

https://dspace.org/

Rotavirus vaccine and diarrhoeal morbidity in South Africa

Groome, Michelle Jennifer

2016-11-04
http://hdl.handle.net/10539/21405

Downloaded from a DSpace repository. https://dspace.org/

ROTAVIRUS VACCINE AND DIARRHOEAL MORBIDITY IN SOUTH AFRICA

Michelle Jennifer Groome

A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Doctor of Philosophy.

Johannesburg, 2016

DECLARATION

I, Michelle Jennifer Groome, declare that this thesis is my own work. It is being submitted for the degree of Doctor of Philosophy 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

10 March 2016

"Science is a way of thinking much more than it is a body of knowledge."

Carl Sagan

"In vaccines, from those people who work at the most local level to those people who develop, who invent, who create vaccines, we all have the power to change the world."

David Salisbury

ABSTRACT

Background

Vaccination against rotavirus, the leading cause of diarrhoea in children under 5 years of age, has the potential to reduce diarrhoeal morbidity and mortality. Lower vaccine efficacy and immunogenicity were observed in clinical studies of oral rotavirus vaccines in low- and middle-income countries in Africa compared to high-income countries. The impact of routine vaccine use in African countries, where almost half of the global rotavirus deaths occur, is yet to be established. In addition, factors affecting immune responses to the rotavirus vaccine warrant further investigation.

Objectives

To assess the effectiveness and public health impact of introduction of the monovalent oral rotavirus vaccine into the national immunisation programme in South Africa, a setting with a high prevalence of human immunodeficiency virus infection; and to determine the effect of maternal rotavirus-specific antibodies and abstention from breastfeeding at the time of rotavirus vaccination on immune responses to the rotavirus vaccine.

Methods

A case-control study was used to estimate vaccine effectiveness in children under 2 years of age, with comparison of rotavirus vaccination status among rotavirus-positive diarrhoeal cases to rotavirus-negative and respiratory controls, respectively. The impact of routine rotavirus vaccination on all-cause diarrhoeal hospitalisations was assessed by comparing the incidence before and after vaccine introduction among HIV-infected and HIV-uninfected children under 5 years of age. HIV-uninfected mother-infant pairs were randomised to either abstention from breastfeeding or unrestricted breastfeeding at the time of rotavirus vaccination to assess the

effect of breast milk on the immune response to the vaccine; in addition maternal rotavirus serum antibodies were measured.

Results

Two doses of rotavirus vaccine provided protection of 57% (95% CI 40–68) against hospitalisation for acute rotavirus diarrhoea. Protection extended through the first 2 years of life and the vaccine protected against different rotavirus strains. Routine vaccine introduction was temporally associated with a 34% to 57% decrease in the overall incidence of all-cause diarrhoeal hospitalisations in children under 5 years of age during 2010–2014 compared to pre-vaccination years ($p<0.001$).The greatest reductions were observed in children under 12 months of age. Reductions were maintained for 5 years post-vaccine introduction. Abstention from breastfeeding for 60 minutes before and after each rotavirus vaccine dose showed no significant improvement in infant immune responses to the vaccine. However, mothers of infants who seroconverted after the first vaccine dose had significantly lower anti-rotavirus immunoglobulin G titres at baseline than those whose infants did not seroconvert.

Conclusion

Rotavirus vaccination was an effective intervention against severe diarrhoea in South African children, preventing hospitalisations due to rotavirus while also reducing diarrhoeal hospitalisations for diarrhoea of any cause. These studies add to the growing body of evidence showing that rotavirus vaccines are reducing diarrhoeal disease in low- and middle-income countries and should form part of comprehensive diarrhoeal disease control and prevention. A change in breastfeeding practice at the time of rotavirus vaccination did not improve immune responses to the vaccine, yet maternal antibodies may play an important role. Continued research is needed to optimise the protection afforded by currently licenced vaccines and to develop novel rotavirus vaccines.

LIST OF ORIGINAL PAPERS

This thesis is based on the following papers:

I. Groome MJ, Page N, Cortese MM, Moyes J, Zar HJ, Kapongo CN, Mulligan M, Diedericks R, Cohen C, Fleming JA, Seheri M, Mphahlele J, Walaza S, Kahn K, Chhagan M, Steele AD, Parashar UD, Zell ER, Madhi SA. Effectiveness of monovalent human rotavirus vaccine against admission to hospital for acute rotavirus diarrhoea in South African children: a case-control study. *Lancet Infect Dis.* 2014;14(11):1096-104.

II. Groome MJ, Zell ER, Solomon F, Izu A, Nzenze S, Parashar UD, Shabir A. Madhi. Temporal association of rotavirus vaccine introduction and reduction in all-cause childhood diarrhoeal hospitalisations in South Africa. *Clin Infect Dis.* 2016. Forthcoming. (Accepted 28 December 2015)

III. Groome MJ, Moon SS, Velasquez D, Jones S, Koen A, van Niekerk N, Jiang B, Parashar UD, Madhi SA. Effect of breastfeeding on immunogenicity of oral live-attenuated human rotavirus vaccine: a randomized trial in HIV-uninfected infants in Soweto, South Africa. *Bull World Health Organ.* 2014;92:238-45.

IV. Moon SS, Groome MJ, Velasquez DE, Parashar UD, Jones S, Koen A, van Niekerk N, Jiang B, Madhi SA. Prevaccination rotavirus serum IgG and IgA are associated with lower immunogenicity of live, oral human rotavirus vaccine in South African infants. *Clin Infect Dis.* 2016;62(2):157-65.

The publishers have given permission for reprinting of published papers. My role in each of the publications and co-author permission are included in Appendix A.

ACKNOWLEDGEMENTS

I have been privileged to have extensive personal and professional support during the past four years as I worked on my thesis. My heartfelt thanks go to:

Chris Groome, my beloved husband – for all your encouragement and love, I could not have done this without you.

Rebecca, Shannon and Julia, my wonderful daughters – for your encouragement and love and allowing me to travel to present my work. I am proud to be your mom.

My extended family – for helping with the girls when I was travelling.

Shabir Madhi, my supervisor – for all the opportunities you have given me to further my career as a vaccinologist and researcher.

Elizabeth Zell, my supervisor – for your help with statistics and so much more. The Skype calls were invaluable.

Kathy Kahn, Wits School of Public Health – for always being available to assist with all aspects of my PhD journey.

Fellow PhD students and staff of the Wits School of Public Health – for the discussions during seminars and writing retreats, and for the support we gave each other. Busi Ngoyi and Paul Bohloko – for administrative assistance.

RMPRU staff – for their dedicated work in conducting these studies to such a high standard.
Clare Cutland – for the discussions, and your encouragement and friendship over the years.

The Soweto community for allowing your children to participate in these studies.

Co-authors on the papers included in this thesis – your contributions were pivotal to the conduct and publication of these studies.

Colleagues at the National Institute for Communicable Diseases, especially Nicola Page – for sharing your knowledge about rotavirus, and for testing all those stool samples!

Colleagues at CDC, Division of Viral Diseases – for being wonderful collaborators on these projects. Margaret Cortese – you were such a great help with the conduct and analysis of the case-control study. Umesh Parashar – for always being just an email away when I needed advice or assistance.

Colleagues at PATH – for your assistance with reports and ethics submissions. Jessica Fleming – for keeping me on track when it came to deadlines.

Esteemed rotavirus colleagues Duncan Steele, Roger Glass and Mathu Santosham – for always being willing to give of your time to support and encourage me.

My fellow ADVAC participants 2013 – for showing me the diversity of the vaccinology field. Elke Leuridan – for your friendship and encouragement. Michael Wacker – for being willing to read and comment on the final draft.

Publishers Oxford University Press, World Health Organization and Elsevier – for permission to use the papers in my thesis.

CONTENTS

DECLARATION ii
ABSTRACT iv
LIST OF ORIGINAL PAPERS vi
ACKNOWLEDGEMENTS vii
ABBREVIATIONS x
PREFACE xi
BACKGROUND 1
- INTRODUCTION 1
- BURDEN OF ROTAVIRUS DISEASE 1
- PREVENTION OF ROTAVIRUS DISEASE 3
- ROTAVIRUS VACCINES IN AFRICA AND ASIA 4
- FACTORS AFFECTING VACCINE EFFICACY IN LOW- AND MIDDLE-INCOME COUNTRIES 5
- THE IMPACT AND EFFECTIVENESS OF ROTAVIRUS VACCINES POST-LICENSURE 12
- DURATION OF PROTECTION 14
- SAFETY OF ROTAVIRUS VACCINES 14

JUSTIFICATION AND OBJECTIVES 16
METHODS 18
- SETTING 18
- STUDY DESIGN 19
- STATISTICAL CONSIDERATIONS 24
- ETHICAL CONSIDERATIONS 27

RESULTS AND DISCUSSION 28
- EFFECTIVENESS AND PUBLIC HEALTH IMPACT OF ROTAVIRUS INTRODUCTION 28
- FACTORS AFFECTING IMMUNOGENICITY OF THE ORAL ROTAVIRUS VACCINE 31
- CONSIDERATIONS WHEN ASSESSING VALIDITY OF RESULTS 38
- OTHER CONSIDERATIONS FOR THE USE OF ROTAVIRUS VACCINES 42

CONCLUSION 49
REFERENCES 50
APPENDICES 72

ABBREVIATIONS

AIDS	acquired immune deficiency syndrome
aOR	adjusted odds ratio
ART	antiretroviral therapy
CDC	Centers for Disease Control and Prevention
CHBAH	Chris Hani Baragwanath Academic Hospital
CI	confidence interval
EIA	enzyme immunoassay
EPI	Expanded Programme on Immunisation
GMT	geometric mean titre
HIV	human immunodeficiency virus
IgA	immunoglobulin A
IgG	immunoglobulin G
IPV	inactivated poliovirus vaccine
OPV	oral polio vaccine
OR	odds ratio
RCT	randomised controlled trial
RMPRU	Respiratory and Meningeal Pathogens Research Unit
PCR	polymerase chain reaction
PCV	pneumococcal conjugate vaccine
US	United States
WHO	World Health Organization

PREFACE

Ten years ago I joined the Respiratory and Meningeal Pathogens Research Unit (RMPRU) based at the Chris Hani Baragwanath Academic Hospital in Soweto and embarked on my research career. Soon after joining the unit, as a medical officer, I was involved in the Phase III clinical trial investigating the efficacy of Rotarix®, never envisioning that I would one day contribute to assessing the impact of introduction of this life-saving vaccine into the national immunization programme in my home country, South Africa.

Immersed in the research-rich environment of the RMPRU, I have grown as a researcher under the mentorship and guidance of our unit director Shabir Madhi. My work on rotavirus vaccines has given me the opportunity to interact with the global rotavirus community which has enriched my knowledge and provided me with endless support. One such opportunity was sharing thoughts and ideas with giants in the field, Umesh Parashar, Roger Glass and Duncan Steele, against the backdrop of the spectacular Victoria Falls. I have been privileged to present my work at conferences in the United States, India and here on the African continent.

Being part of the University of the Witwatersrand's School of Public Health Interdisciplinary PhD programme, under the leadership of Kathleen Kahn, provided me with broad exposure to many areas of public health. The interaction with fellow students and staff members during the past four years has made this PhD journey a fulfilling and memorable experience.

I look forward to continuing my rotavirus work, establishing new collaborations both on the African continent and further afield, as well as broadening my horizons and embracing new opportunities and challenges. To quote one of my favourite authors, Jon Krakauer, "*The joy of life comes from our encounters with new experiences, and hence there is no greater joy than to have an endlessly changing horizon, for each day to have a new and different sun.*"

Michelle Groome 2016

BACKGROUND

INTRODUCTION

With the discovery of rotavirus in 1973, the arduous journey began to find an effective rotavirus vaccine that would prevent diarrhoeal disease due to rotavirus infection and lead to decreased morbidity and mortality in young children (1). Though not without disappointments along the way, such as the withdrawal of the first licenced rotavirus vaccine, Rotashield due to concerns of its association with intussusception, vaccine initiatives culminated in the global licensure of two oral rotavirus vaccines (2). It is an exciting time for rotavirus vaccines as, following World Health Organization (WHO) recommendations, an increasing number of countries have introduced this life-saving vaccine into their national immunisation programmes (3). Eighty countries have introduced the rotavirus vaccine worldwide, as of 1 January 2016, including 32 African countries either with (26 countries) or without (6 countries) support from the GAVI Alliance (4).

BURDEN OF ROTAVIRUS DISEASE

Diarrhoeal disease has long been recognised as a major contributor to the global public health burden, with descriptions of diarrhoea dating back to ancient Greek civilisations and featuring prominently during the modern centuries (5). Children are particularly susceptible to diarrhoeal disease, which remains an leading cause of morbidity and mortality in children <5 years of age worldwide, accounting for approximately 9% of the 6.3 million deaths in this age group in 2013 (6). The burden of diarrhoeal disease is greatest in low- and middle-income countries with the highest incidence rates found among children <12 months of age (7). In South Africa, diarrhoea has been identified as one of the leading causes of death in

children and accounted for 18% of all deaths in those aged <5 years in 2009 according to official statistics, but the true burden of disease is not accurately defined (8).

Diarrhoea can be caused by a number of viral, bacterial and parasitic enteropathogens but rotavirus remains the most important cause of severe diarrhoea in infants and young children globally (9, 10). Global surveillance among children hospitalised for severe diarrhoea in 2001−2008, prior to the introduction of universal rotavirus vaccination programmes, showed that 39% (range 20%−73%) tested positive for rotavirus (11). In 2008, rotavirus-diarrhoea resulted in an estimated 453 000 deaths annually – 37% of all diarrhoeal deaths in children <5 years of age worldwide, with the greatest burden of disease in Africa and Asia (12). The incidence of rotavirus disease was shown to be similar in children in low- and high-income countries. However, children from less-developed countries were more likely to die due to poorer access to health care and comorbidities like malnutrition (10, 12-14).

The rotavirus, a genus of the *Reoviridae* family, comprises an 11-segment double-stranded RNA genome surrounded by an outer capsid, an inner capsid and an internal core. Based on the inner capsid protein (VP6), rotaviruses are classified into seven groups A–G, three of which occur in humans. Group A rotaviruses are responsible for almost all of the disease in humans and are very important from a public health point of view (15, 16). Two structural proteins in the outer capsid, VP7 (a glycosylated protein) and VP4 (a protease-cleaved protein) have been characterised and define the G and P serotypes of the virus, respectively. These differ geographically as well from one season to the next, and in South Africa regional differences in rotavirus strains were observed with the most commonly detected strains being the VP7 serotype G1 rotaviruses, followed by G4 and G2 strains, and the VP4 genotypes P[8], P[4] and P[6] (17). Rotaviruses are classified according to the G-P protein combination, for example G1P[8].

In South Africa, prior to rotavirus vaccine introduction, rotavirus was associated with approximately 25% of diarrhoeal hospitalisations, with the greatest burden of disease (75%) in children <12 months of age (17). The annual incidence of severe rotavirus diarrhoea in children <2 years of age was estimated to be 874−1076 per 100 000 and incidence was highest in children aged 3−11 months (18). Rotavirus infection occurred year round but had a clear peak in the cooler, drier autumn-winter months (17, 18). Multiple infections, with detection of rotavirus and at least one other enteropathogen, were also common (39%) (17).

PREVENTION OF ROTAVIRUS DISEASE

Interventions which have been successful against bacterial and parasitic enteropathogens causing diarrhoeal disease, for example administration of oral rehydration therapy, improvements in management of diarrhoea cases and zinc supplementation, have not had a significant impact on rotavirus disease (19). Transmission of rotavirus occurs through person-to-person contact, thus improvements in sanitation and hygiene have also had limited impact on its prevention, with similar incidence of rotavirus infection, albeit differing severity, reported in children from high-income and lower income countries (10, 14, 20). Vaccination is one of the most cost-effective health interventions available (21). Rotavirus vaccines have the potential to substantially reduce the burden of diarrhoeal disease in young children and in so doing have a tremendous public health impact on children's health, especially in Africa and Asia.

Natural infection with rotavirus was shown to offer protection against subsequent severe acute and recurrent rotavirus diarrhoea, as well as cross protection against multiple circulating strains, and this observation led to development of the currently licenced live-attenuated vaccines, in order to mimic natural infection and develop broad heterotypic (i.e.

against many strains) protective immunity (22, 23). Two oral rotavirus vaccines, Rotarix® − a monovalent human-derived vaccine (GlaxoSmithKline Biologicals, Rixensart, Belgium) and RotaTeq® − a pentavalent bovine-derived vaccine (Merck Vaccines, Whitehouse Station, NJ), are currently licenced in many countries worldwide and recommended for global use in children by the WHO (3). Pre-licensure clinical studies of these vaccines demonstrated very good protective efficacy (85-98%) against severe rotavirus disease in middle- and high-income countries in Latin America, Europe and the United States (24-26). Lower efficacy and immunogenicity have, however, been observed in clinical studies of rotavirus vaccines in low- and middle-income countries in Africa and Asia, including South Africa (27-29). These countries tend to have poorer socioeconomic conditions, high mortality from diarrhoeal disease, high rates of malnutrition, high maternal human immunodeficiency virus (HIV) prevalence and vaccine schedules with co-administration of oral polio vaccine (OPV) and rotavirus vaccine.

ROTAVIRUS VACCINES IN AFRICA AND ASIA

The African study of either two doses (given at 10 and 14 weeks of age) or three doses of the monovalent rotavirus vaccine, Rotarix® (given at 6, 10 and 14 weeks of age) reported vaccine efficacy against severe rotavirus gastroenteritis of 77% (95% confidence interval (CI) 56−88) in South Africa and 49% (95% CI 19−68) in Malawi during the first year of life (27). The pentavalent rotavirus vaccine (RotaTeq®) was evaluated in Ghana, Mali and Kenya, demonstrating a vaccine efficacy of 64% (95% CI 40−79) against severe rotavirus diarrhoea in the first year of life (28), and also in Bangladesh and Vietnam with vaccine efficacy of 51% (95% CI 13−73) (29). This is in keeping with the poorer performance observed in

lower-income countries of other live oral vaccines such as those targeting poliomyelitis, typhoid and cholera, as well as previous rotavirus vaccine candidates (30, 31).

However, vaccine efficacy does not always accurately reflect a vaccine's public health value as it does not account for background disease incidence (32). Although vaccine efficacy was lower in these countries compared to higher income countries, the high incidence of severe rotavirus disease resulted in a considerable vaccine-attributable decrease in severe rotavirus diarrhoea i.e. the number of episodes of severe diarrhoea prevented by rotavirus vaccination was greater (27). Regional differences in immunogenicity to rotavirus vaccine have also been observed, with infants in low-income countries found to have significantly lower rotavirus-specific immunoglobulin A (IgA) titres and rates of seroconversion compared to infants in high-income countries (33).

Locally manufactured oral rotavirus vaccines are available in China (Lanzou lamb rotavirus vaccine; Lanzou Institute of Biological Products), Vietnam (Rotavin-M1; POLYVAC) and India (Rotavac; Bharat Biotech International, Ltd) but the vaccines are only licenced for use within these countries and there are limited efficacy data (34-36).

FACTORS AFFECTING VACCINE EFFICACY IN LOW- AND MIDDLE-INCOME COUNTRIES

Differences in the behaviour of live oral vaccines in the digestive tracts of infants in lower income settings may have an impact on their efficacy. Immune response and efficacy of oral rotavirus vaccine are dose dependent and factors decreasing the dose of the vaccine may impact its immunogenicity and efficacy (33). Rotavirus immunity is not completely understood and there is not an established correlate of protection, but a strong correlation was found between serum rotavirus IgA titres and efficacy after rotavirus vaccination (37). This

suggests that serum rotavirus IgA titres are an important measurable predictor of protection, albeit not the only immunonological determinant of the defense mechanism protecting infants from rotavirus-associated diarrhea.

Immune responses in the infant may be decreased by conditions that lower the effective titre of vaccine delivered to the intestine such as the amount of gastric acid present in the infant's gut, and interference by high levels of rotavirus antibodies acquired transplacentally from the mother during pregnancy or during breastfeeding. Micronutrient deficiency (zinc, vitamin A), malnutrition, interfering microbiota present in the gut, enteric viral and bacterial co-infections and concomitant disease in the infant such as diarrhoea, tuberculosis, malaria or HIV infection as well as co-administration with OPV may also contribute to sub-optimal immune responses among infants in lower-income settings (30, 33). Rotavirus vaccine tends to be administered at a younger age in many lower income countries, where the rotavirus disease burden is high and infection at a young age is more common than higher income countries. The earlier administration of the vaccine is advised to prevent early rotavirus infection but the ability to induce neutralising antibodies against rotavirus is dependent on age, and immunogenicity might be reduced when vaccination occurs at a very young age (38).

Breastmilk and immune responses to rotavirus vaccines

Studies investigating the effects of breast milk on immunogencity and seroconversion to previous rotavirus vaccine candidates suggested a trend toward lower immunogenicity among breastfed infants compared to non-breastfed infants after a single vaccine dose, but this was overcome by increasing the dose and number of doses administered (39-43). Results from the clinical trials of the two currently licenced oral rotavirus vaccines have not shown reduced vaccine efficacy in breastfed infants. The human rotavirus vaccine trial showed a small

difference in immunogenicity but vaccine efficacy was equally high in breastfed and formula-fed infants in the first rotavirus season (44). Similarly, the pentavalent vaccine showed similar vaccine efficacy in infants never breastfed, sometimes breastfed and exclusively breastfed (45). However, these studies did not investigate the interval between breastfeeding and administration of the vaccine. There are no published data on rotavirus vaccine efficacy and breastfeeding from Africa or Asia.

In-vitro studies have been conducted to investigate the role of rotavirus antibodies and neutralising activity in breast milk. Breast milk with low neutralising titres did not significantly reduce the titre of vaccine virus. However, high titres of neutralising activity in breast milk resulted in a reduction of vaccine virus titres. The magnitude of the reduction was dependent on the level of neutralising activity in the breast milk (46). Theoretically, rotavirus antibodies could neutralise virus vaccine if there was breast milk in the stomach of the infant at the time of vaccination. This could decrease the effective titre of vaccine virus reaching the gut thus rendering the vaccine less immunogenic.

Rotavirus-specific IgA titres, lactoferrin levels, lactadherin and neutralising activity in breast milk vary by setting with higher titres found among Indian and South African women compared to those in the United States (47). Both breast milk rotavirus-specific antibodies and neutralising activity are highly prevalent in lower income country settings, where mothers have greater natural exposure to rotavirus infection (48, 49). These high titres present in breast milk consumed at the time of vaccination could explain, in part, the reduced vaccine efficacy in infants in these countries compared to higher income countries. Using this rationale, abstention from breastfeeding near the time of administration of an oral vaccine could theoretically improve the immunogenicity of the oral rotavirus vaccine.

Maternal antibodies and immune responses to rotavirus vaccines

The inhibitory effects of high levels of maternal antibodies on infant immune responses have been reported for live vaccines such as influenza, measles and OPV as well as non-replicating vaccines (50-53). Animal studies demonstrated that high titres of maternal antibodies have substantial effects on immune responses to rotavirus vaccines (54). Earlier studies using reassortant rotavirus vaccine candidates observed that pre-vaccination neutralising antibody titres to different rotavirus strains were negatively correlated with seroconversion, suggesting that maternal immunoglobulin G (IgG) may interfere with immune responses following vaccination (55).

Rotavirus-specific IgG crosses the placenta and a strong correlation between maternal serum and cord serum rotavirus-specific IgG titres has been demonstrated (48, 56). High titres of pre-vaccination IgG decreased the immune response and seroconversion of infants to the oral RV vaccine candidate ORV-116E. However, this effect was overcome by using a higher dose and increased number of doses. Infants with the lowest IgG titres had a more rigorous immune response (57).

There are limited data on the comparisons of maternal serum rotavirus antibody titres between low and high-income countries, with most studies limited to quantification of antibodies in breast milk. Frequent rotavirus re-infection as a result of higher viral loads in the community and greater serotype diversity in lower income countries most likely leads to the higher maternal antibody titres in women from these countries, which results in higher rotavirus-specific IgG titres in their infants. These higher titres could interfere with infant immune responses to the vaccine, especially if the vaccine is administered at a young age e.g. 6 weeks, and this could partially explain the reduced vaccine efficacy in lower income countries. However, the ways by which maternal antibodies impact infant immune responses

are complex and their influence depends on the prevalence of maternal antibodies in a specific population at a specific time as well as the vaccine schedule, number of vaccine doses administered and type, route of administration and antigenicity of the vaccine (54, 58).

HIV exposure and diarrhoeal disease

Although diarrhoeal disease was more common among HIV-infected children than HIV-uninfected children, rotavirus did not cause more frequent or more severe disease in HIV-infected children (59-62). HIV-exposed-uninfected children have been identified as having an increased risk of morbidity and mortality from diarrhoeal disease but there are limited data on rotavirus-specific diarrhoea in these children (63, 64). Rotarix® was safe, well tolerated and immunogenic in a group of HIV-positive South African infants, with no effect on their immunological condition (65). Similarly, a study in Kenya showed Rotateq® to be safe when used in HIV-infected and HIV-exposed infants (66). These studies were not powered to evaluate vaccine efficacy specifically in HIV-infected and HIV-exposed-uninfected infants.

Enteric co-infections

Concurrent infection with enteropathogens at the time of rotavirus vaccination may lead to an impaired immunological response, as was recently described in a systematic review on OPV in which concurrent diarrhoea at the time of vaccination was associated with decreased seroconversion (67, 68). Environmental enteropathy, where there is chronic exposure to enteric pathogens, leads to inflammation and structural changes in the small bowel potentially resulting in functional changes (69). This may influence the immune response to oral vaccines, but has not been specifically evaluated yet in any studies. Prenatal Vitamin A

deficiency may impair responses and decrease efficacy to rotavirus vaccine yet oral supplementation of Vitamin A together with rotavirus vaccine did not increase vaccine efficacy in piglets (70, 71). Colonisation by probiotics affects neonatal immune responses to oral rotavirus vaccine and addition of probiotics to infant formula may act to enhance the efficacy of rotavirus vaccines (72, 73).

Both OPV and rotavirus vaccines are administered orally and replicate in the gut, so the possibility of interference between these two vaccines exist. Co-administration of rotavirus vaccine with OPV did not affect immune responses to OPV but immune responses to rotavirus vaccine were generally lower when the two vaccines were co-administered (74). A reduced immune response was demonstrated following the first dose of the monovalent rotavirus vaccine in those infants who received a concomitant dose of OPV compared to those that did not. However, this effect was overcome with administration of the second rotavirus vaccine dose (75). Concomitant use of OPV and the pentavalent rotavirus vaccine showed some reduction in immune responses to the rotavirus vaccine, but the seroresponse rate was non-inferior compared to that in the group where OPV was not co-administered (76). A recent study from Bangladesh showed decreased immune responses to the monovalent rotavirus vaccine, with no significant difference between the OPV formulation used i.e. children co-administered monovalent, bivalent or trivalent OPV had similar immune responses to the rotavirus vaccine (77). Despite the lower immunogenicity, concomitant administration of rotavirus vaccine and OPV did not seem to affect vaccine efficacy in Latin America, but further data is needed from Africa and Asia to fully evaluate the impact of OPV on rotavirus vaccines (74, 78).

Epidemiology of rotavirus

Differences in the epidemiology of rotavirus and circulating serotypes of rotavirus between low- and high-income settings could lead to differences in vaccine efficacy. Rotavirus tends to occur at a younger age among children in lower income countries compared to high income countries, and up to 80% of children in poorer countries have rotavirus antibodies by 12 months of age (79). In South Africa, rotavirus shedding was detected in over 75% of infants <12 months of age hospitalised with diarrhoeal illness and 95% of hospitalised rotavirus cases occurred in children <18 months old (17). This has implications for the schedule of rotavirus vaccine administration as the vaccine needs to be given at a younger age in order to confer protection to young infants. However, immune responses and seroconversion rates were found to be lower when administered at a younger age (75).

Lower income countries with a tropical climate tend to have year round disease, some having no clear seasonal variation whereas others may have peaks in the cooler, drier months. In South Africa rotavirus infection occurred throughout the year but with increased detection during the autumn-winter months of April through September (17, 18). There is some evidence to suggest that the timing of the infant birth, and thus vaccine administration, in relation to the rotavirus season may impact vaccine efficacy. Bovine rotavirus vaccine was found to be more efficacious when administered immediately before the rotavirus season (80).

Strain type does not seem to determine severity of infection but the diversity of rotavirus strains requires rotavirus vaccines to provide good protection across a variety of strains in order to be maximally effective in preventing rotavirus disease. This includes protection against the strain(s) contained in the vaccine (homotypic strains) as well as cross-protection against strains not contained in the vaccine (heterotypic strains) (16, 81). There was greater

rotavirus strain diversity, with an increased number of unusual strains and mixed infections identified in countries in Africa and Asia which may have implications for vaccine efficacy in these countries (79, 82-84). Cross protection has, however, been observed against the common circulating stains in African infants. Vaccine efficacy against severe gastroenteritis caused by diverse circulating rotavirus stains was demonstrated in South Africa and Malawi, providing support for heterotypic protection provided by rotavirus vaccines even in lower income countries (85). Concerns remain regarding the degree of protection against fully heterotypic strains such as G2P[4], as well as duration of protection and potential waning of immunity (86, 87).

THE IMPACT AND EFFECTIVENESS OF ROTAVIRUS VACCINES POST-LICENSURE

Following the introduction of vaccines into routine immunisation programmes, it is important to monitor their impact on rotavirus-associated diarrhoeal morbidity and mortality in order to demonstrate their public health benefits. Partial vaccination, delays in vaccination, use of differing vaccine schedules, co-administration with other routine infant vaccines, cold chain disruption, changes in rotavirus epidemiology, circulation of differing rotavirus strains, waning immunity and indirect benefits may all impact on the effectiveness of rotavirus vaccine under field conditions once introduced into a national immunisation programme (88). The WHO has emphasised the need for post-licensure monitoring of the impact of rotavirus vaccine on diarrhoeal morbidity and mortality, as well as effectiveness in the setting of routine use which may differ from controlled clinical trial settings (89). This can be achieved by use of a case-control design to assess effectiveness, or by monitoring trends in diarrhoea and rotavirus disease burden through active surveillance systems or use of administrative data sources such as hospital discharge and mortality data (90).

Australia and countries in Europe and the Americas were among the first to introduce rotavirus vaccines into their national immunisation programmes and this has led to substantial decreases in diarrhoea-related hospitalisations (17-50%), diarrhoeal deaths (22-50%) and rotavirus-specific hospitalisations (49-91%) among children <5 years of age in these countries (91-102). Case-control studies have demonstrated good vaccine effectiveness in preventing rotavirus hospitalisations, with high-income countries in Europe and the United States reporting vaccine effectiveness similar to the efficacy observed in the clinical trials (103-106). Lower income countries such as Bolivia, El Salvador and Nicaragua showed good vaccine effectiveness estimates, albeit slightly lower than higher income countries (107-109).

A recent meta-analysis, assessing the strain-specific vaccine effectiveness of the monovalent and pentavalent rotavirus vaccines, found that both vaccines provide similar effectiveness against homotypic and heterotypic rotavirus strains (110). There are limited data from Africa on the impact of rotavirus vaccine, although many countries are presently conducting effectiveness studies. South Africa and Ghana have shown reductions in diarrhoea hospitalisations in children <5 years of age after introduction of rotavirus vaccine but these studies were limited to the first two years post-introduction and did not specifically assess reductions in diarrhoea hospitalisations in HIV-infected children (111, 112).

Vaccine introduction against pathogens such as *Streptococcus pneumoniae* and *Haemophilus influenzae* type b have demonstrated the prevention of disease due to these pathogens in unvaccinated, susceptible children and adults due to vaccine-induced immunity in the population, suggesting indirect protection by vaccines (113, 114). Vaccination of a proportion of the childhood population could lead to an overall decrease in transmission of the targeted pathogen in the community, and thus a decrease in disease risk among unvaccinated individuals. This has also been shown for rotavirus vaccines and many of the early introducing countries have observed indirect benefits for unvaccinated individuals, such as

older children and adults, with decreases in both diarrhoeal and rotavirus hospitalisations observed after rotavirus vaccine introduction (96, 115-117).

DURATION OF PROTECTION

Efficacy trials of the monovalent rotavirus vaccine in Europe and Latin America showed sustained protection through two years of life (26, 118). The South African efficacy trial, while not powered to assess protection against severe rotavirus diarrhoea in the second year of life, showed a lower point-estimate over two consecutive rotavirus seasons compared to during the first rotavirus season, which was particularly evident in children who had received two rather than three doses of vaccine (119). This was also shown in the clinical trials conducted in Malawi, Ghana, Mali and Kenya (28, 120). Rotavirus effectiveness studies in some low-income settings have found protection to be lower among children ≥12 months of age, suggesting the possibility of waning immunity, whereas another showed no difference in effectiveness of rotavirus vaccine between the two age groups (107, 108, 121).

SAFETY OF ROTAVIRUS VACCINES

Intussusception, the telescoping of one segment of the bowel into a more distal bowel segment, is a cause of acute intestinal obstruction in young children and is potentially life-threatening. Intussusception is uncommon and incidence rates vary by age, region, ethnicity, socioeconomic characteristics and feeding patterns (122, 123). The underlying cause of intussusception is unknown in most cases, but there has been suggestion of an association between infectious pathogens, for example adenovirus, and the development of intussusception in young children (124-126). A rhesus-human reassortant oral rotavirus

vaccine (Rotashield, Wyeth Lederle Vaccines, Philadelphia) was licenced and introduced in the United States for routine immunisation in 1998, but withdrawn nine months after its introduction as a result of its association with intussusception (127).

