15). Eighty-one percent of children hospitalized with rotavirus infection were under one year of age.

Other risks for severe acute gastroenteritis include premature birth and low birth weight and a significant association was found between hospitalisation for rotavirus acute gastroenteritis and low birth weight (34, 38). Nosocomially-acquired rotavirus infection in neonates has been shown to be milder and provide natural protection against rotavirus disease later in childhood, although premature infants and those in neonatal intensive care unit (ICU) with rotavirus infection may have a more severe course (34).

Non breastfeeding has been identified as a risk factor for all cause acute gastroenteritis death and severe malnutrition and lack of breastfeeding were found to be important independent risk factors for death in children hospitalised for diarrhoea (39). Observational

studies have shown that exclusive breastfeeding is protective against diarrhoeal disease in both developed and developing countries, whereas the protective effect against rotavirus disease specifically has been variable (40). A study in Europe has shown that exclusive breastfeeding of infants is protective against rotavirus disease (41) but a large study by Glass *et al* concluded that results do not support a protective role for breastfeeding against severe rotavirus disease beyond the first months of life (42). An American study found breast feeding to be protective against hospitalisation in infants under six months of age (43). The protective effect may only be transient and lost when breastfeeding is stopped (44). Breastfeeding may postpone rather than prevent disease.

Malnutrition has been identified as a risk factor for diarrhoeal disease, with a longer duration of diarrhoea and higher incidence of dehydration in malnourished children (39). Animal and human studies have suggested that malnutrition may contribute to the severity of disease caused by rotavirus, in particular increased duration of diarrhoea (34). Malnutrition may interfere with regeneration of the intestinal villi. Malnutrition has been shown to increase the risk of mortality, however, in a study conducted in Malawi, after controlling for age and HIV infection, severity of disease was not influenced by nutritional status (25).

Co-morbidities have also been associated with poor outcome, with presence of pneumonia being a major prognostic indicator for death in children attending a diarrhoea treatment centre in Bangladesh (39). A longer hospital stay was associated with a co-diagnosis of pneumonia in a South African study (22). Respiratory tract infections often occurred together with rotavirus gastroenteritis in a study from Sierra Leone. The most common co-morbidities were malaria followed by respiratory tract infections (17). In a review, 23% of

rotavirus cases had at least one other enteropathogen identified and mixed infections were associated with malnutrition and prolonged diarrhoea (7) Presence of bacteraemia has also been associated with death from diarrhoea (23).

Xerophthalmia and measles infection in the previous 3 months were significantly associated with death from diarrhoea. Duration of diarrhoea between 7 and 14 days, children presenting with moderate or severe dehydration, children of mothers with low education and low household income were also at greater risk of death (39).

1.7 Justification and Study Objectives

There is a lack of burden of disease data for severe acute gastroenteritis in South Africa, especially in HIV-infected children. These data are vital to inform policy regarding the cost-effectiveness of interventions such as vaccines. Especially pertinent to South Africa is the high prevalence of HIV infection with the national prevalence of HIV among antenatal clinic attendees increasing from 22.4% in 1999 to 30.2% in 2005. The prevalence in Gauteng was slightly higher than the national prevalence, with corresponding antenatal HIV prevalence in 2005 of 32.4% (45). The impact of HIV infection on the incidence of acute gastroenteritis is thus important in our community. There is an urgent need for effective interventions to prevent diarrhoeal deaths and morbidity in children under five years of age. The identification of risk factors for severe acute gastroenteritis and poor outcome is the key to targeting interventions aimed at reducing diarrhoeal disease morbidity and mortality.

Childhood mortality and morbidity can be reduced through routine immunization of infants. With effective rotavirus vaccines available the burden of disease needs to be evaluated to aid in determining the cost-effectiveness of vaccination against rotavirus. The identification of rotavirus in the stool is not part of the routine investigations for a child with diarrhoea in most hospitals, as it does not change the management of the disease. Even without microbiological evidence of rotavirus infection, seasonal and age distribution of acute gastroenteritis consistent with rotavirus disease may guide policy decisions for future introduction of a rotavirus vaccine. A study in Italy evaluated the burden of acute gastroenteritis as an indirect measure of rotavirus disease, as rotavirus gastroenteritis is responsible for a large proportion of all cases of acute gastroenteritis (36). Most acute gastroenteritis cases in Italy had the same age and calendar distribution as rotavirus disease. In the same way assessing the overall burden of acute gastroenteritis in Sowetan children may give an estimation of the burden of rotavirus disease.

This study aims to determine the incidence rate of hospitalisation for acute gastroenteritis in a cohort of Sowetan children under five years of age, as well as to determine risk factors associated with poor outcome in children with severe acute gastroenteritis. This will give baseline incidence of severe acute gastroenteritis prior to introduction of the rotavirus vaccine into the routine immunization schedule. Surveillance of acute gastroenteritis post vaccine introduction will then allow investigation of the impact of the vaccine on the incidence of hospitalisation for acute gastroenteritis. Acute episodes of gastroenteritis will be considered, with the exclusion of episodes of persistent diarrhoea. Although persistent diarrhoea contributes largely to the overall burden of diarrhoeal disease, this study focuses on acute severe gastroenteritis and estimates for rotavirus disease burden.

Objectives

1. To determine the incidence of hospitalisation for acute gastroenteritis in a cohort of Soweto children under five years of age, and thus estimate the burden of rotavirus disease.
2. To determine the effect of age and HIV status on incidence of hospitalisation for acute gastroenteritis.
3. To determine the risk factors associated with mortality and prolonged hospitalisation in children hospitalised with acute gastroenteritis over a five-year period.

2. MATERIALS AND METHODS

2.1 Details of the original study

A study investigating the efficacy of a pneumococcal conjugate vaccine (PCV) was conducted in Soweto from 1998-2005 at the CHBH in Soweto. Enrolment began on 2 March 1998, and ended on 30 October 2000. Follow up continued until October 2005. This study was a double-blind, placebo-controlled trial that included 39 836 children who were randomised to receive either a nine-valent pneumococcal conjugate vaccine or placebo at 6, 10 and 14 weeks of age (46). The study was approved by the Committee for the Study of Human Subjects at the University of the Witwatersrand, and permission was obtained from the Medicines Control Council of South Africa (Appendix A). Written informed consent explaining all aspects of the study was obtained from a parent or guardian before enrolment on the trial. Study participants were identified by a unique study number in order to protect confidentiality.

2.2 Study population

The participants were recruited from clinics in the Soweto area. Infants aged 28 to 84 days were eligible for inclusion in the study if they were unvaccinated or had only received OPV and Bacille Calmette-Guérin (BCG) at birth. Exclusion criteria included progressive underlying neurological disorder, a history of seizures or infantile spasms, or children planning to leave the Soweto area and that could not be followed up. In addition to PCV or placebo, all study participants received all other vaccinations which were included in the EPI schedule at the time - vaccination against diphtheria, tetanus, pertussis, Hepatitis B and polio was given. Twenty-four hour passive surveillance was conducted at the paediatric admission ward of the CHBH, a tertiary hospital serving the Soweto area, for the duration of the study period.

During the PCV efficacy trial follow-up period, approximately 24 000 children were born in Soweto annually, approximately 17 000 at the hospital and the remainder at local clinics. It is estimated that approximately 90% of children from the Soweto area requiring hospitalisation are admitted to CHBH. There are 164 beds for general paediatric medical admissions (children < 12 years of age) and 6000 children are admitted annually (19).

All hospitalisations of study participants at CHBH for any cause were identified by study staff, a case report form completed and an examination performed by a study doctor. The case report form included information on symptoms, clinical signs, physician diagnosis, estimated severity of dehydration based on the attending physician assessment, outcome and laboratory results (Appendix B). Blood and respiratory samples were collected on participants by study staff. The study doctors were not involved in the decision to

hospitalise a child, or in the child's care or any management decisions while in hospital. There was no active follow up of enrolled cases.

Standard of care of all children admitted with acute gastroenteritis included rehydration, either oral or intravenous, correction of any electrolyte abnormalities and early feeding. Uncomplicated gastroenteritis cases merely requiring fluid replacement were usually admitted to the gastroenteritis unit (GEU), whereas more complicated cases or those with multiple diagnoses were admitted to the general paediatric wards. Investigations were done as per the attending ward doctor and may have included blood culture, measurement of blood counts, C-reactive protein (CRP), electrolytes and HIV testing as indicated. Antiretroviral therapy (ART) for HIV-infected children was not standard of care in South Africa during this time and most children infected with HIV during the study period did not receive ART. During the recruitment period of this study, antiretrovirals for prevention of mother-to-child transmission of HIV were not routinely given to mothers and infants at delivery in the maternity wards.

Based on the measured prevalence of HIV infection among women attending antenatal clinics during the duration of the study period, it was estimated that 24.87% of the children enrolled onto the study were born to HIV-infected mothers. The vertical transmission rate, in the absence of antiretroviral intervention, from mother to child was estimated to be 26%, and thus, 6.47% of the children recruited into the study were estimated to be HIV-infected (47).

2.3 Study design

Using the existing cohort of children enrolled on the PCV efficacy study, a secondary data analysis was performed. Using a cohort study design, the incidence of hospitalisation for acute gastroenteritis was determined in this cohort of children under five years of age. Total person years of observation were calculated for each study participant. A subset of the total cohort, i.e. those admitted with a primary or secondary diagnosis of acute gastroenteritis, were then analysed to determine risk factors associated with acute gastroenteritis mortality and prolonged hospitalisation. Although incidence calculations made use of the data of the total cohort, this subset of participants looked at data at a specific time point when hospitalised for acute gastroenteritis. These data were, thus, no longer time-dependent but cross-sectional in nature, and analysed as such.

2.4 Study sample

A sample size calculation was not performed as the size of the study population had already been determined. However, calculations were performed to determine the precision of results based on the existing sample size. The incidence of diarrhoea has been estimated as 3.8 episodes per child per year in children under one year of age, and 2.1 episodes per child per year in children aged 1-4 years (10). If this is extrapolated to our cohort of 39 836 children, we would expect about 151540 episodes of diarrhoea in the first year of life, and approximately 334984 episodes of diarrhoea over the remaining 4 year period of follow up. A study from Chile showed that 1.5% of diarrhoeal episodes in infants < 11 months required hospitalisation compared to 0.2% of those aged 1-4 years and these proportions have been used to estimate global diarrhoeal episodes (10). Using the cohort of 39 836 and

assuming hospitalisation rates of acute gastroenteritis as above, the estimation would be within 0.1% of the true proportion hospitalised in those under 1 year and within 0.02% in those aged 1-4 years.

2.5 Data sources

The dataset was made available as a single Stata file, including all variables of participants enrolled on the pneumococcal vaccine trial. A template of the data collection form was made available and variables in the dataset were checked against the data collection form template. Codes were provided for any variables not present on the form but which had been created in the database for previous analyses. There were a total of 43 910 entries and 259 variables. Multiple entries existed for participants with more than one hospitalisation during the study period, so there could be more than one observation per study participant (and study identification number) but with different admission dates reflecting separate admissions. A separate dataset existed recording the weight of the child at admission. These additional data were merged into the master dataset matching entries on study identification number and admission date.

2.6 Exclusions and Definitions

Any admission occurring once a child was five years or older was not included in the analysis. Any re-admission of a child occurring within two weeks of a previous admission in the same child was excluded from the analysis, as such an re-admission was most likely due to the same disease process as the prior admission, a relapse of the previous disease or as a result of a hospital-acquired (nosocomial) infection.

A single episode of acute gastroenteritis is usually defined as ≥ three loose stools in any 24 hour period with duration < 14 days, occurring not less than 30 days after a previous episode of diarrhoea (World Health Organisation (WHO)). However, some of this information was either not collected on the case report form, for example, history of an episode of diarrhoea in the past 30 days, or the number of missing observations for the variable precluded the use of this variable to define an episode of acute gastroenteritis (for example, frequency of diarrhoea in a 24-hour period had a high proportion of missing values). A physician discharge diagnosis was, thus, used to identify all hospitalisations for acute gastroenteritis. Diagnosis fields in the database were both descriptive (text) and numeric codes based on the International Classification of Diseases, 9th Revision (ICD-9) (48), and both fields were used to identify acute gastroenteritis cases. Those with duration of diarrhoea greater than 14 days on admission were then excluded. A child admitted with acute gastroenteritis that went on to have persistent diarrhoea was still identified as an acute diarrhoeal episode. Frequency of diarrhoea was not used for defining an acute gastroenteritis episode, as values were frequently missing for this variable.

Episode of severe acute gastroenteritis: any hospitalisation of a child with a physician diagnosis of acute gastroenteritis. There could be more than one episode per participant, providing subsequent hospitalisations occurred more than two weeks after the previous hospitalisation. All analysis was performed using the number of episodes of acute gastroenteritis, not number of patients.

Physician diagnosis of acute gastroenteritis: A primary or secondary text diagnosis of "dysentery" or "gastroenteritis", and / or a primary or secondary ICD-9 diagnosis code of 003.0, 004, 008.0, 008.5, 008.8, or 009.3 (Appendix C). Those with duration of diarrhoea

≥14 days were excluded as per the WHO definition of persistent diarrhoea. Those with a text diagnosis of "chronic diarrhoea" or "persistent diarrhoea" were not included.

2.7 Measurement of dependent and independent variables

The outcome (dependent) measures considered for this analysis were in-hospital mortality associated with admission for acute gastroenteritis and duration of hospitalisation for acute gastroenteritis in days. Risk factors (independent) measured were age, sex, gestational age, HIV infection status, presence of malnutrition, degree of dehydration, and a concomitant diagnosis of a lower respiratory tract infection (LRTI). Measurement of each of these risk factors is described below.