Currently licenced oral rotavirus vaccines were found to be safe with regards to intussusception and other severe adverse events (24, 25). Post-marketing surveillance in Mexico detected a small yet significant increase in the risk of intussusception among infants in the first week following administration of the first dose of rotavirus vaccine (Rotateq®), while no significant risk was found after the first dose among infants in Brazil. A small increased risk was seen after the second dose in these infants (128). In Australia, both Rotarix® and Rotateq® were associated with an increased risk of intussusception following the first and second doses of vaccine (129). Recent studies conducted in the United States similarly showed a small increased risk of intussusception after the first dose of vaccine for both the monovalent and pentavalent rotavirus vaccines (130, 131).

Global data thus suggests that both rotavirus vaccines are associated with a small risk of intussusception (estimated 0.8–7 cases per 100 000), though of a magnitude substantially less than that associated with Rotashield (10–20 cases per 100 000) (132). However, the benefits of reductions in hospitalisations and death far outweigh the relatively few excess cases of intussusception associated with rotavirus vaccines (128, 129) and thus national immunisation remains a valuable public health intervention, especially in high burden countries in Africa and Asia. There is limited data on rates of intussusception in low and middle-income countries and no studies have yet been completed assessing the intussusception risk following routine introduction of rotavirus vaccine.

JUSTIFICATION AND OBJECTIVES

An increasing number of African countries have introduced or are planning to introduce rotavirus vaccination into their national immunisation programmes and impact data from early adopter countries are pivotal to inform public health decisions and to provide evidence for sustaining policies for vaccine use and investments from governments or donors. As one of the first African countries to introduce rotavirus vaccine into the national immunisation programme, we were able to evaluate effectiveness and impact of this vaccine under routine use in South Africa. In particular, vaccine effectiveness needed evaluation in lower income settings with high HIV prevalence, concurrent OPV use, high rates of malnutrition and enteric co-infections, as well as with schedules different from those used in the efficacy trials.

The immunogenicity and efficacy of a two-dose schedule of Rotarix® at 6 and 14 weeks of age had not been previously studied. Persistence of protection against rotavirus diarrhoea during the second year of life may be suboptimal due to waning immunity and must be assessed post-licensure. The effect of maternal rotavirus antibodies and breastfeeding on immune responses elicited by the rotavirus vaccine in infants may account for differences in efficacy between lower and higher income countries and needed to be better understood. Even small improvements in rotavirus vaccine efficacy could lead to an increase in the number of deaths and hospitalisations prevented by the vaccine.

The overall purpose of this thesis was to assess the effectiveness and public health impact of rotavirus vaccine introduction into the Expanded Programme on Immunisation (EPI) in South Africa and determine the effect of abstention from breastfeeding near the time of rotavirus vaccination on immune responses to the vaccine.

The primary objectives were:

1. To assess the effectiveness of two doses of rotavirus vaccine against severe rotavirus gastroenteritis that required hospitalisation among South African children <2 years of age after its introduction into the EPI (Paper I).

2. To determine the impact of introduction of rotavirus vaccine into the EPI on all-cause diarrhoea hospitalisations in children <5 years of age in Soweto, stratified by age group and HIV infection status (Paper II).

3. To determine whether abstention from breastfeeding for at least 60 minutes pre- and post-rotavirus vaccination affected the serum rotavirus IgA immune response induced by rotavirus vaccine in the infants (Paper III).

4. To examine the association between rotavirus-specific antibodies in maternal sera and breast milk and the serum rotavirus IgA immune response induced by rotavirus vaccine in the infants (Paper IV).

METHODS

SETTING

South Africa is located on the southernmost tip of the African continent, with a population of approximately 54 million people in 2014. The annual birth cohort is about 1.2 million with an estimated 5.7 million children <5 years of age and an under-5 mortality rate of 41 per 1000 in 2014 (133). The national prevalence of HIV infection among pregnant women aged 15–49 years attending antenatal clinics has remained steady at 29−30% since 2004 (134). Among children <5 years estimated national HIV prevalence has decreased over time from 4.4% in 2006 to 3.5% by 2013 due to improvement in the prevention of mother-to-child transmission of HIV – the estimated HIV transmission rate decreased from 9.6% in 2008 to 2.8% in 2011 (135, 136). Access to antiretroviral therapy (ART) has gradually improved since its introduction in the public sector in South Africa in 2004, and the overall estimated coverage in HIV-infected children requiring treatment was 63% in 2012 (137, 138). Although categorised as an upper middle-income country, it is ranked by the World Bank to be the country with the highest income inequality (GINI co-efficient 0.63 in 2011) (139). Certain areas within South Africa may thus be more similar to low- and low-middle income settings.

South Africa introduced the monovalent oral rotavirus vaccine into the EPI on 1 August 2009. Two doses of Rotarix® are recommended at 6 and 14 weeks of age together with other EPI vaccines, including co-administration with trivalent oral polio vaccine (OPV-Merieux; Sanofi Pasteur, Lyon, France) at the 6-week immunisation visit and inactivated poliovirus vaccine (IPV) at the 14-week immunisation visit. The vaccine schedule adopted in the EPI in 2009 (6 and 14 weeks of age) was different to that used in the South African vaccine efficacy trial, i.e. two doses at 10 and 14 weeks or three doses at 6, 10 and 14 weeks and different to that recommended by the WHO (two doses at 6 and 10 weeks of age) (27). Estimates of

coverage rates for the second dose of rotavirus vaccine in South Africa, as per the District Health Information Systems, increased from 67% in 2010 to 96% in 2011, which are higher than the WHO-UNICEF estimates for vaccination coverage for the last dose of rotavirus (66% in 2010; 72% in 2011) (140, 141).

STUDY DESIGN

Randomised controlled trials (RCTs) are regarded as the gold standard for assessing the effect of an intervention (142). Pre-licensure clinical trials of the oral rotavirus vaccines used this study design with infants randomised to receive the rotavirus vaccine or a placebo. Once a vaccine has been introduced into the national immunisation program of a country it is usually considered unethical, except in a few situations, to conduct placebo-controlled trials and other study designs are needed to assess the effect of the vaccine on the disease it prevents (143, 144). Several study methodologies were utilised in order to address the objectives of this thesis. The choice of study design was influenced by the nature of the research question, as well as feasibility of the study, taking into account the advantages and disadvantages of each method. Descriptions of the study methods are discussed in detail within the methods sections of each paper but specific considerations are elaborated on below.

Case-control study

The observational case-control study design has been used successfully to evaluate the protection conferred by the rotavirus vaccine under routine use after introduction in low-middle income settings (108, 109, 145). Vaccine effectiveness is estimated by comparing the

vaccination status among cases that have rotavirus diarrhoea with the vaccination status among controls (those without disease). Case-control studies do not need established surveillance prior to vaccine introduction and are cheaper and quicker to conduct than cohort studies. They are, however, subject to several potential biases and need to be done before vaccine coverage becomes too high. Case-control studies necessitate the controls to represent the source population from which the cases are drawn, and inappropriate selection of controls may introduce bias. The choice of a suitable control group is thus pivotal to the validity of the study results. A number of different control groups have been previously used in rotavirus vaccine effectiveness studies including case-negative controls, community or neighbourhood controls, hospital controls and respiratory controls (86, 103, 105, 107-109, 146, 147).

A case-control study, to estimate the effectiveness of the monovalent rotavirus vaccine against hospitalisation for rotavirus gastroenteritis in children <2 years of age, was conducted at seven hospitals in South Africa from April 2010 to October 2012. The hospitals selected for the study encompassed both urban and rural settings as well as differing HIV infection prevalence. Our primary control group consisted of children hospitalised with acute diarrhoea who tested rotavirus-negative. Three of the hospitals were recruiting children for a concurrent study investigating the effectiveness of the pneumococcal conjugate vaccine (PCV-7) and provided a convenient respiratory control group − children hospitalised with lower respiratory tract infection presumed not to be bacterial pneumonia. All control groups have some advantages and disadvantages so in order to strengthen the reliability our findings we included this second non-diarrhoea control group.

Matching may be utilised to control for known confounders, for example matching on age or HIV status (148). Rotavirus test results were not available immediately and case-control assignment could not occur at the time of enrolment, thus it was more practical to use an unmatched study design i.e. controls were not matched to cases. The unmatched test-negative

design has been used to assess the effectiveness of the influenza vaccine and, more recently, effectiveness of the rotavirus vaccine (149, 150).

Although vaccine effectiveness is a useful determinant of impact, it does not measure impact in absolute terms, only in relative terms. In a case-control study both cases and controls will be protected indirectly and herd (indirect) protection or decreased circulation of disease cannot be assessed (144). It is thus important to assess the change in the absolute amount of disease in the community rather than just comparing the prevalence of vaccination in cases versus controls. This can be done by assessing trends in disease incidence before and after vaccine introduction.

Monitoring trends in diarrheal hospitalisations

Sentinel surveillance for diarrhoeal hospitalisations, including rotavirus-associated hospitalisations, started in South Africa in May 2009, a few months before vaccination introduction in August of that year. We were thus unable to measure absolute reductions in rotavirus-specific diarrhoeal hospitalisations in the post-vaccine period compared to the pre-vaccine period as there were limited data collected prior to vaccine introduction. There was an attempt to assess rotavirus vaccine impact using the rotavirus surveillance data by comparing the number of rotavirus and non-rotavirus diarrhoea hospitalisations from May–December 2010 and 2011 with those from May–December 2009 but there are limitations in this approach (111). Most importantly, rotavirus disease tends to have year-on-year natural seasonal variation with some years having larger seasons than others or different strain circulation, and the WHO recommends 3–5 years of pre-vaccine data collected year round to establish baseline rates (151). Multiple rotavirus seasons prior to vaccine introduction are needed to give a comprehensive overview of the natural variability of rotavirus seasons so

that comparisons of the post-vaccine period to this pre-vaccine period will be accurate. Baseline risk of the disease may differ pre- and post-vaccination and these potential differences should be assessed on a sufficient number of pre-vaccine years (144). For example, the United States' National Respiratory and Enteric Virus Surveillance System has monitored laboratory-confirmed rotavirus diarrhoea over a 15 year period, including multiple pre-vaccine introduction years (2000–2006), and was able to show sustained reductions in rotavirus diarrhoea in the post-vaccine years 2007–2014 (152). This highlights the importance of establishing surveillance systems for a disease several years prior to vaccine implementation.

In the absence of long-term rotavirus surveillance in Soweto, we used all-cause diarrhoeal hospitalisations as a proxy for rotavirus diarrhoeal hospitalisations at the Chris Hani Baragwanath Academic Hospital (CHBAH) and compared incidence rates before and after rotavirus vaccine introduction. This approach has been used in other studies to estimate the impact of rotavirus vaccines on diarrhoeal hospitalisations (92, 95, 112, 153). Rotavirus caused approximately 25–30% of diarrhoeal hospitalisations prior to vaccine introduction and so the impact of vaccination should be observed for rotavirus-specific diarrhoeal hospitalisations as well as all-cause diarrhoeal hospitalisations (17). Previous South African studies have shown that rotavirus disease occurred early in life with 90–95% of children hospitalised for severe rotavirus diarrhoea being <18 months of age and we would expect that the major impact of vaccination would be in children <2 years of age (17, 18).

Randomised, longitudinal cohort study

To investigate the effect of abstention from breastfeeding (intervention) at the time of rotavirus vaccination on the immune response to Rotarix®, we conducted a prospective,

randomised, longitudinal cohort study of healthy, HIV-uninfected, mother-infant pairs. Mothers and their infants were enrolled when they presented for their 6 week immunisation visit at a primary health clinic in Soweto. All vaccines were given according to the EPI schedule, with two doses of Rotarix® given at 6 and 14 weeks of age.

Infants were randomised to one of two groups: 1) infants were not breastfed for 60 minutes before and 60 minutes after the administration of each dose of rotavirus vaccine 2) unrestricted breastfeeding in the infants. RCTs such as this have been used to compare immune responses to rotavirus candidates between breastfed and nonbreastfed children (43). Only HIV-uninfected mothers-infant pairs were eligible for enrolment as maternal HIV infection could reduce transplacental antibody transfer which could affect the immune responses, regardless of the child's HIV status (154, 155). Inclusion of HIV-infected mothers into the study would make the analysis more difficult as one would need to control for differential pre-existing rotavirus immunity in the mothers and infants.

Rotavirus-specific IgA in breast-milk and serum samples and IgG in serum samples were determined by enzyme-linked immunosorbent assays and rotavirus-specific neutralizing activity in breast-milk was measured by a microneutralization assay, as described (46). Despite the lack of an established correlate of protection, serum rotavirus IgA titres are regarded as an important measurable predictor of protection (37).

The study was powered to detect at least a 20% higher frequency of seroconversion among infants abstaining from breastfeeding than infants being breastfed at the time of rotavirus vaccine administration. When formulating a hypothesis it is advisable to be aware of practical and policy issues as well as the scientific basis for potential interventions. For example, although a larger sample size could have detected a smaller difference in seroconversion, from a policy point of view it would be difficult to support a change in breastfeeding

practices based on minimal benefit for the infant in terms of improved immune response. It is also important not to give a negative impression of breastfeeding among mothers, which could potentially impact on the acceptability of breastfeeding in the community. Similarly, we only assessed abstention of breastfeeding for 60 minutes before and 60 minutes after vaccine administration. It is possible that a longer period of abstention may in fact lead to better immune responses in these infants but, on a practical level a longer abstention from breastfeeding in a 6 week old infant is not advisable for physiological reasons and it would be difficult for mothers to accept.

STATISTICAL CONSIDERATIONS

The choice of statistical analysis was influenced by the study design utilised in each paper. Specific statistical considerations are elaborated on below, with full details of the analyses available in the attached papers.

In the case-control study, controls were not matched to cases which was problematic in that hospitalisation for rotavirus disease is associated with the age of the child as well as the season of hospitalisation (17). Vaccination status is also influenced by the age of the child. As a result we decided, *a priori*, to adjust for timing of birth (birth month and year) and hospitalisation (admission quarter and year), and only included children whose vaccine status was unlikely to change i.e. aged ≥18 weeks, which gave children an additional four weeks for vaccination with the second dose. The unmatched design of the case-control study dictated the use of unconditional logistic regression to estimate adjusted odds ratios (aORs) with associated 95% CIs. We adjusted for site *a priori* as protection offered by the vaccine can vary based on geographical location (27). Additional potential confounders were assessed in the unconditional logistic regression models and included in the final models if their

inclusion changed the OR associated with vaccination by >5 percentage points. Interactions between covariates were assessed and interaction terms were included in the finals models if appropriate. Adjusted vaccine effectiveness against hospitalisation for acute rotavirus diarrhoea was calculated as (1–aOR) x 100%. The primary analysis utilised the rotavirus-negative control group, with secondary analyses limited to respiratory controls at the three hospitals where this second control group was available.

For the trend analysis of diarrhoeal hospitalisations in Soweto we estimated the annual incidence of all-cause diarrhoeal hospitalisations (per 1000 population) using the number of children hospitalised for diarrhoea at the CHBAH per year in the numerator and the mid-year population estimates in relevant age categories for Soweto in the denominator (133). HIV prevalence for Soweto was estimated from projections of the Actuarial Society of South Africa's 2008 AIDS and Demographical model (135). Hospitalisation incidence rates were stratified by age group 0–11, 12–23 and 24–59 months, and by HIV infection status. Median annual incidence rates during the pre-vaccine years 2006-2008 were compared to those in the vaccine-era (2010–2014). To determine whether there were any changes in hospital admission practices during the study period we also assessed the incidence of hospitalisation for bronchiolitis, for which there were no preventative intervention strategies implemented over the same period. Mathematical modelling and complex statistical time series analyses have been used in other studies (94, 156). However, the absence of annual Soweto-specific rotavirus vaccine coverage data, ART coverage data and measures of improvement in access to tapped water and improved sanitation limited our ability to attempt more complex analyses to adjust for these potential confounders.

Randomisation is used to ensure similarity between groups with respect to all measured and unmeasured characteristics except the intervention, in this case timing of breastfeeding in

relation to rotavirus vaccination, allowing any difference between the groups to be attributed to the intervention without influence from unmeasured confounders. Mother-infant pairs were randomised to one of two groups (abstention from breastfeeding or unrestricted breastfeeding) in order to assess the effect of breastfeeding on the immune responses to rotavirus vaccine while maintaining a balance of potential confounders between groups. Pre-vaccination (baseline) characteristics and rotavirus-specific titres were compared between groups to ensure that the randomisation had achieved similarity between groups. Seroconversion was defined as ≥ fourfold increase in titres compared to baseline titres prior to the first vaccine dose. The frequency of seroconversion between the two groups was compared one month after the first and second doses of rotavirus vaccine. A significantly higher seroconversion rate in the abstention from breastfeeding group would support the hypothesis that abstention from breastfeeding near the time of administration could improve the immunogenicity of the oral rotavirus vaccine.

Associations between pre-existing maternal and infant rotavirus-specific antibodies and seroconversion following administration of one or two doses of Rotarix® were also examined, using univariate and multivariable logistic regression. The randomisation of mother-infant pairs to one of the two breastfeeding groups allowed us to assess the influence of abstention from breastfeeding on seroconversion without adjusting for confounders (both measured and unmeasured). The additional analyses included the cohort irrespective of breastfeeding group assignment, and thus covariates had to be assessed for confounding and adjusted for in the multivariate models as necessary. Antibody titres are generally not normally distributed and so rotavirus-specific titres of IgA, IgG and neutralising activity were log-transformed in the analyses to give a better approximation to a normal distribution. A *P*-value of <0.05 was considered statistically significant for all analyses.

ETHICAL CONSIDERATIONS

Approval for the thesis was obtained from Human Research Ethics Committee (Medical) of the University of the Witwatersrand - approval number M130273 (Appendix B). In addition, approval for each study which formed part of the thesis was obtained separately from the ethics committees of the University of the Witwatersrand, University of Cape Town, University of KwaZulu-Natal, University of Limpopo and PATH's Research Ethics Committee, as applicable. The US CDC tested only anonymized specimens so this research did not require review by the CDC Institutional Review Board.

Written informed consent was obtained from a parent/guardian of each child enrolled into the case-control study (Paper I) and from each mother enrolled into the randomised trial for her and her infant's participation in the study (Papers III and IV). Participants were reimbursed for travel and incidental costs arising from study visits, if applicable. Consent was waived for the trend analysis (Paper II) as the data was collected routinely by the Paediatric Department, Chris Hani Baragwanath Academic Hospital. All data were anonymised and did not include any personal identifiers.

RESULTS AND DISCUSSION

South Africa has played an instrumental role in influencing policy recommendations for rotavirus vaccine introduction in low- and middle-income countries by conducting pivotal clinical trials which assessed the monovalent oral rotavirus vaccine. Early immunogenicity studies paved the way for a Phase III efficacy study which informed the WHO's global recommendation for use of the vaccine in 2010 (27, 157). As the first African country to introduce the rotavirus vaccine into its national immunisation programme, we explored many of the key issues regarding post-licensure effectiveness and impact of the rotavirus vaccine. The monovalent oral rotavirus vaccine given at 6 and 14 weeks of age was shown to be effective under conditions of routine use and was temporally associated with a decrease in all-cause diarrhoeal hospitalisation rates in HIV-infected and HIV-uninfected children after its introduction into South Africa's EPI. A change in breastfeeding practice at the time of vaccination did not improve immune responses to the rotavirus vaccine in infants. However, high levels of maternal rotavirus antibodies may inhibit responses to a vaccine dose given at an early age.

EFFECTIVENESS AND PUBLIC HEALTH IMPACT OF ROTAVIRUS INTRODUCTION

Our multi-centre case-control study, primarily using rotavirus-negative controls, showed that two doses of rotavirus vaccine provided protection of 57% (95% CI: 40−68) against hospitalisation for acute rotavirus diarrhoea in children <2 years of age. Adjusted vaccine effectiveness was estimated to be 54% (95% CI: 32−68) in children 18 weeks to 11 months of age and similar protection was maintained through the second year of life (61%; 95% CI: 35−77). The trend analysis conducted over the period 2006-2014 showed that the

introduction of the rotavirus vaccine was temporally associated with a 34 to 57% decrease in the overall incidence of all-cause diarrhoeal hospitalisations in children <5 years of age in the urban setting of Soweto, Johannesburg. Reductions were greatest in children <12 months of age and were maintained over a 5-year period post-vaccine introduction in this age group. The hospitalisation incidence decreased from 54 per 1000 pre-vaccination to 30 per 1000 in the first year post-vaccine introduction (incidence reduction of 24 per 1000; percent decrease of 45%; $p<0.001$). Further reductions of 57 to 65% (incidence reduction of 31 to 36 per 1000; $p<0.001$) were maintained through the following four years. Among children in the second year of life, reductions of 40 to 49% (6 to 7 per 1000, $p<0.001$) were observed from the second year post-vaccine introduction compared to pre-vaccination years, and were maintained over time. Among children aged ≥24 months the incidence of all-cause diarrhoeal hospitalisations in the post-vaccine years remained relatively unchanged compared to pre-vaccination years. The reductions thus occurred specifically in the age groups that received rotavirus vaccination and were consistent with increasing vaccine coverage over the study period i.e. reductions observed in children <12 months during the first year and increasing in the second year after vaccine introduction; reductions in the 12−23 month olds from the second year post introduction.

There was a change in the epidemiology of diarrhoeal disease after rotavirus vaccine introduction, with diminished peaks in all-cause diarrhoea hospitalisations during 2010 and near loss of the peaks during 2011−2014, where a bimodal pattern was observed each year. Prior to vaccine introduction diarrhoeal hospitalisations were characterised by peaks during the autumn-winter months of March to May, consistent with published observations of rotavirus seasonality before routine vaccine use (17).

Comparison with results of the Phase III efficacy study in South Africa

It is important to compare the vaccine effectiveness and impact estimates post-vaccine implementation with those obtained from the pre-licensure efficacy study conducted in the same setting. Although unmeasured confounding may affect the results of a case-control study, a comparison of RCTs and observational studies showed that in the majority of cases the estimate of treatment effects from observational studies and RCTs were similar (158). Our point estimate for vaccine effectiveness in the first year of life was lower than the vaccine efficacy observed for the two-dose schedule at 10 and 14 weeks of age in the clinical efficacy trial (54% versus 72% respectively); yet it lay within the 95% confidence interval estimate (40–88%) (24). We showed sustained protection in the second year of life, whereas the Phase III study estimated the vaccine efficacy over two consecutive seasons to be 32% (95% CI -71–75) for the two-dose group. These were, however, exploratory analyses as the study was not powered to assess efficacy of the vaccine in the second year of life (119).

Comparisons between the efficacy trial (a RCT) and our observational study must, however, be made cautiously. Differences in study methodology, such as the definition of the outcome (severe rotavirus diarrhoea as measured by the Vesikari score versus hospitalisation for rotavirus diarrhoea), may have contributed to slightly different point estimates of protection of the rotavirus vaccine in the two studies. There were also differences in enrolment strategies with infants in the efficacy study being vaccinated immediately prior to the onset of the rotavirus season, whilst infants in the case control study were vaccinated throughout the year.

There was a 57 to 65% reduction in all-cause diarrhoeal hospitalisations in children <12 months of age after rotavirus vaccine introduction, which was higher than the vaccine efficacy of 44% (95% CI 19-61) against all-cause severe gastroenteritis demonstrated in this

age group in the clinical trial, although with overlapping confidence intervals. A decrease in transmission due to an overall decrease in circulation of rotavirus in the population could not be accounted for in the efficacy study design, and may account for the greater reductions which we observed.

The overall frequency of seroconversion for rotavirus-specific serum IgA one month after the second rotavirus vaccine dose was 61% (95% CI: 54−68) among the cohort of HIV-uninfected infants enrolled into a longitudinal study assessing immune responses to the rotavirus vaccine, which was similar to that obtained in clinical trial for the two-dose group (57%, 95% CI 45–69). Different assays were, however, used for the immunogenicity assessments in the two studies and direct comparisons should once again be made with caution.

FACTORS AFFECTING IMMUNOGENICITY OF THE ORAL ROTAVIRUS VACCINE

Understanding the reasons behind the lower vaccine efficacy in low- to middle-income countries is critical as even small improvements in efficacy could lead to significant decreases in the number of deaths and hospitalisations caused by rotavirus in countries where disease burden is high (159).

Breast milk antibodies and a change in breastfeeding practice at the time of vaccination

High levels of rotavirus-specific antibodies and other neutralizing factors found in breast-milk from women in lower-income countries, including South Africa, could diminish infant immune responses to the vaccine by lowering the effective titre of vaccine delivered to the intestine. A short abstention from breastfeeding around the time of vaccination may,

therefore, improve immunogenicity (46, 47). We tested this hypothesis by randomising a cohort of infants to either abstention from breastfeeding or unrestricted feeding at the time of rotavirus vaccination and assessed immune responses to the vaccine. Among this cohort of infants, serum anti-rotavirus IgA geometric mean titres (GMTs) increased significantly following administration of two rotavirus vaccine doses, compared to pre-vaccination titres, in both the abstention from breastfeeding group and those with unrestricted breastfeeding. There were no significant differences in seroconversion or serum rotavirus-specific IgA GMTs measured after the first or second doses of Rotarix® between the two groups. The frequency of seroconversion after the second dose was 63% in the abstention from breastfeeding group and 59% in the unrestricted breastfeeding group (p=0.485). Reverse cumulative frequencies of infant serum rotavirus-specific IgA titres in Group-1 (abstention) and Group-2 (unrestricted) are shown below in Figure 1.

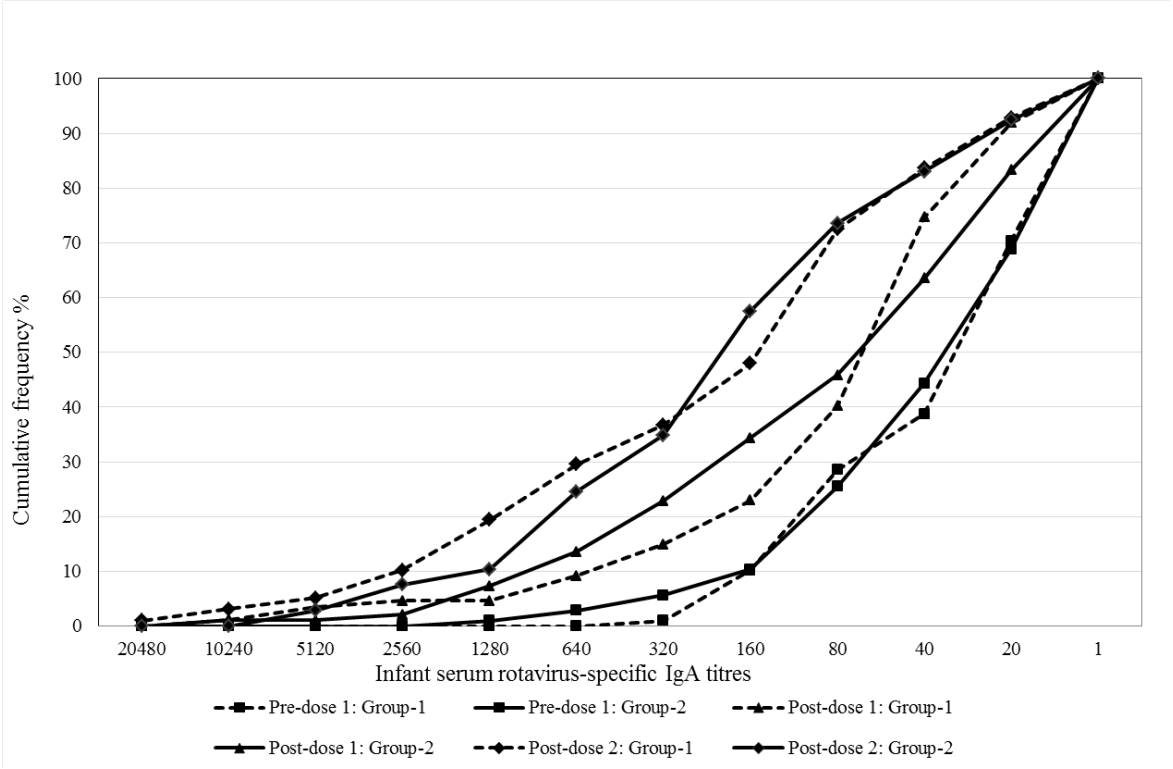


Figure 1: *Reverse cumulative frequency profiles of infant rotavirus-specific IgA titres, by group (Group-1: abstention; Group-2: unrestricted), pre-vaccination, after the first dose and after the second dose of rotavirus vaccine.*

These results are consistent with a study investigating the effect of breastfeeding on antibody response to OPV in infants, in which no difference in seroconversion rates were observed among infants on unrestricted breastfeeds and those abstaining from breastfeeding (160). In addition, studies assessing the impact of breastfeeding on the immunogenicity of rotavirus vaccines in infants have now also been conducted in other settings and support the findings from our study. In India, withholding breastfeeding for 30 minutes before and after each rotavirus vaccine dose (Rotarix®), despite being an acceptable intervention among mothers, similarly showed no improvement in the infant immune response. The frequency of seroconversion was lower than observed in our study but there were no significant differences between the infants in which breastfeeding was withheld and those with unrestricted feeding (26% versus 27% respectively) (161, 162). A study in Pakistan, which assessed withholding breastfeeding for an hour before and after each dose of Rotarix®, also showed no improvement in infant immune responses after three vaccine doses. On the contrary, IgA seroconversion in the group in which breastfeeding was encouraged tended to be higher than in the abstention from breastfeeding group (163). Withholding of breastfeeding for 90 minutes before and 60 min after the first dose of Rotateq® in Nicaraguan infants similarly did not show any significant benefit of withholding breastfeeding (164).

There were no significant differences in baseline maternal rotavirus-specific IgA or neutralising antibodies in breast milk between infants in our cohort who seroconverted and those that did not seroconvert after either the first or second dose, which was supported by similar findings among the Nicaraguan infant cohort (164). There was also no relationship observed between innate immune factors (lactoferrin, lactadherin, and Tenascin-C) in breast milk and immune responses to the rotavirus vaccine in these Nicaraguan infants (165). There is thus overwhelming evidence to suggest that this hypothesis does not hold true *in vivo* and

that withholding breastfeeding at the time of rotavirus vaccination shows no benefit for the infant in terms of improved rotavirus immune responses to the vaccine.

Pre-existing maternal and infant rotavirus antibody levels

It has also been hypothesised that high levels of maternal rotavirus antibodies found in lower income countries are transferred to their infants during pregnancy and breastfeeding, resulting in an inhibitory effect on immune responses to rotavirus vaccines in the infants. Additional analyses of matched serum samples from the mother-infant pairs in our cohort showed that mothers of infants who seroconverted after the first dose had significantly lower rotavirus-specific IgG titres at baseline than those whose infants did not seroconvert (median 5120 vs. 10 240 respectively, p=0.031). A significant difference in maternal IgG rotavirus titres between those who seroconverted and those that did not was no longer observed following the second dose of vaccine, suggesting that the inhibitory effect of maternal rotavirus antibodies may be overcome by administration of the second dose at 14 weeks of age when maternal antibody levels are lower. This is consistent with the current understanding of the mechanisms of maternal antibody inhibition, whereby the influence of maternal antibodies depends on the ratio of maternal antibody to vaccine antigen at the time of vaccination. The second dose of vaccine could induce an infant response because the maternal antibody levels declined beyond a certain threshold, decreasing the inhibitory effect and enabling a better immune response to the second dose (58). Infants who seroconverted after the second dose had lower rotavirus IgA titres at baseline compared to those who did not seroconvert, which suggests that pre-existing infant rotavirus antibodies, most likely from exposure to natural rotavirus infection prior to vaccination, may also have an inhibitory effect on the immune response to the vaccine.

Our findings are consistent with those from Nicaragua, where infants who seroconverted after the first Rotateq® dose had mothers with significantly lower baseline serum rotavirus-specific IgG titres compared with infants who did not seroconvert. In addition, infant baseline IgA titres were significantly lower in the seroconverted compared with non-seroconverted infants (164). These associations were, however, not assessed after subsequent doses of Rotateq®, so the effects of further doses could not be evaluated. In contrast, in the Indian study lower odds of seroconversion after the second Rotarix® dose were observed with increasing titres of baseline maternal serum rotavirus-specific IgG, similar to what was observed with the Indian rotavirus vaccine ORV 116E vaccine (57, 161).

Further investigation of rotavirus exposure in infants prior to vaccination as well as the impact of maternal rotavirus antibodies on the immune response is needed. The above-mentioned studies all measured maternal antibody titres at the time of infant vaccination, not at birth. We showed moderate correlation (r=0.56) between maternal and infant rotavirus-specific IgG titres at baseline i.e. infant aged 6 weeks, similar to that in the Nicaraguan study (r=0.57) (164). This is likely due to decreasing levels of transplacentally-derived rotavirus-specific IgG in infant serum which occurred since birth. Studies assessing rotavirus antibody levels in maternal serum and infant cord blood may be useful to tease out the relative effects of transplacental transfer of antibodies from the mother versus rotavirus exposure in the infant.

The influence of maternal antibody titres on infant immune responses depends on the distribution of the titres in a specific population at a specific time point (58). The introduction of a rotavirus vaccine could itself influence levels of pre-existing rotavirus-specific antibodies in infants at the time of vaccination. As a vaccine program matures, diminished circulation of rotavirus in the community may lead to lower rotavirus antibody titres in women of child-bearing age, resulting in lower transplacental transfer from mothers to their

infants. Infants would also be less likely to be exposed to natural rotavirus infection in the first 6 weeks of life, further lowering pre-existing rotavirus antibodies and leading to an overall improvement in the immune response following the first rotavirus vaccine dose.

We observed an increase in maternal rotavirus-specific IgA GMTs following the first dose of rotavirus vaccine in their infants. This was not shown in any of the other studies as maternal serum samples were only obtained at baseline and not after vaccination in the infant as in our study. The majority of the maternal samples were obtained prior to the classic peak rotavirus season, indicating possible boosting in the mother through acquiring rotavirus vaccine from the shedding in their infants. We do not, however, know how this increase in maternal titres following infant vaccination compares to natural boosting of maternal antibody titres and further evaluation is necessary to determine whether this might affect infant immune responses.

HIV infection

Exposure to HIV infection *in-utero* remains an important consideration among South African children. The phase III clinical trial included HIV-infected children, but was not powered to specifically address the question of efficacy in this sub-group children and data are limited to safety and immunogenicity of the rotavirus vaccine among HIV-infected children (27, 65). While our case-control study did not enrol sufficient HIV-infected children to assess rotavirus vaccine effectiveness in this group, the adjusted two-dose vaccine effectiveness was similar in HIV-exposed-uninfected and HIV-unexposed-uninfected children (64%; 95% CI: 34−80 and 54%; 31−69 respectively). With just under a third of infants born to HIV-infected mothers, it is reassuring that rotavirus vaccine was equally effective in the HIV-exposed infants. The hospitalisation incidence among HIV-infected children aged <12 months

decreased from 133 per 1000 in pre-vaccination years to 104, 58, 39, 50 and 31 per 1000 during 2010−2014 respectively (incidence decrease of 29 to 102 per 1000, percent decrease of 22 to 78%, $p<0.05$ for all comparisons). Percent reductions in incidence in the post-vaccine years 2011–2014 compared to pre-vaccine years among those aged 12-23 months ranged from 45 to 65% (incidence decrease of 17 to 24 per 1000, $p<0.05$ for all comparisons). There were significant reductions in hospitalisation incidence in older HIV-infected children aged 24–59 months in most post-vaccine years compared to pre-vaccine years, in contrast to the relatively unchanged hospitalisation incidence among older HIV-uninfected children.

It is more difficult to attribute reductions in the HIV-infected children to the rotavirus vaccine. Firstly, although rotavirus was shown to be the most common organism causing diarrhoea among HIV-infected children <5 years of age in this setting, the prevalence of rotavirus detection among HIV-infected children hospitalised with diarrhoea was lower than that among HIV-uninfected children hospitalised for diarrhoea (34.7% versus 14.8% respectively) (166). Reductions in diarrhoea attributable to rotavirus may thus be diluted, as rotavirus is responsible for proportionally less diarrhoea hospitalisations among these children. Secondly, the upscaling of ART has led to a decrease in HIV-related hospitalisations and overall mortality at CHBAH with HIV prevalence among paediatric admission peaking at 31.7% in 2005 and decreasing to 19.3% by 2010/2011 (167). ART may thus account for some of the reductions in all-cause diarrhoea hospitalisations which we observed. In the absence of accurate ART coverage data among children in the age groups that were assessed, it is difficult to quantify the reduction due to ART and that due to rotavirus introduction. ART coverage data stratified by year and age group would allow statistical modelling to be performed which could provide estimates of the individual effects of interventions.

We were unable to assess the immune response to the two dose rotavirus vaccine schedule among HIV-exposed infants, as only HIV-unexposed infants were enrolled into the longitudinal cohort. In the case-control study, point estimates for adjusted vaccine effectiveness after one dose were higher in HIV-exposed uninfected children than HIV-unexposed children (61% (95% CI 22−81) versus 24% (95% CI -17−51) respectively), albeit with overlapping confidence intervals. This may suggest lower transplacental transfer of rotavirus-specific antibodies from HIV-infected mothers to their infants, with a more robust immune response after the first dose in HIV-exposed infants. South African HIV-exposed uninfected infants were shown to have lower antibody levels to pertussis and pneumococcus at birth than unexposed infants, with higher antibody responses to pertussis and pneumococcal vaccination (155). Further investigation is needed to see whether this holds true for rotavirus antibodies. The similar vaccine effectiveness point estimates between HIV-exposed and HIV-unexposed uninfected children after two doses suggest that any differences in pre-existing rotavirus-specific antibodies are overcome after the second dose is given at 14 weeks when maternal ant-rotavirus antibody levels are lower.

CONSIDERATIONS WHEN ASSESSING VALIDITY OF RESULTS

The limitations of the individual studies have been highlighted within the papers but a summary of limitations and their potential threat to the validity of the conclusions are discussed below.

Methodological

An ecological study is limited in its ability to attribute causality. We used all-cause diarrhoeal hospitalisations as a proxy for rotavirus hospitalisations, in the absence of long-term

pathogen-specific diarrhoea surveillance, and it is thus difficult to definitively conclude that a causal relationship exists between rotavirus vaccine introduction and reductions in diarrhoeal hospitalisations. There may be secular trends in diarrhoeal hospitalisation incidence unrelated to the vaccine, including introduction of non-vaccine prevention measures, such as improvement in access to fresh water, sanitation and refuse removal in the population, which may contribute to the observed decline in diarrhoeal hospitalisations in the post-vaccine period. Optimally one would require accurate annual data on improvements in access to clean water, sanitation and other socio-economic indicators but in Soweto these data are limited to that obtained from 5-yearly census data. Although sub-district level information is lacking, there were some improvements in these indicators over the study period in the Johannesburg region (168). These changes would, however, have been more consistent over time and unlikely to account for the dramatic decline observed in the vaccine-era years. Changes in hospital referral practices could also account for changes in hospitalisation rates but the incidence of bronchiolitis hospitalisations were relatively unchanged during 2006-2014, giving reassurance that this was unlikely to account for the reductions we observed in diarrheal hospitalisations.

Reliance on census data to obtain annual population estimates for Soweto and extrapolation of provincial model projections to obtain HIV prevalence estimates for Soweto are limitations and may have influenced the validity of our diarrhoeal hospitalisation incidence estimates. However, the population in Soweto had been relatively stable over the years included in the study and the method used for producing the population estimates has been consistent over time, so the decreases in incidence of diarrhoeal hospitalisation observed post-vaccine introduction, compared to pre-vaccine introduction, should be valid. Children who were not tested for HIV infection were considered to be HIV-uninfected, assuming that physicians were less inclined to test for HIV in the absence of clinical stigmata. This may have

underestimated the disease burden among HIV-infected children. However, an HIV-infected child would have had several hospitalisations, prompting HIV testing and thus our assumption most likely approximates the true prevalence of HIV-infection among children hospitalised for diarrhoea.

The choice of the control group in a case-control study may influence the validity of the results obtained. We used rotavirus-negative diarrhoea controls as the primary control group and performed secondary analyses using respiratory controls enrolled from a subset of the study hospitals. These analyses gave similar estimates of protection of the vaccine against hospitalisation for rotavirus diarrhoea, which gave us some reassurance that the effectiveness estimates we obtained were valid.

We were reliant on the results of a commercially available enzyme immunoassay (EIA; ProSpecT ELISA, Oxoid, UK) to define rotavirus-positive cases and rotavirus-negative controls which, when compared to reverse-transcriptase-PCR as the gold standard, had a sensitivity of 75% and specificity of 100% (169). There was thus potential for misclassification of the case status as children with a false negative EIA test would have been defined as a control rather than a case. We also assumed that rotavirus was the cause of the hospitalisation if it was detected in the stool, which may not necessarily have been the case, and we may have falsely attributed disease to rotavirus instead of a co-infecting viral or bacterial pathogen. These misclassifications would likely have biased estimates toward the null.

Vaccine uptake increased quickly in South Africa reaching a high, steady rate soon after introduction as has been seen in settings where vaccination programmes are well-established , for example in the Americas (170). Estimates of coverage rates for the second dose of rotavirus vaccine in South Africa increased from 67% in 2010, the first year after vaccine

introduction, to 96% in 2011 (140). High coverage can be problematic when using a case-control design as the sample size is dependent on the vaccine coverage and once it reaches beyond 80% the required the sample size can become prohibitively high (151). We enrolled a sufficient number of cases when coverage rates were <80% and were adequately powered for the analyses we conducted. In contrast, high coverage is preferable when looking at the impact of an intervention on disease incidence. If rotavirus vaccination coverage had remained low, it would have been difficult to observe reductions in a non-specific outcome such as all-cause diarrhoeal hospitalisations.

We excluded HIV-infected mothers in the breastfeeding study so we could not assess maternal rotavirus-specific antibody titres in HIV-infected mothers and compare these titres to those measured in HIV-uninfected women.

Generalisability of results

The public health impact of rotavirus vaccines may differ between countries as well as between regions in the same country but we were not powered to assess site-specific vaccine effectiveness in the case-control study. The trend analysis was restricted to one low-income, urban community in South Africa and may not be generalisable to all areas of the country. A similar analysis of all-cause hospitalisation trends should be replicated in other hospitals in South Africa, which would strengthen the evidence supporting vaccine introduction in geographical and socially diverse settings.

Vaccine effectiveness estimates from Malawi, a very low-income African country, have recently been published with a similar vaccine effectiveness estimate (64%; 95% CI 24–83) observed against rotavirus gastroenteritis for two doses of Rotarix® as observed in our study.

The vaccine schedule introduced, a two-dose schedule given at 6 and 10 weeks of age, as well as the study methodology was slightly different and age-specific estimates were not provided (150). Case-control studies are underway in several other African countries and as additional post-implementation data become available, the body of evidence supporting rotavirus vaccine introduction in the African region will grow.

OTHER CONSIDERATIONS FOR THE USE OF ROTAVIRUS VACCINES

Optimising the dosing schedule of oral rotavirus vaccines

Alternative dosing schedules of the vaccine should also be explored in view of the potential inhibition by maternal rotavirus antibodies with an early vaccine dose. There does not appear to be any benefit from addition of a third dose of Rotarix® as a study in Pakistan recently showed that immunogenicity did not improve significantly with three doses administered at 6, 10 and 14 weeks compared to two doses given at 6 and 10 weeks. In addition, a delayed two-dose schedule (10 and 14 weeks) did not result in improved seroconversion compared to two doses given at 6 and 10 weeks (171). This supports data from the clinical efficacy trial in South Africa which showed no significant difference in immunogenicity or efficacy against severe rotavirus diarrhoea between the two and three-dose arms in the first year of life, although the study was not powered to detect a difference between these two arms (27). We also observed similar immunogenicity of the 6 and 14 week schedule compared to that of the 6 and 10 week schedule in the clinical trial (27).

Due to initial concerns about the age-specific risk of intussusception associated with use of rotavirus viruses, WHO initially recommended that the last dose of vaccine be administered by 32 weeks of age, but subsequently these recommendations have been revised and the age

restrictions removed (3, 172). A study in Bangladesh investigated the administration of a booster dose of Rotarix® at 9 months of age, following two primary doses at 6 and 10 weeks of age. Concomitant administration of measles-rubella vaccine and rotavirus vaccine was safe with no negative effect of the rotavirus vaccine on measles and rubella immunity. Serum rotavirus-specific IgA and IgG GMTs measured two months following the booster dose increased significantly from pre-vaccination levels measured at 9 months of age. Increases were mainly observed among infants with low pre-vaccination titres, suggesting that this additional dose at the 9 month routine immunisation visit could have public health benefit (173).

Rotavirus infection occurs at an early age in lower income countries and some infants may be exposed to natural rotavirus and acquire disease prior to their first vaccination, even with a dose given as early as 6 weeks of age. One alternative approach is to use a birth dose of rotavirus vaccine. This would enable completion of three doses prior to 3 months of age i.e. 0, 6 and 10 weeks and in addition minimise any potential risk of intussusception associated with the first dose as intussusception risk is low in the neonatal period. The neonatal vaccine candidate RV3-BB was found to be immunogenic and well-tolerated in Phase I and II trials and this birth-dose strategy potentially provides a way to improve the safety and effectiveness of rotavirus vaccines in lower income countries (174, 175).

Season of birth and vaccination

Seasonal factors may influence rotavirus vaccine performance as evidenced by a recent pooled analysis of results from case-control studies performed in the Americas which found that vaccine effectiveness was significantly reduced among children aged <12 months born during the rotavirus season compared to those born outside of these months (176). Infections

with other enteric pathogens at the time of vaccination may vary by season, for example bacterial diarrhoeal infections are generally more common in the warmer summer months outside of the peak rotavirus season, and may affect the immune response in infants differently depending on when they are born (177). Maternal rotavirus-specific antibody titres are likely to be higher during the rotavirus season resulting in higher levels of transplacental transfer of antibodies to their infants, with greater potential to inhibit the immune response to the vaccine (48). In addition, children born in the rotavirus season are likely to be vaccinated just after the rotavirus season with several months delay until their first exposure to natural rotavirus infection in the following rotavirus season, which may also result in waning of immunity.

We did not assess vaccine effectiveness by season of birth or season of vaccine administration, but additional analyses of the case-control data are underway. An additional year of enrolment was conducted at all seven sites and while the coverage may be too high to allow additional sub-analyses of vaccine effectiveness against hospitalisation for rotavirus diarrhoea, a closer look at factors associated with vaccine failure (i.e. rotavirus diarrhoea in children who had received two doses of rotavirus vaccine) are warranted. New methods such as the propensity score design and analysis could also be utilised in further analysis of the data, where traditional analysis methods are not appropriate (178).

Waning of immunity and indirect protection

We did not observe any waning of vaccine effectiveness and similar estimates were observed in children <12 months as children aged 12-23 months. In addition, there was no shift towards increased incidence of diarrhoeal hospitalisation in this age group, which is encouraging. Some reduction in all-cause diarrhoeal hospitalisations was observed in the first

post-vaccination year with greater reductions observed from the second year of vaccine introduction. Reductions were maintained in all four subsequent years, suggesting that the vaccine affords protection through the second year of life. We did not see any clear evidence of indirect protection i.e. protection in older unvaccinated children, as has been observed in some high-income settings (96, 115). In our setting very little rotavirus-associated hospitalisation occurs in children >2 years of age, so any indirect protection in this age group would be minimal.

Impact on mortality

Trends in all-cause diarrhoea deaths should also be assessed post-vaccine introduction, especially in countries where diarrhoeal mortality rates are high, as many policy makers and national health departments have prioritised decreasing overall mortality in their countries. Although the number of children hospitalised, together with the diagnoses, were consistently recorded at CHBAH during the study period, data on the outcome of the hospitalisation were less accurately recorded with many of hospitalised children missing outcome data. We were thus unable to assess trends in all-cause diarrhoeal deaths in addition to the hospitalisation trends. In addition, diarrhoeal mortality rates are much lower than hospitalisation rates making it difficult to interpret when numbers are small. Plans are underway to analyse vital registration data in South Africa to measure the impact of rotavirus vaccine introduction on all-cause diarrhoeal mortality at a national level. This may, however, be difficult to interpret in the context of reduction in under-5 childhood mortality specifically related to declines in death due to HIV/AIDS.

Strain replacement

There is a concern that selective pressure from strain-specific rotavirus vaccines may impact strain circulation after vaccine introduction and that rotavirus vaccines may not provide protection against emergent strains. Our case-control study showed effectiveness of the rotavirus vaccine against homotypic, partly heterotypic and fully heterotypic strains but was not powered to assess protection against specific strains, except for G12P[8], the dominant strain during the study period. An integrated analysis of all the clinical trials using the monovalent rotavirus vaccine showed comparable clinical protection against severe rotavirus diarrhoea caused by multiple rotavirus strains (179). A recent systematic review found that both the monovalent and pentavalent rotavirus vaccines had similar effectiveness against homotypic and heterotypic strains and that predominance of specific strains was not observed after routine vaccine introduction (110). In particular, initial concern over the predominance of genotype G2P[4] after introduction of the monovalent vaccine was shown to disappear over time (180). Continued diarrhoeal surveillance and monitoring of rotavirus strains is warranted in South Africa to detect the emergence of new and unusual genotypes, especially with the high genetic strain diversity observed in Africa, as well as to monitor the potential for replacement by other viruses, for example norovirus (82).

Safety considerations

The overwhelming benefit of rotavirus vaccination compared to the low intussusception risk, has allowed policy makers and global health authorities, for example WHO, to support routine vaccination of infants with rotavirus vaccine, albeit with recommendations for continued safety monitoring (132, 181). While we have demonstrated significant benefits of rotavirus vaccine introduction in South Africa, it is important to monitor vaccine safety

following rotavirus vaccine introduction in order to better evaluate the risk-benefit ratio specifically in African countries.

Once again South Africa is in a prime position to provide post-implementation safety data from Africa, and active prospective surveillance for intussusception was implemented at 8 hospitals, which include the majority of the larger paediatric surgical departments, in South Africa since October 2013 (principal investigators M.J. Groome, S.A. Madhi). This surveillance will use a case-series as well as a case-control methodology and generate data on the risk of intussusception following rotavirus vaccination, other potential risk factors for intussusception, and explore the mechanisms of disease. These methods have both been successfully used to assess intussusception risk, with consistency of estimates observed in studies using both methodologies (128, 129). Serum, stool and tissue samples will be collected on both cases and control to investigate possible aetiological agents and the role of C-reactive protein in the pathogenesis of intussusception in children (124).

The future of rotavirus vaccines

Despite the success of the currently licenced rotavirus vaccines, continued research and development of new live-attenuated rotavirus vaccines is warranted to ensure a sustainable and uninterrupted vaccine supply as the demand for rotavirus vaccines increase. Issues associated with the live-attenuated oral vaccines may be circumvented by the development of "new generation" inactivated rotavirus vaccines, which are administered parenterally and may have superior efficacy, have less safety risks, be less expensive to produce and could be added to existing EPI vaccines, thereby facilitating delivery (182).

Vaccine candidates include protein subunit vaccines, inactivated virus and virus-like particle vaccines. There is a rich rotavirus vaccine pipeline with several vaccine candidates under development, and one such vaccine candidate is currently in Phase1/II human trials in Soweto, South Africa (principal investigator M.J. Groome; ClinicalTrials.gov number, NCT02109484). This monovalent P2-VP8 subunit vaccine was shown to be well tolerated and immunogenic in healthy adults in the US (183). Preliminary results from the current clinical trial in South African toddlers and infants showed that the vaccine was well-tolerated in the target population, and, pending favourable immunogenicity results, a Phase I/II trial of the trivalent P2-VP8 subunit vaccine will commence at three sites in South Africa in 2016 (184).

CONCLUSION

Through the work undertaken in this doctoral thesis, the monovalent oral rotavirus vaccine was shown to be an effective intervention against hospitalisation for rotavirus diarrhoea in children <2 years of age in a middle-income setting with a high prevalence of HIV infection among pregnant women, high rates of malnutrition and enteric co-infections, as well as with a schedule different to that used in the efficacy trials of rotavirus vaccine. Despite lower rotavirus vaccine efficacy and effectiveness estimates observed in South Africa, compared to higher income countries, there was a significant impact of the vaccine on all-cause diarrhoeal hospitalisations in both HIV-infected and HIV-uninfected children <5 years of age which was maintained for five years after vaccine introduction. As an increasing number of African countries introduce or plan introduction of this life saving vaccine into their national immunisation programmes, our data are pivotal to inform public health decisions and to provide evidence for sustained vaccine use and continued investment from governments and donors.

Although abstention from breastfeeding at the time of vaccination has been conclusively shown not to improve the immune response to the rotavirus vaccine, several key factors surrounding optimal use of the oral rotavirus vaccines still need further evaluation. Further studies are needed to better define the role of maternal rotavirus antibodies as well as the impact of enteric co-infections, concurrent medical conditions, zinc supplementation and probiotic administration with the vaccine. Economic impact assessments, such as cost effectiveness studies, are essential to inform decisions in countries yet to introduce the rotavirus vaccine and support decisions made in countries already using the vaccine. Furthermore, success of the currently licenced vaccines should not deter future vaccine research or comprehensive strategies to control diarrhoeal disease including promotion of oral rehydration and improvements in water and sanitation.

REFERENCES

1. Bishop R. Discovery of rotavirus: Implications for child health. *J Gastroenterol Hepatol.* 2009;24 Suppl 3:S81-5.
2. Glass RI, Parashar UD, Bresee JS, et al. Rotavirus vaccines: current prospects and future challenges. *Lancet.* 2006;368(9532):323-32.
3. Rotavirus vaccines:an update. *Wkly Epidemiol Rec.* 2009;84(50):533-40.
4. PATH. Rotavirus Vaccine Country Introduction Spreadsheet. Available: http://sites.path.org/rotavirusvaccine/country-introduction-maps-and-spreadsheet [accessed 03 March 2016].
5. Henock Blaise NY, Dovie DB. Diarrheal diseases in the history of public health. *Arch Med Res.* 2007;38(2):159-63.
6. Liu L, Oza S, Hogan D, et al. Global, regional, and national causes of child mortality in 2000-13, with projections to inform post-2015 priorities: an updated systematic analysis. *Lancet.* 2015;385(9966):430-40.
7. Fischer Walker CL, Perin J, Aryee MJ, et al. Diarrhea incidence in low- and middle-income countries in 1990 and 2010: a systematic review. *BMC Public Health.* 2012;12:220.
8. Statistics South Africa. Levels and trends of morbidity and mortality among children aged under-five years in South Africa, 2006-2010. Available: http://www.statssa.gov.za/publications/Report-03-09-10/Report-03-09-102010.pdf [accessed 14 March 2015].
9. Kotloff KL, Nataro JP, Blackwelder WC, et al. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. *Lancet.* 2013;382(9888):209-22.

10. Walker CL, Rudan I, Liu L, et al. Global burden of childhood pneumonia and diarrhoea. *Lancet.* 2013;381(9875):1405-16.
11. Widdowson MA, Steele D, Vojdani J, et al. Global rotavirus surveillance: determining the need and measuring the impact of rotavirus vaccines. *J Infect Dis.* 2009;200 Suppl 1:S1-8.
12. Tate JE, Burton AH, Boschi-Pinto C, et al. 2008 estimate of worldwide rotavirus-associated mortality in children younger than 5 years before the introduction of universal rotavirus vaccination programmes: a systematic review and meta-analysis. *Lancet Infect Dis.* 2012;12(2):136-41.
13. 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.
14. Parashar UD, Hummelman EG, Bresee JS, et al. Global illness and deaths caused by rotavirus disease in children. *Emerg Infect Dis.* 2003;9(5):565-72.
15. Sanchez-Padilla E, Grais RF, Guerin PJ, et al. Burden of disease and circulating serotypes of rotavirus infection in sub-Saharan Africa: systematic review and meta-analysis. *Lancet Infect Dis.* 2009;9(9):567-76.
16. Santos N, Hoshino Y. Global distribution of rotavirus serotypes/genotypes and its implication for the development and implementation of an effective rotavirus vaccine. *Rev Med Virol.* 2005;15(1):29-56.
17. 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.
18. Mapaseka SL, Dewar JB, van der Merwe L, et al. Prospective hospital-based surveillance to estimate rotavirus disease burden in the Gauteng and North West Province of South Africa during 2003-2005. *J Infect Dis.* 2010;202 Suppl:S131-8.

19. UNICEF/WHO. 2009. Diarrhoea: Why children are still dying and what can be done. Available: http://www.unicef.org/media/files/Final_Diarrhoea_Report_October_2009_final.pdf [accessed 11 November 2015].

20. Vesikari T. Rotavirus vaccines against diarrhoeal disease. Lancet. 1997;350(9090):1538-41.

21. WHO, UNICEF, World Bank. State of the world's vaccines and immunization, 3rd ed. Geneva, World Health Organization, 2009. Available: http://www.who.int/immunization/sowvi/en/ [accessed 25 Feb 2016].

22. Velazquez FR, Matson DO, Calva JJ, et al. Rotavirus infections in infants as protection against subsequent infections. *N Engl J Med.* 1996;335(14):1022-8.

23. Fischer TK, Valentiner-Branth P, Steinsland H, et al. Protective immunity after natural rotavirus infection: a community cohort study of newborn children in Guinea-Bissau, West Africa. *J Infect Dis.* 2002;186(5):593-7.

24. 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.

25. 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.

26. Vesikari T, Karvonen A, Prymula R, et al. Efficacy of human rotavirus vaccine against rotavirus gastroenteritis during the first 2 years of life in European infants: randomised, double-blind controlled study. *Lancet.* 2007;370(9601):1757-63.

27. Madhi SA, Cunliffe NA, Steele D, et al. Effect of human rotavirus vaccine on severe diarrhea in African infants. *N Engl J Med.* 2010;362(4):289-98.

28. Armah GE, Sow SO, Breiman RF, et al. Efficacy of pentavalent rotavirus vaccine against severe rotavirus gastroenteritis in infants in developing countries in sub-Saharan Africa: a randomised, double-blind, placebo-controlled trial. *Lancet.* 2010;376(9741):606-14.

29. Zaman K, Dang DA, Victor JC, et al. Efficacy of pentavalent rotavirus vaccine against severe rotavirus gastroenteritis in infants in developing countries in Asia: a randomised, double-blind, placebo-controlled trial. *Lancet.* 2010;376(9741):615-23.

30. Levine MM. Immunogenicity and efficacy of oral vaccines in developing countries: lessons from a live cholera vaccine. *BMC biology.* 2010;8:129. doi: 10.1186/1741-7007-8-129.

31. Patriarca PA, Wright PF, John TJ. Factors affecting the immunogenicity of oral poliovirus vaccine in developing countries: review. *Rev Infect Dis*; 1991;13(5):926-39.

32. Gessner BD, Feikin DR. Vaccine preventable disease incidence as a complement to vaccine efficacy for setting vaccine policy. *Vaccine.* 2014;32(26):3133-8.

33. Patel M, Shane AL, Parashar UD, et al. Oral rotavirus vaccines: how well will they work where they are needed most? *J Infect Dis.* 2009;200 Suppl 1:S39-48.

34. Fu C, He Q, Xu J, et al. Effectiveness of the Lanzhou lamb rotavirus vaccine against gastroenteritis among children. *Vaccine.* 2012;31(1):154-8.

35. Dang DA, Nguyen VT, Vu DT, et al. A dose-escalation safety and immunogenicity study of a new live attenuated human rotavirus vaccine (Rotavin-M1) in Vietnamese children. *Vaccine.* 2012;30 Suppl 1:A114-21.

36. Bhandari N, Rongsen-Chandola T, Bavdekar A, et al. Efficacy of a monovalent human-bovine (116E) rotavirus vaccine in Indian infants: a randomised, double-blind, placebo-controlled trial. *Lancet.* 2014;383(9935):2136-43.

37. Patel M, Glass RI, Jiang B, et al. A systematic review of anti-rotavirus serum IgA antibody titer as a potential correlate of rotavirus vaccine efficacy. *J Infect Dis.* 2013;208(2):284-94.

38. Ward RL, Kirkwood CD, Sander DS, et al. Reductions in cross-neutralizing antibody responses in infants after attenuation of the human rotavirus vaccine candidate 89-12. *J Infect Dis.* 2006;194(12):1729-36.

39. Glass RI, Ing DJ, Stoll BJ, et al. Immune response to rotavirus vaccines among breast-fed and nonbreast-fed children. *Adv Exp Med Biol.* 1991;310:249-54.

40. Pichichero ME, Losonsky GA, Rennels MB, et al. Effect of dose and a comparison of measures of vaccine take for oral rhesus rotavirus vaccine. The Maryland Clinical Studies Group. *Pediatr Infect Dis J.* 1990;9(5):339-44.

41. Rennels MB. Influence of breast-feeding and oral poliovirus vaccine on the immunogenicity and efficacy of rotavirus vaccines. *J Infect Dis.* 1996;174 Suppl 1:S107-11.

42. Pichichero ME. Effect of breast-feeding on oral rhesus rotavirus vaccine seroconversion: a metaanalysis. *J Infect Dis.* 1990;162(3):753-5.

43. Rennels MB, Wasserman SS, Glass RI, et al. Comparison of immunogenicity and efficacy of rhesus rotavirus reassortant vaccines in breastfed and nonbreastfed children. US Rotavirus Vaccine Efficacy Group. *Pediatrics.* 1995;96(6):1132-6.

44. Vesikari T, Prymula R, Schuster V, et al. Efficacy and Immunogenicity of Live-attenuated Human Rotavirus Vaccine in Breast-fed and Formula-fed European Infants. *Pediatr Infect Dis J.* 2012;31(5):509-13.

45. Goveia MG, DiNubile MJ, Dallas MJ, et al. Efficacy of pentavalent human-bovine (WC3) reassortant rotavirus vaccine based on breastfeeding frequency. *Pediatr Infect Dis J.* 2008;27(7):656-8.

46. Moon SS, Wang Y, Shane AL, et al. Inhibitory effect of breast milk on infectivity of live oral rotavirus vaccines. *Pediatr Infect Dis J.* 2010;29(10):919-23.

47. Moon SS, Tate JE, Ray P, et al. Differential Profiles and Inhibitory Effect on Rotavirus Vaccines of Nonantibody Components in Breast Milk From Mothers in Developing and Developed Countries. *Pediatr Infect Dis J.* 2013;32(8):863-70.

48. Chan J, Nirwati H, Triasih R, et al. Maternal antibodies to rotavirus: could they interfere with live rotavirus vaccines in developing countries? *Vaccine.* 2011;29(6):1242-7.

49. Tino De Franco M, Vieira PD, Santos SM, et al. Neutralizing activity and secretory IgA antibodies reactive with rotavirus SA-11 (serotype G3) in colostrum and milk from Brazilian women. *Paediatr Int Child Health.* 2013;33(2):102-7.

50. Albrecht P, Ennis FA, Saltzman EJ, et al. Persistence of maternal antibody in infants beyond 12 months: mechanism of measles vaccine failure. *J Pediatr.* 1977;91(5):715-8.

51. Perkins FT, Yetts R, Gaisford W. A comparison of the responses of 100 infants to primary poliomyelitis immunization with two and with three doses of vaccine. *Br Med J.* 1959;1(5129):1083-6.

52. Karron RA, Steinhoff MC, Subbarao EK, et al. Safety and immunogenicity of a cold-adapted influenza A (H1N1) reassortant virus vaccine administered to infants less than six months of age. *Pediatr Infect Dis J.* 1995;14(1):10-6.

53. Booy R, Aitken SJ, Taylor S, et al. Immunogenicity of combined diphtheria, tetanus, and pertussis vaccine given at 2, 3, and 4 months versus 3, 5, and 9 months of age. *Lancet.* 1992;339(8792):507-10.

54. Nguyen TV, Yuan L, Azevedo MS, et al. High titers of circulating maternal antibodies suppress effector and memory B-cell responses induced by an attenuated rotavirus

priming and rotavirus-like particle-immunostimulating complex boosting vaccine regimen. *Clin Vaccine Immunol.* 2006;13(4):475-85.

55. Ward RL, Knowlton DR, Zito ET, et al. Serologic correlates of immunity in a tetravalent reassortant rotavirus vaccine trial. US Rotavirus Vaccine Efficacy Group. *J Infect Dis.* 1997;176(3):570-7.

56. McLean B, Holmes IH. Transfer of antirotaviral antibodies from mothers to their infants. *J Clin Microbiol.* 1980;12(3):320-5.

57. Appaiahgari MB, Glass R, Singh S, et al. Transplacental rotavirus IgG interferes with immune response to live oral rotavirus vaccine ORV-116E in Indian infants. *Vaccine.* 2014;32(6):651-6.

58. Siegrist CA. Mechanisms by which maternal antibodies influence infant vaccine responses: review of hypotheses and definition of main determinants. *Vaccine.* 2003;21(24):3406-12.

59. Steele AD, Cunliffe N, Tumbo J, et al. A review of rotavirus infection in and vaccination of human immunodeficiency virus-infected children. *J Infect Dis.* 2009;200 Suppl 1:S57-62.

60. 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.

61. van Eijk AM, Brooks JT, Adcock PM, et al. Diarrhea in children less than two years of age with known HIV status in Kisumu, Kenya. *Int J Infect Dis.* 2010;14(3):e220-5. doi: 10.1016/j.ijid.2009.06.001.

62. Groome MJ, Madhi SA. Five-year cohort study on the burden of hospitalisation for acute diarrhoeal disease in African HIV-infected and HIV-uninfected children: potential benefits of rotavirus vaccine. *Vaccine.* 2012;30 Suppl 1:A173-8.

63. Marinda E, Humphrey JH, Iliff PJ, et al. Child mortality according to maternal and infant HIV status in Zimbabwe. *Pediatr Infect Dis J.* 2007;26(6):519-26.

64. 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.

65. Steele AD, Madhi SA, Louw CE, et al. Safety, Reactogenicity, and Immunogenicity of Human Rotavirus Vaccine RIX4414 in Human Immunodeficiency Virus-positive Infants in South Africa. *Pediatr Infect Dis J.* 2011;30(2):125-30.

66. Laserson KF, Nyakundi D, Feikin DR, et al. Safety of the pentavalent rotavirus vaccine (PRV), RotaTeq((R)), in Kenya, including among HIV-infected and HIV-exposed infants. *Vaccine.* 2012;30 Suppl 1:A61-70.

67. Parker EP, Kampmann B, Kang G, et al. Influence of enteric infections on response to oral poliovirus vaccine: a systematic review and meta-analysis. *J Infect Dis.* 2014;210(6):853-64. doi: 10.1093/infdis/jiu182.

68. Wang H, Moon S, Wang Y, et al. Multiple virus infection alters rotavirus replication and expression of cytokines and Toll-like receptors in intestinal epithelial cells. *Virus Res.* 2012;167(1):48-55.

69. Korpe PS, Petri WA, Jr. Environmental enteropathy: critical implications of a poorly understood condition. *Trends Mol Med.* 2012;18(6):328-36. doi: 10.1016/j.molmed.2012.04.007.

70. Kandasamy S, Chattha KS, Vlasova AN, et al. Prenatal vitamin A deficiency impairs adaptive immune responses to pentavalent rotavirus vaccine (RotaTeq(R)) in a neonatal gnotobiotic pig model. *Vaccine.* 2014;32(7):816-24.