Outcome of a study participant was described as either death during hospitalisation for an episode of acute gastroenteritis, or discharge from the hospital. Mortality was classified as a dichotomous variable whereby participants were assigned to either death in hospital or discharge from hospital. Duration of hospitalisation for an episode of acute gastroenteritis was defined as the number of days from the date of admission to the date of outcome (discharge or death). i.e. date of outcome minus date of admission. Duration of hospitalisation was a continuous variable in the dataset; and could have been treated as such in the analysis. However, duration of hospitalisation was rather represented as a dichotomous variable, as this has more clinical application for the physician who may not want to predict the length of hospitalisation but merely to determine whether, on admission, children with certain risk factors may be more likely to be hospitalised for a longer period. Those with duration of hospitalisation greater than the median were considered to have a prolonged hospitalisation.

Age at study enrolment was calculated as date of first study visit minus date of birth. Age at admission was calculated as date of admission minus date of birth. Age at admission was categorised into age groups for incidence rate calculations. Age groups were generated as follows: < 6 months, 6-11.9 months, 12-23.9 months, and 24-59.9 months. Age at admission, in months, was also considered as a continuous variable in univariate and multivariate analysis to investigate the association between age and the above-mentioned outcomes. Questions on both birth weight and gestational age were included in the case report form, however, birth weight was missing for most patients in the dataset. Gestational age was, thus used as a risk factor and categorised in those who were born preterm (< 37 weeks gestation at birth) and those who were term (≥37 weeks gestation at birth).

Although HIV enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) results were available for some participants (obtained during the hospital admission), at this time routine HIV testing was not done in all paediatric patients admitted to CHBH. Blood drawn by study doctors was, therefore, tested to determine HIV status, as per study protocol approved by the Ethics Committee. An HIV ELISA antibody test was performed in children 18 months of age and older, and an HIV PCR in those under 18 months of age with a reactive HIV ELISA test or clinical suspicion of HIV-infection. These results were recorded in the database and used in this analysis.

Those with a positive HIV ELISA (≥ 18 months) or a positive PCR (< 18 months) were considered as HIV-infected. Children with a negative HIV ELISA or PCR were considered HIV-uninfected. Those with a missing or indeterminate HIV result were considered to have unknown HIV status. There was no information in the database on whether infants were born to HIV-infected mothers or not. For incidence rate calculations, stratified by

HIV infection status, those with unknown HIV status were considered to be HIV-uninfected. Any misclassification of HIV-infected children as HIV-uninfected in those with unknown HIV status would underestimate the incidence in HIV-infected children. For all other analysis involving stratification by HIV status, those with unknown HIV status were excluded from the analysis.

Although the Vesikari severity scoring scale is a tool frequently used to evaluate the severity of gastroenteritis in children in clinical trials, all the variables required to calculate this score were not available in the dataset. The scoring scale includes duration of diarrhoea, maximum number of stools per 24 hours, duration of vomiting, maximum number of vomiting episodes per 24 hours, temperature, dehydration and treatment (35). This score has been used extensively in efficacy and safety clinical trials evaluating the two new rotavirus vaccines. In a hospital setting, this scoring system is not practical as it is based on indicators spanning the entire duration of the acute gastroenteritis episode. Clinicians usually assess the level of dehydration at admission as an indicator of severity of the acute gastroenteritis episode, and express this as a percentage (commonly 2.5%, 5%, 7.5%, 10% or greater than 10%). In the Integrated Management of Childhood Illness Guidelines the WHO gives guidance on assessing dehydration in children. For this analysis the degree of dehydration was categorised into those who were ≤2.5% dehydrated, >2.5% but ≤5%, >5% but ≤7.5%, and >7.5% dehydrated. Presence of severe dehydration was defined as a level of dehydration, as assessed clinically by the hospital physician on admission, of greater than 5%.

Weight-for-age Z-scores for boys and girls respectively, from birth to five years (WHO child growth standards), were used to classify children as being malnourished or not. The

weight and age of each participant on admission was compared to the corresponding weight-for-age Z-score. Those with weight-for-age less than minus two standard deviations were classified as being malnourished on admission. Physicians in the hospital diagnosed malnutrition based on the Welcome classification (49). Diagnosis fields in the database were both descriptive (text) and codes based on the ICD-9 classification of disease (48). The case report form also recorded the presence or absence of kwashiorkor. Those recorded as presenting with kwashiorkor were also classified as being malnourished. In those participants in whom a weight on admission was not available, malnutrition was considered present if the physician diagnosed kwashiorkor, marasmus or marasmic-kwashiorkor at admission. This included a descriptive diagnosis as above or an ICD-9 code of 260, 261, or 262 (Appendix C). Those missing an admission weight with no diagnosis of malnutrition by the physician were considered to be adequately nourished.

Descriptive diagnosis and diagnosis codes by hospital physicians were used to categorise participants as having a concomitant LRTI or not on admission. LRTI included a descriptive diagnosis of pneumonia, bronchopneumonia, bronchiolitis and /or the following diagnosis codes: 466.1, 480, 481, 482, or 485 (Appendix C). Those with asthma, bronchiectasis, aspiration pneumonia or chronic pneumonia were not classified as having a LRTI. Patients with positive blood culture of a bacterial pathogen were defined as having bacteraemia.

2.8 Data processing methods

All data was cleaned and analyzed using STATA version 11.0 (StataCorp, Texas, USA). Variables which were not needed for the present analysis were dropped. Dates were checked to ensure that they were formatted correctly, then edited for consistency and changed, if it was possible to verify these dates against another variable or the original data. The date of the first visit was considered the date of entry into the trial. If the first visit date was missing, the child was assumed not to have been enrolled on the trial and was not included in the analysis. The censoring date was calculated as date of birth plus (5 x 365.25) days (5th birthday). Text diagnosis variables were edited for spelling errors and recoded if necessary. ICD-9 codes were cross-checked for relevant diagnoses as these were needed to classify the participant as having acute gastroenteritis, malnutrition or a concomitant diagnosis of LRTI.

Data was cleaned by performing logic, range and skip checks. Completeness of data was assessed by checking for missing data. The number of missing values was documented for each variable. Categorical variables were tabulated to check for invalid options. These were corrected if possible, for example from small letters to capitals, or set to missing if entries were invalid and could not be verified. Continuous variables were summarised and the range of values checked to ensure these values were within possible limits. Entries that were not possible, for example gestational age of 50 weeks, were checked if this was possible or set to missing if the value could not be verified. Continuous variables were assessed for normality, as this determined whether parametric or non-parametric tests were used for analysis. New variables were created for defining and categorising independent and dependent variables as necessary, based on definitions as stated previously.

2.9 Data analysis

The total number of admissions was counted, regardless of admission diagnosis and the proportion of these with a primary or secondary diagnosis of acute gastroenteritis was calculated. In those with a repeat admission within two weeks of an admission, the subsequent admission was excluded as this admission would most likely reflect the same disease process or a nosocomial infection. The first admission for the child was included. Incidence rates were calculated, together with a 95% confidence interval ($CI_{95\%}$), using person time analysis. The censoring point was the date the participant turned five or death (whichever occurred first), and follow up times were calculated accordingly. The total number of acute gastroenteritis episodes was used in the numerator and the total person years contributed by all those in the cohort in the denominator. Children can have more than one episode of diarrhoea so are at risk for the entire follow up period unless they were lost to follow up or died during the study period. Incidence rates in the placebo and vaccine groups were compared and incidence was stratified by age category (as previously defined).

HIV infection status was not determined in all those enrolled on the study, only those who had been hospitalised during the study period. It was, therefore, not possible to calculate incidence rates stratified by HIV infection status using person time analysis, as above. The HIV prevalence in the cohort had been estimated to be 6.5% in the original study (47). This estimation was used to determine the denominators for cumulative incidence calculations stratified by HIV infection status in this analysis. Those with an indeterminate or unknown HIV status were considered HIV-uninfected for the purposes of cumulative incidence calculations.

The initial number of children at risk was the number of children at enrolment. For each age group the denominator was estimated as the number of children still alive at the midpoint of the interval (calculated as the average of those alive at the beginning and end of the period). These estimations for HIV-infected and uninfected children are shown in Appendix D. The number of acute gastroenteritis episodes in each interval was used in the numerator. A relative risk (RR) was calculated (with $CI_{95\%}$) to assess significant differences in the incidence of acute gastroenteritis between those who were HIV-infected and HIV-uninfected. Incidence could not be compared across age categories as the time interval varied in each age category.

The number of episodes of acute gastroenteritis was plotted by month to investigate seasonality of acute gastroenteritis during the study period, and this was compared to that of total hospital admissions for the same month and year.

All episodes of acute gastroenteritis (as previously defined) were analysed as cross-sectional data. The descriptive component included a description of cases by various demographic factors. Each admission was regarded as a separate event for the analysis, even if the participant had been admitted previously or was admitted subsequently during the study follow up period, provided the time between admissions was greater than 2 weeks. The mean (± standard deviation (SD)) was calculated for normally distributed data, the median and interquartile range (IQR) for skewed data, and frequencies for categorical data. Characteristics of all children admitted with acute gastroenteritis were determined and then stratified by HIV infection status to investigate any differences between HIV-infected and HIV-uninfected children. Only children with a known HIV result were included in the analysis. Presenting symptoms and severity indicators were also compared

between HIV-infected and HIV-uninfected children. Laboratory results were analysed if these were available.

Continuous variables were compared using a *t* test (difference between the means) for normally distributed data or Wilkoxon Ranksum test (Mann Whitney) for data which was not normally distributed. The association between categorical variables was tested using the chi square test or Fisher's exact test. All tests were 2-sided and a p-value < 0.05 was considered statistically significant.

The analytical component included both univariate and multivariate analysis. Risk factors associated with mortality and prolonged hospitalisation were first investigated using univariate analysis. The chi square test was used to assess the relationship between categorical risk factors and mortality, as well as between categorical risk factors and prolonged hospitalisation. The *t* test (for comparison of normally distributed continuous variables) or Mann-Whitney test (for comparison of continuous variables not normally distributed) was used to test differences in continuous variables by outcome. Multivariate analysis was done using logistic regression models to predict risk factors for mortality and prolonged hospitalisation. Forward selection was used to determine inclusion of risk factors in the final model, with the most significant variable in the univariate analysis introduced into the model first. Risk factors were then introduced into the model one by one, and retained in the model if there was significant improvement (as assessed by improvement in the -2 log likelihood) in the model after addition of the risk factor. Adjusted and unadjusted odds ratios, together with estimated 95% confidence intervals were calculated.

Confounding refers to a mixing of effects and there is distortion of the estimated effect of an exposure on an outcome caused by the presence of a third variable, which is associated with both the exposure and the outcome. This third variable must not be an intermediate step in the causal pathway between exposure and outcome. Effect measure modification is a situation where the association between an exposure and an outcome varies by levels of a third factor. Confounding can be controlled for in the analysis, whereas effect modification is a real effect and needs to be reported. A variable can be both a confounder and an effect modifier. Stratification is useful to detect both confounding and effect modification. Effect measure modification and confounding was evaluated using the Mantel Hanzel method. If the stratified odds ratios were heterogenous but the combined OR was different to the crude OR, confounding was considered to be present. Confounding could be adjusted for in the multivariate analysis and a combined OR reported. If the odds ratios were not heterogenous ($p<0.05$), the odds ratios could not be combined and effect measure modification between the variables was considered to be present. A stratified analysis, with separate logistic regression models for each group, might then be indicated.

2.10 Ethics approval

This secondary data analysis was approved by the Human Research Ethics Committee (Medical) of the University of Witwatersrand on 28/11/2008 (Appendix E).

3. RESULTS

3.1 General description of hospital admissions

Using the dataset, a total of 39 879 children were identified as being enrolled on the study. The first patients were enrolled on 2 March 1998 and enrolment concluded on 29 September 2000. Mean age at enrolment was 46 days (± SD 8.94), so on average the participants were 6 weeks or older at enrolment. The total study follow up period was from initial enrolment (first study visit) until 18 September 2005. After excluding admissions occurring over the age of 5 years and those within 2 weeks of a previous admission, there were 9108 admissions to the paediatric wards at CHBH over the study period, occurring in 6328 patients. Admissions included those to the four general admission wards, the short-stay ward and the gastroenteritis unit (GEU). Median age at admission was 11.96 months (IQR 5.09-23.97 months, range 1.22-59.99).

There were 2006 admissions with a primary or secondary diagnosis of gastroenteritis. Of these 57 were excluded as duration of diarrhoea was 14 days or longer, leaving a total of 1949 admissions for acute gastroenteritis in 1761 participants. Twenty-one percent of all admissions were, therefore, due completely or in part to acute gastroenteritis. Of the 1949 acute gastroenteritis admissions, 609 (31.25%) occurred in those < 6 months of age (> 6 weeks), 635 (32.58%) in those aged 6-11.9 months, 473 (24.27%) in those between 1 and 2 years and 232 (11.90%) in those between 2 and 5 years. The majority of acute gastroenteritis admissions (88.90%) occurred in children less than 2 years, with 63.83% in those less than one year of age. Figure 1 shows the number of admissions for acute gastroenteritis as a proportion of total hospital admissions, stratified by age group. In those

under 6 months of age 23.05% of total admissions in that age group were due to acute gastroenteritis, 32.99% in those aged between 6 and 12 months, 20.86% in those aged between 1 and 2 years and 10.20% in those aged between 2 and 5 years.