71. Chattha KS, Kandasamy S, Vlasova AN, et al. Vitamin A deficiency impairs adaptive B and T cell responses to a prototype monovalent attenuated human rotavirus vaccine

and virulent human rotavirus challenge in a gnotobiotic piglet model. *PloS One.* 2013;8(12):e82966. doi: 10.1371/journal.pone.0082966.

72. Chattha KS, Vlasova AN, Kandasamy S, et al. Probiotics and colostrum/milk differentially affect neonatal humoral immune responses to oral rotavirus vaccine. *Vaccine.* 2013;31(15):1916-23.

73. Holscher HD, Czerkies LA, Cekola P, et al. Bifidobacterium lactis Bb12 enhances intestinal antibody response in formula-fed infants: a randomized, double-blind, controlled trial. *J Parenter Enteral Nutr.* 2012;36(1 Suppl):106S-17S.

74. Patel M, Steele AD, Parashar UD. Influence of oral polio vaccines on performance of the monovalent and pentavalent rotavirus vaccines. *Vaccine.* 2012;30 Suppl 1:A30-5.

75. Steele AD, De Vos B, Tumbo J, et al. Co-administration study in South African infants of a live-attenuated oral human rotavirus vaccine (RIX4414) and poliovirus vaccines. *Vaccine.* 2010;28(39):6542-8.

76. Ciarlet M, Sani-Grosso R, Yuan G, et al. Concomitant use of the oral pentavalent human-bovine reassortant rotavirus vaccine and oral poliovirus vaccine. *Pediatr Infect Dis J.* 2008;27(10):874-80.

77. Emperador DM, Velasquez DE, Estivariz CF, et al. Interference of monovalent, bivalent, and trivalent oral poliovirus vaccines on monovalent rotavirus vaccine immunogenicity in rural Bangladesh. *Clin Infect Dis.* 2016;62(2):150-6. doi:10.1093/cid/civ807.

78. Tregnaghi MW, Abate HJ, Valencia A, et al. Human rotavirus vaccine is highly efficacious when coadministered with routine expanded program of immunization vaccines including oral poliovirus vaccine in Latin America. *Pediatr Infect Dis J.* 2011;30(6):e103-8.

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

80. Ruuska T, Vesikari T, Delem A, et al. Evaluation of RIT 4237 bovine rotavirus vaccine in newborn infants: correlation of vaccine efficacy to season of birth in relation to rotavirus epidemic period. *Scand J Infect Dis.* 1990;22(3):269-78.

81. Kirkwood CD. Genetic and antigenic diversity of human rotaviruses: potential impact on vaccination programs. *J Infect Dis.* 2010;202 Suppl:S43-8.

82. Seheri M, Nemarude L, Peenze I, et al. Update of rotavirus strains circulating in Africa from 2007 through 2011. *Pediatr Infect Dis J.* 2014;33 Suppl 1:S76-84.

83. Gentsch JR, Laird AR, Bielfelt B, et al. Serotype diversity and reassortment between human and animal rotavirus strains: implications for rotavirus vaccine programs. *J Infect Dis.* 2005;192 Suppl 1:S146-59.

84. Todd S, Page NA, Duncan Steele A, et al. Rotavirus strain types circulating in Africa: Review of studies published during 1997-2006. *J Infect Dis.* 2010;202 Suppl:S34-42.

85. Steele AD, Neuzil KM, Cunliffe NA, et al. Human rotavirus vaccine Rotarix provides protection against diverse circulating rotavirus strains in African infants: a randomized controlled trial. *BMC Infect Dis.* 2012;12:213. doi: 10.1186/1471-2334-12-213.

86. Correia JB, Patel MM, Nakagomi O, et al. Effectiveness of monovalent rotavirus vaccine (Rotarix) against severe diarrhea caused by serotypically unrelated G2P[4] strains in Brazil. *J Infect Dis.* 2010;201(3):363-9.

87. Snelling TL, Andrews RM, Kirkwood CD, et al. Case-control evaluation of the effectiveness of the G1P[8] human rotavirus vaccine during an outbreak of rotavirus G2P[4] infection in central Australia. *Clin Infect Dis.* 2011;52(2):191-9.

88. Patel MM, Steele D, Gentsch JR, et al. Real-world impact of rotavirus vaccination. *Pediatr Infect Dis J.* 2011;30(1 Suppl):S1-5.

89. Meeting of the immunization Strategic Advisory Group of Experts, April 2009-conclusions and recommendations. *Wkly Epidemiol Rec.* 2009;84(23):220-36.

90. Patel MM, Parashar UD. Assessing the effectiveness and public health impact of rotavirus vaccines after introduction in immunization programs. *J Infect Dis.* 2009;200 Suppl 1:S291-9.

91. Yen C, Armero Guardado JA, Alberto P, et al. Decline in rotavirus hospitalizations and health care visits for childhood diarrhea following rotavirus vaccination in El Salvador. *Pediatr Infect Dis J.* 2011;30(1 Suppl):S6-S10.

92. Quintanar-Solares M, Yen C, Richardson V, et al. Impact of rotavirus vaccination on diarrhea-related hospitalizations among children < 5 years of age in Mexico. *Pediatr Infect Dis J.* 2011;30(1 Suppl):S11-5.

93. Richardson V, Hernandez-Pichardo J, Quintanar-Solares M, et al. Effect of rotavirus vaccination on death from childhood diarrhea in Mexico. *N Engl J Med.* 2010;362(4):299-305.

94. do Carmo GM, Yen C, Cortes J, et al. Decline in diarrhea mortality and admissions after routine childhood rotavirus immunization in Brazil: a time-series analysis. *PLoS Med.* 2011;8(4):e1001024. doi: 10.1371/journal.pmed.1001024.

95. Bayard V, DeAntonio R, Contreras R, et al. Impact of rotavirus vaccination on childhood gastroenteritis-related mortality and hospital discharges in Panama. *Int J Infect Dis.* 2012;16(2):e94-8. doi: 10.1016/j.ijid.2011.09.003.

96. Clarke MF, Davidson GP, Gold MS, et al. Direct and indirect impact on rotavirus positive and all-cause gastroenteritis hospitalisations in South Australian children following the introduction of rotavirus vaccination. *Vaccine.* 2011;29(29-30):4663-7.

97. Raes M, Strens D, Vergison A, et al. Reduction in pediatric rotavirus-related hospitalizations after universal rotavirus vaccination in Belgium. *Pediatr Infect Dis J.* 2011;30(7):e120-5.

98. Curns AT, Steiner CA, Barrett M, et al. Reduction in acute gastroenteritis hospitalizations among US children after introduction of rotavirus vaccine: analysis of hospital discharge data from 18 US states. *J Infect Dis.* 2010;201(11):1617-24.

99. Yen C, Tate JE, Wenk JD, et al. Diarrhea-associated hospitalizations among US children over 2 rotavirus seasons after vaccine introduction. *Pediatrics.* 2011;127(1):e9-e15.

100. Dey A, Wang H, Menzies R, et al. Changes in hospitalisations for acute gastroenteritis in Australia after the national rotavirus vaccination program. *Med J Aust.* 2012;197(8):453-7.

101. Paulke-Korinek M, Kollaritsch H, Aberle SW, et al. Sustained low hospitalization rates after four years of rotavirus mass vaccination in Austria. *Vaccine.* 2013;31(24):2686-91.

102. Tate JE, Panozzo CA, Payne DC, et al. Decline and change in seasonality of US rotavirus activity after the introduction of rotavirus vaccine. *Pediatrics.* 2009;124(2):465-71.

103. Braeckman T, Van Herck K, Meyer N, et al. Effectiveness of rotavirus vaccination in prevention of hospital admissions for rotavirus gastroenteritis among young children in Belgium: case-control study. *BMJ.* 2012;345:e4752.

104. Castilla J, Beristain X, Martinez-Artola V, et al. Effectiveness of rotavirus vaccines in preventing cases and hospitalizations due to rotavirus gastroenteritis in Navarre, Spain. *Vaccine.* 2012;30(3):539-43.

105. Boom JA, Tate JE, Sahni LC, et al. Effectiveness of pentavalent rotavirus vaccine in a large urban population in the United States. *Pediatrics.* 2010;125(2):e199-207.

106. Payne DC, Boom JA, Staat MA, et al. Effectiveness of pentavalent and monovalent rotavirus vaccines in concurrent use among US children <5 years of age, 2009-2011. *Clin Infect Dis.* 2013;57(1):13-20.

107. Patel MM, Patzi M, Pastor D, et al. Effectiveness of monovalent rotavirus vaccine in Bolivia: case-control study. *BMJ.* 2013;346:f3726. doi: 10.1136/bmj.f3726.

108. de Palma O, Cruz L, Ramos H, et al. Effectiveness of rotavirus vaccination against childhood diarrhoea in El Salvador: case-control study. *BMJ.* 2010;340:c2825. doi: 10.1136/bmj.c2825.

109. Patel M, Pedreira C, De Oliveira LH, et al. Association between pentavalent rotavirus vaccine and severe rotavirus diarrhea among children in Nicaragua. *JAMA.* 2009;301(21):2243-51.

110. Leshem E, Lopman B, Glass R, et al. Distribution of rotavirus strains and strain-specific effectiveness of the rotavirus vaccine after its introduction: a systematic review and meta-analysis. *Lancet Infect Dis.* 2014;14(9):847-56.

111. Msimang VM, Page N, Groome MJ, et al. Impact of rotavirus vaccine on childhood diarrheal hospitalization after introduction into the South African public immunization program. *Pediatr Infect Dis J.* 2013;32(12):1359-64.

112. Enweronu-Laryea CC, Boamah I, Sifah E, et al. Decline in severe diarrhea hospitalizations after the introduction of rotavirus vaccination in Ghana: a prevalence study. *BMC Infect Dis.* 2014;14:431. doi: 10.1186/1471-2334-14-431.

113. Griffin MR, Zhu Y, Moore MR, et al. U.S. hospitalizations for pneumonia after a decade of pneumococcal vaccination. *N Engl J Med.* 2013;369(2):155-63.

114. Adegbola RA, Secka O, Lahai G, et al. Elimination of Haemophilus influenzae type b (Hib) disease from The Gambia after the introduction of routine immunisation with a Hib conjugate vaccine: a prospective study. *Lancet.* 2005;366(9480):144-50.

115. Lopman BA, Curns AT, Yen C, et al. Infant rotavirus vaccination may provide indirect protection to older children and adults in the United States. *J Infect Dis.* 2011;204(7):980-6.

116. Gastanaduy PA, Curns AT, Parashar UD, et al. Gastroenteritis hospitalizations in older children and adults in the United States before and after implementation of infant rotavirus vaccination. *JAMA.* 2013;310(8):851-3.

117. Paulke-Korinek M, Kundi M, Rendi-Wagner P, et al. Herd immunity after two years of the universal mass vaccination program against rotavirus gastroenteritis in Austria. *Vaccine.* 2011;29(15):2791-6.

118. Linhares AC, Velazquez FR, Perez-Schael I, et al. Efficacy and safety of an oral live attenuated human rotavirus vaccine against rotavirus gastroenteritis during the first 2 years of life in Latin American infants: a randomised, double-blind, placebo-controlled phase III study. *Lancet.* 2008;371(9619):1181-9.

119. Madhi SA, Kirsten M, Louw C, et al. Efficacy and immunogenicity of two or three dose rotavirus-vaccine regimen in South African children over two consecutive rotavirus-seasons: A randomized, double-blind, placebo-controlled trial. *Vaccine.* 2012;30 Suppl 1:A44-51.

120. Cunliffe NA, Witte D, Ngwira BM, et al. Efficacy of human rotavirus vaccine against severe gastroenteritis in Malawian children in the first two years of life: a randomized, double-blind, placebo controlled trial. *Vaccine.* 2012;30 Suppl 1:A36-43.

121. Patel M, Pedreira C, De Oliveira LH, et al. Duration of protection of pentavalent rotavirus vaccination in Nicaragua. *Pediatrics.* 2012;130(2):e365-72.

122. Johnson B, Gargiullo P, Murphy TV, et al. Sociodemographic and dietary risk factors for natural infant intussusception in the United States. *J Pediatr Gastroenterol Nutr.* 2010;51(4):458-63.

123. Jiang J, Jiang B, Parashar U, et al. Childhood intussusception: a literature review. *PloS One.* 2013;8(7):e68482. doi: 10.1371/journal.pone.0068482.

124. Okimoto S, Hyodo S, Yamamoto M, et al. Association of viral isolates from stool samples with intussusception in children. *Int J Infect Dis.* 2011;15(9):e641-5. doi: 10.1016/j.ijid.2011.05.008.

125. Mansour AM, El Koutby M, El Barbary MM, et al. Enteric viral infections as potential risk factors for intussusception. *J Infect Dev Ctries.* 2013;7(1):28-35. doi: 10.3855/jidc.2321.

126. Nylund CM, Denson LA, Noel JM. Bacterial enteritis as a risk factor for childhood intussusception: a retrospective cohort study. *J Pediatr.* 2010;156(5):761-5.

127. 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.

128. Patel MM, Lopez-Collada VR, Bulhoes MM, et al. Intussusception risk and health benefits of rotavirus vaccination in Mexico and Brazil. *N Engl J Med.* 2011;364(24):2283-92.

129. Carlin JB, Macartney KK, Lee KJ, et al. Intussusception risk and disease prevention associated with rotavirus vaccines in Australia's National Immunization Program. *Clin Infect Dis.* 2013;57(10):1427-34.

130. Yih WK, Lieu TA, Kulldorff M, et al. Intussusception risk after rotavirus vaccination in U.S. infants. *N Engl J Med.* 2014;370(6):503-12.

131. Weintraub ES, Baggs J, Duffy J, et al. Risk of intussusception after monovalent rotavirus vaccination. *N Engl J Med.* 2014;370(6):513-9.

132. Buttery JP, Standish J, Bines JE. Intussusception and rotavirus vaccines: consensus on benefits outweighing recognized risk. *Pediatr Infect Dis J.* 2014;33(7):772-3.

133. Statistics South Africa (STATSSA). Mid-year population estimates. 2006-2014. Pretoria: Statistics South Africa. Available: http://www.statssa.gov.za [accessed 26 Feb 2016].

134. National Department of Health, South Africa.The national antenatal sentinel HIV and syphilis prevalence survey, South Africa. 2011. Available: http://www.health.gov.za/index.php/2014-03-17-09-09-38/reports/category/99-2013re. [accessed 13 January 2016].

135. Actuarial Society of South Africa (ASSA). AIDS and Demographic Model 2008. Available: http://aids.actuarialsociety.org.za [accessed 22 Jul 2015].

136. Barron P, Pillay Y, Doherty T, et al. Eliminating mother-to-child HIV transmission in South Africa. *Bull World Health Organ.* 2013;91(1):70-4.

137. Johnson L. Access to antiretroviral treatment in South Africa, 2004 - 2011. *South Afr J HIV Med.* 2012;13:22-27. Available: http://www.sajhivmed.org.za/index.php/hivmed/article/view/156/261 [accessed 26 Feb 2016].

138. Global report: UNAIDS report on the global AIDS epidemic 2013. Available: http://www.unaids.org/sites/default/files/media_asset/UNAIDS_Global_Report_2013_en_1.pdf [accessed 2 February 2015].

139. The World Bank. World Development Indicators - South Africa. Available: http://data.worldbank.org/country/south-africa [accessed 19 September 2015].

140. Madhi SA, Bamford L, Ngcobo N. Effectiveness of pneumococcal conjugate vaccine and rotavirus vaccine introduction into the South African public immunisation programme. *S Afr Med J.* 2014;104(3 Suppl 1):228-34.

141. WHO. WHO vaccine-preventable diseases: monitoring system. 2015 global summary. Available: http://apps.who.int/immunization_monitoring/globalsummary [accessed 15 September 2015].

142. Hennekens CH, Buring JE. Epidemiology in medicine. 1st ed. Boston: Little, Brown; 1987.

143. Rid A, Saxena A, Baqui AH, et al. Placebo use in vaccine trials: Recommendations of a WHO expert panel. *Vaccine.* 2014;32(37):4708-12.

144. Hanquet G, Valenciano M, Simondon F, et al. Vaccine effects and impact of vaccination programmes in post-licensure studies. *Vaccine.* 2013;31(48):5634-42.

145. Mast TC, Khawaja S, Espinoza F, et al. Case-control study of the effectiveness of vaccination with pentavalent rotavirus vaccine in Nicaragua. *Pediatr Infect Dis J.* 2011;30(11):e209-15.

146. Boom JA, Tate JE, Sahni LC, et al. Sustained protection from pentavalent rotavirus vaccination during the second year of life at a large, urban United States pediatric hospital. *Pediatr Infect Dis J.* 2010;29(12):1133-5.

147. Yen C, Figueroa JR, Uribe ES, et al. Monovalent rotavirus vaccine provides protection against an emerging fully heterotypic G9P[4] rotavirus strain in Mexico. *J Infect Dis.* 2011;204(5):783-6.

148. Rose S, Laan MJ. Why match? Investigating matched case-control study designs with causal effect estimation. *Int J Biostat.* 2009;5(1):Article 1. doi: 10.2202/1557-4679.1127.

149. Jackson ML, Nelson JC. The test-negative design for estimating influenza vaccine effectiveness. *Vaccine.* 2013;31(17):2165-8.

150. Bar-Zeev N, Kapanda L, Tate JE, et al. Effectiveness of a monovalent rotavirus vaccine in infants in Malawi after programmatic roll-out: an observational and case-control study. *Lancet Infect Dis.* 2015;15(4):422-8.

151. World Health Organization. Generic protocol for monitoring impact of rotavirus vaccination on gastroenteritis disease burden and viral strains. 2008. Available: http://www.who.int/immunization/documents/monitoring/en/ [accessed 26 Feb 2016].

152. Aliabadi N, Tate JE, Haynes AK, et al. Sustained decrease in laboratory detection of rotavirus after implementation of routine vaccination-United States, 2000-2014. *MMWR. Morb Mortal Wkly Rep.* 2015;64(13):337-42.

153. Esparza-Aguilar M, Gastanaduy PA, Sanchez-Uribe E, et al. Diarrhoea-related hospitalizations in children before and after implementation of monovalent rotavirus vaccination in Mexico. *Bull World Health Organ.* 2014;92(2):117-25.

154. de Moraes-Pinto MI, Almeida AC, Kenj G, et al. Placental transfer and maternally acquired neonatal IgG immunity in human immunodeficiency virus infection. *J Infect Dis.* 1996;173(5):1077-84.

155. Jones CE, Naidoo S, De Beer C, et al. Maternal HIV infection and antibody responses against vaccine-preventable diseases in uninfected infants. *JAMA.* 2011;305(6):576-84.

156. De Oliveira LH, Giglio N, Ciapponi A, et al. Temporal trends in diarrhea-related hospitalizations and deaths in children under age 5 before and after the introduction of the rotavirus vaccine in four Latin American countries. *Vaccine.* 2013;31 Suppl 3:C99-108.

157. Steele AD, Reynders J, Scholtz F, et al. Comparison of 2 different regimens for reactogenicity, safety, and immunogenicity of the live attenuated oral rotavirus vaccine

RIX4414 coadministered with oral polio vaccine in South African infants. *J Infect Dis.* 2010;202 Suppl:S93-100.

158. Benson K, Hartz AJ. A comparison of observational studies and randomized, controlled trials. *N Engl J Med.* 2000;342(25):1878-86.
159. Atherly D, Dreibelbis R, Parashar UD, et al. Rotavirus vaccination: cost-effectiveness and impact on child mortality in developing countries. *J Infect Dis*. 2009;200 Suppl 1:S28-38.
160. John TJ, Devarajan LV, Luther L, et al. Effect of breast-feeding on seroresponse of infants to oral poliovirus vaccination. *Pediatrics.* 1976;57(1):47-53.
161. Rongsen-Chandola T, Strand TA, Goyal N, et al. Effect of withholding breastfeeding on the immune response to a live oral rotavirus vaccine in North Indian infants. *Vaccine.* 2014;32 Suppl 1:A134-9.
162. Rongsen-Chandola T, Winje BA, Goyal N, et al. Compliance of mothers following recommendations to breastfeed or withhold breast milk during rotavirus vaccination in North India: a randomized clinical trial. *Trials.* 2014;15:256.
163. Ali A, Kazi AM, Cortese MM, et al. Impact of withholding breastfeeding at the time of vaccination on the immunogenicity of oral rotavirus vaccine-a randomized trial. *PloS One.* 2015;10(6):e0127622. doi: 10.1371/journal.pone.0127622.
164. Becker-Dreps S, Vilchez S, Velasquez D, et al. Rotavirus-specific IgG antibodies from mothers' serum may inhibit infant immune responses to the pentavalent rotavirus vaccine. *Pediatr Infect Dis J.* 2015;34(1):115-6.
165. Becker-Dreps S, Choi WS, Stamper L, et al. Innate immune factors in mothers' breast milk and their lack of association with rotavirus vaccine immunogenicity in Nicaraguan infants. *J Pediatric Infect Dis Soc.* 2015. doi:10.1093/jpids/piv076.

166. 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.

167. Meyers T, Dramowski A, Schneider H, et al. Changes in pediatric HIV-related hospital admissions and mortality in Soweto, South Africa, 1996-2011: light at the end of the tunnel? *J Acquir Immune Defic Syndr.* 2012;60(5):503-10.

168. Statistics South Africa (STATSSA). Census 2011 municipal report—Gauteng. Pretoria: Statistics South Africa, 2012. Available: http://www.statssa.gov.za/census/census_2011/census_products/GP_Municipal_Report.pdf [accessed 26 Feb 2016].

169. Gautam R, Lyde F, Esona MD, et al. Comparison of Premier Rotaclone(R), ProSpecT, and RIDASCREEN(R) rotavirus enzyme immunoassay kits for detection of rotavirus antigen in stool specimens. *J Clin Virol.* 2013;58(1):292-4.

170. de Oliveira LH, Danovaro-Holliday MC, Matus CR, et al. Rotavirus vaccine introduction in the Americas: progress and lessons learned. *Expert review of vaccines.* 2008;7(3):345-53.

171. Ali SA, Kazi AM, Cortese MM, et al. Impact of different dosing schedules on the immunogenicity of the human rotavirus vaccine in infants in Pakistan: a randomized trial. *J Infect Dis.* 2014;210(11):1772-9.

172. Rotavirus vaccines. WHO position paper - January 2013. *Wkly Epidemiol Rec.* 2013;88(5):49-64.

173. Zaman K, Fleming JA, Victor JC, et al. Non-interference of rotavirus vaccine with measles-rubella vaccine at 9 months and improvements in anti-rotavirus immunity: a randomized trial. *J Infect Dis.* 2016. doi:10.1093/infdis/jiw024.

174. Danchin M, Kirkwood CD, Lee KJ, et al. Phase I trial of RV3-BB rotavirus vaccine: a human neonatal rotavirus vaccine. *Vaccine.* 2013;31(23):2610-6.

175. Bines JE, Danchin M, Jackson P, et al. Safety and immunogenicity of RV3-BB human neonatal rotavirus vaccine administered at birth or in infancy: a randomised, double-blind, placebo-controlled trial. *Lancet Infect Dis.* 2015(DOI:10.1016/S1473-3099(15)00227-3).

176. Premkumar PS, Parashar UD, Gastanaduy PA, et al. Reduced rotavirus vaccine effectiveness among children born during the rotavirus season: a pooled analysis of 5 case-control studies from the Americas. *Clin Infect Dis.* 2015;60(7):1075-8.

177. Guerrant RL, Lohr JA, Williams EK. Acute infectious diarrhea. I. Epidemiology, etiology and pathogenesis. *Pediatr Infect Dis.* 1986;5(3):353-9.

178. Pattanayak CW, Rubin DB, Zell ER. [Propensity score methods for creating covariate balance in observational studies]. *Rev Esp Cardiol.* 2011;64(10):897-903.

179. De Vos B, Han HH, Bouckenooghe A, et al. Live attenuated human rotavirus vaccine, RIX4414, provides clinical protection in infants against rotavirus strains with and without shared G and P genotypes: integrated analysis of randomized controlled trials. *Pediatr Infect Dis J.* 2009;28(4):261-6.

180. Gurgel RQ, Alvarez Ade J, Rodrigues A, et al. Incidence of rotavirus and circulating genotypes in Northeast Brazil during 7 years of national rotavirus vaccination. *PloS One.* 2014;9(10):e110217. doi: 10.1371/journal.pone.0110217.

181. Global Advisory Committee on Vaccine Safety, 11-12 December 2013. *Wkly Epidemiol Rec.* 2014;89(7):53-60.

182. Jiang B, Gentsch JR, Glass RI. Inactivated rotavirus vaccines: a priority for accelerated vaccine development. *Vaccine.* 2008;26(52):6754-8.

183. Fix AD, Harro C, McNeal M, et al. Safety and immunogenicity of a parenterally administered rotavirus VP8 subunit vaccine in healthy adults. *Vaccine.* 2015;33(31):3766-72.

184. Groome M, Koen A, Jose L. A Phase I/II descending age, double-blinded, randomized, placebo-controlled dose escalation study to examine the safety, tolerability and immunogenicity of the P2-VP8 subunit parenteral rotavirus vaccine in healthy South African toddlers and infants. Paper presented at the 8th Conference on Vaccines for Enteric Diseases; 2015 July 8-10; Edinburgh, Scotland.

APPENDICES

Appendix A - Role of the student

Appendix B - Ethical clearance certificate

Paper I

Paper II

Paper III

Paper IV

Roles played by the student in the papers 09 November 2015

Michelle Jennifer Groome, student number 9200682V, had the following roles in the studies and the writing of the papers forming part of her thesis entitled: "Rotavirus vaccine and diarrhoeal morbidity in South Africa".

Paper I

I. Groome MJ, Page N, Cortese MM, Moyes J, Zar HJ, Kapongo CN, Mulligan M, Diedericks R, Cohen C, Fleming JA, Seheri M, Mphahlele J, Walaza S, Kahn K, Chhagan M, Steele AD, Parashar UD, Zell ER, Madhi SA. Effectiveness of monovalent human rotavirus vaccine against admission to hospital for acute rotavirus diarrhoea in South African children: a case-control study. Lancet Infect Dis. 2014;14(11):1096-104.

Project title: *Case-Control Study to Assess the Effectiveness of Rotarix Vaccine in HIV infected and HIV uninfected Children in South Africa.*

Role of the student: Conception and design of the study, development of the protocol, ethics application, case report form design, overall co-ordination of the study including oversight of all study sites, data collation and cleaning, data analysis, interpretation of the data, drafting of paper and revising drafts of the paper.

Paper II

II. Groome MJ, Zell ER, Solomon F, Izu A, Nzenze S, Parashar UD, Shabir A. Temporal association of rotavirus vaccine introduction and reduction in all-cause childhood diarrhoeal hospitalisations in South Africa. Under review *Clin Infect Dis.*

Project title: *A Time Series Analysis: All-Cause and Disease Specific In-Hospital Mortality and Hospitalisation Rates Pre and Post Introduction of Pneumococcal and Rotavirus Vaccination*

Role of the student: Conception and design of the study, development of the protocol, ethics application of the protocol, data collation and cleaning, data analysis, interpretation of the data, drafting of paper and revising drafts of the paper.

Paper III and IV

III. Groome MJ, Moon SS, Velasquez D, Jones S, Koen A, van Niekerk N, Jiang B, Parashar UD, Madhi SA. Effect of breastfeeding on immunogenicity of oral live-attenuated human rotavirus vaccine: a randomized trial in HIV-uninfected infants in Soweto, South Africa. Bull World Health Organ 2014;92:238-45.

IV. Moon SS, Groome MJ, Velasquez DE, Parashar UD, Jones S, Koen A, van Niekerk N, Jiang B, Madhi SA. Pre-vaccination rotavirus serum IgG and IgA are associated with lower immunogenicity of live-oral human rotavirus vaccine in South African infants. *Clin Infect Dis* 2015. Published online 23 Sept 2015

Project title: *Immunogenicity of Pentaxim, Prevnar, Rotarix and other childhood vaccines included in the new immunization schedule for South African infants.*

Role of the student: Design of the study, development of the protocol, ethics application of the protocol, case report form design, overall co-ordination of the study including enrolment and follow up of participants, data collation and cleaning, data analysis, interpretation of the data, drafting of papers and revising drafts of the papers. Sung-sil Moon is the first author on Paper IV, but the student was integrally involved in the project as a whole as outlined above, and contributed extensively to this paper.

All co-authors have been informed that the papers are to be used in a PhD thesis and agree on their use within the thesis.

Shabir A Madhi (supervisor)

Elizabeth R Zell (supervisor)

Michelle J Groome (student)

Sungsil-sil Moon (first author Paper IV)

R14/49 Dr Michelle Groome

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M130273

NAME: Dr Michelle Groome
(Principal Investigator)

DEPARTMENT: School of Public Health
Medical School

PROJECT TITLE: Rotavirus Vaccine and Diarrhoeal Morbidity in South Africa (under protocol M110528)

DATE CONSIDERED: Ad hoc

DECISION: Approved unconditionally

CONDITIONS:

SUPERVISOR: Professor shabir Madhi

APPROVED BY:

Professor PE Cleaton-Jones, Chairperson, HREC (Medical)

DATE OF APPROVAL: 22/02/2013

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**.

Principal Investigator Signature

Date 19/03/2013.

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

PAPER I

Effectiveness of monovalent human rotavirus vaccine against admission to hospital for acute rotavirus diarrhoea in South African children: a case-control study

Michelle J Groome, Nicola Page, Margaret M Cortese, Jocelyn Moyes, Heather J Zar, Constant N Kapongo, Christine Mulligan, Ralph Diedericks, Cheryl Cohen, Jessica A Fleming, Mapaseka Seheri, Jeffrey Mphahlele, Sibongile Walaza, Kathleen Kahn, Meera Chhagan, A Duncan Steele, Umesh D Parashar, Elizabeth R Zell, Shabir A Madhi

Lancet Infect Dis 2014; 14: 1096–104

Published Online October 8, 2014 http://dx.doi.org/10.1016/S1473-3099(14)70940-5

This online publication has been corrected. The corrected version first appeared at thelancet.com/infection on October 20, 2014

See Comment page 1031

Department of Science and Technology/National Research Foundation: Vaccine Preventable Diseases (M J Groome MBBCh, Prof S A Madhi PhD), Medical Research Council: Respiratory and Meningeal Pathogens Research Unit (M J Groome, Prof S A Madhi), School of Public Health, Faculty of Health Sciences (J Moyes MBBCh, C Cohen MBBCh), and MRC/Wits Rural Public Health and Health Transitions Research Unit (Agincourt), School of Public Health, Faculty of Health Sciences (K Kahn PhD), University of the Witwatersrand, Johannesburg, South Africa; National Institute for Communicable Diseases: a Division of National Health Laboratory Service, Sandringham, South Africa (N Page PhD, J Moyes, C Cohen, S Walaza MBBCh, Prof S A Madhi); Division of Viral Diseases, Centers for Disease Control and Prevention, Atlanta, GA, USA (M M Cortese MD, U D Parashar MBBS); Department of Paediatrics and Child Health, Red Cross War Memorial Children's Hospital, University of Cape Town, Cape Town, South Africa (Prof H J Zar PhD, C Mulligan MBChB, R Diedericks FCP[Paed]); Department of Paediatrics, Ngwelezane Hospital, Empangeni, South Africa (C N Kapongo FCPaeds[SA]); PATH, Seattle, WA, USA

Summary

Background The effectiveness of the rotavirus vaccine under conditions of routine use in an African setting with a high prevalence of HIV infection needs to be established. We assessed the vaccine effectiveness of monovalent human rotavirus vaccine in preventing admission to hospital for acute rotavirus diarrhoea, after its introduction at age 6 and 14 weeks into South Africa's national immunisation programme.

Methods This case-control study was done at seven hospitals in South Africa between April 19, 2010, and Oct 31, 2012. The hospitals were located in a range of urban, peri-urban, and rural settings, with varying rates of population HIV infection. Cases were children aged from 18 weeks to 23 months who were age-eligible to have received at least one dose of the human rotavirus vaccine (ie, those born after June 14, 2009) admitted to hospital with laboratory-confirmed acute rotavirus diarrhoea, and the primary control group was children admitted to hospital with diarrhoea testing negative for rotavirus. A second control group comprised children admitted to a subset of three of the seven hospitals with respiratory illness. The primary endpoint was adjusted vaccine effectiveness (1−adjusted odds ratio×100%) in children aged from 18 weeks to 23 months and was calculated by unconditional logistic regression. This study is registered on the South African National Clinical Trial Register, number DOH-27-0512-3247.

Findings Of 540 rotavirus-positive cases, 278 children (52%) received two doses, 126 (23%) one dose, and 136 (25%) no doses of human rotavirus vaccine, compared with 1434 rotavirus-negative controls of whom 856 (60%) received two doses, 334 (23%) one dose, and 244 (17%) no doses. Adjusted vaccine effectiveness using rotavirus-negative controls was 57% (95% CI 40–68) for two doses and 40% (16–57) for one dose; estimates were similar when respiratory controls were used as the control group. Adjusted vaccine effectiveness for two doses was similar between age groups 18 weeks–11 months (54%, 95% CI 32–68) and 12–23 months (61%, 35–77), and was similar in HIV-exposed-uninfected (64%, 95% CI 34–80) and HIV-unexposed-uninfected children (54%, 31–69).

Interpretation Human rotavirus vaccine provided sustained protection against admission to hospital for acute rotavirus diarrhoea during the first and second years of life. This finding is encouraging and establishes the public health value of rotavirus vaccine in an African setting, especially as rotavirus vaccines are introduced into an increasing number of African countries.

Funding GAVI Alliance (with support from PATH).

Introduction

Rotavirus is the leading cause of diarrhoeal morbidity and mortality in children younger than 5 years, accounting for an estimated 453 000 global deaths in 2008, with more than 90% of deaths occurring in low-income countries in Africa and Asia.[1,2] In 2009, diarrhoea was a leading cause of death in South African children younger than 5 years,[3] and rotavirus was detected in about 25% of children admitted to hospital for diarrhoea before the rotavirus vaccine was introduced in August, 2009.[4]

Two oral rotavirus vaccines—a monovalent human rotavirus vaccine (Rotarix, GlaxoSmithKline Biologicals, Rixensart, Belgium) and a pentavalent bovine–human reassortant rotavirus vaccine (RotaTeq, Merck Vaccines, Whitehouse Station, NJ, USA)—are recommended for global use by WHO.[5] Both vaccines showed high efficacy against severe rotavirus diarrhoea in trials undertaken in middle-income and high-income countries in Europe and Latin America.[6,7] However, lower immunogenicity and vaccine efficacy were shown in subsequent trials in low-income and middle-income countries in Asia and Africa.[8–10]

Efficacy trials of the monovalent human rotavirus vaccine showed sustained protection through 2 years,[11,12] but some post-licensure studies in low-income and middle-income countries have recorded lower degrees of protection in children aged 12 months and older, suggesting the possibility of waning immunity.[13,14] In a trial of the human rotavirus vaccine in South Africa, vaccine efficacy against severe rotavirus diarrhoea in the composite group of vaccinees who received either two

doses (at 10 and 14 weeks of age) or three doses (at 6, 10, and 14 weeks of age) of human rotavirus vaccine was 77% (95% CI 56–88) during the first year of life and 59% (1–83) during two consecutive rotavirus seasons.[8,15] However, the study was not powered to specifically assess efficacy in the second year of life.

The performance of rotavirus vaccines post-licensure has been assessed in countries in the Americas, Australia, and Europe,[16] but no published data assessing the effectiveness of these vaccines are yet available from Africa. It is important to establish the effectiveness of rotavirus vaccination in an African setting, under conditions of routine use, including durability of protection in the second year of life for the two-dose series of the monovalent human rotavirus vaccine.

South Africa was the first African country to introduce rotavirus vaccine into its national immunisation programme, starting in August, 2009, with vaccination recommended at 6 and 14 weeks of age. Children receive trivalent oral polio vaccine (Merieux; Sanofi Pasteur, Lyon, France) concurrent with human rotavirus vaccine at the 6-week immunisation visit.[17] District Health Information Systems estimates of coverage rates for the second dose of rotavirus vaccine increased from 67% in 2010 to 96% in 2011.[18] In the same year, the estimated national HIV prevalence was 30% in pregnant women aged 15–49 years.[19]

We did a case-control study to assess the vaccine effectiveness of human rotavirus vaccine in preventing admission to hospital for acute rotavirus diarrhoea in children younger than 2 years in a setting with a high prevalence of HIV exposure in infants.

Methods

Study design and participants

The study was done between April 19, 2010, and Oct 31, 2012, at seven hospitals in South Africa, including urban, peri-urban, and rural settings, with varying rates of population HIV infection. The hospitals were: Chris Hani Baragwanath Academic Hospital, Mapulaneng Hospital, Matikwane Hospital, Dr George Mukhari Academic Hospital, Edendale Hospital, Ngwelezane Hospital, and Red Cross War Memorial Children's Hospital (figure 1 and appendix pp 1–3). All hospitals have inpatient and outpatient facilities and care is provided free of charge. Children with diarrhoea present either to community clinics, where hospital referral is based on health-care worker assessment, or directly to the hospital. All decisions regarding admission, investigations, and treatment were made by the attending physician, independent of study staff.

Children admitted to hospital overnight with physician-diagnosed acute diarrhoea who were age-eligible to have received at least one dose of human rotavirus vaccine (ie, those born after June 14, 2009) were invited to participate. Written consent was obtained from a parent or guardian. A stool specimen was collected within 48 h of admission

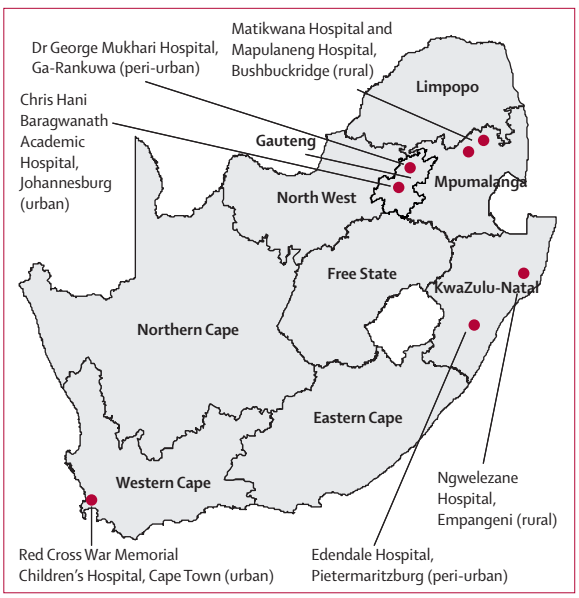


Figure 1: **Location of study hospitals in South Africa**
Antenatal HIV prevalence in each province in 2011: Gauteng 29% (95% CI 27–30); Mpumalanga 37% (34–39), KwaZulu-Natal 37% (36–39), Western Cape 18% (14–23).[19]

and was stored at 2–8°C before transfer to the National Institute for Communicable Diseases (Sandringham, South Africa) or MRC Diarrhoeal Pathogens Research Unit (Pretoria, South Africa), where rotavirus testing was done by a commercially available enzyme immunoassay (ProSpecT; ELISA, Oxford, UK). Rotavirus-positive samples were genotyped with standardised methods.[20] Stool samples from all hospitals except for Dr George Mukhari Academic Hospital were also tested for the presence of adenovirus, norovirus genogroup I and II, astrovirus, and sapovirus by real-time PCR detection assays (in accordance with published methods; see appendix p 4).

Children fulfilling the case definition for acute diarrhoea (≤7 days duration at admission and three or more loose stools in a 24-h period) and EIA-confirmed rotavirus infection were classified as cases. Children with acute diarrhoea testing rotavirus negative by EIA served as the primary control group. A second control group was selected from children enrolled into a concurrent study investigating the effectiveness of a pneumococcal conjugate vaccine at three of the hospitals (Chris Hani Baragwanath Academic, Ngwelezane, and Red Cross War Memorial Children's hospitals). Age-eligible children admitted to hospital with physician-diagnosed lower respiratory tract infection were assessed with C-reactive protein and chest radiograph.[21] Children who were presumed not to have bacterial pneumonia (ie, those with C-reactive protein <381 nmol/L [<40 mg/L] and without radiologically confirmed pneumonia) were eligible for inclusion into the respiratory control group, since they could be judged unlikely to have a vaccine-preventable

(J A Fleming PhD, A D Steele PhD); **MRC/Diarrhoeal Pathogens Research Unit, Department of Virology, Medunsa Campus, University of Limpopo/National Health Laboratory Service, Pretoria, South Africa** (M Seheri PhD, Prof J Mphahlele PhD, A D Steele); **Centre for Global Health Research, Umeå University, Umeå, Sweden** (K Kahn); **INDEPTH Network, Accra, Ghana** (K Kahn); **Department of Paediatrics and Child Health, University of KwaZulu-Natal, South Africa** (M Chhagan*); **Bill & Melinda Gates Foundation, Seattle, Washington, USA** (A D Steele†); **and Stat-Epi Associates Inc, FL, USA** (E R Zell MStat)

*Dr Chhagan died in August, 2014

†Denotes current affiliation

Correspondence to:
Dr Michelle J Groome, New Nurses Residence, 11th Floor West Wing, Chris Hani Baragwanath Academic Hospital, Chris Hani Road, Soweto, 2013, South Africa
groomem@rmpru.co.za

See Online for appendix

disease. At the time of study initiation, few published studies were available that used rotavirus-negative children with diarrhoea as controls in assessments of rotavirus vaccine effectiveness. Because of the importance of studying effectiveness in an African setting and the fact that every control group has some advantages and disadvantages for its use, we felt it prudent to include a second non-diarrhoea control group because overall consistency in results would strengthen our findings.

We obtained ethical approvals from the ethics committees of the University of the Witwatersrand, University of KwaZulu-Natal, University of Cape Town, and University of Limpopo, and from PATH's Research Ethics Committee. Respiratory controls consented as part of the pneumococcal conjugate vaccine effectiveness study with separate ethical approval. This study is registered on the South African National Clinical Trial Register, number DOH-27-0512-3247.

Procedures

Demographic characteristics, medical history, clinical presentation, and outcome were obtained by interview with each child's parent or guardian and by hospital record review. Vaccination history was ascertained by review of the vaccination card, a photocopy of which was obtained when possible, before determination of case or control status. If the vaccination card was unavailable but the parent reported that the child had not received any vaccines other than those given at birth, the child was included and judged not to have received any doses of human rotavirus vaccine. When a photocopy of the vaccination card was available, discrepancies between the photocopy and transcribed vaccination data were reviewed, after which a final vaccination status was assigned.

HIV testing included HIV ELISA testing for children aged 18 months or older and those born to women not infected with HIV; or HIV PCR testing in children younger than 18 months born to women known to be infected with HIV or if maternal HIV status was unknown. HIV PCR testing was done for children who tested HIV positive on ELISA. An HIV-uninfected child was judged to be HIV-exposed if the mother gave a history of testing HIV positive during pregnancy, or HIV-unexposed if the mother had a history of negative HIV tests during pregnancy.

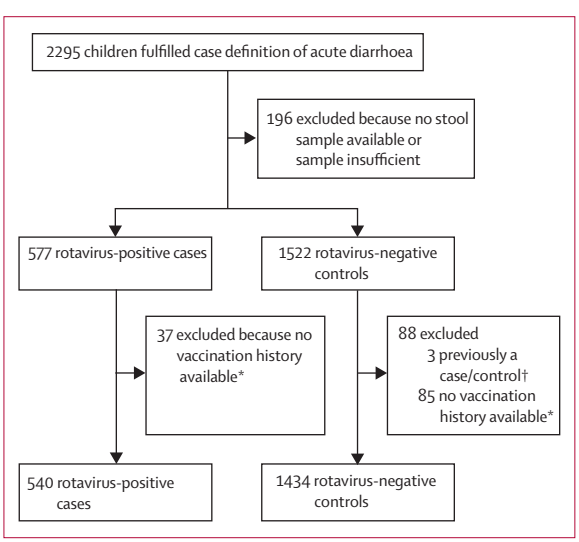


Figure 2: **Enrolment of children aged from 18 weeks to 23 months with acute diarrhoea, and age-eligible to have received rotavirus vaccine (ie, born after June 14, 2009) at seven hospitals in South Africa**

Enrolled children age-eligible to receive rotavirus vaccine and aged <18 weeks and ≥24 months are not included in the enrolment figure. *539 of 540 rotavirus-positive cases and 1427 of 1434 rotavirus-negative controls had a vaccination card available for review. †A child enrolled more than once into the study for acute diarrhoea with any stool testing positive for rotavirus was defined as a case and any other admissions for that child were excluded from the analysis. If all stool results were rotavirus negative, the first admission to hospital for diarrhoea contributed to the control data.

Statistical analysis

In the primary analysis, we assessed the primary endpoint of adjusted vaccine effectiveness of two human rotavirus vaccine doses compared with no vaccination in the prevention of admission to hospital for acute rotavirus diarrhoea in children aged from 18 weeks to 23 months, irrespective of their HIV infection status, with use of rotavirus-negative controls. We excluded children without an adequate stool sample or available vaccination history. We counted a dose of human rotavirus vaccine if it was given 14 days or more before admission to hospital. Our secondary analyses were limited to children from the three hospitals where respiratory controls were enrolled.

We calculated that a minimum sample size of 148 cases with rotavirus diarrhoea was needed for the primary analysis, based on an estimated two-dose vaccine effectiveness of 60%,[8,15] with a control-to-case ratio of 2:1, and two-dose estimated vaccine coverage in controls of 80%. We enrolled additional cases to enable the stratified analyses specified in the secondary objectives.

We used unconditional logistic regression to estimate adjusted odds ratios (aOR) with associated 95% CIs, and we calculated adjusted vaccine effectiveness as $(1-\text{aOR})\times 100\%$. We included hospital, birth month, birth year, month by year of birth (interaction term), quarter of hospital admission, year of hospital admission, and quarter by year of hospital admission (interaction term) in the models a priori. We included other covariates in the models if their inclusion changed the OR associated with vaccination by more than 5%. We did stratified analyses to assess adjusted vaccine effectiveness by age (18 weeks–11 months and 12–23 months) and by HIV exposure status in HIV-uninfected children. We included interaction terms for age or HIV exposure and vaccination status in the model for these analyses. All reported adjusted vaccination effectiveness estimates were against admission to hospital for acute rotavirus diarrhoea. We used STATA version 12.1 for all analyses.

Role of the funding source

The funder had no role in the writing of the report or the decision to submit for publication. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

1974 children with acute diarrhoea aged from 18 weeks to 23 months fulfilled the study case definition and had a stool sample and vaccination history available: 540 rotavirus-positive cases and 1434 rotavirus-negative controls (figure 2). 37 (6%) of 577 rotavirus cases and 85 (6%) of 1522 rotavirus-negative controls did not have a vaccination history available and were therefore excluded from analyses. We recorded no significant differences in characteristics between children with and without an available vaccination history (data not shown). In the subset of three hospitals with respiratory controls (Chris Hani Baragwanath Academic Hospital, Ngwelezane Hospital, and Red Cross War Memorial Children's Hospital), 370 rotavirus-positive cases, 1024 rotavirus-negative controls, and 1069 respiratory controls were included in the analysis (appendix pp 5–6). Table 1 shows comparisons of characteristics between cases and controls.

	All hospitals			Subset of hospitals*				
	Rotavirus-positive cases (n=540)	Rotavirus-negative controls (n=1434)	p value	Rotavirus-positive cases (n=370)	Rotavirus-negative controls (n=1024)	p value	Respiratory controls (n=1069)	p value
Hospital			0·029			0·279		<0·0001
Chris Hani Baragwanath Academic	101 (19%)	283 (20%)	..	101 (27%)	283 (28%)		555 (52%)	
Matikwane and Mapulaneng†	72 (13%)	167 (12%)	..	..	..	..	..	..
Dr George Mukhari Academic	67 (12%)	200 (14%)	..	..	..	..	..	..
Edendale	31 (6%)	43 (3%)	..	..	..	..	..	..
Red Cross War Memorial Children's	197 (36%)	504 (35%)	..	197 (53%)	504 (49%)	..	401 (38%)	..
Ngwelezana	72 (13%)	237 (17%)	..	72 (20%)	237 (23%)	..	113 (11%)	..
Age, months	9 (7–13)	10 (7–14)	0·004	9 (7–12)	10 (7–14)	0·002	8 (6–13)	0·601
Male sex	304 (56%)	810 (56%)	0·940	199 (54%)	575 (56%)	0·432	604/1068 (57%)	0·355
Black ethnic origin	499 (92%)	1327/1431 (93%)	0·805	329 (89%)	921 (90%)	0·580	960/1068 (90%)	0·598
Breastfeeding before age 4 months			0·218			0·562		0·807
Exclusive breastfeeding	303/539 (56%)	799/1432 (56%)	..	202/369 (55%)	558 (55%)	..	563/1066 (53%)	..
Mixed breast and formula	47/539 (9%)	95/1432 (7%)	..	31/369 (8%)	70 (7%)	..	91/1066 (9%)	..
Formula only	189/539 (35%)	538/1432 (38%)	..	136/369 (37%)	396 (39%)	..	412/1066 (39%)	..
House built of brick	341/537 (64%)	865/1431 (60%)	0·215	211/368 (57%)	558 (55%)	0·347	734/1062 (69%)	<0·0001
Electricity available	475 (88%)	1221 (85%)	0·109	323 (87%)	874 (85%)	0·357	988 (92%)	0·003
Indoor water source	227 (42%)	571/1432 (40%)	0·383	141 (38%)	364 (36%)	0·380	431 (40%)	0·454
Flush toilet available	313/538 (58%)	791/1432 (55%)	0·241	249/368 (68%)	627/1023 (61%)	0·030	866/1066 (81%)	<0·0001
Number of people sleeping in same room as the child	2 (2–3)	2 (2–3)	0·412	2 (2–4)	2 (2–4)	0·763	3 (2–4)	0·268
Daycare attendance	85/536 (16%)	296/1431 (21%)	0·016	70/366 (19%)	237/1023 (23%)	0·110	115/1064 (11%)	<0·0001
Maternal education			0·170			0·410		0·770
None	5/524 (1%)	29/1422 (2%)	..	4/366 (1%)	21/1020 (2%)	..	11/1051 (1%)	..
Primary	44/524 (8%)	128/1422 (9%)	..	29/366 (8%)	86/1020 (8%)	..	69/1051 (7%)	..
Secondary	461/524 (88%)	1208/1422 (85%)	..	322/366 (88%)	869/1020 (85%)	..	932/1051 (89%)	..
Higher education	14/524 (3%)	57/1422 (4%)	..	11/366 (3%)	44/1020 (4%)	..	39/1051 (4%)	..
Low birthweight (<2·5 kg)	92/529 (17%)	226/1400 (16%)	0·510	61/363 (17%)	158/1002 (16%)	0·645	222/1045 (21%)	0·069
HIV infection status			0·267			0·163		<0·0001
Infected	45 (8%)	155 (11%)	..	27 (7%)	83 (8%)	..	36 (3%)	..
Uninfected	454 (84%)	1174 (82%)	..	318 (86%)	897 (88%)	..	1000 (94%)	..
Status unknown	41 (8%)	105 (7%)	..	25 (7%)	44 (4%)	..	33 (3%)	..
Days in hospital	2 (1–5)	2 (1–5)	0·097	2 (1–4)	2 (1–4)	0·118	1 (1–3)	<0·0001
Died	7/533 (1%)	31/1412 (2%)	0·210	1/367 (<1%)	7/1017 (<1%)	0·689	2/1059 (<1%)	>0·999

Data are n (%), median (IQR), or n/N (%). *Hospitals with respiratory controls: Chris Hani Baragwanath Academic Hospital, Ngwelezane Hospital, and Red Cross War Memorial Children's Hospital. †Matikwane Hospital and Mapulaneng Hospital are two small hospitals that both serve the Bushbuckridge district; therefore, for the analysis these two hospitals were grouped together. All continuous variables were not normally distributed and were therefore assessed with the Wilcoxon rank-sum test; p<0·05 was judged to be significant. The χ^2 test or Fisher's exact test was used to assess dichotomous and categorical variables; p<0·05 was judged to be significant. Missing values: totals are given for each characteristic. Those with a missing value for any covariate were excluded for that particular bivariate analysis.

Table 1: **Baseline characteristics of rotavirus-positive cases, rotavirus-negative controls, and respiratory controls in children aged from 18 weeks to 23 months**

	All hospitals			Subset of hospitals*				
	Rotavirus-positive cases (n=540)	Rotavirus-negative controls (n=1434)	Adjusted vaccine effectiveness, % (95% CI)†	Rotavirus-positive cases (n=370)	Rotavirus-negative controls (n=1024)	Adjusted vaccine effectiveness, % (95% CI)†	Respiratory controls (n=1069)	Adjusted vaccine effectiveness, % (95% CI)†
0 doses (reference)	136 (25%)	244 (17%)	..	94 (25%)	181 (18%)	..	140 (13%)	..
1 dose	126 (23%)	334 (23%)	40% (16–57)	87 (24%)	238 (23%)	35% (4–56)	232 (22%)	54% (31–69)
2 doses	278 (52%)	856 (60%)	57% (40–68)	189 (51%)	605 (59%)	52% (29–67)	697 (65%)	63% (45–75)

Data are n (%) unless otherwise shown. A dose of human rotavirus vaccine was counted if it was administered ≥14 days before the date of hospital admission. *Hospitals with respiratory controls: Chris Hani Baragwanath Academic Hospital, Ngwelezane Hospital, and Red Cross War Memorial Children's Hospital. †Unconditional logistic regression was used to calculate adjusted vaccine effectiveness. Models that included sex, race, history of breastfeeding, maternal education, daycare attendance, birthweight, and variables of household characteristics did not change the adjusted odds ratio by >5% and were therefore not included in the final models. Hospital, birth month, birth year, month by year of birth (interaction term), quarter of hospital admission, year of hospital admission, and quarter by year of hospital admission (interaction term) were included in the models a priori.

Table 2: **Effectiveness of one and two doses of rotavirus vaccine in preventing hospital admission for acute rotavirus diarrhoea in children aged from 18 weeks to 23 months**

	All hospitals			Subset of hospitals*				
	Rotavirus-positive cases	Rotavirus-negative controls	Adjusted vaccine effectiveness, % (95% CI)†	Rotavirus-positive cases	Rotavirus-negative controls	Adjusted vaccine effectiveness, % (95% CI)†	Respiratory controls	Adjusted vaccine effectiveness, % (95% CI)†
Age 18 weeks–11 months								
0 doses (reference)	90/389 (23%)	149/947 (16%)	..	62/274 (23%)	107/671 (16%)	..	80/749 (11%)	..
1 dose	92/389 (24%)	231/947 (24%)	39% (9 to 59)	65/274 (24%)	168/671 (25%)	35% (–4 to 60)	162/749 (22%)	60% (34 to 76)
2 doses	207/389 (53%)	567/947 (60%)	54% (32 to 68)	147/274 (54%)	396/671 (59%)	47% (18 to 66)	507/749 (68%)	66% (46 to 79)
Age 12–23 months								
0 doses (reference)	46/151 (30%)	95/487 (20%)	..	32/96 (33%)	74/353 (21%)	..	60/320 (19%)	..
1 dose	34/151 (23%)	103/487 (21%)	40% (–7 to 66)	22/96 (23%)	70/353 (20%)	32% (–35 to 65)	70/320 (22%)	41% (–17 to 71)
2 doses	71/151 (47%)	289/487 (59%)	61% (35 to 77)	42/96 (44%)	209/353 (59%)	60% (26 to 79)	190/320 (59%)	60% (21 to 80)

A single logistic regression model was used, with introduction of an interaction term for age group by vaccination status. A dose of human rotavirus vaccine was counted if it was given ≥14 days before the date of admission to hospital. *Hospitals with respiratory controls: Chris Hani Baragwanath Academic Hospital, Ngwelezane Hospital, and Red Cross War Memorial Children's Hospital. †Unconditional logistic regression was used to calculate adjusted vaccine effectiveness. Models that included sex, ethnic origin, history of breastfeeding, maternal education, daycare attendance, birthweight, and variables of household characteristics did not change the adjusted odds ratio by >5% and were therefore not included in the final models. Hospital, birth month, birth year, month by year of birth (interaction term), quarter of hospital admission, year of hospital admission, and quarter by year of hospital admission (interaction term) were included in the models a priori.

Table 3: **Effectiveness of one and two doses of rotavirus vaccine in preventing admission to hospital for acute rotavirus-diarrhoea in children aged from 18 weeks to 23 months, stratified by age group**

When we include all 3043 children who were available for analysis (540 rotavirus-positive cases, 1434 rotavirus-negative controls, and 1069 respiratory controls), the median age at receipt of human rotavirus vaccine was 6 weeks (IQR 6–8) for the first dose and 15 weeks (14–17) for the second dose. Of the 1868 children who received two doses of the vaccine, 1553 (83%) completed vaccination by 18 weeks and 1779 (95%) did so by age 24 weeks (appendix p 7). Oral polio vaccine was given concurrently with the first dose of rotavirus vaccine in 1869 (74%) of 2532 vaccinated children, with no significant differences between cases and controls. Children who received only one dose of the human rotavirus vaccine received it at an older age (median 8 weeks [IQR 6–14]) than the first dose in two-dose recipients (6 weeks [6–7]; p<0·0001), and this dose was less likely to have been given with oral polio vaccine than was the first dose in two-dose recipients (371/664 [56%] of one-dose recipients received concomitant oral polio vaccine *vs* 1498/1868 [80%] of two-dose recipients; p<0·0001).

In children aged from 18 weeks to 23 months, the overall adjusted vaccine effectiveness was higher for two doses of human rotavirus vaccine than for one dose (table 2). In the age-stratified analyses, protection after two doses of the vaccine was similar in the two age groups (table 3). HIV exposure status was available for 2570 (98%) of 2628 HIV-uninfected children (including all analysable rotavirus-positive cases, rotavirus-negative controls, and respiratory controls); 807 (31%) of 2570 were HIV-exposed-uninfected and 1763 (69%) were HIV-unexposed-uninfected. In all HIV-uninfected children (including those both exposed and not exposed to the virus), protection after two doses was 57% (95% CI 39–70; table 4). The adjusted vaccine effectiveness for two doses of human rotavirus vaccine was similar in HIV-exposed-

	All hospitals			Subset of hospitals†				
	Rotavirus-negative cases	Rotavirus-negative controls	Adjusted vaccine effectiveness, % (95% CI)‡	Rotavirus-positive cases	Rotavirus-negative controls	Adjusted vaccine effectiveness, % (95% CI)‡	Respiratory controls	Adjusted vaccine effectiveness, % (95% CI)‡
All HIV-uninfected children*								
0 doses (reference)	110/454 (24%)	191/1174 (16%)	..	77/318 (24%)	152/897 (17%)	..	133/1000 (13%)	..
1 dose	106/454 (23%)	265/1174 (23%)	37% (10 to 56)	75/318 (24%)	201/897 (22%)	32% (–4 to 56)	215/1000 (22%)	52% (27 to 69)
2 doses	238/454 (52%)	718/1174 (61%)	57% (39 to 70)	166/318 (52%)	544/897 (61%)	53% (30 to 69)	652/1000 (65%)	60% (39 to 74)
HIV-exposed, HIV-uninfected children§								
0 doses (reference)	30/131 (23%)	50/396 (13%)	..	21/94 (22%)	40/295 (14%)	..	28/280 (10%)	..
1 dose	26/131 (20%)	90/396 (23%)	61% (22 to 81)	21/94 (22%)	67/295 (23%)	54% (–1 to 79)	69/280 (25%)	73 (38 to 88)
2 doses	75/131 (57%)	256/396 (65%)	64% (34 to 80)	52/94 (55%)	188/295 (64%)	58% (16 to 79)	183/280 (65%)	67 (31 to 84)
HIV-unexposed, HIV-uninfected children§								
0 doses (reference)	79/320 (25%)	132/741 (18%)	..	55/222 (25%)	107/584 (18%)	..	100/702 (14%)	..
1 dose	79/320 (25%)	166/741 (22%)	24% (–17 to 51)	54/222 (24%)	133/584 (23%)	25 (–25 to 54)	144/702 (21%)	45 (9 to 67)
2 doses	162/320 (51%)	443/741 (60%)	54% (31 to 69)	113/222 (51%)	344/584 (59%)	52 (23 to 70)	458/702 (65%)	60 (36 to 75)

A single logistic regression model was used, with introduction of an interaction term for HIV exposure status by vaccination status. A dose of human rotavirus vaccine was counted if it was administered ≥14 days before the date of admission to hospital. *The total number of HIV-uninfected children was 2628 (454 rotavirus-positive cases, 1174 rotavirus-negative controls, and 1000 respiratory controls). Children who tested positive for HIV and those with unknown HIV status were excluded. †Hospitals with respiratory controls: Chris Hani Baragwanath Academic Hospital, Ngwelezane Hospital, and Red Cross War Memorial Children's Hospital. ‡Unconditional logistic regression was used to calculate adjusted vaccine effectiveness. Models that included sex, ethnic origin, history of breastfeeding, maternal education, daycare attendance, birthweight, and variables of household characteristics did not change the adjusted odds ratio by >5% and were therefore not included in the final models. Hospital, birth month, birth year, month by year of birth (interaction term), quarter of hospital admission, year of hospital admission, and quarter by year of hospital admission (interaction term) were included in the models a priori. §HIV exposure status in HIV-uninfected children was not available for three rotavirus-positive cases, 37 rotavirus-negative controls, and 18 respiratory controls.

Table 4: **Effectiveness of one and two doses of rotavirus vaccine in preventing admission to hospital for acute rotavirus-diarrhoea in children aged from 18 weeks to 23 months, stratified by HIV exposure in HIV-uninfected children only***

uninfected and HIV-unexposed-uninfected children (table 4). The study was not powered to assess effectiveness in HIV-infected children (45 cases).

The most common rotavirus strains (data for which are available for 538 patients) during the study period were G12P[8] (n=230), G2P[4] (n=76), G1P[8] (n=64), G9P[8] (n=42), G8P[4] (n=38), and G2P[6] (n=28). Other strains were detected infrequently (n=45) or were mixed strains (n=15). Strain-specific adjusted vaccine effectiveness for two doses of human rotavirus vaccine was 71% (95% CI 55–82) against the dominant G12P[8] strain; 62% (45–74) against any homotypic or partly heterotypic strains (in which G protein or P protein were of the same type as the vaccine strain [369 cases]); and 52% (20–72) against any fully heterotypic strains (in which both G and P proteins were different from those of the vaccine strain [155 cases]).

We did additional viral testing for 464 (86%) of 540 rotavirus-positive cases and one or more viruses were detected in 140 (30%) of these children. The two-dose adjusted vaccine effectiveness with use of only rotavirus cases without an additional virus detected in the stool (54%, 95% CI 31–69) was similar to that using rotavirus cases with an additional virus detected (62%, 36–77).

In the subset of three hospitals with respiratory controls, the two-dose adjusted vaccine effectiveness estimates were similar when either rotavirus-negative controls or respiratory controls were used as the control group (tables 2–4).

Discussion

This case-control study is the first to assess the effectiveness of rotavirus vaccination under routine conditions of a national immunisation programme in an African country, which included a range of sociodemographic and HIV prevalence settings (panel). Two doses of human rotavirus vaccine provided protection against admission to hospital for acute rotavirus diarrhoea in children younger than 2 years of age using rotavirus-negative controls, with similar effectiveness recorded with use of respiratory controls in a subset of hospitals. Vaccine effectiveness seemed to be sustained through the second year of life and was similar in children both exposed and unexposed to HIV.

The rationale for implementation of a 6-week and 14-week human rotavirus vaccine schedule was to induce immunity from an early age, which was why the first dose was given at 6 rather than 10 weeks, and the 14-week dose logistically helped concurrent implementation of pneumococcal conjugate vaccine and human rotavirus vaccine into the immunisation programme (since the pneumococcal conjugate vaccine is also administered at 6 and 14 weeks).[18] Our present study showed an overall adjusted vaccine effectiveness (54%) in the first year of life that seems to be lower than the efficacy of two doses at 10 and 14 weeks shown in the clinical trial (72%).[8] Differences in study methods might have contributed to

Panel: **Research in context**

Systematic review

A recent review of the present status of rotavirus vaccines was done by Catherine Yen and colleagues in 2014.[22] This review lists all published rotavirus vaccine impact and effectiveness studies (up to April, 2014) undertaken in countries that have introduced rotavirus vaccination into their national immunisation programmes.

Interpretation

Studies of the effects of the rotavirus vaccine, mainly done in Australia and countries in Europe and the Americas, have shown reductions in diarrhoea-associated mortality, diarrhoea-associated hospital admissions, and rotavirus-specific hospital admissions in children younger than 5 years. Rotavirus vaccine effectiveness studies have been undertaken in high-income and upper-middle-income countries, and in low-income countries outside Africa, and good protection against rotavirus-associated hospital admission has been shown. One assessment of vaccine impact has been done in South Africa and showed a reduction in diarrhoea-associated and rotavirus-specific admissions to hospital after rotavirus vaccine introduction, but until the present study there have been no published rotavirus vaccine effectiveness studies from an African country. We present the first rotavirus vaccine effectiveness data from Africa, showing sustained protection against admission to hospital for acute rotavirus diarrhoea during the first 2 years of life.

this outcome. Furthermore, infants enrolled in the efficacy study were vaccinated before the onset of the rotavirus season, whereas in our study we included children vaccinated throughout the year, which might affect the amount of protection especially if infants were vaccinated soon after the peak rotavirus season. Vaccine efficacy might differ depending on the season of birth and time of vaccination, and was greater when given immediately before the rotavirus season in an early rotavirus vaccine trial.[23]

Nevertheless, our findings suggest that the vaccine provides sustained protection through the second year of life, which is important because the efficacy trial, although not powered to assess protection against severe rotavirus diarrhoea in the second year of life, showed a lower point estimate across two consecutive rotavirus seasons than the first rotavirus season in the group who had been randomly assigned to receive the two-dose schedule but not in those allocated to the three-dose schedule.[15] A recent post-licensure assessment of the effect of human rotavirus vaccine in South Africa that used data from a hospital-based rotavirus surveillance programme estimated that the reduction in rotavirus diarrhoea hospital admissions was 45–50% greater than the reduction in rotavirus-negative admissions to hospital for infants—but not for children aged 12–23 months—since vaccine introduction.[24] This finding corroborates our results in children younger than 12 months, but we showed continued protection in the second year of life. Our results are similar to those from a study in Bolivia, which showed similar effectiveness of the Rotarix human rotavirus vaccine in preventing admission to hospital for rotavirus between the younger (64%) and older age groups (72%).[25]

Human rotavirus vaccine has been shown to be safe and immunogenic in HIV-infected children,[26] but insufficient numbers of these children were enrolled in the present study to assess effectiveness in this group. Improvement in access to antiretroviral treatment and success of programmes for the prevention of mother-to-child transmission of HIV have reduced HIV prevalence in children in South Africa—between 2008 and 2011, the proportion of infants who were HIV infected decreased from 9·6% to 2·8%.[27] However, the antenatal HIV prevalence in women of childbearing age remains high at 30% in South Africa.[19] Furthermore, HIV-exposed-uninfected children have emerged as a high-risk group for morbidity and mortality, including from diarrhoeal disease.[28,29] We showed that the two-dose effectiveness of the rotavirus vaccine was similar in HIV-exposed-uninfected and HIV-unexposed-uninfected children. However, one-dose adjusted vaccine effectiveness point estimates were higher in HIV-exposed-uninfected than in HIV-unexposed-uninfected children, and could suggest lower transplacental transmission of rotavirus-specific antibodies in HIV-exposed-uninfected children, leading to a more robust immune response in these infants. Although some evidence exists to support this theory in HIV-exposed-uninfected children receiving pneumococcal conjugated vaccine,[30] further studies are needed to test this hypothesis.