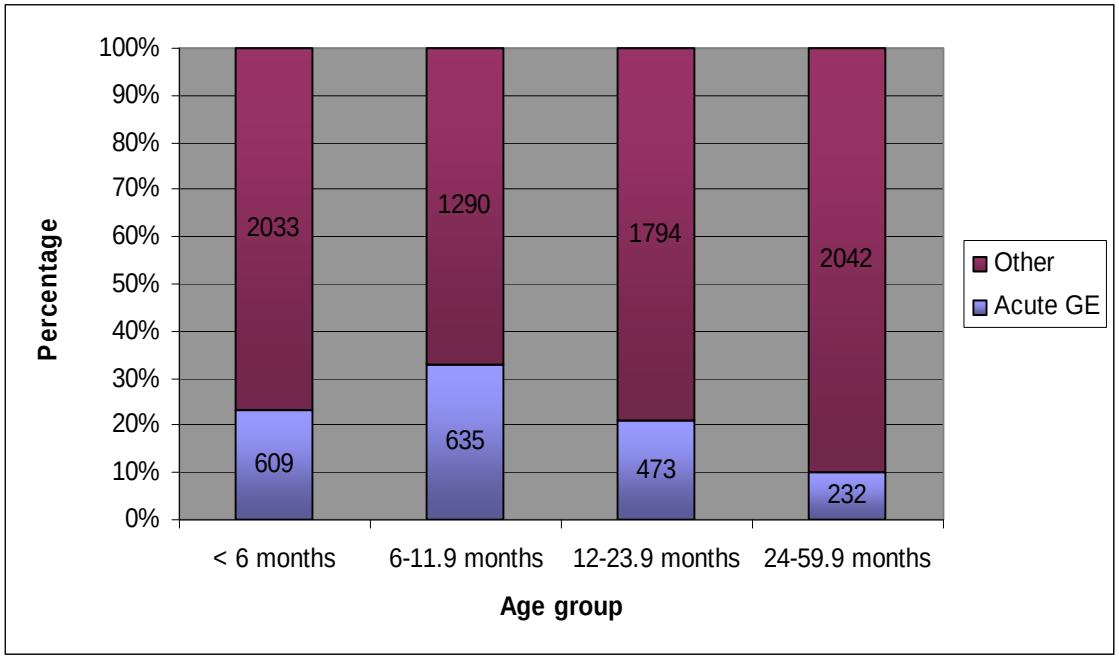

***Figure 1**: Number of admissions over the study period due to acute gastroenteritis as a proportion of total hospital admissions, stratified by age group.*

Of the 1761 participants admitted with acute gastroenteritis, 156 had multiple admissions for acute gastroenteritis. Eighty-four (53.85%) of these children were HIV-infected. Those children with more than one admission for acute gastroenteritis were 4.97 ($CI_{95\%}$ 2.85, 5.81) times more likely to be HIV-infected than those with only one admission for acute gastroenteritis.

Of the 1949 admissions for acute gastroenteritis, 504 (25.86%) occurred in children found to be HIV-infected , with 29.56% of those under 6 months testing HIV-positive, 24.57% of

those between 6 and 12 months, 23.26% between 1 and 2 years, and 25.0% of those between 2 and 5 years. Figure 2 shows the proportion of admissions due to acute gastroenteritis (stratified by age group) of children who were HIV-infected, HIV-uninfected and those in whom HIV status was unknown. HIV status was not known in 12.52% of total participants. The age group with the most patients with unknown HIV status was those between 2 and 5 years, with 34.05% not tested for HIV. In those admitted in the general paediatric wards 247/562 (43.95%) were HIV-infected compared to 251/1127 (22.27%) in the short-stay ward / GEU. The HIV status was unknown in 208/1335 (15.58%) of those admitted to the short-stay ward compared to 31/593 (5.23%) in the general wards.

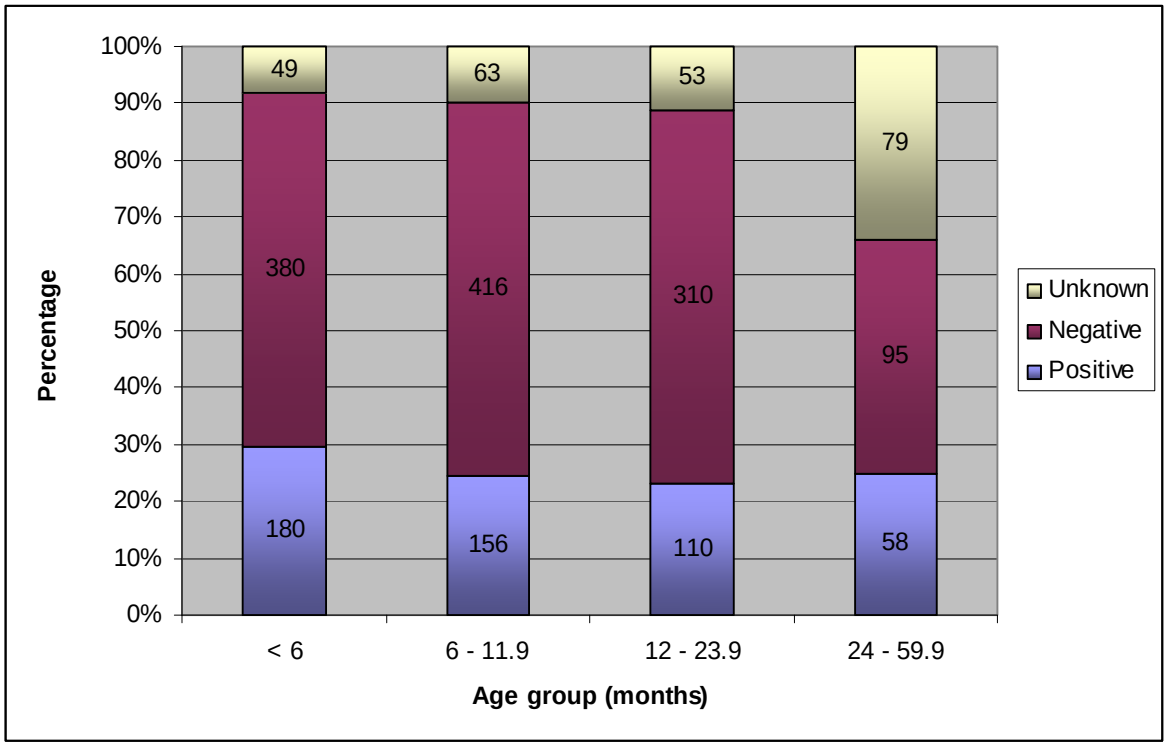

***Figure 2**: HIV infection status of children hospitalised due to acute gastroenteritis, stratified by age group.*

3.2 Seasonality of admissions

The number of admissions for acute gastroenteritis as well as the total number of admissions during the study period were stratified by month of admission and depicted graphically over the study follow up time. Figure 3 shows seasonal trends over time for children under 5 years of age. There appears to be a peak in acute gastroenteritis admissions in March to May in the years 1999, 2000 and 2001. The initiation of these peaks are mirrored in the graph for total admissions, however, total admissions then remain high until a dip in December, reflecting the increased winter admissions due to respiratory tract infections. The peaks in acute gastroenteritis admissions are most apparent in those aged under two years. Admission numbers decrease with increasing time as the cohort is aging and the five year individual follow up period stretches over a total follow up period of eight years.

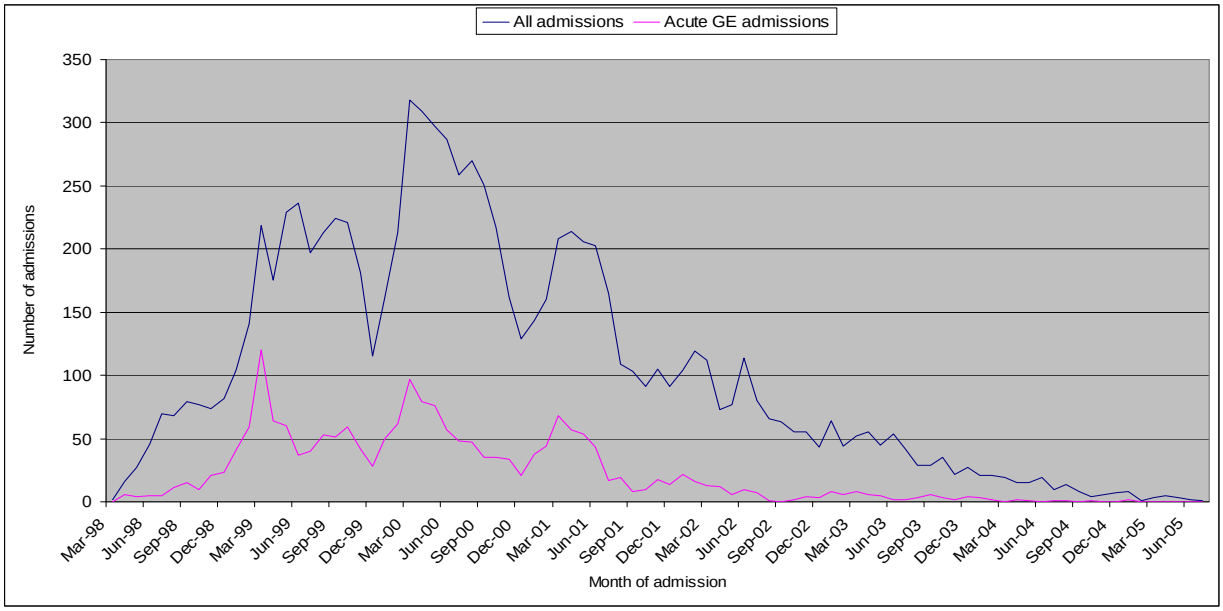

Figure 3: *Distribution of total admissions and acute gastroenteritis admissions over time in children under five years of age.*

3.3 Presenting symptoms on admission

Common symptoms at admission included fever, loss of weight, refusal to feed, vomiting, and diarrhoea (Figure 4). Sixty-four percent of children admitted with acute gastroenteritis presented with both vomiting and diarrhoea. There was no significant difference in the proportion presenting with fever, vomiting or diarrhoea (p=0.12, p=0.19 and p=0.13 respectively) between HIV-infected and uninfected children. HIV-infected children were more likely to present with refusal to feed and loss of weight (p<0.001 for both).

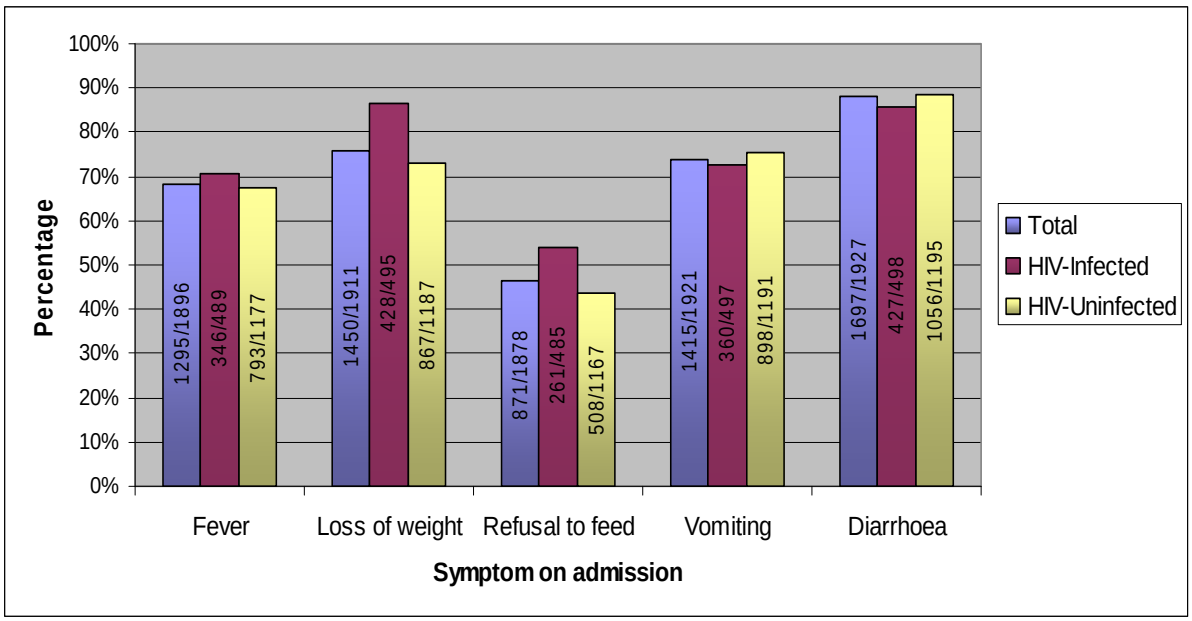

***Figure 4**: Presenting symptoms on admission for acute gastroenteritis by HIV status. (Numbers of children presenting with each symptom are enumerated on the bars. Denominators may be different to the total number due to missing data. Denominators reflect number in whom data were available.)*

The mean duration of diarrhoea was 3.05 days (±1.86) with duration of diarrhoea significantly longer in HIV-infected children compared to HIV–uninfected children (3.40 and 2.99 days respectively, p<0.001). Seizures were more common in HIV-uninfected children with 5.56% presenting with this symptom compared to 1.83% in the HIV-infected (p=0.001).

3.4 Characteristics of children admitted with an episode of acute gastroenteritis

The characteristics of children admitted with an episode of acute gastroenteritis are shown in Table 1. This includes total acute gastroenteritis admissions as well as characteristics stratified by HIV infection status. The median age at admission was 9.06 months, with 50% of admissions occurring in children between 5.09 and 15.50 months of age. There was no significant difference in median age between HIV-infected and HIV-uninfected children. Fifty-four percent of those admitted were male, with no significant difference between those HIV-infected and uninfected. Mean gestational age was 38.08 weeks (±2.06) with 12.65% of children having a gestational age of < 37 weeks at birth and defined as preterm. Gestational age did not differ significantly when comparing HIV-infected and HIV–uninfected children (p=0.52), but 15.51% of HIV-infected children had been born preterm compared to 12% of HIV-uninfected children (p=0.05).

A large proportion of children were admitted to the short-stay ward or GEU and only 30.76% were admitted in the general paediatric wards. HIV-infected children were almost three times more likely to be admitted to a general ward than uninfected children (OR 2.74; $CI_{95\%}$ 2.19, 3.42). Just under thirty-three percent of children were classified as malnourished on admission and 13.32% were clinically assessed as being severely dehydrated. HIV-infected children were eight times more likely to be malnourished at the time of admission (OR 8.39; $CI_{95\%}$ 6.60, 10.69) and 1.6 times more likely to be assessed as having severe dehydration (OR 1.56; $CI_{95\%}$1.15, 2.10). A co-diagnosis of LRTI and acute gastroenteritis was also more likely in HIV-infected children, with 31.75% of these children presenting with acute gastroenteritis as well as a LRTI on admission diagnosis, compared to 9.83% in uninfected children (OR 4.27; $CI_{95\%}$ 3.24, 5.63).

Table 1: *Characteristics of children hospitalised with an episode of acute gastroenteritis – total numbers and stratified by HIV infection status.*

Characteristic	Total n=1949	HIV Uninfected * n= 1201	HIV Infected * n=504	Odds Ratio ($CI_{95\%}$)	P value
Median age in months (IQR)	9.06 (5.09, 15.50)	8.97 (5.12, 14.19)	8.57 (4.57, 14.77)	-	0.75
Male Sex (%)	1060/1947 (54.44)	649/1200 (54.08)	275/503 (54.67)	1.02 (0.83, 1.27)	0.82
Preterm (%)	246/1944 (12.65)	144/1200 (12.00)	78/503 (15.51)	1.35 (0.99, 1.83)	0.05
General ward (%)	593/1928 (30.76)	315/1191 (26.45)	247/498 (49.60)	2.74 (2.19, 3.42)	<0.001
Malnutrition (%)	642/1949 (32.94)	254/1201 (21.15)	349/504 (69.25)	8.39 (6.60, 10.69)	<0.001
Level of dehydration	n=1885	n=1174	n=488		
≤2.5%	1107 (58.73)	713 (60.73)	235 (48.16)	0.60 (0.48, 0.75)	<0.001
>2.5%-≤5%	527 (27.96)	316 (26.92)	165 (33.81)	1.39 (1.10, 1.75)	0.005
>5%-≤7.5%	115 (6.10)	64 (5.45)	45 (9.22)	1.76 (1.16, 2.66)	0.005
>7.5%	136 (7.21)	81 (6.90)	43 (8.81)	1.30 (0.86, 1.94)	0.177
Severe Dehydration (%)	251/1885 (13.32)	145/1174 (12.35)	88/488 (18.03)	1.56 (1.15, 2.10)	0.002
Co-diagnosis of LRTI (%)	282/1949 (14.47)	118/1201 (9.83)	160/504 (31.75)	4.27 (3.24, 5.63)	<0.001
Bacteraemia (%) #	77/855 (9.01)	28/480 (5.8)	45/293 (15.36)	2.93 (1.74, 5.00)	<0.001

IQR – interquartile range, LRTI – lower respiratory tract infection

** Only includes those who had an HIV result available. Those with an equivocal or unknown HIV status were included in the total number but not included in the comparison between HIV-infected and HIV-uninfected children.*

Denominators in each cell may be different to total number HIV-infected and –uninfected due to missing data. Denominators reflect number in whom data were available.

 # Excluding the following contaminants: Bacillus, Coagulase negative *Staphylococcus, Corynebacterium, Micrococcus* or *Propionibacterium.*

3.5 Laboratory indicators

Laboratory indicators were not available in all patients. Most of the patients admitted to the short-stay ward did not have any investigations done during the admission. A white cell count (WCC) was only available in 223 (11.44%) patients with an episode of acute gastroenteritis. Median WCC was 12.5 (IQR 9.3, 17.9) x109/ l. Platelets were measured in 595 (30.53 %) with a mean platelet count of 384 (SD ±175) x109/ l. C-reactive protein, an acute inflammatory marker, was available in 590 (30.27%) with a median value of 16 (IQR 4, 54) mg/l. There was a discrepancy between the number of WCC and platelet counts available. A full blood count usually reports both WCC and platelet count, however, in the dataset there were more platelet results than WCC results.

Blood cultures were done in 855 (43.87%) patients and 665 (77.78%) of these were reported as negative. Of the 190 remaining blood cultures where an organism grew, 113 grew an organism considered likely to be a contaminant i.e. *Bacillus*, Coagulase negative *Staphylococcus, Corynebacterium, Micrococcus* or *Propionibacterium.* The remaining 77 were considered to have cultured a significant pathogen and the following were isolated: *Escherichia coli* (n=20), *Streptococcus pneumoniae* (n=14), *Salmonella spp* (n=10), *Staphylococcus aureus* (n=7), *Haemophilus influenzae* (n=4), *Campylobacter spp* (n=2), *Shigella spp* (n=1) and other pathogens (n=19). HIV-infected children were more likely to have a significant positive blood culture on admission than HIV-uninfected children (OR 2.93; $CI_{95\%}$ 1.74, 5.00; Table 1).

3.6 Incidence rates of acute gastroenteritis

The incidence of acute gastroenteritis is shown in Table 2, with a total incidence in the cohort of 10.13 ($CI_{95\%}$ 9.68, 10.58) episodes of acute gastroenteritis per 1000 person years. Incidence rates were also stratified by age group. The incidence was highest in those aged between 6 weeks and 6 months, with a slightly lower incidence in those aged between 6 and 12 months compared to the first six months of life. Incidence decreased with increasing age and was lowest in those between 2 and 5 years of age. There was no difference in the incidence rate in the placebo group (10.03; $CI_{95\%}$ 9.40, 1.07 per 1000 person years) compared to those receiving the PCV (10.18; $CI_{95\%}$ 9.56, 10.84; Incidence rate ratio (IRR) 0.98; $CI_{95\%}$ 0.90, 1.08).

Table 2: *Incidence rates per 1000 person years (95% confidence interval) of acute gastroenteritis episodes among children under 5 years of age, stratified by age group.*

Age group	Number of acute GE episodes	Total person years in age interval	Incidence rate ($CI_{95\%}$) (per 1000 person years)
6 weeks - 6 months	609	14840	41.04 (37.90, 44.35)
6 - 11.9 months	635	19782	32.10 (29.69, 34.65)
12 - 23.9 months	473	39498	11.97 (10.93, 13.10)
24 – 59.9 months	232	118334	1.96 (1.72, 2.23)
Total	1949	192454	10.13 (9.68, 10.58)

Incidence risk of acute gastroenteritis stratified by HIV infection status is shown in Table 3. These results are calculated per 1000 persons over the age interval, not per 1000 person years, and are thus not comparable across age categories. The total incidence of acute gastroenteritis over the five year study period was 5.42 times ($CI_{95\%}$ 4.89, 6.01) higher in those infected with HIV compared to that in HIV-uninfected children. The incidence was higher in the HIV-infected compared to the HIV-uninfected children across all age groups. Over the five year follow up period the excess occurrence of acute gastroenteritis among HIV-infected children attributable to their HIV infection was 171.56 ($CI_{95\%}$ 153.09, 190.04) per 1000 persons.

Table 3: *Incidence rates per 1000 persons per time period (95% confidence interval) of acute gastroenteritis episodes among children under 5 years of age, stratified by age group and HIV infection status.*

	HIV-Infected			HIV-Uninfected				
Age group	Number of acute GE episodes	Number of persons	Incidence risk per 1000 persons per time period	Number of acute GE episodes	Number of persons	Incidence risk per 1000 persons per time period	Relative risk	Attributable risk
6 weeks- 6 months	180	2468	72.93 (62.98, 83.91)	429	37279	11.51 (10.45, 12.64)	6.33 (5.30, 7.56)	61.43 (50.72, 72.14)
6 - 11.9 months	156	2319	67.27 (57.41, 78.24)	479	37247	12.86 (11.74, 14.06)	5.23 (4.34, 6.28)	54.41 (43.79, 65.03)
12 - 23.9 months	110	2264	48.59 (40.10, 58.27)	363	37229	9.75 (8.78, 10.80)	4.98 (3.99, 6.18)	38.84 (29.70, 47.97)
24 – 59.9 months	58	2230	26.01 (19.81, 33.49)	174	37214	4.68 (4.01, 5.42)	5.56 (4.06, 7.53)	21.33 (14.61, 28.07)
Total	504	2396	210.35 (194.18, 227.23)	1445	37254	38.79 (36.85, 40.80)	5.42 (4.89, 6.01)	171.56 (153.09, 190.04)

Attributable risk = risk difference

See Appendix D for tables illustrating the estimations for the denominators.

3.7 Risk factors for mortality

There were 68 deaths among those admitted with acute gastroenteritis during the follow up period, so mortality was 3.49%. The month of the year in which the admission occurred did not have any significant effect on mortality (p=0.63 for trend), nor did the year of admission have any significant effect on mortality (p=0.27 for trend). There was no difference in mortality between those who received the pneumococcal conjugate vaccine and those that received placebo (p=0.50).

The results of a univariate analysis of risk factors associated with death are shown in Table 4. Age (in months) was significantly associated with death (p=0.004), and the odds of dying decreased by 5% ($CI_{95\%}$ 2, 8%) for each month increase in age. Age was also considered categorically (not shown), with age < 6 months (but >6 weeks) as the reference age group. Children in age groups 6-11.9 months, 12-23.9 months and 24-59.9 months were all less likely to die than those aged < 6 months (OR 0.44 $CI_{95\%}$: 0.24, 0.79; OR 0.41 $CI_{95\%}$: 0.21, 0.81; OR 0.21 $CI_{95\%}$: 0.06, 0.68 respectively). However, these confidence intervals were wide due to the small numbers in some of the cells. Age was thus re-categorised as a dichotomous variable comparing those aged 6 weeks to 6 months to those aged 6 months or older. Those aged under 6 months were 2.57 times ($CI_{95\%}$ 1.53, 4.31) more likely to die than those aged 6 months or above.

Sex, prematurity and duration of diarrhoea preceding hospitalisation were not significantly associated with death. The odds of dying were increased in those presenting with malnutrition (OR 6.55; $CI_{95\%}$ 3.68, 12.18) and HIV-infection (OR 8.32; $CI_{95\%}$ 4.52, 16.15). Compared to the reference group (those with dehydration ≤2.5%) children with >2.5% but

≤5%, were not more likely to die (OR 1.10; $CI_{95\%}$ 0.53, 2.30). However, children presenting with dehydration of >5% but ≤7.5%, and those with >7.5% dehydration were more likely to die compared to the reference group (OR 3.87; $CI_{95\%}$ 1.67, 8.94 and OR 11.08; $CI_{95\%}$ 5.98, 20.5 respectively). Once again the confidence intervals were large due to small numbers in cells after categorising the variable. Dehydration was, thus, considered as a dichotomous variable with the latter two levels of dehydration combined and considered as severely dehydrated, and the former as not severely dehydrated. The odds of dying were increased in those presenting with dehydration >5% (severe dehydration) compared to those who were less than 5% dehydrated (OR 7.31; $CI_{95\%}$ 4.24, 12.58).

Table 4: *Clinical risk factors for death among children hospitalised for acute gastroenteritis: univariate analysis.*

Risk factor	Died n=68*	Discharged n=1881*	Unadjusted odds ratio	$CI_{95\%}$	p -value
Age in months median (IQR)	5.68 (3.25 – 10.87)	9.19 (5.12 – 15.57)	0.95	0.92, 0.98	0.004
Age > 6 weeks < 6 months	36/68 (52.94)	573/1881 (30.46)	2.57	1.53, 4.31	<0.001
Male Sex (%)	36/68 (52.94)	1024/1879 (54.50)	0.94	0.56, 1.58	0.80
Prematurity	7/68 (10.29)	239/1876 (12.74)	0.79	0.30, 1.75	0.55
Duration of diarrhoea at admission (%)	3.47 (± 2.17)	3.04 (± 1.84)	1.11	0.98, 1.28	0.10
Malnutrition (%)	51/68 (75.00)	591/1881 (31.42)	6.55	3.68, 12.18	<0.001
HIV infection (%)	48/63 (76.19)	456/1642 (27.77)	8.32	4.52, 16.15	<0.001
Severe dehydration (%)	32/64 (50.00)	219/1821 (12.03)	7.31	4.24, 12.58	<0.001
Bacteraemia	13/58 (22.41)	64/797 (8.03)	3.31	1.55, 6.63	<0.001
Co-diagnosis of LRTI (%)	31/68 (45.59)	251/1881 (13.34)	5.44	3.19, 9.18	<0.001

**Denominators in each cell may be different to total number HIV-infected and –uninfected due to missing data. Denominators reflect number in whom data were available.*

A concomitant diagnosis of LRTI was significantly associated with death (OR 5.44; $CI_{95\%}$ 3.19, 9.18), and those with significant bacteraemia (excluding contaminants) also had increased odds of dying (OR 3.31; $CI_{95\%}$ 1.55, 6.63).

For the multivariate analysis variables were introduced into the logistic regression model one by one using forward selection, and improvement in the model was assessed by significant improvement in the -2 log likelihood ratio. Age and level of dehydration were considered as dichotomous variables as described above. The final multiple logistic regression model included age, presence of malnutrition, severe dehydration, HIV infection, and concomitant diagnosis of LRTI. Sex, prematurity and duration of diarrhoea were not included as these were not significantly associated with death in the univariate analysis and when included in the logistic regression model did not improve the fit of the model. Bacteraemia was not included in the model due to the number of children in which a culture was not done. The results of the multivariate analysis are shown in Table 5.