One dose of human rotavirus vaccine provided protection against admission to hospital for rotavirus diarrhoea, which is an encouraging finding because children in low-income countries might not return for the second dose and therefore can be protected early in life by a single dose. Infants receiving only one dose tended to be older and less likely to receive their dose with oral polio vaccine than were those who received two doses.

Studies have shown that the human rotavirus vaccine protects against several rotavirus strains;[31] however, potential waning of immunity might be a concern against illness caused by strains that share neither the G nor P type with the vaccine strain.[32] We showed protection against homotypic, partly heterotypic, and fully heterotypic strains, but our analyses were not powered to assess strain-specific protection, except for G12P[8], or the durability of this protection into the second year of life.

Lower effectiveness estimates in low-income countries could result from misattribution of rotavirus as the pathogen responsible for the illness necessitating admission to hospital, especially if vaccination reduced the severity of illness but not the actual rate of infection. Previous assessments have not directly addressed this possibility; we showed similar results using cases for which additional testing for viruses did not yield other pathogens and cases that did yield additional pathogens, suggesting that this possible misclassification of illness as rotavirus disease did not substantially bias our results. However, we did not uniformly assess for bacterial pathogens in our study.

The limitations of our study include the fact that we excluded children in whom vaccination cards were

unavailable, apart from those reporting no vaccination since the birth dose. However, the baseline characteristics of these children were similar to those included in the analysis. Study staff were also unaware of the child's case or control status when their vaccination history was recorded. HIV status in the mother was ascertained by history alone, not by HIV testing, and might have led to misclassification of exposure status in the children. The proportion of children reported as having been exposed to HIV (31%) was, however, consistent with antenatal HIV prevalence data from South Africa. We included rural and urban communities from four of the nine provinces to best estimate the effectiveness in our population but province-specific variations exist (eg, differing rotavirus strains in circulation),[4] which we have not accounted for and could affect the generalisability of our results. Cases and controls were not matched for age so we included only children in whom vaccine status was unlikely to change. Use of a cutoff of 18 weeks of age gave children an additional 4 weeks to be vaccinated with the second dose. A main reason for the unmatched design was a practical one, because rotavirus testing was batched every 2 weeks so results were not available immediately. An unmatched test-negative design has been used in published literature when assessing influenza vaccine effectiveness and, more recently, rotavirus vaccine effectiveness.[33] We adjusted for birth month and year, and admission quarter and year in the analyses to address residual confounding by age and rotavirus season. Effectiveness estimates were similar when we restricted our analyses to children aged from 24 weeks to 23 months (data not shown).

In summary, two doses of human rotavirus vaccine provided sustained protection against admission to hospital for acute rotavirus diarrhoea during the first and second years of life under conditions of routine use, in a setting with a high prevalence of maternal HIV infection. These findings are encouraging and establish the public health value of rotavirus vaccine in an African setting, especially as rotavirus vaccines are being introduced into an increasing number of African countries.

Contributors

MJG, MMC, JM, CC, ADS, UDP, and SAM conceived and designed the study. NP, MS, and JM acquired the laboratory data. JM, CC, HJZ, CNK, CM, RD, MS, SW, KK, and MC acquired the clinical data. MJG, MMC, and ERZ analysed the data. MJG, MMC, JAF, ADS, UDP, ERZ, and SAM interpreted the data. MJG drafted the report. All authors critically reviewed drafts of the report and gave final approval of the submitted version.

Declaration of interests

MJG has received grants from the GAVI Alliance, grants and personal fees from GlaxoSmithKline, and personal fees from Sanofi Pasteur and MSD. NP has received grants from GlaxoSmithKline, and personal fees from GlaxoSmithKline, Merck Pty (Ltd), and Aspen Pharma. JM has received grants from GlaxoSmithKline and the GAVI Alliance. JAF has received a grant from the GAVI Alliance. CC has received grants from GlaxoSmithKline and the GAVI Alliance. SAM has received grants from the GAVI Alliance Special Studies, GlaxoSmithKline, and Novartis; and grants and personal fees from GlaxoSmithKline, Pfizer, and Sanofi Pasteur. All other authors declare no competing interests.

Acknowledgments

Support for this project was provided by PATH through funding from the GAVI Alliance. The views expressed by the authors do not necessarily reflect the views of GAVI, PATH, or the Centers for Disease Control and Prevention. The authors have not been paid to write this report by a pharmaceutical company or other agency.

References

1 Tate JE, Burton AH, Boschi-Pinto C, Steele AD, Duque J, Parashar UD. 2008 estimate of worldwide rotavirus-associated mortality in children younger than 5 years before the introduction of universal rotavirus vaccination programmes: a systematic review and meta-analysis. *Lancet Infect Dis* 2012; **12:** 136–41.

2 Mwenda JM, Ntoto KM, Abebe A, et al. Burden and epidemiology of rotavirus diarrhea in selected African countries: preliminary results from the African Rotavirus Surveillance Network. *J Infect Dis* 2010; **202** (suppl)**:** S5–11.

3 Levels and trends of morbidity and mortality among children aged under-five years in South Africa, 2006–2010. Pretoria: Statistics South Africa, 2010. http://www.statssa.gov.za/publications/Report-03-09-10/Report-03-09-102010.pdf (accessed March 27, 2014).

4 Steele AD, Peenze I, de Beer MC, et al. Anticipating rotavirus vaccines: epidemiology and surveillance of rotavirus in South Africa. *Vaccine* 2003; **21:** 354–60.

5 WHO. Rotavirus vaccines: an update. *Wkly Epidemiol Rec* 2009; **84:** 533–40.

6 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:** 23–33.

7 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:** 11–22.

8 Madhi SA, Cunliffe NA, Steele D, et al. Effect of human rotavirus vaccine on severe diarrhea in African infants. *N Engl J Med* 2010; **362:** 289–98.

9 Armah GE, Sow SO, Breiman RF, et al. Efficacy of pentavalent rotavirus vaccine against severe rotavirus gastroenteritis in infants in developing countries in sub-Saharan Africa: a randomised, double-blind, placebo-controlled trial. *Lancet* 2010; **376:** 606–14.

10 Zaman K, Dang DA, Victor JC, et al. Efficacy of pentavalent rotavirus vaccine against severe rotavirus gastroenteritis in infants in developing countries in Asia: a randomised, double-blind, placebo-controlled trial. *Lancet* 2010; **376:** 615–23.

11 Linhares AC, Velazquez FR, Perez-Schael I, et al. Efficacy and safety of an oral live attenuated human rotavirus vaccine against rotavirus gastroenteritis during the first 2 years of life in Latin American infants: a randomised, double-blind, placebo-controlled phase III study. *Lancet* 2008; **371:** 1181–89.

12 Vesikari T, Karvonen A, Prymula R, et al. Efficacy of human rotavirus vaccine against rotavirus gastroenteritis during the first 2 years of life in European infants: randomised, double-blind controlled study. *Lancet* 2007; **370:** 1757–63.

13 de Palma O, Cruz L, Ramos H, et al. Effectiveness of rotavirus vaccination against childhood diarrhoea in El Salvador: case-control study. *BMJ* 2010; **340:** c2825.

14 Patel M, Pedreira C, De Oliveira LH, et al. Duration of protection of pentavalent rotavirus vaccination in Nicaragua. *Pediatrics* 2012; **130:** e365–72.

15 Madhi SA, Kirsten M, Louw C, et al. Efficacy and immunogenicity of two or three dose rotavirus-vaccine regimen in South African children over two consecutive rotavirus-seasons: a randomized, double-blind, placebo-controlled trial. *Vaccine* 2012; **30** (suppl 1)**:** A44–51.

16 Patel MM, Glass R, Desai R, Tate JE, Parashar UD. Fulfilling the promise of rotavirus vaccines: how far have we come since licensure? *Lancet Infect Dis* 2012; **12:** 561–70.

17 Expanded programme on immunisation (EPI) SA – Revised childhood immunisation schedule from April 2009. Department of Health South Africa,2009. http://www.health.gov.za/docs/notices/2013/EPI_SA_Immunisation_Schedule.pdf (accessed March 27, 2014).

18 Madhi SA, Bamford L, Ngcobo N. Effectiveness of pneumococcal conjugate vaccine and rotavirus vaccine introduction into the South African public immunisation programme. *S Afr Med J* 2014; **104** (3 suppl 1)**:** 228–34.

19 The National Antenatal Sentinel HIV and Syphilis Prevalence Survey, South Africa. Department of Health South Africa, 2011. http://www.health.gov.za/docs/reports/2013/Antenatal_survey_report_2012_web_optimized.pdf (accessed March 27, 2014).

20 Manual of rotavirus detection and characterization methods. World Health Organization, 2009. http://whqlibdoc.who.int/hq/2008/who_ivb_08.17_eng.pdf (accessed March 27, 2014).

21 Cherian T, Mulholland EK, Carlin JB, et al. Standardized interpretation of paediatric chest radiographs for the diagnosis of pneumonia in epidemiological studies. *Bull World Health Organ* 2005; **83:** 353–59.

22 Yen C, Tate JE, Hyde TB, et al. Rotavirus vaccines: current status and future considerations. *Hum Vaccine Immunother* 2014; **10:** 1436–48.

23 Ruuska T, Vesikari T, Delem A, Andre FE, Beards GM, Flewett TH. Evaluation of RIT 4237 bovine rotavirus vaccine in newborn infants: correlation of vaccine efficacy to season of birth in relation to rotavirus epidemic period. *Scand J Infect Dis* 1990; **22:** 269–78.

24 Msimang VM, Page N, Groome MJ, et al. Impact of rotavirus vaccine on childhood diarrheal hospitalization following introduction into the South African public immunization program. *Pediatr Infect Dis J* 2013; **32:** 1359–64.

25 Patel MM, Patzi M, Pastor D, et al. Effectiveness of monovalent rotavirus vaccine in Bolivia: case-control study. *BMJ* 2013; **346:** f3726.

26 Steele AD, Madhi SA, Louw CE, et al. Safety, reactogenicity, and immunogenicity of human rotavirus vaccine RIX4414 in human immunodeficiency virus-positive infants in South Africa. *Pediatr Infect Dis J* 2011; **30:** 125–30.

27 Barron P, Pillay Y, Doherty T, et al. Eliminating mother-to-child HIV transmission in South Africa. *Bull World Health Organ* 2013; **91:** 70–74.

28 Marinda E, Humphrey JH, Iliff PJ, et al. Child mortality according to maternal and infant HIV status in Zimbabwe. *Pediatr Infect Dis J* 2007; **26:** 519–26.

29 Slogrove A, Reikie B, Naidoo S, et al. HIV-exposed uninfected infants are at increased risk for severe infections in the first year of life. *J Trop Pediatr* 2012; **58:** 505–08.

30 Madhi SA, Adrian P, Cotton MF, et al. Effect of HIV infection status and anti-retroviral treatment on quantitative and qualitative antibody responses to pneumococcal conjugate vaccine in infants. *J Infect Dis* 2010; **202:** 355–61.

31 De Vos B, Han HH, Bouckenooghe A, et al. Live attenuated human rotavirus vaccine, RIX4414, provides clinical protection in infants against rotavirus strains with and without shared G and P genotypes: integrated analysis of randomized controlled trials. *Pediatr Infect Dis J* 2009; **28:** 261–66.

32 Correia JB, Patel MM, Nakagomi O, et al. Effectiveness of monovalent rotavirus vaccine (Rotarix) against severe diarrhea caused by serotypically unrelated G2P[4] strains in Brazil. *J Infect Dis* 2010; **201:** 363–69.

33 Foppa IM, Haber M, Ferdinands JM, Shay DK. The case test-negative design for studies of the effectiveness of influenza vaccine. *Vaccine* 2013; **31:** 3104–09.

THE LANCET Infectious Diseases

Supplementary webappendix

This webappendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Groome MJ, Page N, Cortese MM, et al. Effectiveness of monovalent human rotavirus vaccine against admission to hospital for acute rotavirus diarrhoea in South African children: a case-control study. *Lancet Infect Dis* 2014; published online Oct 8. http://dx.doi.org/10.1016/S1473-3099(14)70940-5.

Study population and study hospitals - Supplementary Information

The study was conducted at seven hospitals in order to include a diversity of sites in South Africa, encompassing both urban and rural sites as well as differing HIV infection prevalence. The main criteria for selection included number of diarrhoeal admissions and ability and willingness of the site to accommodate the study. These included 5 sites which were currently involved in the rotavirus surveillance program in South Africa: Chris Hani Baragwanath Academic Hospital (Gauteng), Edendale Hospital (Kwazulu-Natal), Mapulaneng Hospital and Matikwana Hospital (Mpumalanga), and Dr George Mhukari Hospital (North West Province). Two additional sites Ngwelezane Hospital (Kwazulu-Natal) and the Red Cross War Memorial Children's Hospital (Western Cape) were then included. The two Mpumalanga hospitals, Matikwana and Mapulaneng are small hospitals and were considered one site and referred to as the Agincourt site. The sites Chris Hani Baragwanath Academic Hospital, Ngwelezane and Red Cross War Memorial Children's Hospital were recruiting patients for a concurrent study investigating the effectiveness of the PCV-7 vaccine. At these sites patients already enrolled as part of the PCV-7 effectiveness study were included for the second group of controls in this rotavirus effectiveness study if eligible.

Chris Hani Baragwanath Academic Hospital

CHBH is located in an urban setting with a defined catchment population. It is the only public hospital which serves the population of Soweto and it is estimated that 90% of all hospitalisations in children from the area occur in this hospital. The population of Soweto includes an estimated 1.5 million people, including 120 000 children under five years of age.

Red Cross Children's Hospital

Red Cross hospital provides secondary-tertiary level care to the majority of children utilizing the public health-care sector in the Cape Town Metropolis. The birth cohort for the Cape Town Metropolis is approximately 22 000 per annum.

Ngwelezana Hospital

This provincial hospital is the only regional referral centre for the eighteen district rural hospitals of the north eastern region of KwaZulu-Natal (KZN).The hospital has 150 paediatric beds. The catchment population as a regional hospital is estimated at 2.3 million (including 295 000 under 5 years of age).

Agincourt site

The two hospitals Mapulaneng and Matikwana service the district of Bushbuckridge, Ehlanzeni and Mbombela with a combined population of more than 1 million. The Agincourt demographic and surveillance site (DSS) is situated between the hospitals and DSS activities have been ongoing in this area from 1994. The data collected by the DSS provides some population estimates. The population in the DSS is 58401, and reports under five mortality rates of 17 per 1000 live births. Total births in 2008 were 1389.

Edendale hospital

This regional and district hospital with 900 beds is situated near Pietermaritzburg, and serves a population of approximately 1.6 million, including urban and periurban populations.

Dr George Mukhari Academic Hospital

DGMH is a tertiary care facility of 1600 beds that renders a service to about 6 million people and serves as a referral hospital to 43 communities and 4 provinces. The hospital serves catchment areas including Ga-Rankuwa, Soshanguve and Mabopane districts in the Gauteng province and parts of the Madibeng district in the North-West Province. In the catchment areas, there were 84 484 children under 5 years of age from the two provinces, divided as follows: 6 218 in Ga-Rankuwa, 19 318 in Mabopane, 28 882 in Soshanguve, and 30 066 in Madibeng, with approximately equal numbers of children in each 1-year age category. The areas include urban, rural and informal communities with moderate to low socio-economic status.

Supplementary information: genotyping methods

For the RNA viruses, cDNA was synthesised using random primers and the Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics GmbH, Mannheim, Germany), according to the manufacturer's instructions. The Lightcycler Taqman Master Kit (Roche Diagnostics GmbH) was used according to the manufacturer's instructions and primers and probes from the following: norovirus genogroup I, norovirus, sapovirus, astrovirus and adenovirus.[1–7]

1 Van Stelten A, Kreman TM, Hall N, Desjardin LE. Optimization of a real-time RT-PCR assay reveals an increase of genogroup I norovirus in the clinical setting. *J Virol Methods* 2011; **175:** 80–84.

2 Svraka S, Duizer E, Vennema H, et al. Etiological role of viruses in outbreaks of acute gastroenteritis in The Netherlands from 1994 through 2005. *J Clin Microbiol* 2007; **45:** 1389–94.

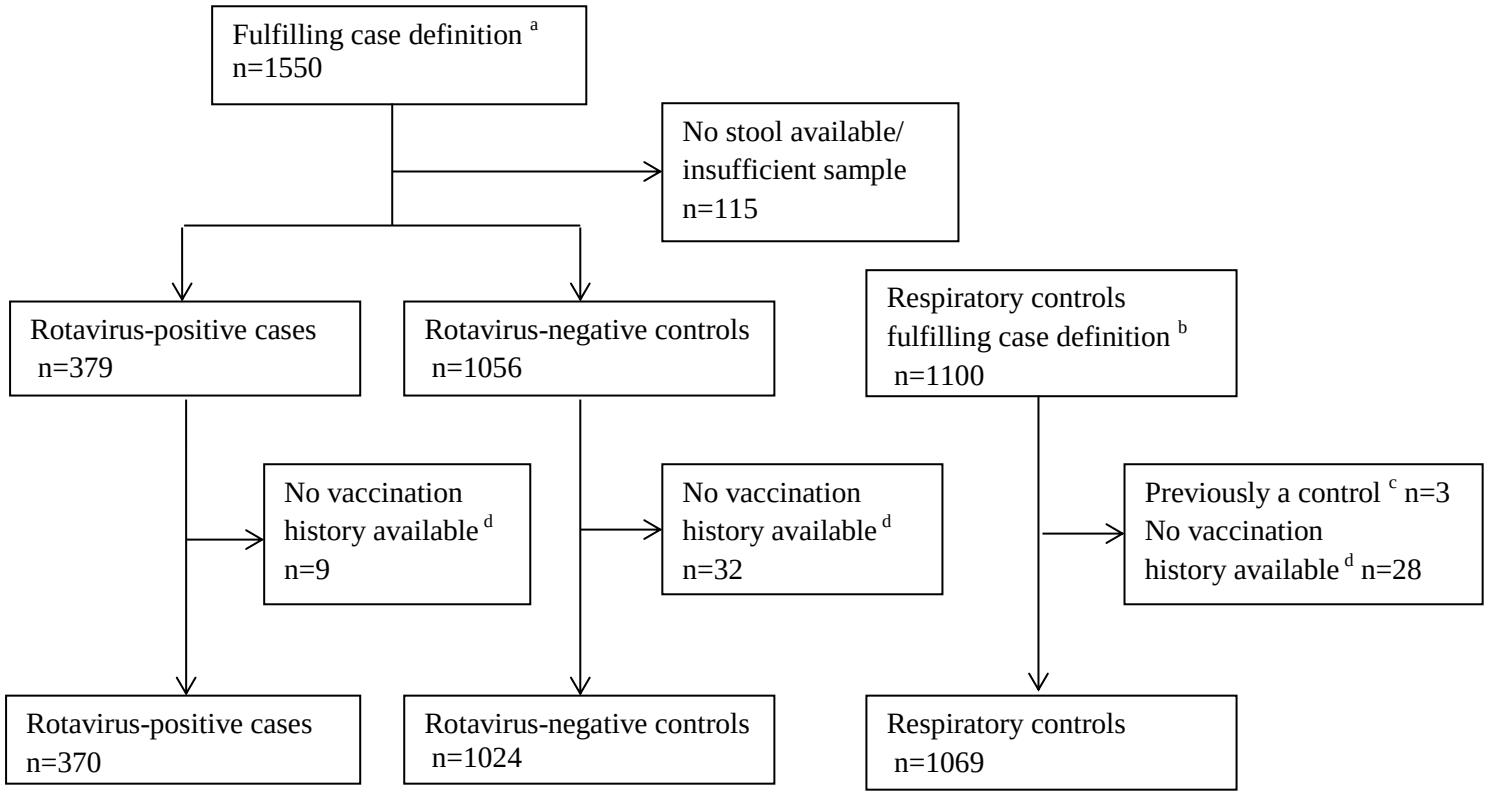
3 Loisy F, Atmar RL, Guillon P, Le Cann P, Pommepuy M, Le Guyader FS. Real-time RT-PCR for norovirus screening in shellfish. *J Virol Methods* 2005; **123:** 1–7.

4 Kageyama T, Kojima S, Shinohara M, et al. Broadly reactive and highly sensitive assay for Norwalk-like viruses based on real-time quantitative reverse transcription-PCR. *J Clin Microbiol* 2003; **41:** 1548–57.

5 Chan MC, Sung JJ, Lam RK, Chan PK, Lai RW, Leung WK. Sapovirus detection by quantitative real-time RT-PCR in clinical stool specimens. *J Virol Methods* 2006; **134:** 146–53.

6 Le Cann P, Ranarijaona S, Monpoeho S, Le Guyader F, Ferre V. Quantification of human astroviruses in sewage using real-time RT-PCR. *Res Microbiol* 2004; **155:** 11–15.

7 Heim A, Ebnet C, Harste G, Pring-Akerblom P. Rapid and quantitative detection of human adenovirus DNA by real-time PCR. *J Med Virol* 2003; **70:** 228–39.



Supplementary Figure 1: Enrolment of children ≥18 weeks and <24 months of age, and age-eligible to have received rotavirus vaccine (born i.e. after 14 June 2009) from the three hospitals where respiratory controls were enrolled (Chris Hani Baragwanath Academic Hospital, Ngwelezane Hospital, Red Cross War Memorial Children's Hospital).

[a] Acute diarrhoea: ≥3 loose stools in a 24 hour period, diarrhoea duration ≤7 days at admission.

[b] Enrolled into a concurrent study investigating the effectiveness of a pneumococcal conjugate vaccine. Age-eligible children hospitalised with physician-diagnosed lower respiratory tract infection were assessed by C-reactive protein (CRP) and chest X-ray. Children who were presumed not to have bacterial pneumonia i.e. with CRP <40 mg/l and without radiological-confirmed pneumonia, were eligible for inclusion into the respiratory-control group.

[c] A child enrolled more than once into the study for acute diarrhoea with any stool testing positive for rotavirus was defined as a case and the other hospitalisation excluded from the analysis. If all stool results were rotavirus-negative, the first diarrhoeal hospitalisation contributed to the control data. A rotavirus-negative control re-enrolled as a respiratory control could be included in the analysis, and vice versa, but a control re-enrolled into the same control group was excluded.

[d] Vaccination history was ascertained by review of the vaccination card, a photocopy of which was obtained when possible. If the vaccination card was unavailable but the parent indicated that the child had not received any vaccines other than those given at birth, the child was included and considered not to have received any human rotavirus vaccine doses. Rotavirus-positive cases – 369/370 had a vaccination card available for review; rotavirus-negative controls – 1021/1024 had a vaccination card available for review; all respiratory controls had a vaccination card available for review.

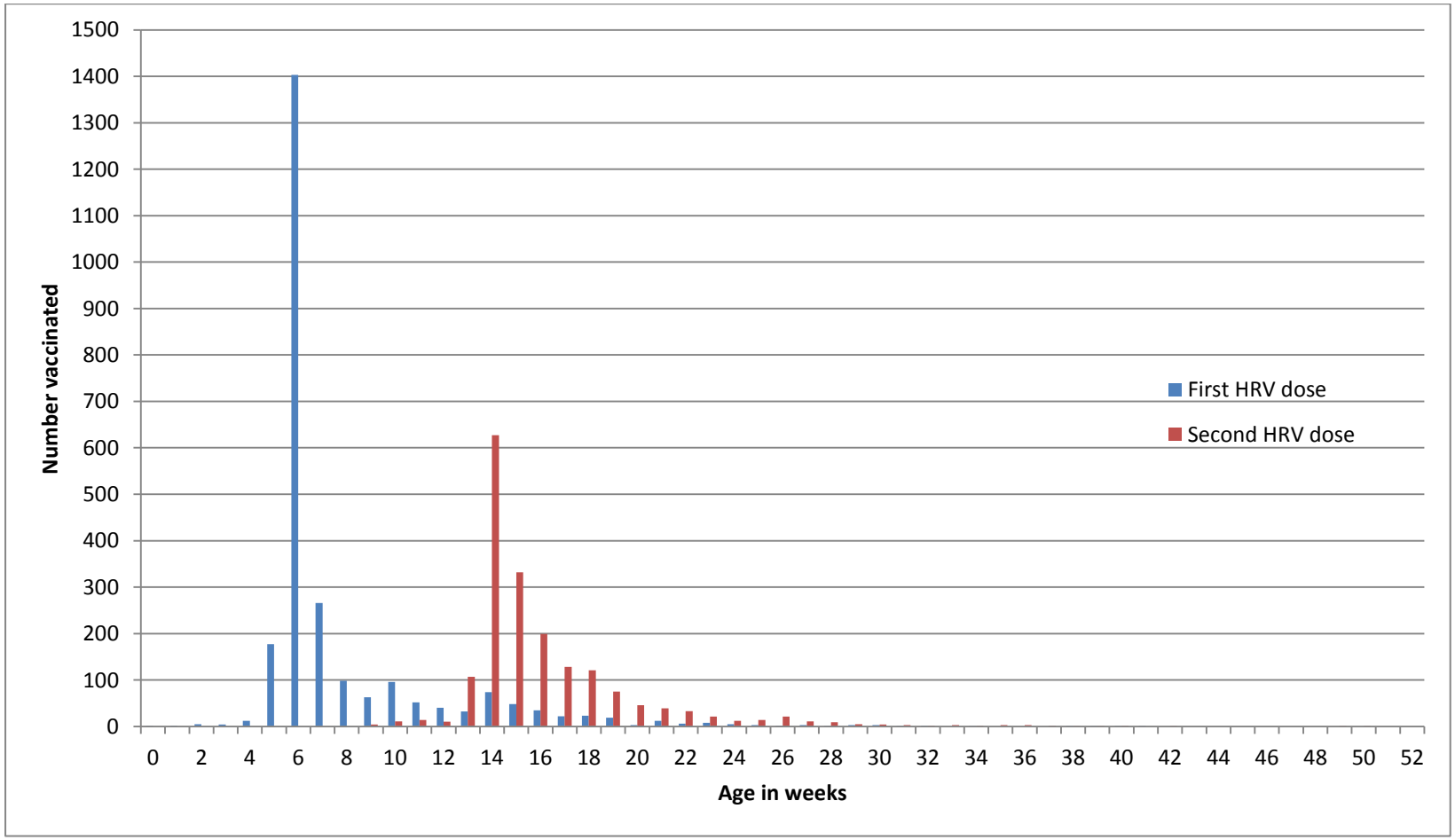


Figure 2: Age at vaccination in weeks (up until 52 weeks) for the first and second HRV doses in children aged 18 weeks–23 months.

Note: Total number of children available for the analysis (n=3043) included 540 rotavirus-positive cases, 1434 rotavirus-negative controls and 1069 respiratory controls. Three children received their first HRV dose at 53, 78 and 102 weeks of age respectively, and were not included in the graph. HRV=human rotavirus vaccine.

PAPER II

Temporal association of rotavirus vaccine introduction and reduction in all-cause childhood diarrhoeal hospitalisations in South Africa

Michelle J. Groome[1,2,3], Elizabeth R. Zell[4], Fatima Solomon[1,2], Susan Nzenze[1,2], Umesh D. Parashar[5], Alane Izu[1,2] , Shabir A. Madhi[1,2,6]

[1]Medical Research Council: Respiratory and Meningeal Pathogens Research Unit, University of the Witwatersrand, Johannesburg, South Africa

[2]Department of Science and Technology/National Research Foundation: Vaccine Preventable Diseases, University of the Witwatersrand, Johannesburg, South Africa

[3]School of Public Health, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

[4]Stat-Epi Associates Inc., Florida, USA

[5]Division of Viral Diseases, Centers for Disease Control and Prevention, Atlanta, USA;

[6]National Institute for Communicable Diseases: a division of National Health Laboratory Service, Sandringham, South Africa;

Corresponding Author: Michelle J. Groome, MD

Medical Research Council: Respiratory and Meningeal Pathogens Research Unit; DST/NRF: Vaccine Preventable Diseases

New Nurses Residence, 11th Floor West Wing, Chris Hani Baragwanath Academic Hospital, Soweto, South Africa, 2013, South Africa

Phone: +27-11-983-4283

E-mail: groomem@rmpru.co.za

Alternate corresponding Author: Shabir A. Madhi, MD

Medical Research Council: Respiratory and Meningeal Pathogens Research Unit / DST/NRF: Vaccine Preventable Diseases

New Nurses Residence, 11th Floor West Wing, Chris Hani Baragwanath Academic Hospital, Soweto, South Africa, 2013, South Africa

Phone: +27-11-983-4283

E-mail: ShabirM@nicd.ac.za

Running header: Reduction in diarrhoeal hospitalisations

Abstract 247 words

Manuscript 3014 words

Abstract

Background: The public health impact of rotavirus vaccination in African settings with a high HIV infection prevalence is yet to be established. We evaluated trends in all-cause diarrhoeal hospitalisations in Soweto, Johannesburg before and after the introduction of rotavirus vaccine into South Africa's national immunisation programme in August 2009.

Methods: Hospitalisations in children <5 years of age with a diagnosis of diarrhoea, defined by one of the following ICD-10 codes A00-A05, A06.0-A06.3, A06.9, A07.0-A07.2, A07.9, A08-A09, were identified at the Chris Hani Baragwanath Academic Hospital from 1 January 2006−31 December 2014. The median annual pre-vaccine (2006−2008) hospitalisation incidence was compared to the vaccine-era (2010−2014), and stratified by age group and HIV infection status.

Results: Incidence reductions (per 1000 population) were greatest in children aged <12 months: 54.4 in the pre-vaccine era compared to 30.0, 23.6, 20.0, 18.8 and 18.9 in the post-vaccine years 2010 to 2014 respectively; a 44.9% to 65.4% reduction. Lower incidence reductions (39.8% to 49.4%) were observed among children aged 12−24 months from the second year post-vaccine introduction onward. Reductions were observed in both HIV-infected and HIV-uninfected children. There was a change in the seasonal pattern of diarrhoeal hospitalisations post-vaccine introduction, with flattening of the autumn-winter peaks seen in the pre-vaccine years.

Discussion: An accelerated and sustained decline in all-cause diarrhoeal hospitalisations, temporally associated with rotavirus vaccine introduction, was observed in children <2 years of age. However, the impact of other interventions such as improved sanitation and changes in HIV management cannot be discounted.

Key words: rotavirus vaccine, diarrhoea, HIV, children

Introduction

Rotavirus is the leading cause of diarrhoea among children <5 years of age, accounting for approximately 27% of all severe diarrhoea episodes worldwide in 2011. Rotavirus vaccines have the potential to reduce diarrhoeal morbidity and mortality, especially in Africa where almost half of the global rotavirus deaths occur.[1,2] Following demonstration of high vaccine efficacy of Rotarix® (GlaxoSmithKline Biologicals, Rixensart, Belgium) and RotaTeq® (Merck Vaccines, Whitehouse Station, NJ) in pre-licensure clinical trials in Latin America, Europe and the United States, introduction of rotavirus vaccines into national immunisation programmes resulted in a substantial decrease in diarrhoea-related hospitalisations and deaths in these countries.[3-9] Lower vaccine efficacy was, however, observed in clinical trials in low-middle income countries in Africa, and there are limited data evaluating the impact of rotavirus vaccines on the burden of diarrhoeal hospitalisations in African settings.[10,11]

The effectiveness of rotavirus vaccines in Malawi and South Africa has been evaluated by case-control studies but these studies were not able to fully address the public health impact of this intervention.[12,13] Sentinel surveillance in children <5 years in South Africa showed that diarrhoeal hospitalisations decreased by about a third in 2010 and 2011 compared to 2009, the year of vaccine introduction.[14] A study from Ghana reported a 52-59% reduction in all-cause diarrhoea hospitalisations in children <5 years in the year following rotavirus vaccine introduction compared to the immediate pre-vaccination year.[15] These studies were, however, limited to the first two years post-introduction and did not specifically assess reductions in diarrhoea hospitalisations in HIV-infected children, who are at fivefold greater risk of diarrheal hospitalisation compared to HIV-uninfected children.[16]

We investigated the impact of routine infant rotavirus vaccination on all-cause diarrhoeal hospitalisations by comparing the incidence before (2006−2008) and after (2010−2014) vaccine

introduction among HIV-infected and HIV-uninfected children <5 years of age in Soweto, South Africa.

Methods

Study setting

The Chris Hani Baragwanath Academic Hospital (CHBAH) was the only public hospital serving the urban community of Soweto, Johannesburg, South Africa, among whom <10% had private medical insurance. As a result the majority of children who required hospitalisation were admitted to this hospital, where care was provided free of charge to children <5 years. The prevalence of HIV infection among mothers attending antenatal clinics in the Gauteng province remained steady at 30% since 2006, however, due to improvement in the prevention of mother-to-child transmission of HIV, the HIV prevalence among children <5 years decreased from 5.0% in 2006 to 3.8% by 2013.[17,18] Access to antiretroviral therapy (ART) improved since its introduction into the public sector in 2004, with the estimated coverage in HIV-infected children requiring treatment being 54% in 2009 and 63% in 2012.[19,20] Rotarix® was introduced into the national immunisation programme on 1 August 2009, and is provided at no cost at primary healthcare facilities. Two doses are recommended at 6 and 14 weeks of age and children received trivalent oral polio vaccine (OPV-Merieux; Sanofi Pasteur, Lyon, France) concurrent with rotavirus vaccine at the 6-week immunisation visit. District Health Information Systems estimates of coverage rates for the second dose of rotavirus vaccine increased from 67% in 2010 to 96% in 2011.[21]

Study participants

Children presenting with diarrhoea either came directly from the community or were referred from community clinics. Decisions regarding hospitalisation, investigations and treatment were at the discretion of the attending physicians. Patient discharge diagnoses were obtained from a discharge summary completed by the attending physician on discharge/death of the child or the

ward admission registry. A study physician coded the discharge diagnoses using the 10th revision of the International Classification of Diseases (ICD-10). HIV infection status was obtained from HIV test results recorded in the discharge summary, ward registry or from the hospital laboratory. Children ≥9 months were considered to be HIV-infected if either HIV ELISA or HIV PCR test was positive; and HIV-uninfected if HIV ELISA or HIV PCR test was negative. Children <9 months were considered to be HIV-infected if HIV PCR test was positive and HIV-uninfected if HIV ELISA or HIV PCR test was negative. If a child was hospitalised more than once, results of any HIV test performed during these hospitalisations were used to assign HIV status to that child.