Table 5: *Clinical risk factors for death among children hospitalised for acute gastroenteritis: multivariate analysis*

Risk factor	Unadjusted OR	Adjusted OR *
Age > 6 weeks < 6 months	2.57 (1.53, 4.31)	2.11 (1.21, 3.69)
Malnutrition	6.55 (3.68, 12.18)	2.17 (1.11, 4.23)
Severe dehydration	7.31 (4.24, 12.58)	5.78 (3.23, 10.33)
HIV Infection	8.32 (4.52, 16.15)	3.99 (2.04, 7.81)
Co-diagnosis of LRTI	5.44 (3.19, 9.18)	3.57 (1.99, 6.42)

** Adjusted OR: OR after adjusting for age, malnutrition, severe dehydration, HIV infection and concomitant diagnosis of lower respiratory tract infection (LRTI).*

After adjustment for other factors included in the model, associations between the variables and the odds of dying were diminished but remained significant. Those children greater than 6 weeks to less than 6 months were 2.11 times ($CI_{95\%}$ 1.21, 3.69) more likely to die than those 6 months and older. HIV-infected children were almost four times more likely to die during the admission than HIV-uninfected children, those presenting with malnutrition were approximately twice as likely to die compared to those classified as adequately nourished. The odds of dying were 3.57 ($CI_{95\%}$ 1.99, 6.42) times greater in those with a concomitant diagnosis of LRTI and 5.78 times ($CI_{95\%}$3.23, 10.33) greater in those presenting with severe dehydration.

The possibility of effect measure modification was investigated in the logistic regression model. HIV infection was an effect modifier when investigating the association between malnutrition and mortality. When stratifying the association between malnutrition and death by HIV status, the odds ratios obtained were not homogenous with an OR of 1.77 (CI_{95} 0.86, 3.66) for those HIV-infected compared to 7.72 ($CI_{95\%}$ 2.59, 23.01) in the HIV-uninfected (Mantel-Haenszel method, test for homogeneity of ORs p=0.02). The combined OR (2.61) was also different from the crude OR (6.55). This suggests both confounding of the relationship between malnutrition and death by HIV infection as well as possible effect modification. The addition of a multiplicative interaction term (HIV status x malnutrition) into the model was also significant. A stratified logistic regression model may, therefore, have been indicated with two separate models generated, one for HIV-infected and one for HIV-uninfected children. The adjusted OR for malnutrition in the model for the HIV-infected was 1.34 ($CI_{95\%}$ 0.63, 2.89) and in the model for the HIV-uninfected the adjusted OR was 4.60 ($CI_{95\%}$ 1.48, 14.34). In the uninfected, malnutrition was a significant risk

factor for death in those with acute gastroenteritis, while this association was not significant in the HIV-infected children.

The reason for this may lie in the classification of malnutrition used. In this classification those children under 80% of expected weight were classified as under-weight for age. HIV leads to low weight for age by pathways other than malnutrition, and HIV-infected children may be misclassified as malnourished (under-weight for age or marasmic) in the absence of true malnourishment. The HIV disease process itself is accounting for the lower weight in these children. However, the stratified model for the HIV-uninfected children led to small numbers in some of the cells leading to large standard errors and wide confidence intervals. It was finally decided not to stratify and thus one logistic regression model was presented (Table 5) with HIV-infected and uninfected children included in one model.

3.8 Risk factors for prolonged hospitalisation

The median duration of hospitalisation was 2 days (IQR 1-4 days). There were 1233 (63.26%) children admitted with an acute gastroenteritis episode who had a duration of hospitalisation of 2 days or less, and 716 (36.74%) with duration of hospitalisation greater than the median of 2 days. There was no difference in the duration of hospitalisation between those receiving PCV and those receiving placebo ($p=0.94$). The median duration of hospitalisation in HIV-uninfected children admitted with acute gastroenteritis was 2 days (IQR 1-3) compared to 3 days (IQR 2-7) in the HIV-infected children with acute gastroenteritis ($p<0.001$).

The results of a univariate analysis of risk factors associated with hospitalisation greater than 2 days are shown in Table 6. Age (in months) was significantly associated with prolonged hospitalisation ($p<0.001$). Age was also considered categorically (not shown), with age < 6 months (but >6 weeks) as the reference age group. Children in age groups 6-11.9 months, 12-23.9 months and 24-59.9 months were all less likely to be hospitalised for longer than 2 days compared to those aged < 6 months (OR 0.55 $CI_{95\%}$: 0.43, 0.69; OR 0.44 $CI_{95\%}$: 0.34, 0.57; OR 0.41 $CI_{95\%}$: 0.30, 0.27 respectively). Age was re-categorised as a dichotomous variable comparing those aged less than 6 months (> 6 weeks) with the older age categories combined. Those aged less than 6 months were 2.07 times ($CI_{95\%}$ 1.69, 2.53) more likely to be hospitalised for longer than 2 days compared to those aged 6 months or older. Sex was not significantly associated with death ($p=0.31$). Those who were born at a gestational age of less than 37 weeks were at a slightly increased risk of prolonged hospitalisation (OR 1.33; $CI_{95\%}$ 1.00, 1.76, $p=0.04$).

The odds of prolonged hospitalisation were increased in those presenting with malnutrition (OR 3.78; CI95% 3.08, 4.63) and HIV-infection (OR 3.00; $CI_{95\%}$ 2.41, 3.74). Compared to the reference group, those with dehydration ≤2.5%, children with >2.5% but ≤5%, were more likely to have a prolonged hospitalisation (OR 1.68; $CI_{95\%}$ 1.34, 2.09). Children presenting with dehydration of >5% but ≤7.5%, and those with >7.5% dehydration were much more likely to be hospitalised for longer than 2 days compared to the reference group (OR 14.90; $CI_{95\%}$ 8.86, 25.08 and OR 12.28; $CI_{95\%}$ 7.80, 19.33). But once again the confidence intervals were large due to small numbers in cells after categorising the variable. Dehydration was, thus, considered as a dichotomous variable with the latter two levels of dehydration combined and considered as severely dehydrated, and the former as not severely dehydrated. The odds of a prolonged hospitalisation were increased in those

presenting with dehydration >5% (severe dehydration) compared to those who were less than 5% dehydrated (OR 11.20; $CI_{95\%}$ 7.87, 16.18). A concomitant diagnosis of LRTI was significantly associated with prolonged hospitalisation (OR 3.47; $CI_{95\%}$ 2.65, 4.55), and those with significant bacteraemia (excluding contaminants) had increased odds of a prolonged hospitalisation (OR 2.49; $CI_{95\%}$ 1.38, 4.70).

Table 6: *Clinical risk factors for prolonged hospitalisation (> 2 days) among children hospitalised for acute gastroenteritis: univariate analysis.*

Risk factor	> 2 days n=716	≤ 2 days n=1233	Unadjusted OR	95% CI	p -value
Age in months median (IQR)	7.52 (3.96, 13.03)	9.79 (5.94, 17.58)	0.98	0.97, 0.98	<0.001
Age > 6 weeks < 6 months	296/716 (41.34)	313/1233 (25.39)	2.07	1.69, 2.53	<0.001
Male Sex (%)	400/715 (55.94)	660/1232 (53.57)	1.10	0.91, 1.33	0.31
Prematurity	105/715 (14.69)	141/1229 (11.47)	1.33	1.01- 1.76	0.04
Malnutrition (%)	370/716 (51.68)	272/1233 (22.06)	3.78	3.08, 4.63	<0.0001
HIV infection (%)	294/676 (43.49)	210/1029 (20.41)	3.00	2.41, 3.74	<0.0001
Severe dehydration (%)	208/701 (29.67)	43/1184 (3.63)	11.20	7.87, 16.18	<0.0001
Co-diagnosis of LRTI (%)	176/716 (24.58)	106/1233 (8.60)	3.47	2.65, 4.55	<0.0001
Bacteraemia	61/532 (11.47)	16/323 (4.95)	2.49	1.38, 4.70	0.001

For the multivariate analysis variables were introduced into the logistic regression model one by one using forward selection, and improvement in the model was assessed by significant improvement in the -2 log likelihood ratio. Age and level of dehydration were considered as dichotomous variables.

The final multiple logistic regression model included age, presence of malnutrition, severe dehydration, HIV infection, and concomitant diagnosis of LRTI. Sex and bacteraemia were not included in the model as these variables were not significantly associated with prolonged hospitalisation in the univariate analysis and when included in the logistic regression model did not improve the fit of the model. Prematurity, although significantly associated with prolonged hospitalisation in the univariate analysis, did not significantly improve the model and was not included in the final model. The results of the multivariate analysis are shown in Table 7.

Table 7: *Clinical risk factors for prolonged hospitalisation among children hospitalised for acute gastroenteritis: multivariate analysis*

Risk factor	Unadjusted OR	Adjusted OR *
Age > 6 weeks < 6 months	2.07 (1.69, 2.53)	1.67 (1.31, 2.12)
Malnutrition	3.78 (3.08, 4.63)	2.32 (1.80, 3.00)
Severe dehydration	11.20 (7.87, 16.18)	11.58 (7.82, 17.13)
HIV Infection	3.00 (2.41, 3.74)	1.81 (1.38, 2.38)
Co-diagnosis of LRTI	3.47 (2.65, 4.55)	2.80 (2.06, 3.79)

* *Adjusted OR: OR after adjusting for age, malnutrition, severe dehydration, HIV infection and concomitant diagnosis of lower respiratory tract infection (LRTI).*

After adjustment for other factors included in the model, associations between the variables and the odds of being hospitalised for longer than 2 days are shown in Table 7. Those children greater than 6 weeks (on average) but < 6 months were 1.67 times ($CI_{95\%}$ 1.31, 2.12) more likely to have prolonged hospitalisation than those 6 months and older. HIV-infected children were almost twice as likely to have a prolonged hospitalisation as HIV-uninfected children, and those presenting with malnutrition were almost 2.32 times as

likely to have a prolonged hospitalisation compared to those classified as adequately nourished. The odds of remaining in hospital for longer than 2 days were 2.80 ($CI_{95\%}$ 2.06, 3.79) times greater in those with a concomitant diagnosis of LRTI and 11.58 times ($CI_{95\%}$ 7.82, 17.13) greater in those presenting with severe dehydration.

The possibility of effect measure modification was investigated in the logistic regression model. HIV infection was an effect modifier when investigating the association between dehydration and prolonged hospitalisation. When stratifying the association between dehydration and prolonged hospitalisation by HIV status, the odds ratios obtained were not homogenous with an OR of 4.91 ($CI_{95\%}$ 2.59, 9.3) for those HIV-infected compared to 17.42 ($CI_{95\%}$ 10.29, 29.49) in the HIV-uninfected (Mantel-Haenszel method, test for homogeneity of ORs p=0.002). The addition of a multiplicative interaction term (HIV status x dehydration) into the model was also significant. A stratified logistic regression model may, therefore, have been indicated with two separate models generated, one for HIV-infected and one for HIV-uninfected children. The adjusted OR for dehydration in the model for the HIV-infected was 5.31 ($CI_{95\%}$ 2.79, 10.09) and in the model for the HIV-uninfected the adjusted OR was 16.33 ($CI_{95\%}$ 10.03, 26.57). It was finally decided not to stratify due to the large standard errors and confidence intervals in this model, and thus one logistic regression model was presented (Table 7) with HIV-infected and uninfected children included in one model.

4. DISCUSSION

The aim of this project was to determine the burden of disease caused by severe acute gastroenteritis and the influence of age and HIV infection status on incidence rates, as well as to identify risk factors associated with death and prolonged hospitalisation in children admitted with acute gastroenteritis. Acute gastroenteritis was identified as an important cause of admission to the CHBH with 21% of the total admissions over the study period due totally or partly to acute gastroenteritis. HIV infection remains a major challenge to clinicians with just over 25% of children admitted with acute gastroenteritis being identified as HIV-infected despite only an estimated 6.47% of the enrolled cohort being HIV-infected. Incidence of severe acute gastroenteritis was highest in the under 6 month age group, with almost 90% of admissions occurring in those under 2 years of age. The incidence rate was five times greater in HIV-infected children compared to those children who were HIV-uninfected. Those presenting with malnutrition, severe dehydration, HIV infection and a concomitant diagnosis of LRTI were more likely to die during the hospitalisation, and more likely to be hospitalised for longer than two days compared to those children who did not have these risk factors,. However, in considering the findings of this study there are several limitations to consider.

The original study was primarily a vaccine efficacy trial with invasive pneumococcal disease and pneumonia as the measured outcomes, and the data collected was not done with the present study objectives in mind. There are some risk factors or potential confounders which should have been considered for inclusion in the analysis, yet were not measured and could not be adjusted for in the multivariate analysis. Information on maternal education and household income or socioeconomic status was not collected. Non breastfeeding has been identified risk factor for death from acute gastroenteritis (39) yet a

feeding history was not available in the dataset and could not be investigated as a potential risk factor for death or prolonged hospitalisation, or adjusted for in the logistic regression model. In an urban area with high HIV prevalence breastfeeding is generally discouraged in HIV-infected mothers, and there is a possibility that these infants are at greater risk of diarrhoeal disease especially in the first few months of life.

Low birth weight has been associated with hospitalisation for acute gastroenteritis (34, 38), yet birth weight was only available in a very small proportion (169/1949, 8.7%) of the participants and, thus, this risk factor could not be adjusted for in the multivariate analysis. Gestational age was available on all participants and was used as a proxy for birth weight. Birth weight is correlated with gestational age, and those infants born prematurely tend to have a lower birth weight. However, estimation of gestational age may be inaccurate if the date of the last menstruation is not known, early estimation of gestational age by sonar is not done and gestation is only determined late in the pregnancy. Birth weight is a more accurate and verifiable measure as it is measured at birth in all children and the measurement tool (a baby scale) can be validated. So although gestational age at birth was available, birth weight would have been preferable.

More detailed data on duration of vomiting and maximum number of episodes of diarrhoea and vomiting in a 24-hour period was needed in order to calculate a severity score. A score was not applied in this analysis to grade the severity of episodes, as outlined by Vesikari (35), as some pertinent data was not available.

The diagnosis of malnutrition could be made from weight-for-age criteria in the majority of patients (97%) and diagnosis of marasmus or kwashiorkor by a hospital physician was

used if no weight-for-age measure was available. Physician diagnosis is more subjective as there may be differences in the way hospital physicians record a diagnosis of malnutrition. Weight was measured on admission to the ward, and was not adjusted for the level of dehydration present. Acute gastroenteritis presents with dehydration which can cause acute weight loss. Some of the participants may have been misclassified as having malnutrition, as the corrected weight (after rehydration) may have been slightly higher, and may no longer have met the criteria (< 2 SD weight-for-age) for malnutrition. There was also the possibility of falsely classifying ex-premature infants as being malnourished when using their chronological age. This may have overestimated the number of participants classified as having malnutrition on admission, and thus overestimated the true prevalence of malnutrition. However, this is likely to be non-differential misclassification and would cause any estimated association to be biased towards the null. The percentage dehydration on admission needed to be considered, and the admission weight corrected in order to minimise misclassification. The corrected age in ex-premature infants should have been used in order to classify their nutritional status more accurately. Stunting may be considered as a more accurate measure.

HIV results were not available for any of the participants in the cohort who were not hospitalised, and an estimated HIV prevalence was used based on certain assumptions on maternal HIV prevalence and transmission of HIV. These assumptions may lead to an inaccurate estimate of the true incidence of acute gastroenteritis based on HIV infection status. For incidence calculations, those with an unknown HIV result were considered to be HIV-uninfected. There was thus a risk of misclassification as some of these may actually have been HIV-infected. However, any misclassification of children as HIV-uninfected who were truly HIV-infected would have led to an underestimation of the true incidence of

acute gastroenteritis in the HIV-infected cohort. HIV results were also not available in all hospitalised participants with acute gastroenteritis (missing in 12.52%), and excluding those without a known result in the comparative analysis may have lead to incorrect measures of association between risk factors and outcomes.

Although rotavirus infection did not affect HIV viral load or CD4 counts in a Malawian study (50), the numbers in the study were small. With the data available in this analysis, the effect of diarrhoea on CD4 counts and viral loads could not be investigated.

There were no stool samples collected on admission and so no stool identification of pathogens was possible. As a result the true proportion of severe acute gastroenteritis caused by rotavirus could not be determined. Inferences can be made based on data from surveillance programmes studies, where rotavirus was the most important cause of severe acute gastroenteritis accounting for approximately 40% of hospitalisations for diarrhoea in children less than 5 years (12). Results from Dr George Mukhari hospital in Gauteng found 24% of patients presenting with diarrhoea to be rotavirus positive, but included both in-patients and out-patients. Rotavirus gastroenteritis was more frequently detected among in-patients (personal communication Dr Seheri, Medunsa).

There were also limitations related to the original study design. There was no active follow up of participants, only passive surveillance of hospitalisations of study participants. Participants may have moved from the area or died at home, and thus no longer be contributing to the total follow up time, yet as this information was not available, it was assumed that these participants had contributed the full 5 years of follow up time. This would have led to underestimation of incidence rates as the denominator would be inflated.

In a longitudinal birth cohort study conducted in Soweto, Johannesburg (Birth to Twenty), where children were followed up for more than 12 years, an average attrition rate of less than 3% per annum was found with most attrition occurring in the first two years of the study (51).

Although CHBH is the referral hospital for all local clinics in Soweto, there is a chance that some participants may have consulted a private practitioner and had an admission at a private hospital. There is also the possibility that those with very severe acute gastroenteritis may have died in the community before arriving at the hospital. These cases would not have been identified as an episode of acute gastroenteritis and, therefore, not included in the numerator in incidence calculations but would have contributed to total person time. This would also lead to an underestimation of the number of admissions for severe acute gastroenteritis and the incidence rates.

Deaths occurring at home after discharge would also not have been recorded as such, but as having been discharged. These deaths as well as those dying in the community prior to hospitalisation would lead to underestimation of mortality rates. Incidence of acute gastroenteritis varies with geographical area so these results may not be generalisable to other areas in South Africa.

Despite these limitations the results of this secondary data analysis are consistent with findings from previous studies (13, 14, 19, 22, 23, 39). This study also provides unique information on disease burden estimates in HIV-infected children. The burden of disease due to severe acute gastroenteritis is greatest in young children, with incidence decreasing with increasing age. Eighty-nine percent of admissions for acute gastroenteritis occurred in

children less than 2 years of age, with 31% in those less than 6 months. This is similar to studies where rotavirus infection was shown to be most common in children under 2 years (13). Steele *et al* also showed the < 6 months age group to have a significant proportion of disease (14).

In children over 2 years of age, the incidence falls dramatically and interventions should be focused in those under 2 years. Disease burden is high in those under 6 months of age, and these children had increased odds of dying and having a prolonged hospitalisation compared to older children, which confirms the need for early introduction of a rotavirus vaccine, and early completion of vaccination course in developing countries. All the infants in this study were on average 6 weeks old so disease in neonates and preterm infants could not be investigated. Incidence calculations, therefore, only included those aged six weeks and upwards and we would have missed any admissions occurring prior to study enrolment. The incidence rate in the under six month age group may have been an underestimation if many hospitalisations for acute gastroenteritis occurred in the first six weeks of life. Admission criteria in the hospital may also have influenced the incidence calculated in those under six months of age. Children under six months may be selectively admitted by the admitting doctor when compared to an older child with a similar clinical picture and severity of diarrhoea. This might lead to inflation of the number of admissions in children under six months, and overestimate the incidence in this age group.

The total burden of acute gastroenteritis (including mild and moderate acute gastroenteritis) could not be determined with the current study, as data were only available on those hospitalised with acute gastroenteritis. Less severe cases of acute gastroenteritis will present and be treated at local clinics or general practitioners, may not present to a

health care facility and just be treated at home. The economic burden of acute gastroenteritis extends beyond the costs associated with hospitalisation for severe acute gastroenteritis, and includes costs at primary health care level as well as the economic implications of parents who need to take time off work to look after their ill children.

HIV infection remains a concern and is a frequent underlying illness, with just over a quarter of children admitted with acute gastroenteritis found to be infected with HIV. The percentage admitted in the general wards that were HIV-infected approached 45%. In a previous study conducted at CHBH, prevalence of HIV among all hospitalised children, not just those with acute gastroenteritis was 26.2%, with the same assumption that those with unknown status were uninfected (19). A similar study of diarrhoeal disease done at CHBH in 1996-1997 showed that 17.6% of children admitted for gastroenteritis were classified as HIV-infected, which is much lower than the proportion in our study and likely reflects the rising prevalence of HIV among attendees at antenatal clinics in the time between these studies and the greater proportion of children born that were HIV-infected. The Johnson *et al* study was conducted in the late 1990's and the HIV infection rate was lower at that time (22). HIV prevalence at antenatal clinics was 22.8% in 1998 compared to 30.2 % in 2005 (45). HIV infection among children has been demonstrated to have increased the number of admissions in HIV-infected children (52).

Although our data includes acute gastroenteritis from any cause, there does appear to be a peak in acute gastroenteritis admissions in March – May in 1999 to 2001. This is the period when most of the cohort was aged < 2 years and may reflect the increase due to rotavirus infections in the winter months. Unfortunately stool samples were not taken on any of the children and a definitive diagnosis of rotavirus disease could not be confirmed.

This would have enabled us to link the peak in acute gastroenteritis admissions to rotavirus infection. Data on seasonality from Dr George Mukhari Hospital between 2005 and 2008 (Appendix F) shows increased rotavirus isolation (as a percentage of total diarrhoea) from March to August with a peak in May of each year.

Concomitant diagnoses included HIV infection, malnutrition and lower respiratory tract infection which is in line with findings from other studies where infectious diseases and malnutrition have been identified as the most common reasons for admission in HIV-infected children in developing countries (19, 53, 54). HIV-infected children were almost five times more likely than HIV-uninfected children to have multiple admissions for acute gastroenteritis. This once again highlights the extra burden of disease created by the HIV epidemic in this country, with multiple admissions and multiple illnesses diagnosed at each admission. Concurrent blood cultures were only submitted in just over 40% of those admitted for acute gastroenteritis. A high number of blood cultures grew suspected contaminants (113/190 [59.5%]). Concurrent significant bacteraemia was found in 9% of those in whom a blood culture was done. Bacteraemia has been found previously to be a significant risk factor for death (23). In our study the odds of dying and having a prolonged hospitalisation were increased in those with significant bacteraemia in the univariate analysis but this risk factor was not included in the multivariate analysis as many children had not had a blood culture performed. The presentation of invasive pneumococcal disease as severe gastroenteritis has been previously described and fourteen patients were found to have a blood culture positive for *Streptococcus pneumoniae (*55).

The overall mortality rate was 3.49% among those admitted with acute gastroenteritis, which is lower than that found in a Natal study in 2001 where paediatric diarrhoeal

admissions had an inpatient mortality of 11% (23). However, this study included both acute and persistent cases of diarrhoea. These differing mortality rates may also reflect HIV infection prevalence, with prevalence in Natal being higher than in Gauteng, accounting for the increased mortality. HIV prevalence among antenatal clinic attendees in 2001 was estimated at 33.5% in KwaZulu Natal compared to 29.8% in Gauteng (56). Mortality was only 2.2% in the acute gastroenteritis study from CHBH done in 1997 (22). Mortality rates may have risen from 1997 due to the increasing prevalence of HIV in Gauteng, accounting for increased mortality compared to Johnson et *al*'s study at CHBH (22). In my analysis, after adjustment, those who were HIV-infected were almost four times more likely to die than those who were HIV-uninfected, which is slightly higher than that found in the Natal gastroenteritis study (23).

In children hospitalised for acute gastroenteritis there are likely to be many mediators and confounders in the association between HIV infection and mortality. Some of these have been accounted for in the analysis including malnutrition and infectious co-morbidities. But HIV infection in itself affects mortality and increased mortality occurs in HIV infected children irrespective of the admission diagnosis. Higher mortality in HIV infected children may be due to underlying HIV disease and concomitant infections. Acute gastroenteritis may not be the sole contributor to death as other aggravating factors may be present in these patients. HIV infection was also an important risk factor in predicting prolonged hospitalisation in children admitted with acute gastroenteritis and HIV-infected children were almost twice as likely to have a hospital stay of longer than 2 days.

Prevention and treatment of HIV infection in children is an important way to decrease mortality and duration of hospitalisation. The rollout of ART is likely to have a profound

effect on mortality. ART suppresses HIV viral replication, allowing the immune system to reconstitute and to reverse immunodeficiency in children. This will lead to a decrease in paediatric hospitalisations and a decrease in morbidity and mortality in general (57). There is need for earlier diagnosis and treatment of HIV-infected children, as well as effective prevention of mother-to-child transmission of HIV infection.

Malnutrition was identified as a significant risk factor for both mortality and prolonged hospitalisation as has been found in other studies (22, 39). The relationship between HIV and malnutrition is not a simple one. Effect measure modification was demonstrated between HIV status and malnutrition; even though data were presented without stratifying by HIV status. The aetiology of malnutrition is multifactorial and there is a complicated relationship between HIV and malnutrition making it difficult to determine independent contributions of HIV infection and malnutrition on mortality (23). There is an adverse effect of infections on the nutritional status as well as the greater susceptibility to infection of malnourished children, and the effects of malnutrition and infection combined are greater than the sum of the two. Nutritional deficiencies have effects on both humoral and cell-mediated immune function (58), and the interaction between HIV-infection and malnutrition is, therefore, also likely to be synergistic.

HIV/AIDS is associated with both biological and social factors that may affect a child's ability to consume and utilise food. Poor nutritional status and weight loss, common in HIV-infected children, is an important cause of morbidity and mortality in these children (59). In children < 60 months mortality was associated with decreasing weight-for-age Z score. About half of the children in a Zambian study of HIV-infected children on ART were more than 2 standard deviations below their expected weight for age, and this was

associated with higher mortality rates (60). Children receiving ART showed significant improvement in weight gain, as well as an increase in absolute CD4 cell count. A study from Natal, investigating a cohort of children receiving ART, showed that 96.6% had an increase in CD4% from baseline, and 73.8% had a significant increase in weight-for-age Z score after the first month following HAART (61).

Malnourished children can be identified and can thus be targeted for early intervention, for example, initiation of antibiotics and nutritional therapies. Treatment of HIV infection will lead to an improvement in weight and both nutritional supplements and ART are likely to decrease malnutrition and, therefore, decrease mortality and duration of hospitalisation in children admitted with acute gastroenteritis.

Severe dehydration was identified as a significant risk factor for both death and prolonged hospitalisation. Early identification of those with severe dehydration, together with early introduction of intravenous fluids is needed to prevent an adverse outcome in children admitted with acute gastroenteritis. Effect measure modification was demonstrated between HIV status and dehydration when investigating their effect on prolonged hospitalisation; even though data were presented without stratifying by HIV status. As with malnutrition there may be additional factors that need to be considered in the relationship between dehydration and prolonged hospitalisation in HIV-infected children. HIV-infected children were more likely to present with severe dehydration than uninfected children. The wellbeing of HIV-infected children relies on the mothers remaining alive and well and maternal death has been shown to have a negative impact on a child's health (62, 63). There may be delays in bringing the child to a health care facility if the mother herself is not well and there may still be feelings of stigmatisation by the mother regarding the HIV

infection status of her and her child. Delays in accessing care may lead to the child being more severely dehydrated on admission.

Concomitant infection with a lower respiratory tract infection was also a significant risk factor for both mortality and prolonged hospital stay in this analysis, which is once again in keeping with previous studies from South Africa (22, 23). Concurrent infections, especially lower respiratory tract infections, need to be identified on admission and treated appropriately with antibiotics early on to prevent the effects on mortality and prolonged hospitalisation.

5. CONCLUSION

Acute gastroenteritis is an important cause of hospitalisation, especially in children under 2 years of age and those with concomitant HIV infection. Presence of malnutrition and severe dehydration on admission and a concomitant diagnosis of HIV infection and LRTI were all significant risk factors for both mortality in children admitted with an acute gastroenteritis episode, as well as predictors of a prolonged hospital stay.