Data analysis

All-cause diarrhoeal hospitalisations were defined by the following ICD-10 diagnosis codes: A00-A05, A06.0-A06.3, A06.9, A07.0-A07.2, A07.9, A08-A09. Children <5 years of age hospitalised with a primary or secondary diagnosis of diarrhoea from 1 January 2006−31 December 2014 were included in this study. Any hospitalisations with a co-diagnosis of nosocomial infection (ICD-10 code Y95) or occurring within 14 days of the discharge date of a previous admission in the same child were excluded. All data were anonymised for personal identifiers of patients.

Monthly counts of all-cause diarrhoeal hospitalisations were plotted against time for age groups 0–11, 12–23 and 24–59 months for the period 2006–2014. A general strike of hospital staff occurred during a three-week period in June 2007, resulting in only one paediatric ward remaining functional during this time. We adjusted for the resulting decrease in hospitalisations by calculating the ratio of hospitalisations, by age group, in June compared to May in 2006 and 2008, and multiplying the number of hospitalisations in May 2007 by this factor. Annual incidence of all-cause diarrhoeal hospitalisation (per 1000 population) was estimated using the annual number of children hospitalised for diarrhoea in the numerator and the mid-year population estimate in the denominator. Population denominators for Soweto (sub-district D and

G, Johannesburg) were obtained from Statistics South Africa and HIV prevalence was estimated from projections of the Actuarial Society of South Africa's (ASSA) 2008 AIDS and Demographical model.[17] The median annual incidence during the pre-vaccine years 2006-2008 was compared to the incidence in the vaccine-era (2010-2014), and stratified by age group and HIV status. Children who were not tested for HIV infection were assumed to be HIV-uninfected, on the assumption that physicians were less inclined to test for HIV in the absence of clinical stigmata. We assessed the incidence of hospitalisation for bronchiolitis (ICD-10 codes J21.0, J21.1, J21.8, J21.9), for which there were no preventative intervention strategies implemented over the same period, to determine whether there were any changes in hospital admission practices. Confidence intervals for incidence estimates were calculated using the Poisson distribution.

Ethics statement

Approval for the study was obtained from Human Research Ethics Committee (HREC approval number: M110528) of the University of the Witwatersrand. There was a waiver of consent for this observational study.

Results

Trends in diarrhoeal hospitalisations

A total of 16 800 diarrhoeal hospitalisations occurred in children <5 years from 1 January 2006−31 December 2014. There was a downward trend in the number of diarrhoeal hospitalisations after rotavirus vaccine introduction, especially in children aged 0−11 months (Figure 1). Before rotavirus vaccine introduction, 68.6% of diarrhoeal hospitalisations occurred in children 0−11 months, 20.9% in those 12−23 months and 10.5% in those 24−59 months of age, compared to the vaccine-era where 58.8%, 24.3% and 16.9% of hospitalisations occurred in the respective age groups ($p<0.001$). During the pre-vaccine period there were distinct annual peaks in the number of diarrhoeal hospitalisations during the autumn and early winter months of

March−May in children <24 months, albeit this varied in magnitude and timing from year to year. In contrast, the peaks in diarrhoeal hospitalisations during the vaccine-era were less pronounced and had a bimodal pattern.

Annual diarrhoeal hospitalisation rates

The estimated annual incidence (per 1000 population) of diarrhoeal hospitalisations among children <5 years decreased from 14.7 (median 2006−2008) to 9.7 in 2010; a 33.8% reduction (Table 1). Significant incidence reductions of 47.6% to 56.6% (7.0 to 8.3 per 1000) were maintained through the following four years (2011−2014). Reductions were most pronounced in children 0−11 months and were evident from the first year post-vaccine introduction (incidence reduction 24.4 per 1000; 44.9% in 2010 compared to the pre-vaccine era; Table 1). Further reduction was seen in 2011, the second post-vaccine year (30.8 per 1000, 55.6%), compared to pre-vaccine years and reductions of 63.2% to 65.2% (34.4 to 35.5 per 1000) were maintained during 2012−2014 in this age group. Among children 12−23 months, there was a marginal reduction in hospitalisation incidence in the first year post-vaccine introduction, and reductions of 39.8% to 49.4% were observed during the subsequent four years. Hospitalisation incidence among children 24−59 months remained relatively constant with minimal reductions in the vaccine-era compared to pre-vaccine years (Table1). In contrast to the vaccine-era declines in diarrhoeal hospitalisations, the incidence of bronchiolitis hospitalisations remained relatively constant throughout the observation period (Supplementary Figure).

HIV-infected and HIV-uninfected children

Of the total diarrhoeal hospitalisations, 1743 (10.4%) occurred in children diagnosed with HIV-infection. The prevalence of HIV infection among children <5 years hospitalised for diarrhoea decreased from 14.1% in 2006 to 5.3% in 2014. Overall only 50% of children were tested for HIV infection, which varied by age group (older children were less likely to be tested) and year of hospitalisation (increased testing in the latter years; data not shown).

Among children assumed to be HIV-uninfected, significant declines in diarrhoeal hospitalisation incidence in the vaccine-era compared to pre-vaccine years, ranging from 23.2 to 32.8 per 1000 (45.8% to 64.9%), were observed in those 0−11 months (Figure 2; Supplementary Table 1). In children 12−23 months, reductions of 36.5% to 49.5% were observed from the second year post-vaccine introduction onward. There were minimal or no incidence reductions in children 24−59 months.

The hospitalisation incidence among HIV-infected children 0−11 months decreased by 102.0 per 1000 (77%) in 2014 compared to the pre-vaccine years, with reductions ranging from 21.8% to 70.8% during 2010−2013 (Figure 3; Supplementary Table 2). In children 12-23 months, reductions in diarrhoeal hospitalisations in the vaccine-era ranged from 45.0% to 64.8% (16.9 to 24.4 per 1000). There were significant reductions (41.6% to 56.7%) in incidence in HIV-infected children 24-59 months in most post-vaccine years.

Discussion

The introduction of an oral live-attenuated rotavirus vaccine into the South African national immunisation programme was temporally associated with a 34% to 57% decrease in the overall incidence of all-cause diarrhoeal hospitalisations in children <5 years in the urban setting of Soweto, Johannesburg. Reductions were greatest among children <12 months and maintained over a 5-year period post-vaccine introduction. A decrease of 45% in incidence was observed in the first year post-vaccine introduction in this age group, with further reductions of 57% to 65% maintained through the subsequent four years. Among children 12−24 months, reductions of 40% to 49% were observed from the second year post-vaccine introduction and were also maintained over time. These trends were observed among both HIV-infected and HIV-uninfected children. The incidence of all-cause diarrhoeal hospitalisations in the post-vaccine years remained relatively unchanged or changed minimally among children >24 months.

Rotavirus testing was not conducted, either routinely or as part of a surveillance program until diarrhoeal surveillance was established at CHBAH in May 2009. We could, therefore, not access the impact of rotavirus vaccine introduction on rotavirus-specific diarrhoeal hospitalisations and used all-cause diarrhoeal hospitalisations as a proxy. Our findings do, however, support the hypothesis that the observed decline in all-cause diarrhoeal hospitalisations can be attributed partly to the introduction of rotavirus vaccination into the routine immunisation program. The reductions in incidence that we observed occurred specifically in the age groups that received the vaccine and were consistent with increasing vaccine coverage. Children <12 months would have been eligible to receive rotavirus vaccine from August 2009 and a 45% reduction was observed in the first post-vaccination year (2010). As coverage increased to >90% at the end of 2010, consistent reductions of ~65% were observed from 2011 to 2014. Some of the children aged 12-23 months and hospitalised in the latter half of 2010 may have received rotavirus vaccine, and there is a small incidence reduction observed in the first post-vaccination year with greater reductions observed from the second post-vaccination year in this age group. Previous South African studies showed that rotavirus disease occurred early in life, with 90-95% of children hospitalised for severe rotavirus diarrhoea being <18 months of age, hence we would expect that the major public health impact of vaccination would be in children <24 months.[22,23] We did not see any clear evidence of indirect protection i.e. protection in older unvaccinated children, as has been observed in some high-income settings.[24,25] In our setting very little rotavirus-associated hospitalisation occurred in children >2 years of age prior to vaccine introduction, so any indirect protection in this age group would likely be minimal. Encouragingly, we did not see a shift toward increased incidence of diarrhoeal hospitalisations in these older children either.

There was a change in the epidemiology of diarrhoeal disease after rotavirus vaccine introduction. The pre-vaccine years were characterised by peaks in diarrhoeal hospitalisations among children <24 months during the autumn-winter months of March to May. This seasonal pattern is consistent with what was known about rotavirus epidemiology in South Africa prior to

vaccine introduction. Although rotavirus disease occurred year round, increases in rotavirus shedding had been observed during the cool, drier months resulting in autumn-winter peaks in rotavirus-associated hospitalisations.[23] We observed a diminished peak in all-cause diarrhoeal hospitalisations during 2010 and these peaks were less pronounced during 2011−2014, where a bimodal pattern was observed.

The Phase III clinical trial, which included both HIV-infected and HIV-uninfected children, demonstrated efficacy of the rotavirus vaccine against all-cause severe gastroenteritis of 44% (95% CI 19-61) during the first year of life, whereas our trend analysis showed reductions of 57-65% in children <12 months.[10] Although the confidence intervals do overlap, the point estimates we observed were higher. A decrease in transmission due to an overall decrease in circulation of rotavirus in the population could not be accounted for in the efficacy study design, and may account for the greater reductions which we observed in children <12 months. Our observed reductions are also greater than estimates of the reductions in all-cause diarrhoeal hospitalisations in children <12 months obtained from sentinel surveillance conducted in South Africa: 38% in the first and 43% in the second year post vaccine introduction.[14] However, these data were based on comparison with pre-vaccine estimates from 2009, the year of vaccine introduction, with no data available prior to vaccine introduction and analyses were also limited to the months of May-December.

The oral rotavirus vaccine has been shown to be safe and immunogenic in HIV-infected children, but vaccine efficacy against diarrhoea has never been specifically assessed in this sub-group.[26] The rotavirus vaccine effectiveness study conducted in South Africa observed similar effectiveness in HIV-exposed uninfected and HIV-unexposed children but was not able to assess effectiveness specifically among HIV-infected children.[12] To our knowledge, this is the first study to show the impact of rotavirus vaccine introduction on diarrhoeal hospitalisations in HIV-infected children. The reductions we observed may have been confounded by interventions other than rotavirus vaccine introduction, most notably the expanded use of ART. During the study

period there was increased uptake of ART among HIV-infected children as well as several changes in the South African guidelines for ART use. In 2010 and 2013 the HIV management guidelines were revised to include initiation of ART in all children <12 months and <5 years of age, respectively, irrespective of immunological status.[27,28] The monitoring of ART access and usage in South Africa is challenging, with changes in reporting practices, lack of data on age of patients, changes in treatment guidelines leading to changes in the denominator for estimation of treatment coverage and debate over the best measures to use to access coverage. It is thus difficult to tease out the specific impact of ART versus rotavirus vaccine among these children, and it is likely that both interventions contributed to the reduced diarrhoeal hospitalisations observed in HIV-infected children.

An ecological study such as this has inherent limitations, including the ability to attribute causality. We used all-cause diarrhoeal hospitalisations as a proxy for rotavirus-associated hospitalisations but without pathogen-specific testing it is difficult to definitively conclude on a causal relationship between rotavirus vaccine introduction and reductions in diarrhoeal hospitalisations. We cannot exclude other possible reasons for the decline in diarrhoeal hospitalisations such as changes in the socioeconomic factors in the population or variability in the natural occurrence of rotavirus or other enteric pathogens, most notably norovirus and bacteria. Furthermore, vaccine coverage data were not available specifically for the Soweto population, only on a national level. Comparing census data from 2001 and 2011 respectively, there were improvements in the proportion of households with access to tapped water (84.5% to 91.6%) and flush toilets (86.5% to. 90.5%) as well as a decrease in the average household size (3.1 to 2.9) in the Johannesburg region, although no sub-district level or year-on-year data were available.[29] One would assume that changes in sanitation would improve consistently over the years leading to a steadier decline in annual diarrhoeal hospitalisations rather than the more accelerated decline that we observed in the vaccine-era.

Our incidence calculations relied on population estimates that are based on census data (2001 and 2011) with extrapolation to other years and this may have led to under- or over-estimation of the incidence. HIV prevalence was determined using the ASSA model with extrapolation of provincial estimates to the Soweto population, which may also have influenced our incidence calculations. Our assumption that children without a documented HIV status were HIV-uninfected may have underestimated the disease burden among HIV-infected children. We believe, however, that this assumption likely approximates the true prevalence of HIV among diarrhoeal hospitalisations in that clinicians generally only tested children they suspected of being HIV-infected. An HIV-infected child would most likely have had more than one hospitalisation, prompting HIV testing and if a child tested positive on a subsequent admission, we assumed that the child would have been positive on all admissions. Lastly, our study was restricted to one urban community in South Africa and may not be generalizable to other African settings.

While the impact of other interventions such as improvement in socio-economic conditions and changes in HIV management cannot be discounted, the accelerated decline in all-cause diarrhoeal hospitalisations after rotavirus vaccine introduction and the observed changes in diarrhoeal epidemiology are suggestive of a significant public health impact of rotavirus vaccine introduction in an African setting with a high prevalence of HIV infection.

NOTES

Author contributions

MJ Groome (MJG) ER Zell (ERZ) F Solomon (FS) S Nzenze (SN) UD Parashar (UDP) Alane Izu (AI) SA Madhi (SAM)

Conception and design of the study – MJG, UDP, SAM

Acquisition of data – MJG, FS, SN

Analysis of data – MJG, ERZ, AI

Interpretation of data – MJG, ERZ, UDP, SAM

Drafting of article - MJG

Critically revising drafts of article – MJG, ERZ, FS, SN, UDP, AI, SAM

Final approval of submitted version – MJG, ERZ, FS, SN, UDP, AI, SAM

Acknowledgements

We would like to thank the Department of Paediatrics, Chris Hani Baragwanath Academic Hospital for assistance with collection of the data.

Funding

Support for this project was provided by PATH through funding from the GAVI Alliance. The funding source had no role in the writing of the manuscript or the decision to submit it for publication. The views expressed by the authors do not necessarily reflect the views of GAVI or the Centers for Disease Control and Prevention. The authors have not been paid to write this article by a pharmaceutical company or other agency. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Supplement Sponsorship: This article appeared as part of the supplement “Health Benefits of Rotavirus Vaccination in Developing Countries,” sponsored by the CDC Foundation.

Conflicts of Interest

M.J. Groome reports grants from GAVI Alliance, during the conduct of the study; personal fees from GlaxoSmithKline, personal fees from Sanofi Pasteur, outside the submitted work.

Dr Solomon reports grants from GAVI Alliance, during the conduct of the study.

Dr Nzenze reports grants from GAVI Alliance, during the conduct of the study.

Dr. Madhi reports grants from GAVI AVI Special Studies, during the conduct of the study; grants and personal fees from GSK, personal fees from Pfizer, grants from Novartis, personal fees from Medimmune, grants from BMGF, outside the submitted work.

The other authors report no conflicts of interest.

References

1. Tate JE, Burton AH, Boschi-Pinto C, Steele AD, Duque J, Parashar UD. 2008 estimate of worldwide rotavirus-associated mortality in children younger than 5 years before the introduction of universal rotavirus vaccination programmes: a systematic review and meta-analysis. *Lancet Infect Dis* 2012; **12**(2): 136-41.
2. Walker CL, Rudan I, Liu L, et al. Global burden of childhood pneumonia and diarrhoea. *Lancet* 2013; **381**(9875): 1405-16.
3. 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.
4. 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.
5. Vesikari T, Karvonen A, Prymula R, et al. Efficacy of human rotavirus vaccine against rotavirus gastroenteritis during the first 2 years of life in European infants: randomised, double-blind controlled study. *Lancet* 2007; **370**(9601): 1757-63.
6. Rha B, Tate JE, Payne DC, et al. Effectiveness and impact of rotavirus vaccines in the United States - 2006-2012. *Expert review of vaccines* 2014; **13**(3): 365-76.
7. Yen C, Armero Guardado JA, Alberto P, et al. Decline in rotavirus hospitalizations and health care visits for childhood diarrhea following rotavirus vaccination in el salvador. *Pediatr Infect Dis J* 2011; **30**(1 Suppl): S6-S10.
8. Quintanar-Solares M, Yen C, Richardson V, Esparza-Aguilar M, Parashar UD, Patel MM. Impact of rotavirus vaccination on diarrhea-related hospitalizations among children < 5 years of age in Mexico. *Pediatr Infect Dis J* 2011; **30**(1 Suppl): S11-5.
9. Richardson V, Hernandez-Pichardo J, Quintanar-Solares M, et al. Effect of rotavirus vaccination on death from childhood diarrhea in Mexico. *N Engl J Med* 2010; **362**(4): 299-305.

10. Madhi SA, Cunliffe NA, Steele D, et al. Effect of human rotavirus vaccine on severe diarrhea in African infants. *N Engl J Med* 2010; **362**(4): 289-98.
11. Armah GE, Sow SO, Breiman RF, et al. Efficacy of pentavalent rotavirus vaccine against severe rotavirus gastroenteritis in infants in developing countries in sub-Saharan Africa: a randomised, double-blind, placebo-controlled trial. *Lancet* 2010; **376**(9741): 606-14.
12. Groome MJ, Page N, Cortese MM, et al. Effectiveness of monovalent human rotavirus vaccine against admission to hospital for acute rotavirus diarrhoea in South African children: a case-control study. *Lancet Infect Dis* 2014; **14**(11): 1096-104.
13. Bar-Zeev N, Kapanda L, Tate JE, et al. Effectiveness of a monovalent rotavirus vaccine in infants in Malawi after programmatic roll-out: an observational and case-control study. *Lancet Infect Dis* 2015; **15**(4): 422-8.
14. Msimang VM, Page N, Groome MJ, et al. Impact of rotavirus vaccine on childhood diarrheal hospitalization after introduction into the South African public immunization program. *Pediatr Infect Dis J* 2013; **32**(12): 1359-64.
15. Enweronu-Laryea CC, Boamah I, Sifah E, Diamenu SK, Armah G. Decline in severe diarrhea hospitalizations after the introduction of rotavirus vaccination in Ghana: a prevalence study. *BMC Infect Dis* 2014; **14**: 431.
16. Groome MJ, Madhi SA. Five-year cohort study on the burden of hospitalisation for acute diarrhoeal disease in African HIV-infected and HIV-uninfected children: potential benefits of rotavirus vaccine. *Vaccine* 2012; **30 Suppl 1**: A173-8.
17. Actuarial Society of South Africa (ASSA). AIDS and Demographic Model 2008. Available at: http://aids.actuarialsociety.org.za. Accessed 22 July 2015.
18. National Department of Health South Africa.The National Antenatal Sentinel HIV and Syphilis Prevalence Survey, South Africa, 2011. Available at: http://www.health.gov.za/index.php/2014-03-17-09-09-38/reports/category/99-2013re. Accessed 13 January 2016.

19. Johnson L. Access to antiretroviral treatment in South Africa, 2004 - 2011. *The Southern African Journal of HIV Medicine* 2012; **13**: 22-7.
20. Global report: UNAIDS report on the global AIDS epidemic 2013. Available at: http://www.unaids.org/sites/default/files/media_asset/UNAIDS_Global_Report_2013_en_1.pdf. Accessed 2 February 2015.
21. Madhi SA, Bamford L, Ngcobo N. Effectiveness of pneumococcal conjugate vaccine and rotavirus vaccine introduction into the South African public immunisation programme. *S Afr Med J* 2014; **104**(3 Suppl 1): 228-34.
22. Mapaseka SL, Dewar JB, van der Merwe L, et al. Prospective hospital-based surveillance to estimate rotavirus disease burden in the Gauteng and North West Province of South Africa during 2003-2005. *J Infect Dis* 2010; **202 Suppl**: S131-8.
23. 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.
24. Clarke MF, Davidson GP, Gold MS, Marshall HS. Direct and indirect impact on rotavirus positive and all-cause gastroenteritis hospitalisations in South Australian children following the introduction of rotavirus vaccination. *Vaccine* 2011; **29**(29-30): 4663-7.
25. Lopman BA, Curns AT, Yen C, Parashar UD. Infant rotavirus vaccination may provide indirect protection to older children and adults in the United States. *J Infect Dis* 2011; **204**(7): 980-6.
26. Steele AD, Madhi SA, Louw CE, et al. Safety, Reactogenicity, and Immunogenicity of Human Rotavirus Vaccine RIX4414 in Human Immunodeficiency Virus-positive Infants in South Africa. *Pediatr Infect Dis J* 2011; **30**(2): 125-30.
27. National Department of Health South Africa. Guidelines for the Management of HIV in Children, 2nd Edition, 2010. Available at: http://www.sahivsoc.org/practise-guidelines/national-dept-of-health-guidelines. Accessed 13 January 2016.

28. National Department of Health South Africa. The South African Antiretroviral Treatment Guidelines, 2013. Available at: http://www.sahivsoc.org/practise-guidelines/national-dept-of-health-guidelines. Accessed 13 January 2016.

29. Statistics South Africa (STATSSA). Census 2011 Municipal report - Gauteng. Pretoria, 2012.

Figure legends

Figure 1: Monthly count of all-cause diarrhoeal hospitalisations in children <5 years of age at Chris Hani Baragwanath Academic Hospital, Soweto, South Africa: 2006–2014.

Note: vertical line represents introduction of rotavirus vaccine into the national immunisation program in August 2009.

Figure 2: Annual incidence (per 1000 population, 95% confidence interval) of diarrhoeal hospitalisations among HIV-uninfected children <5 years of age in Soweto, 2006-2014.

Values are incidence difference = incidence in vaccine-era years 2010, 2011, 2012, 2013 and 2014 respectively minus median incidence in the pre-vaccine years 2006-2008 (percent change). A negative value indicates a reduction in incidence; a positive value indicates an increase in incidence.

* $p<0.05$

Note: 95% confidence intervals and p-values are provided in Supplementary Table 1.

Figure 3: Annual incidence (per 1000 population, 95% confidence interval) of diarrhoeal hospitalisations among HIV-infected children <5 years of age in Soweto, 2006-2014.

Values are incidence difference = incidence in vaccine-era years 2010, 2011, 2012, 2013 and 2014 respectively minus median incidence in the pre-vaccine years 2006-2008 (percent change). A negative value indicates a reduction in incidence; a positive value indicates an increase in incidence.

* $p<0.05$

Note: 95% confidence intervals and p-values are provided in Supplementary Table 2.

Table 1: Incidence of all-cause diarrhoeal hospitalisations (per 1000 population) pre-vaccine introduction (2006−2008) compared to vaccine-era (2010−2014) among children <5 years of age in Soweto, South Africa

Year	Hospitalisation incidence (per 1000 population)	Incidence difference[a] (95% CI[b])	Percent change in incidence[c] (95% CI[b])	P value
0−59 months				
2006−2008[d]	14.7 (14.1, 15.3)			
2010	9.7 (9.3, 10.2)	-5.0 (-5.7, -4.2)	-33.8 (-37.8, -29.7)	<0.001
2011	7.7 (7.3, 8.1)	-7.0 (-7.7, -6.3)	-47.6 (-51.0, -44.1)	<0.001
2012	6.7 (6.3, 7.1)	-8.0 (-8.7, -7.3)	-54.5 (-57.6, -51.3)	<0.001
2013	6.4 (6.0, 6.8)	-8.3 (-9.0, -7.6)	-56.6 (-59.5, -53.4)	<0.001
2014	6.9 (6.6, 7.3)	-7.8 (-8.5-7.1)	-52.9 (-56.0, -49.6)	<0.001
0−11 months				
2006−2008[d]	54.4 (51.9, 57.0)			
2010	30.0 (28.2, 31.9)	-24.4 (-27.6, -21.3)	-44.9 (-49.0, -40.4,)	<0.001
2011	23.6 (22.1, 25.3)	-30.8 (-33.8, -27.8)	-56.6 (-60.0, -52.8)	<0.001
2012	20.0 (18.6, 21.5)	-34.4 (-37.3, -31.5)	-63.2 (-66.3, -59.8)	<0.001
2013	18.8 (17.4, 20.3)	-35.6 (-38.5, -32.7)	-65.4 (-68.4, -62.2)	<0.001
2014	18.9 (17.6, 20.4)	-35.5 (-38.4, -32.6)	-65.2 (-68.2, -62.0)	<0.001
12−23 months				
2006-2008[d]	14.9 (13.6, 16.2)			
2010	13.0 (11.8, 14.2)	-1.9 (-3.6, -0.1)	-12.7 (-23.2, -0.8)	0.035
2011	8.7 (7.7., 9.7)	-6.2 (-7.8, -4.6)	-41.6 (-49.4, -32.6)	<0.001
2012	7.6 (6.7, 8.5)	-7.3 (-8.9, -5.7)	-49.0 (-56.2, -40.9)	<0.001
2013	7.5 (6.7, 8.5)	-7.3 (-8.9, -5.8)	-49.4 (-56.4, -41.2)	<0.001
2014	8.9 (8.0, 10.0)	-5.9 (-7.5, -4.3)	-39.8 (-47.7, -30.7)	<0.001
24−59 months				
2006-2008[d]	2.5 (2.2, 2.8)			
2010	2.3 (2.1, 2.7)	-0.2 (-0.6, 0.3)	-6.2 (-21.2, 11.6)	0.461
2011	2.2 (1.9, 2.5)	-0.3 (-0.7, 0.1)	-13.0 (-27.1, 3.9)	0.116
2012	2.0 (1.7, 2.2)	-0.5 (-0.9, -0.1)	-21.7 (-34.9, -6.1)	0.007
2013	1.8 (1.6, 2.1)	-0.7 (-1.1, -0.3)	-26.5 (-39.0, -11.5)	<0.001
2014	2.2 (1.9, 2.5)	-0.3 (-0.7, 0.1)	-12.9 (-27.1, 4.0)	0.118

[a] Incidence in vaccine-era years 2010, 2011, 2012, 2013 and 2014 respectively compared to median incidence in the pre-vaccine years 2006-2008. A negative value indicates a reduction in incidence; a positive value indicates an increase in incidence

[b] Confidence interval

[c] A negative percent change indicates a reduction in incidence; a positive percent change indicates an increase in incidence
[d] Median hospitalisation incidence 2006-2008

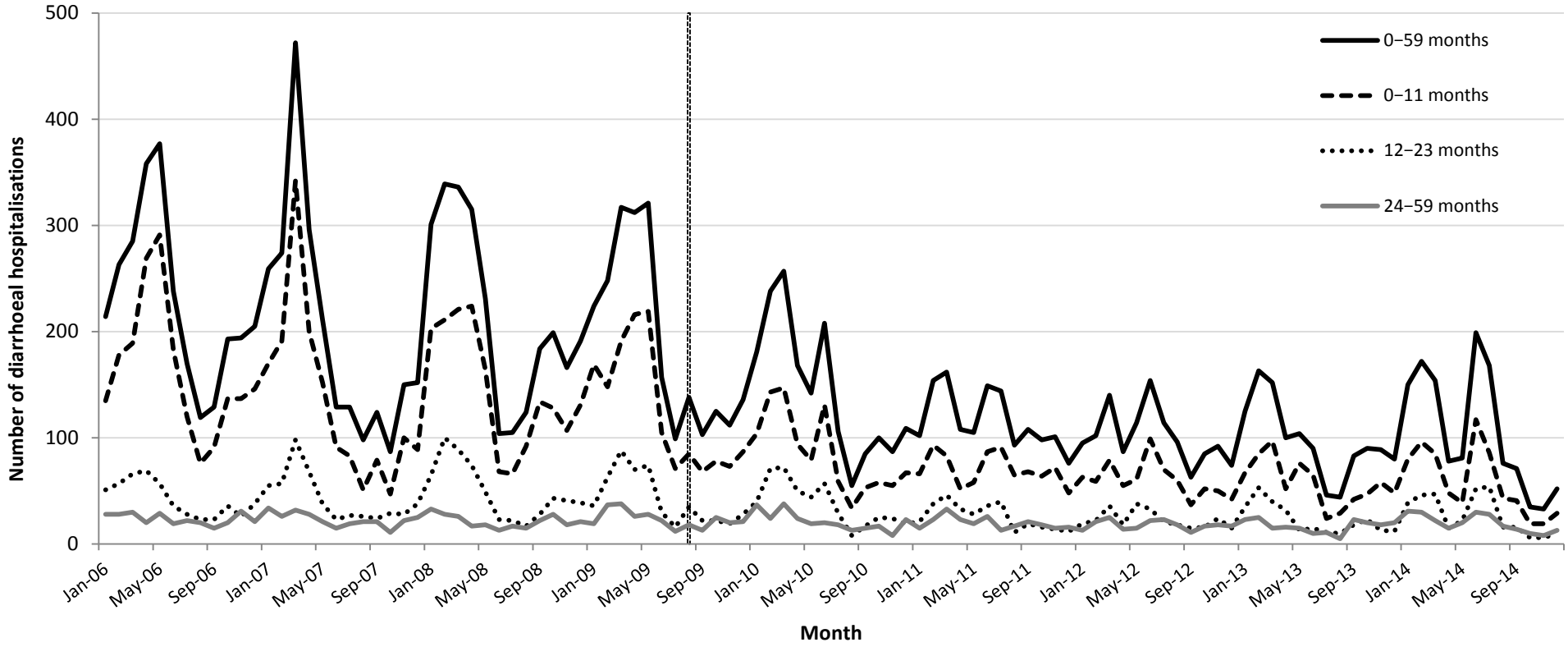
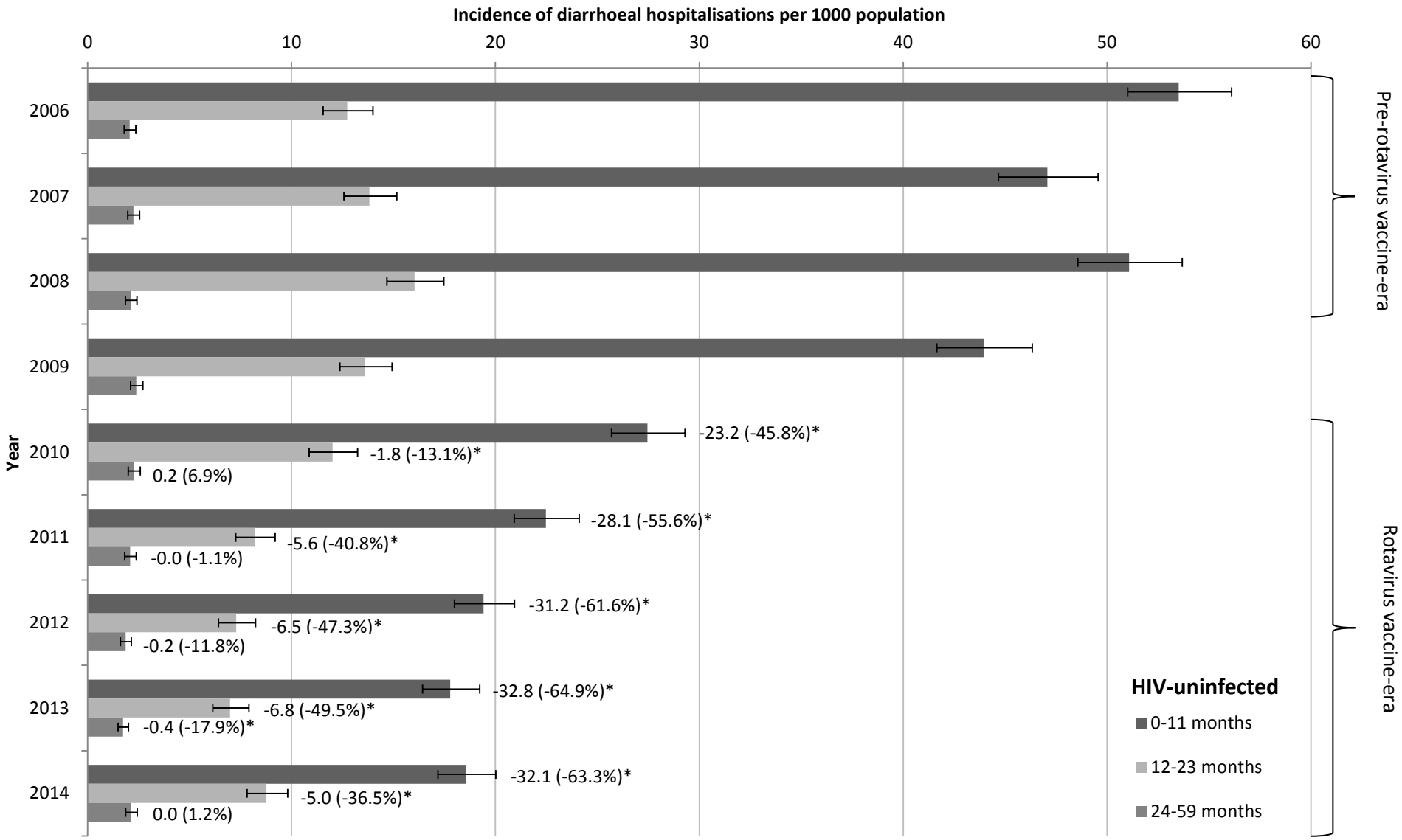
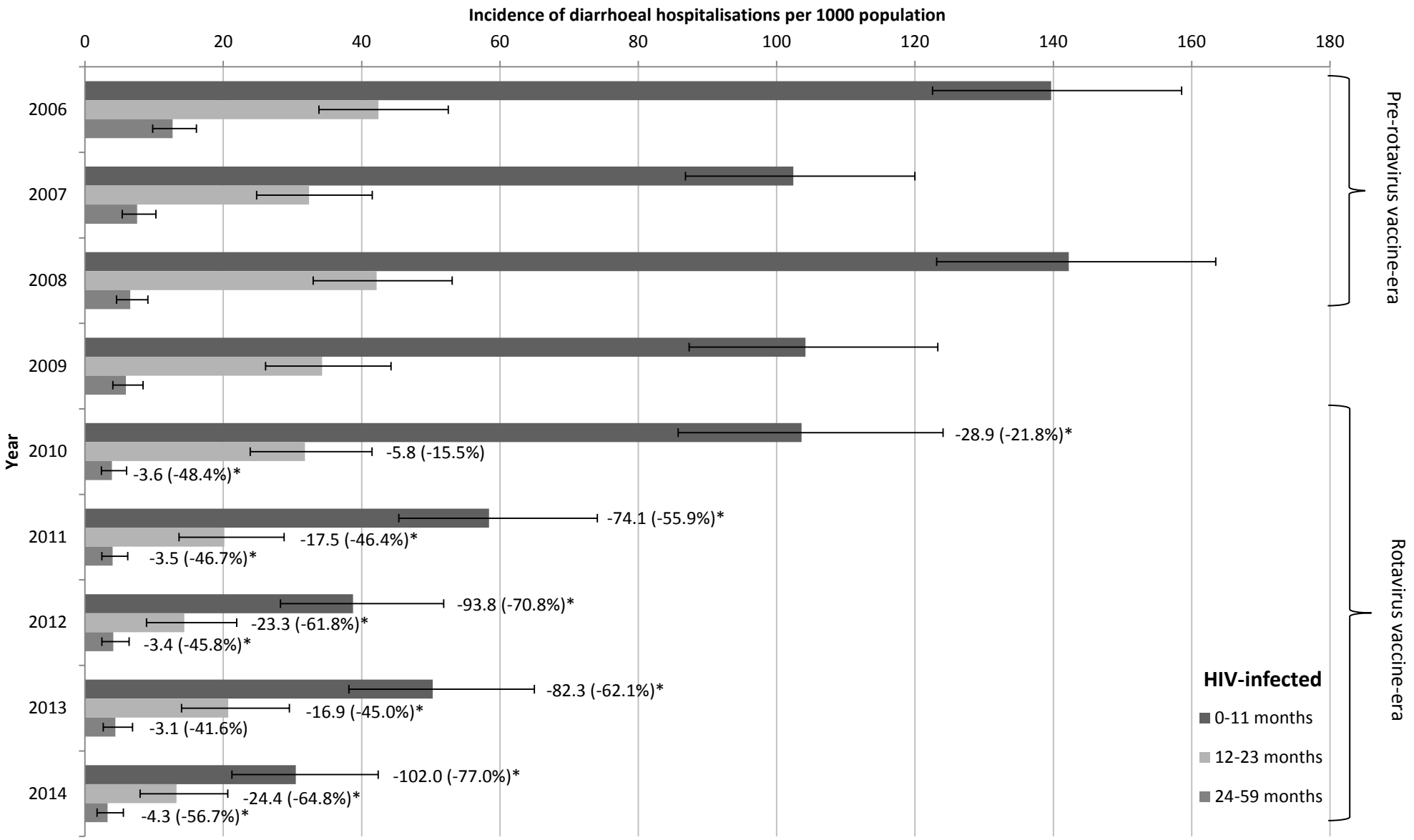


Figure 1: Monthly count of all-cause diarrhoeal hospitalisations in children <5 years of age at Chris Hani Baragwanath Academic Hospital, Soweto, South Africa: 2006–2014.