Interventions to prevent gastroenteritis and thus decrease mortality in children presenting with severe acute gastroenteritis, as well as to shorten the duration of the hospitalisation, can be targeted based on the abovementioned findings. Children with severe dehydration should be treated timeously with intravenous fluids. Underlying conditions, such as HIV infection, malnutrition and LRTI should be identified early and treated accordingly. HIV testing should be offered to all children and HIV-infected children should be referred for ART on discharge, if this has not already been introduced. Increasing access to ART plays

an important role in reducing co-morbidities and mortality. Nutritional interventions are paramount especially in HIV-infected children, where this should form an adjunct to ART. Mothers should be educated on the importance of proper nutrition in preventing infection.

Although there was no specific identification of rotavirus infection in this analysis, rotavirus is likely to be the main cause of acute gastroenteritis in this cohort, based on results of previous studies as well as the seasonal distribution of the cases. The introduction of rotavirus vaccine into the EPI in South Africa is likely to decrease the burden of acute gastroenteritis in general, both in HIV-infected and HIV-uninfected children. With the high burden of disease in those under 6 months of age, early vaccination at 6 weeks and the booster dose at 14 weeks, should allow better protection against rotavirus at an early age.

Further studies are needed to investigate the effect of the rotavirus vaccine once this is introduced at a population level. Although immunogenicity studies suggest that rotavirus vaccine is immunogenic in HIV-infected children, this needs to be confirmed in effectiveness trials. The vaccine needs to be assessed in different geographical locations as well as in situations with concomitant malnutrition and co-infection with other bacterial pathogens. Increasing ART usage in children is likely to impact the burden of acute gastroenteritis in a positive way and time series analyses will be useful in monitoring trends in acute gastroenteritis admissions at this and other hospitals in future.

APPENDICES

Appendix A: Ethics clearance certificate for original study by Drs Klugman and Madhi

APPENDIX 4C

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

COMMITTEE FOR RESEARCH ON HUMAN SUBJECTS (MEDICAL)
Ref: R14/49 Klugman

CLEARANCE CERTIFICATE	PROTOCOL NUMBER M970916
PROJECT	Double blind, randomized trial of nonavalent pneumococcal conjugate vaccine to reduce the incidence of invasive pneumococcal disease and pneumonia requiring hospitalization in infants
INVESTIGATORS	Professor K Klugman
DEPARTMENT	Microbiology, Pathology/SAIMR
DATE CONSIDERED	970926

1997 -12- 01

DECISION OF THE COMMITTEE *

Approved unconditionally

DATE 971125 CHAIRMAN ..... (Professor P E Cleaton-Jones)

* Guidelines for written "informed consent" attached where applicable.

c c Supervisor: Professor K Klugman
Dept of Med Microbiology, SAIMR

Works2\Jan\C015\HumEth97.wpd\M 970916

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DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and ONE COPY returned to the Secretary at Room 10001, 10th Floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee.

DATE 2/12/97 SIGNATURE .....

PROTOCOL NO.: M 970916

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

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Appendix B: Case report form

Vaccine patient data form:

1. Adm Date: __/__/__ 2. Unit:___ 3. Ex-unit:___
4. DoBirth: __/__/__ 5. Sex: M/F 6. Hosp No.:_____
7. Surname:_____
8. Study number:_____–_____
9. Dates of doses: 1. __/__/__ 2. __/__/__ 3. __/__/__

History:

10. Cough duration: _____ days
11. Fever: Y N 12. Fever duration:____ days
13. Refusing feeds: Y N
14. Vomiting: Y N
15. Diarrhea: Y N 16. Frequency: ____/day 17. Duration: _____days
18. Loss of weight: Y N 19. Duration: _____days
20. Night sweats: Y N
21. Seizures: Y N
22. Irritable/Excessive crying: Y N 23. Duration: __ days
24. Hospitalization in previous 2 weeks: Y N 25. Diagnoses: _____
26. > 2 admissions for pneumonia: Y N 27. Number of admissions for pneumonia: _____
28. On oral antibiotics: Y N 29. Name: _____ 30. Date started: __/__/____
31. Bactrim prophylaxis: Y N 32. date started __/__/____
33. TB contact: Y N 34. Who: _____ 35. When: _____
36. Predisposing medical cause for pneumonia: Y N
37. Diagnosis: __ 1 = Ex-prem 2. = cardiac condition 3 = RVD 4. = Chronic lung 5 = TB
37a. Neonatal History: Birth weight _____ Gestational age: _____Weeks
Ventilated: Y N

Examination details:

38. Weight: _____ Kg 39. Centile: _____th 40. Height: _____ cm 41. Centile: _____ th
42. Head: ____ cm 43. Centile: ____th
44. Temp: ____ (thermoscan) 45. Temp: ____ axillary
46. Resp rate: ____ /min 47. Sats: ____ % (room air) 48. Sats. ____ % (on 02)
49. Pulse rate: ____ /min
50. Kwash: Y N
51. Cyanosis: Y N
52. Clubbing: Y N
53. Intercostal recession: Y N
54. Subcostal retractions: Y N
55. Crepitations: Y N
56. Wheezing: Y N
57. Stridor: Y N
58. Bronchial breathing: Y N
59. Oral candidiasis: Y N
60. Perineal candidiasis: Y N
61. Ch Discharging ears Y N
62. Ch sinusitis: Y N
63. Acute Otitis media Y N
64. Hepar (exclude hyperinflation): Y N
65. Spleno: Y N
66. LN: Y N
67. Dermatitis: Y N
68. CDC HIV class: N__ A____ B____ C____
69. Neck stiffness: Y N
70. Bulging fontanelle: Y N
71. **GCS**:___ {E:__ M:__ V:__}
72. % dehydration:____%
73. Other:_____

Rosside 31106

74.Outcome:____ 1= Discharged 2= Demised 3=RHT 75.Date of Dish: ___/___/___
76.ICU admission:___ 1=no 2=yes 3= not considered re:HIV 4= no bed 5= in 36
Disch diag:771. _____ **78.** 2._____ **79.** 3._____

Result sheet:
FBC: 78.WCC:____ 79. Hb:____ 80.MCV:____ 81.Plt:____ 82. N:_____ 83.L:_____
84.M:____ 85. E:___
86.CRP:____
87.LDH:____

88.HIV ELISA:___ 1=pos 2= neg 3= not done
89.HIV PCR: ____ 1=pos 2=neg 3=not done

90.Blood cultures: ____ 1= Neg 2= Pneumococcus 3= Hib 4= S aureus 5= Not done 6=other _____

CSF results
Results:91. N:___ 92.L:___ 93.RBC:___ 94. TP:___ 95.Gluc:___ 96. Cl:___
97.LDH:___
98.G-Stain:_____ 1.GPC 2.GNB 3:GNDC 4.Other_____ 5.Neg
99.Latex:____ 1.Pn 2.Hib 3.NMen 4.Neg 5. Not done
100.Culture:___ 1.Pn 2.Hib 3.NMen 4. Neg 5.Not done

Other fluids:101.____ 1.Pleural 2.Other____
Results:102. N:___ 103. L:___ 104.RBC:___ 105. TP:___ 106. Gluc:___ 107. Cl:___
108.LDH:___
109.G-Stain:_____ 1.GPC 2.GNB 3:GNDC 4.Other_____ 5.Neg
110.Culture:___ 1.Pn 2.Hib 3.NMen 4. Neg 5.Not done

111.PPD:_____mm
112.Gastric washings:___ 1= 1 done 2= 2 done 3=3 done 4=> 3 done 5=not done
113.Ao Results:____ 1= neg 2= pos 3= not done
114.Bactec Result:___ 1=neg 2=pos 3=not done

115.Nasopharyngeal aspirates:___ 1= done 2=not done
116.Result:____ 1=Neg 2= RSV 3= Inf A 4= Inf B 5= Para1-3 6=Adeno
117. PCP: Y N
118. PCP results: 1 = Positive 2 = Negative 3 Not done

CXR:
Parenchymal changes: **117.**1.____ **118.** 2.____
1. Patchy infiltrate 2. Interstitial infiltrate 3. Dense non-lobar 4. Lobar/multi
5. Poor quality 6. Normal 7. Not done
Secondary: **119.**1.____ **120.** 2. ___
1. Effusion 2. Cavitation 3. Atelectasis 4. Hilar LN 5. Hyperinflation

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Appendix C: Excerpt from International Disease Classification Code Book, Department of Paediatrics, Baragwanath Hospital. Based on ninth revision of WHO codes edition. 1990.

003.0	Salmonella gastroenteritis
003.1	Salmonella septicaemia
004	Shigella gastroenteritis
008.0	Enteropathic E coli gastroenteritis
008.5	Bacterial gastroenteritis
008.8	Viral gastroenteritis
009.3	Presumed infectious gastroenteritis
466.1	Bronchiolitis
480	Viral pneumonia
481	Lobar pneumonia
482	Bacterial pneumonia
485	Bronchopneumonia
485.0	Bronchopneumonia
260	Kwashiorkor
261	Marasmus
262	Marasmic Kwashiorkor

Appendix D – Denominator estimations for cumulative incidence calculations stratified by HIV status.

HIV-Infected – assuming a prevalence of 6.47% at enrolment

Age interval	Total alive at beginning of age interval	Number of deaths in age interval	Total alive at end of the age interval	Average number alive during age interval
6 weeks- 5.9 months	2579	223	2356	2468
6 - 11.9 months	2356	74	2282	2319
12 - 23.9 months	2282	36	2246	2264
24 – 59.9 months	2246	33	2213	2230
Total	2579	366	2213	2396

HIV-Uninfected

Age interval	Total alive at beginning of age interval	Number of deaths in age interval	Total alive at end of the age interval	Average number alive during age interval
6 weeks- 5.9 months	37300	43	37257	37279
6 - 11.9 months	37257	20	37237	37247
12 - 23.9 months	37237	17	37220	37229
24 – 59.9 months	37220	12	37208	37214
Total	37300	92	37208	37254

Appendix E: Ethics certificate for current secondary data analysis.

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UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Groome

CLEARANCE CERTIFICATE	**PROTOCOL NUMBER M081142**
PROJECT	The Burden of Severe Acute Gastroenteritis and Risk Factors Associated with Poor Outcome in a Cohort of Sowetan Children Under Five Years of Age
INVESTIGATORS	Dr MJ Groome
DEPARTMENT	School of Public Health
DATE CONSIDERED	08.11.28
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 08.11.28 **CHAIRPERSON** .....
(Professor P E Cleaton Jones)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Prof SA Madhi

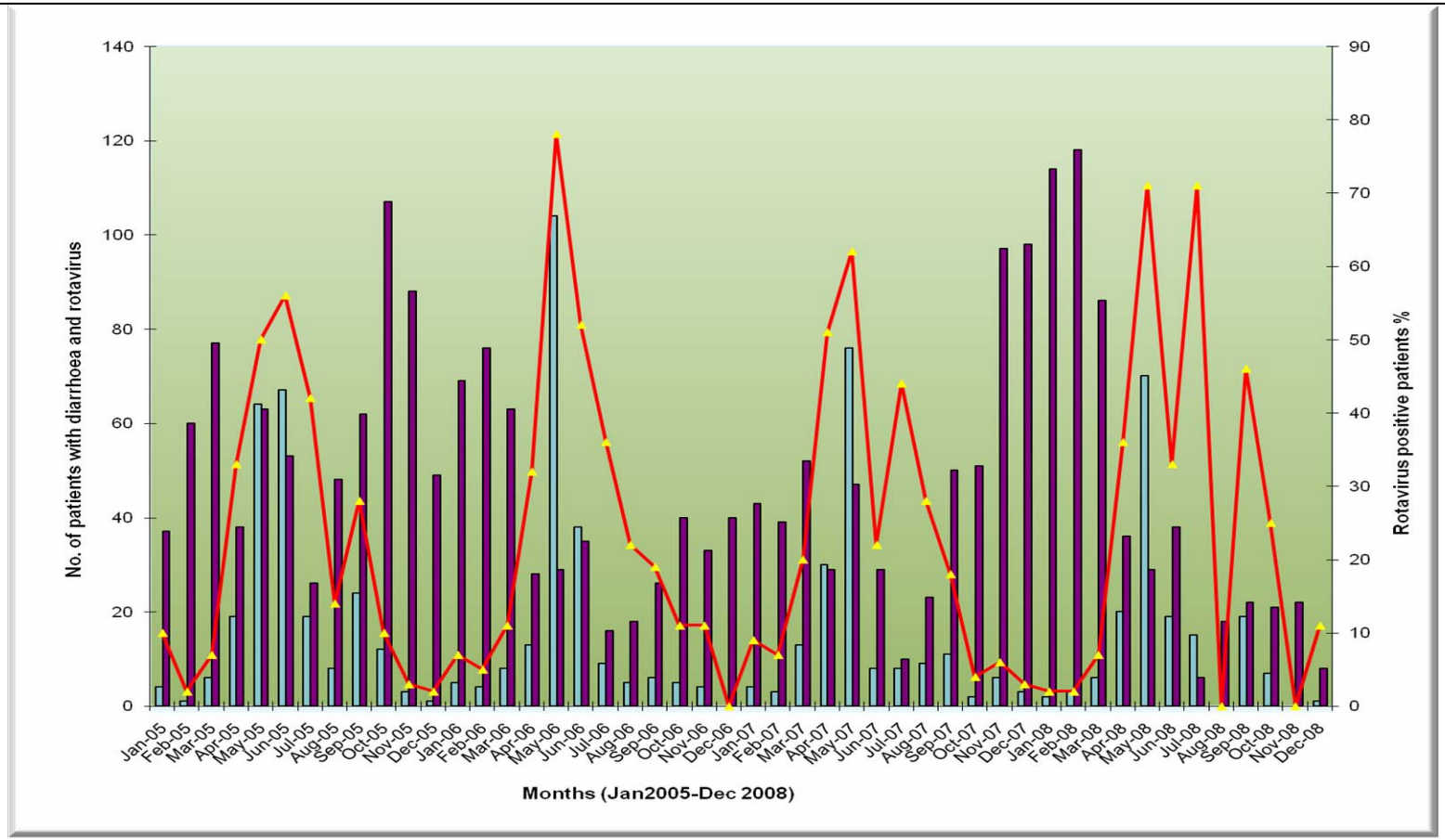
DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...

.....

Appendix F: Seasonality of rotavirus diarrhoea at Dr George Mukhari Hospital 2005 - 2008



Purple bar – number of patients with non-rotavirus diarrhoea

Blue bar – number of patients with rotavirus

Line plot – percentage of patients that were rotavirus positive

Data provided by Dr LM Seheri – MRC Diarrhoeal Pathogens Research Unit, University of Limpopo, Medunsa Campus, Department of Virology, South Africa. Presented at Vaccinology 2009, Hermanus, South Africa.

REFERENCES

1. Bryce J, Boschi-Pinto C, Shibuya K, Black RE. WHO estimates of the causes of death in children. Lancet 2005;365(9465):1147-52.

2. Kosek M, Bern C, Guerrant RL. The global burden of diarrhoeal disease, as estimated from studies published between 1992 and 2000. Bull World Health Organ 2003;81(3):197-204.

3. Moore SR, Lima AA, Conaway MR, Schorling JB, Soares AM, Guerrant RL. Early childhood diarrhoea and helminthiases associate with long-term linear growth faltering. Int J Epidemiol 2001;30(6):1457-64.

4. Niehaus MD, Moore SR, Patrick PD *et al*. Early childhood diarrhea is associated with diminished cognitive function 4 to 7 years later in children in a northeast Brazilian shantytown. Am J Trop Med Hyg 2002;66(5):590-3.

5. Bradshaw D, Groenewald P, Laubscher R *et al*. Initial burden of disease estimates for South Africa, 2000. Cape Town: South African Medical Research Council, 2003. <www.mrc.ac.za/bod/reports.htm> [Accessed 30 July 2009].

6. Cunliffe N, Nakagomi O. Introduction of rotavirus vaccines in developing countries: remaining challenges. Ann Trop Paediatr 2007;27(3):157-67.

7. Cunliffe NA, Kilgore PE, Bresee JS *et al*. Epidemiology of rotavirus diarrhoea in Africa: a review to assess the need for rotavirus immunization. Bull World Health Organ 1998;76(5):525-37.

8. Reither K, Ignatius R, Weitzel T *et al*. Acute childhood diarrhoea in northern Ghana: epidemiological, clinical and microbiological characteristics. BMC Infect Dis 2007;7:104.

9. Parashar UD, Burton A, Lanata C, *et al*. Global mortality associated with rotavirus disease among children in 2004. J Infect Dis 2009;200 Suppl 1:S9-S15.

10. Parashar UD, Hummelman EG, Bresee JS, Miller MA, Glass RI. Global illness and deaths caused by rotavirus disease in children. Emerg Infect Dis 2003; 9(5):565-72.

11. Parashar UD, Gibson CJ, Bresse JS, Glass RI. Rotavirus and severe childhood diarrhea. Emerg Infect Dis 2006;12(2):304-6.

12. World Health Organisization. Global networks for surveillance of rotavirus gastroenteritis, 2001-2008. Weekly epidemiological record 2008;47:421-8.

13. Bresee JS, Glass RI, Ivanoff B, Gentsch JR. Current status and future priorities for rotavirus vaccine development, evaluation and implementation in developing countries. Vaccine 1999;17(18):2207-22.

14. Steele AD, Alexander JJ, Hay IT. Rotavirus-associated gastroenteritis in black infants in South Africa. J Clin Microbiol 1986;23(5):992-4.

15. Steele AD, Peenze I, de Beer MC *et al*. Anticipating rotavirus vaccines: epidemiology and surveillance of rotavirus in South Africa. Vaccine 2003; 21(5-6):354-60.

16. Kavaliotis I, Papaevangelou V, Aggelakou V *et al*. ROTASCORE Study: epidemiological observational study of acute gastroenteritis with or without rotavirus in Greek children younger than 5 years old. Eur J Pediatr 2008; 167(6):707-8.

17. De Villiers FPR, Sawyerr TN, de Villiers GK. The incidence and clinical presentation of infantile rotavirus diarrhoea in Sierra Leone. S Afr Med J 2009; 99(4):249-52.

18. Yeung S, Wilkinson D, Escott S, Gilks CF. Paediatric HIV infection in a rural South African district hospital. J Trop Pediatr 2000;46(2):107-10.

19. Meyers TM, Pettifor JM, Gray GE, Crewe-Brown H, Galpin JS. Pediatric admissions with human immunodeficiency virus infection at a regional hospital in Soweto, South Africa. J Trop Pediatr 2000;46(4):224-30.

20. Thea DM, St Louis ME, Atido U *et al*. A prospective study of diarrhea and HIV-1 infection among 429 Zairian infants. N Engl J Med 1993;329(23):1696-702.

21. Pavia AT, Long EG, Ryder RW *et al*. Diarrhea among African children born to human immunodeficiency virus 1-infected mothers: clinical, microbiologic and epidemiologic features. Pediatr Infect Dis J 1992;11(12):996-1003.

22. Johnson S, Hendson W, Crewe-Brown H *et al*. Effect of human immunodeficiency virus infection on episodes of diarrhea among children in South Africa. Pediatr Infect Dis J 2000;19(10):972-9.

23. Chhagan MK, Kauchali S. Comorbidities and mortality among children hospitalized with diarrheal disease in an area of high prevalence of human immunodeficiency virus infection. Pediatr Infect Dis J 2006;25(4):333-8.

24. Liste MB, Natera I, Suarez JA, Pujol FH, Liprandi F, Ludert JE. Enteric virus infections and diarrhea in healthy and human immunodeficiency virus-infected children. J Clin Microbiol 2000;38(8):2873-7.

25. Cunliffe NA, Gondwe JS, Kirkwood CD *et al*. Effect of concomitant HIV infection on presentation and outcome of rotavirus gastroenteritis in Malawian children. Lancet 2001;358(9281):550-5.

26. Villa S, Guiscafre H, Martinez H, Munoz O, Gutierrez G. Seasonal diarrhoeal mortality among Mexican children. Bull World Health Organ 1999; 77(5):375-80.

27. de Zoysa I, Feachem RG. Interventions for the control of diarrhoeal diseases among young children: rotavirus and cholera immunization. Bull World Health Organ 1985;63(3):569-83.

28. Murphy TV, Gargiullo PM, Massoudi MS *et al*. Intussusception among infants given an oral rotavirus vaccine. N Engl J Med 2001;344(8):564-72.

29. Ruiz-Palacios GM, Perez-Schael I, Velazquez FR *et al*. Safety and efficacy of an attenuated vaccine against severe rotavirus gastroenteritis. N Engl J Med 2006;354(1):11-22.

30. Vesikari T, Matson DO, Dennehy P *et al*. Safety and efficacy of a pentavalent human-bovine (WC3) reassortant rotavirus vaccine. N Engl J Med 2006; 354(1):23-33.

31. McCormack PL, Keam SJ, Bernstein DI *et al*. Rotavirus vaccine RIX4414 (Rotarix): a review of its use in the prevention of rotavirus gastroenteritis. Paediatr Drugs 2009;11(1):75-88.

32. Steele A. Reactogenicity and Immunogenicity of Live Attenuated Human Rotavirus Vaccine RIX4414 in HIV+ Infants in South Africa. [Poster G1-442B]. In: 48th Interscience Conference on Antimicrobial Agents and Chemotherapy/ Infectious Diseases Society of America 46th Annual Meeting, Washington DC, October 25-28, 2008.

33. Cunliffe N, Kirsten M, Madhi SA *et al*. Efficacy of human rotavirus vaccine RIX4414 in Africa during the first year of life. [Poster P524]. In: 27th Annual Meeting of the European Society for Paediatric Infectious Diseases, Brussels, Belgium,June 9-13 2009.

34. Huppertz H, Salman N, Giaquinto C. Risk Factors for Severe Rotavirus Gastroenteritis. Pediatr Infect Dis J 2008; 27(1 Suppl):S11-9.

35. Ruuska T, Vesikari T. Rotavirus disease in Finnish children: use of numerical scores for clinical severity of diarrhoeal episodes. Scand J Infect Dis 1990; 22(3):259-67.

36. Sturkenboom M, Soriano-Gabarró M, Picelli G, Scamarcia A, Fregonese F, Cantarutti L. Incidence and outcomes of acute gastroenteritis in Italian children. Pediatr Infect Dis J 2008;27:S42-7.

37. Van Damme P, Giaquinto C, Huet F, Gothefors L, Maxwell M, Van der Wielen M. Multicenter prospective study of the burden of rotavirus acute gastroenteritis in Europe, 2004-2005: the REVEAL study. J Infect Dis 2007;195 Suppl 1:S4-S16.

38. Newman RD, Grupp-Phelan J, Shay DK, Davis RL. Perinatal risk factors for infant hospitalization with viral gastroenteritis. Pediatrics 1999;103(1):E3.

39. Teka T, Faruque AS, Fuchs GJ. Risk factors for deaths in under-age-five children attending a diarrhoea treatment centre. Acta Paediatr 1996;85(9):1070-5.

40. Mrukowicz J, Szajewska H, Vesikari T. Options for the prevention of rotavirus disease other than vaccination. J Pediatr Gastroenterol Nutr 2008;46 Suppl 2:S32-7.

41. Sethi D, Cumberland P, Hudson MJ *et al*. A study of infectious intestinal disease in England: risk factors associated with group A rotavirus in children. Epidemiol Infect 2001;126(1):63-70.

42. Glass RI, Stoll BJ, Wyatt RG, Hoshino Y, Banu H, Kapikian AZ. Observations questioning a protective role for breast-feeding in severe rotavirus diarrhea. Acta Paediatr Scand 1986;75(5):713-8.

43. Dennehy PH, Cortese MM, Begue RE *et al*. A case-control study to determine risk factors for hospitalization for rotavirus gastroenteritis in U.S. children. Pediatr Infect Dis J 2006;25(12):1123-31.

44. Ruuska T. Occurrence of acute diarrhea in atopic and nonatopic infants: the role of prolonged breast-feeding. J Pediatr Gastroenterol Nutr 1992;14(1):27-33.

45. Department of Health: Report - National HIV and Syphilis Prevalence Survey 2006 [Department of Health web site]. <www.doh.gov.za/docs/reports/2007/hiv/index.html> [Accessed 7 May 2009].

46. Klugman KP, Madhi SA, Huebner RE, Kohberger R, Mbelle N, Pierce N. A trial of a 9-valent pneumococcal conjugate vaccine in children with and those without HIV infection. N Engl J Med 2003;349(14):1341-8.

47. Madhi SA, Kuwanda L, Cutland C, Klugman KP. The impact of a 9-valent pneumococcal conjugate vaccine on the public health burden of pneumonia in HIV-infected and -uninfected children. Clin Infect Dis 2005;40(10):1511-8.

48. International Disease Classification Code Book, Department of Paediatrics, Baragwanath Hospital. Based on ninth revision of WHO codes edition. 1990.

49. No authors listed. Classification of infantile malnutrition. Lancet 1970;2(7667): 302-3.

50. Jere C, Cunliffe NA, Hoffman IF *et al*. Plasma HIV burden in Malawian children co-infected with rotavirus. AIDS 2001;15(11):1439-42.

51. Richter LM, Norris SA, De Wet T. Transition from Birth to Ten to Birth to Twenty: the South African cohort reaches 13 years of age. Paediatr Perinat Epidemiol 2004; 18(4):290-301.

52. Zwi KJ, Pettifor JM, Soderlund N. Paediatric hospital admissions at a South African urban regional hospital: the impact of HIV, 1992-1997. Ann Trop Paediatr 1999;19(2):135-42.

53. Vetter KM, Djomand G, Zadi F *et al*. Clinical spectrum of human immunodeficiency virus disease in children in a west African city. Pediatr Infect Dis J 1996; 15(5):438-42.

54. Lucas SB, Peacock CS, Hounnou A *et al*. Disease in children infected with HIV in Abidjan, Cote d'Ivoire. BMJ 1996;312(7027):335-8.

55. Madhi SA, Petersen K, Madhi A, Wasas A, Klugman KP. Impact of human immunodeficiency virus type 1 on the disease spectrum of Streptococcus pneumoniae in South African children. Pediatr Infect Dis J 2000; 19(12):1141-7.

56. Department of Health: Report - National HIV and Syphilis Prevalence Survey 2003 [Department of Health web site]. <www.doh.gov.za/docs/reports/2003/hiv/p1-23.pdf> [Accessed 1 July 2010].

57. Riordan A, Bugembe T. Update on antiretroviral therapy. Arch Dis Child 2009; 94(1):70-4.

58. Scrimshaw NS, SanGiovanni JP. Synergism of nutrition, infection, and immunity: an overview. Am J Clin Nutr 1997;66(2):464S-77S.

59. Colecraft E. HIV/AIDS: nutritional implications and impact on human development. Proc Nutr Soc 2008;67(1):109-13.

60. Bolton-Moore C, Mubiana-Mbewe M, Cantrell RA *et al*. Clinical outcomes and CD4 cell response in children receiving antiretroviral therapy at primary health care facilities in Zambia. JAMA 2007;298(16):1888-99.

61. Reddi A, Leeper SC, Grobler AC *et al*. Preliminary outcomes of a paediatric highly active antiretroviral therapy cohort from KwaZulu-Natal, South Africa. BMC Pediatr 2007;7:13.

62. Kurewa EN, Gumbo FZ, Munjoma MW *et al*. Effect of maternal HIV status on infant mortality: evidence from a 9-month follow-up of mothers and their infants in Zimbabwe. J Perinatol 2009 Aug 20. [Epub ahead of print].

63. Rollins N, Mphatswe W. From prevention of mother-to-child transmission to child survival... and back. Curr Opin HIV AIDS 2008;3(2):180-5.