Note: vertical line represents introduction of rotavirus vaccine into the national immunisation program in August 2009.


Incidence of diarrhoeal hospitalisations per 1000 population
0
10
20
30
40
50
60
Year
2006
2007
2008
2009
2010
2011
2012
2013
2014
-23.2 (-45.8%)*
-1.8 (-13.1%)*
0.2 (6.9%)
-28.1 (-55.6%)*
-5.6 (-40.8%)*
-0.0 (-1.1%)
-31.2 (-61.6%)*
-6.5 (-47.3%)*
-0.2 (-11.8%)
-32.8 (-64.9%)*
-6.8 (-49.5%)*
-0.4 (-17.9%)*
-32.1 (-63.3%)*
-5.0 (-36.5%)*
0.0 (1.2%)
HIV-uninfected
0-11 months
12-23 months
24-59 months
Pre-rotavirus vaccine-era
Rotavirus vaccine-era


Incidence of diarrhoeal hospitalisations per 1000 population
0
20
40
60
80
100
120
140
160
180
Year
2006
2007
2008
2009
2010
2011
2012
2013
2014
-28.9 (-21.8%)*
-5.8 (-15.5%)
-3.6 (-48.4%)*
-74.1 (-55.9%)*
-17.5 (-46.4%)*
-3.5 (-46.7%)*
-93.8 (-70.8%)*
-23.3 (-61.8%)*
-3.4 (-45.8%)*
-82.3 (-62.1%)*
-16.9 (-45.0%)*
-3.1 (-41.6%)
-102.0 (-77.0%)*
-24.4 (-64.8%)*
-4.3 (-56.7%)*
HIV-infected
0-11 months
12-23 months
24-59 months
Pre-rotavirus vaccine-era
Rotavirus vaccine-era

Supplementary Table 1: Incidence of all-cause diarrhoeal hospitalisations (per 1000 population) pre-vaccine introduction (2006−2008) compared to vaccine-era (2010−2014) among HIV-uninfected children <5 years of age in Soweto, South Africa

Year	Hospitalisation incidence (per 1000 population)	Incidence difference[a] (95% CI[b])	Percent change in incidence[c] (95% CI[b])	P value
0−11 months				
2006−2008[d]	50.6 (48.1, 53.2)			
2010	27.5 (25.7, 29.3)	-23.2 (-26.3, -20.1)	-45.8 (-50.1, -41.1)	<0.001
2011	22.5 (20.9, 24.1)	-28.1 (-31.1, -25.2)	-55.6 (-59.3, -51.6)	<0.001
2012	19.4 (18.0, 20.9)	-31.2 (-34.1, -28.3)	-61.6 (-65.0, -58.0)	<0.001
2013	17.8 (16.4, 19.2)	-32.8 (-35.7, -30.0)	-64.9 (-68.0, -61.4)	<0.001
2014	18.6 (17.2, 20.0)	-32.1 (-34.9, -29.2)	-63.3 (-66.6, -59.8)	<0.001
12−23 months				
2006-2008[d]	13.8 (12.6, 15.2)			
2010	12.0 (10.9, 13.2)	-1.8 (-3.5, -0.1)	-13.1 (-24.2, -0.4)	0.041
2011	8.2 (7.3, 9.2)	-5.6 (-7.2, -4.0)	-40.8 (-49.1, -31.1)	<0.001
2012	7.3 (6.4, 8.2)	-6.5 (-8.1, -5.0)	-47.3 (-55.0, -38.4)	<0.001
2013	7.0 (6.1, 7.9)	-6.8 (-8.4, -5.3)	-49.5 (-57.0, -40.9)	<0.001
2014	8.8 (7.8, 9.8)	-5.0 (-6.7, -3.4)	-36.5 (-45.3, -26.5)	<0.001
24−59 months				
2006-2008[d]	2.1 (1.8, 2.4)			
2010	2.3 (2.0, 2.6)	0.2 (-0.2, 0.6)	6.9 (-12.2, 22.9)	0.443
2011	2.1 (1.8, 2.4)	-0.0 (-0.4, 0.4)	-1.1 (-18.3, 19.8)	0.910
2012	1.9 (1.6, 2.1)	-0.2 (-0.6, 0.1)	-11.8 (-27.6, 7.4)	0.201
2013	1.7 (1.5, 2.0)	-0.4 (-0.8, -0.0)	-17.9 (-32.9, 0.3)	0.048
2014	2.1 (1.9, 2.4)	0.0 (-0.4, 0.4)	1.2 (-19.5, 18.3)	0.902

[a] Incidence in vaccine-era years 2010, 2011, 2012, 2013 and 2014 respectively compared to median incidence in the pre-vaccine years 2006-2008. A negative value indicates a reduction in incidence; a positive value indicates an increase in incidence

[b] Confidence interval

[c] A negative percent change indicates a reduction in incidence; a positive percent change indicates an increase in incidence

[d] Median hospitalisation incidence 2006-2008

Supplementary Table 2: Incidence of all-cause diarrhoeal hospitalisations (per 1000 population) pre-vaccine introduction (2006−2008) compared to vaccine-era (2010−2014) among known HIV-infected children <5 years of age in Soweto, South Africa

Year	Hopsitalisation incidence (per 1000 population)	Incidence difference [a] (95% CI[b])	Percent change in incidence[c] (95% CI[b])	P value
0−11 months				
2006−2008[d]	132.5 (114.7, 152.3)			
2010	103.6 (85.8, 124.1)	-28.9 (-55.2, -2.7)	-21.8 (-38.3, -1.3)	0.033
2011	58.4 (45.4, 74.1)	-74.1 (-97.2, -51.0)	-55.9 (-67.0, -41.7)	<0.001
2012	38.8 (28.3, 51.9)	-93.8 (-115.4, -72.1)	-70.8 (-79.3, -59.4)	<0.001
2013	50.3 (38.2, 65.0)	-82.3 (-104.8, -59.7)	-62.1 (-72.2, -48.9)	<0.001
2014	30.5 (21.2, 42.4)	-102.0 (-123.1, -81.0)	-77.0 (-84.4, -66.9)	<0.001
12−23 months				
2006-2008[d]	37.6 (29.4, 47.4)			
2010	31.8 (23.9, 41.5)	-5.8 (-18.0, 6.3)	-15.5 (-41.7, 22.0)	0.352
2011	20.2 (13.6, 28.8)	-17.5 (-28.8, -6.2)	-46.4 (-66.2, -16.9)	0.003
2012	14.4 (8.9, 22.0)	-23.3 (-33.9, -12.6)	-61.8 (-77.7, -37.2)	<0.001
2013	20.7 (14.0, 29.6)	-16.9 (-28.3, -5.5)	-45.0 (-65.3, -14.6)	0.005
2014	13.2 (8.0, 20.7)	-24.4 (-34.9, -13.9)	-64.8 (-80.0, -41.0)	<0.001
24−59 months				
2006-2008[d]	7.5 (5.4, 10.3)			
2010	3.9 (2.4, 6.0)	-3.6 (-6.5, -0.8)	-48.4 (-71.4, -9.6)	0.014
2011	4.0 (2.5, 6.2)	-3.5 (-6.4, -0.6)	-46.7 (-70.5, -6.6)	0.020
2012	4.1 (2.5, 6.4)	-3.4 (-6.4, -0.5)	-45.8 (-70.3, -4.1)	0.025
2013	4.4 (2.6, 6.9)	-3.1 (-6.2, -0.1)	-41.6 (-68.0, 3.3)	0.051
2014	3.3 (1.7, 5.6)	-4.3 (-7.2, -1.3)	-56.7 (-78.8, -17.4)	0.006

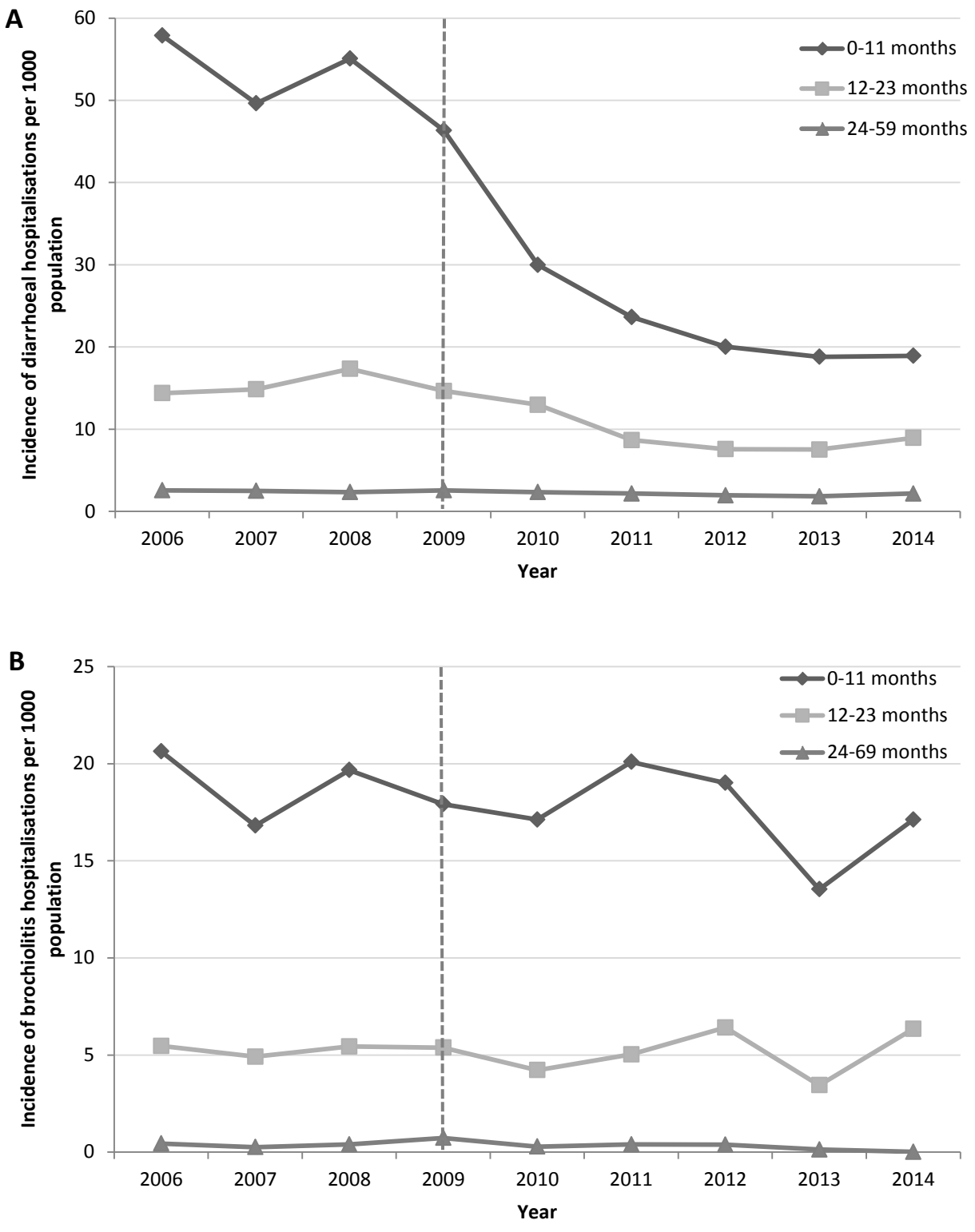
[a] Incidence in vaccine-era years 2010, 2011, 2012, 2013 and 2014 respectively compared to median incidence in the pre-vaccine years 2006-2008. A negative value indicates a reduction in incidence; a positive value indicates an increase in incidence

[b] Confidence interval

[c] A negative percent change indicates a reduction in incidence; a positive percent change indicates an increase in incidence

[d] Median hospitalisation incidence 2006-2008

Supplementary Figure: Annual incidence (per 1000 population) of diarrhoeal hospitalisations (A) compared to bronchiolitis hospitalisations (B) among children <5 years of age in Soweto, 2006-2014.



Supplementary Figure: Annual incidence (per 1000 population) of diarrhoeal hospitalisations (A) compared to bronchiolitis hospitalisations (B) among children <5 years of age in Soweto, 2006-2014.

PAPER III

Effect of breastfeeding on immunogenicity of oral live-attenuated human rotavirus vaccine: a randomized trial in HIV-uninfected infants in Soweto, South Africa

Michelle J Groome,[a] Sung-Sil Moon,[b] Daniel Velasquez,[b] Stephanie Jones,[a] Anthonet Koen,[a] Nadia van Niekerk,[a] Baoming Jiang,[b] Umesh D Parashar[b] & Shabir A Madhi[a]

Objective To investigate the effect of abstention from breastfeeding, for an hour before and after each vaccination, on the immune responses of infants to two doses of rotavirus vaccine.

Methods In Soweto, South Africa, mother–infant pairs who were uninfected with human immunodeficiency virus (HIV) were enrolled as they presented for the "6-week" immunizations of the infants. Each infant was randomly assigned to Group 1 – in which breastfeeding was deferred for at least 1 h before and after each dose of rotavirus vaccine – or Group 2 – in which unrestricted breastfeeding was encouraged. Enzyme-linked immunosorbent assays were used to evaluate the titres of rotavirus-specific IgA in samples of serum collected from each infant immediately before each vaccine dose and 1 month after the second dose. Among the infants, a fourfold or greater increase in titres of rotavirus-specific IgA following vaccination was considered indicative of seroconversion.

Findings The evaluable infants in Group 1 ($n = 98$) were similar to those in Group 2 ($n = 106$) in their baseline demographic characteristics and their pre-vaccination titres of anti-rotavirus IgA. After the second vaccine doses, geometric mean titres of anti-rotavirus IgA in the sera of Group-1 infants were similar to those in the sera of Group-2 infants ($P = 0.685$) and the frequency of seroconversion in the Group-1 infants was similar to that in the Group-2 infants ($P = 0.485$).

Conclusion Among HIV-uninfected South African infants, abstention from breastfeeding for at least 1 h before and after each vaccination dose had no significant effect on the infants' immune response to a rotavirus vaccine.

Abstracts in عربي, 中文, Français, Русский and Español at the end of each article.

Introduction

Rotavirus is the leading cause of severe gastroenteritis in young children.[1] It was estimated that 453 000 of the deaths – or 37% of all of the diarrhoea-related deaths – that occurred globally in 2008 were attributable to rotavirus infection.[1] In South Africa in 2009, diarrhoea was the leading cause of death among children younger than 5 years and accounted for 18% of the deaths in this age group.[2] In a review of relevant published studies, the median rate of rotavirus detection reported among children hospitalized for diarrhoea in South Africa was found to be 24% (range: 13–55%).[3,4]

Two live oral vaccines against rotavirus – Rotarix® (GlaxoSmithKline Biologicals, Rixensart, Belgium) and RotaTeq® (Merck Vaccines, Whitehouse Station, United States of America) – have been licensed for international use. Both are being introduced into national immunization programmes, in accordance with World Health Organization recommendations.[5] Clinical trials have demonstrated that the two vaccines are highly effective against severe rotavirus gastroenteritis in middle- and high-income countries in Europe and Latin America.[6,7] However, the vaccines appear to show relatively lower efficacy and immunogenicity in some low- to middle-income countries in Africa and Asia.[8–11] In an African study, two or three doses of Rotarix® were found to reduce the incidence of severe rotavirus gastroenteritis during the first year of life by 77% in South Africa and 49% in Malawi.[8] RotaTeq® was found to have vaccine efficacies – again measured against severe rotavirus disease in the first year of life – of 64% in a multicentre trial in Ghana, Kenya and Mali[9] and of 51% in a separate study based in Bangladesh and Viet Nam.[10] Other live oral vaccines, such as those against polio and cholera and earlier potential rotavirus vaccines, have also shown relatively lower efficacies when tested in low-income countries.[12]

There are several possible reasons why rotavirus vaccines might show relatively lower efficacies in low- and middle-income countries in Africa and Asia. These include interference from high levels of rotavirus-specific antibodies that infants may acquire – either transplacentally or via breastfeeding – from their mothers; the co-administration of oral polio vaccine; micronutrient deficiency; enteric co-infections and other concurrent diseases, such as infection with human immunodeficiency virus (HIV).[11] In trials of earlier vaccine candidates for the control of rotavirus infection, seroconversion was sometimes found to be less common among breastfed infants than among non-breastfed infants who had been given the same number of doses of the vaccine.[13–15] Although there appears to be no evidence to indicate that, during the first year of life, Rotarix® or RotaTeq® shows lower efficacy among breastfed infants than among infants given only formula milk, there has been no detailed attempt to explore this possibility.[16,17] Titres of anti-rotavirus IgA in sera of infants and levels of lactoferrin and rotavirus-specific neutralizing activity in the breast milk of mothers have been shown to vary by setting. For example, breast milk samples from Indian and South African women with low socioeconomic status tend to have

[a] Department of Science and Technology/National Research Foundation, Vaccine Preventable Diseases, University of the Witwatersrand, PO Box 90753, Bertsham, 2013, Gauteng, South Africa.

[b] Division of Viral Diseases, Centers for Disease Control and Prevention, Atlanta, United States of America.

Correspondence to Michelle J Groome (e-mail: groomem@rmpru.co.za).

(*Submitted: 19 August 2013 – Revised version received: 10 December 2013 – Accepted: 10 December 2013 – Published online: 4 February 2014*)

higher titres of anti-rotavirus IgA and neutralizing activity than those collected from women in the United States.[18–20] Such differences may partially explain why current rotavirus vaccines have relatively poor efficacy in low-income settings. Among infants, the efficacies of both Rotarix® and RotaTeq® appear to be positively correlated with serum titres of rotavirus-specific IgA.[20]

The main objectives of the present study were to determine the titres of rotavirus-specific IgA in infant serum, maternal serum and maternal breast milk in a cohort of HIV-uninfected South African mother–infant pairs, and to investigate the effect of abstention from breastfeeding at the time of vaccination on the immunogenicity of the Rotarix® vaccine.

Methods

Participants and specimen collection

We conducted a prospective, randomized, longitudinal cohort study of consenting, healthy, HIV-uninfected mother–infant pairs. The mothers and infants were enrolled – between 2 December 2009 and 9 April 2010 – as they presented for their "6-week" routine immunization visits at Diepkloof Primary Health Clinic, Soweto, South Africa. Follow-up continued until August 2010. Infants were considered for enrolment if they were aged 5 to 8 weeks, were being breastfed, had received no vaccines except oral polio vaccine and bacille Calmette–Guérin (BCG) at birth and had mothers who had been found seronegative for HIV after week 24 of gestation. Infants with known underlying immunosuppressive conditions and infants showing failure to thrive – that is, infants who fell below the third centile for weight-for-age or had dropped more than two weight-for-age centiles since birth – were excluded. Enrolled infants were randomized into two groups, known simply as Group 1 and Group 2. Mothers of Group 1 infants were instructed not to breastfeed their infants for at least 1 h before and after administration of each dose of rotavirus vaccine. Unrestricted breastfeeding was encouraged among the mothers of Group 2 infants. Breastfeeding of the enrolled infants was monitored at the study clinic as the mother–infant pairs presented for vaccination. The laboratory personnel who tested sera and breast-milk samples were blind to the group allocation.

All vaccines were provided according to the standard schedule of the expanded programme on immunization in South Africa. According to this schedule, each South African infant should be given trivalent oral polio vaccine (OPV-Merieux; Sanofi Pasteur, Lyon, France) at 6 weeks of age, a combination diphtheria and tetanus toxoids and acellular pertussis adsorbed, inactivated poliovirus and *Haemophilus influenzae* type b conjugate vaccine (Pentaxim; Sanofi Pasteur) and a hepatitis B vaccine (Heberbiovac HB; Heber Biotec, Havana, Cuba) at 6, 10 and 14 weeks, and rotavirus vaccine (Rotarix®) and a heptavalent pneumococcal conjugate vaccine (Prevenar; Wyeth, Madison, USA) at 6 and 14 weeks.

Serum samples were collected from the infants immediately before the first and second doses of Rotarix® and 1 month after the second dose. Samples of serum and breast milk were collected from the mothers shortly before their infants received each dose of Rotarix®. All of the sera and breast-milk samples were given code numbers to hide the identities of their donors. The samples were kept frozen at −70 °C until they were shipped – on solid carbon dioxide – to the Division of Viral Diseases of the United States Centers for Disease Control and Prevention, in Atlanta, United States, where they were investigated. Titres of anti-rotavirus IgA in all of the sera and breast milk samples, titres of anti-rotavirus IgG in the sera collected from the infants before the first dose of Rotarix®, and levels of rotavirus-specific neutralizing antibodies in the breast-milk samples were evaluated.

The protocol was reviewed and approved by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand (M090824). Written informed consent was obtained from a parent of each enrolled infant. The trial was registered on the South African National Clinical Trial Register (DOH-27–0511–2991).

Specimen testing

Titres of rotavirus-specific IgA were determined in enzyme-linked immunosorbent assays.[18] For these assays, microplate wells were coated with rabbit hyperimmune serum to rhesus rotavirus and incubated either with diluted Rotarix® strain or 5% (v/v) skim milk in phosphate-buffered saline (PBS). After washing, breast milk diluted 1:5–1:5120 in diluent buffer – PBS supplemented with 1% (v/v) skim milk and 0.5% (v/v) of a 10% solution of polyoxyethylene ether W1 – or serum diluted 1:20–1:10 240 in the same buffer – was added to each well, followed by biotin-conjugated goat anti-human IgA antibodies (KPL, Gaithersburg, USA). After incubation and washing, Extravidin (Sigma-Aldrich, St Louis, USA) was added to the wells and incubated, and then the reactions were developed with 3,3′,5,5′-tetramethylbenzidine (Sigma-Aldrich) and stopped with 1 M HCl. The optical density of the contents of each well was determined, at 450 nm, in a plate reader (MRX Revelation; Dynex Technologies, Chantilly, USA).

Anti-rotavirus IgA titres were calculated as the reciprocals of the highest dilutions that gave mean optical densities that were greater than the cut-off value. The cut-off value was set three standard deviations above the mean optical density for the negative-control wells. Rotavirus-specific IgG was tested and analysed in the same manner as IgA except that biotin-conjugated goat anti-human IgG antibodies (KPL) were used and 0.5% (v/v) normal rabbit serum was added to the biotin-conjugate solution.

The level of rotavirus-specific neutralizing activity in each breast-milk sample was evaluated in a microneutralization assay.[18] For this, a twofold serial dilution of each breast-milk sample was prepared and 50 µl of each dilution were mixed with an equal volume of trypsin-activated Rotarix® vaccine virus in the well of a microtitre plate – to yield a concentration of 4000 focus-forming units per well – and incubated at 37 °C for 1 h. The contents of each well were then placed on a PBS-washed monolayer of MA104 cells that had been grown in a 96-well plate. After another incubation at 37 °C for 1 h, the plate was washed with PBS and 100 µl of Iscove's modified Dulbecco's medium (Invitrogen, Carlsbad, USA) containing 5 µg trypsin per ml were added to each well. After incubation at 37 °C for 20 hours, 15 µl of 37% (w/v) formaldehyde were added to each well and left at 4 °C for 30 minutes. Rotavirus antigen in the MA104 cells was detected by incubating plates with a rabbit anti-rhesus-rotavirus hyperimmune serum, then anti-rabbit IgG labelled with horseradish peroxidase, and finally with 3,3′,5,5′-tetramethyl-

benzidine. The neutralizing titre in a breast milk sample was determined as the reciprocal of the highest dilution that showed a reduction in the absorbance value – relative to that in the virus-only controls – of more than 70%.

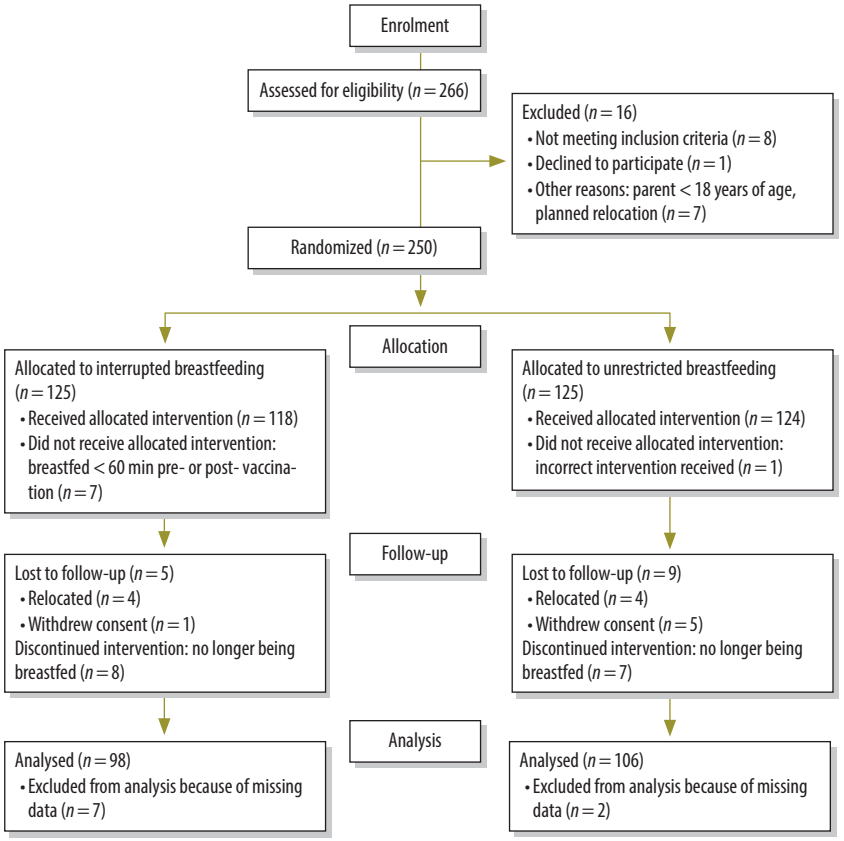
Sample size

The study was powered to detect at least a 20% higher frequency of seroconversion among the Group 1 infants than among the Group 2 infants. It was assumed that about 55% of the Group-2 infants would seroconvert in terms of their anti-rotavirus IgA, since 57% of infants given two doses of the vaccine had previously shown such seroconversion in a trial of the efficacy of Rotarix® in South Africa.[8] After setting a 5% significance level and 80% power and allowing for 20% loss to follow-up, our aim was to enrol at least 123 infants into each of the two study groups.

Statistical analysis

For the allocation of infants to each study group, a randomization list was generated using the SAS software package (SAS Institute, Cary, USA). Data analysis was performed using version 12.1 of the STATA package (StataCorp. LP, College Station, USA). A *P*-value of 0.05 or lower was considered statistically significant. Titres were log-transformed to give a better approximation to a normal distribution. Geometric mean titres were calculated. Continuous variables were compared using two-sample Student's *t*-tests – if the data were normally distributed – or Wilcoxon rank-sum tests. Temporal changes in the log-transformed titres within a single study group were investigated in *t*-tests for matched pairs. Categorical variables were compared using χ^2 tests. For the calculation of geometric mean titres, titres of anti-rotavirus IgA in serum that fell below 20 and titres of anti-rotavirus IgA in breast milk that fell below 5 were each assigned a value of 1. Even the data for infants who had pre-vaccination titres of anti-rotavirus IgA in serum that exceeded 20 were included in the final analyses. Seroconversion was defined as a fourfold or greater increase in anti-rotavirus IgA titre compared with the titre recorded before the first dose of Rotarix®.

Fig. 1. **Flowchart of mother–infant pair enrolment and follow-up in study of the effect of breastfeeding on the immunogenicity of rotavirus vaccine, South Africa, 2009–2010**



Results

In total, 250 infants were enrolled at a median age of 6.1 weeks. At enrolment, each of these infants received a first dose of Rotarix®, oral polio vaccine and other, scheduled, childhood vaccines. Although 125 infants were allocated to each study group, only 98 (78%) of those allocated to Group 1 and 106 (85%) of those allocated to Group 2 were fully adherent to the study protocol and included in the final analysis (Fig. 1). No significant differences were observed – in infant age at enrolment, sex or birth weight or baseline titres of anti-rotavirus IgA and IgG in the infants and their mothers – between the "evaluable" infants who were included in the final analysis and the other enrolled infants (data not shown).

In terms of sex, birth weight and age at vaccination, the evaluable infants in Group 1 were similar to those in Group 2 (Table 1). The second Rotarix® dose was administered at a median of 9.1 weeks (range: 5.4–16.9) after the first dose and subsequent immunogenicity was measured a median of 4.5 weeks (range: 3.9–8.3) later. The timing of these events was similar in the two study groups. When measured before the first vaccine dose, the geometric mean titres of anti-rotavirus IgG and IgA in infant sera and anti-rotavirus IgA in maternal sera and breast milk samples and the geometric mean neutralizing titres in breast milk were also similar in the two study groups (Table 1).

There were no significant between-group differences in the geometric mean titres of anti-rotavirus IgA in infant sera measured after the first ($P = 0.612$) or second doses ($P = 0.685$) of Rotarix® (Table 1). However, in both the Group 1 infants and the Group 2 infants, such mean titres were higher after both the first dose of Rotarix® (53.4 and 46.0, respectively) and the second (137.7 and 122.7, respectively) than the titres recorded before the first dose (14.2 and 14.6, respectively). These within-group temporal changes were all statistically significant ($P < 0.001$).

Compared with the values recorded at baseline, the maternal sera and breast milk samples collected after the infants had received their first dose of Rotarix® also showed significantly higher geometric mean titres – of anti-rotavirus

Table 1. **Comparison of mother–infant pairs assigned to two arms of a study on the effect of breastfeeding on the immunogenicity of rotavirus vaccine, South Africa, 2009–2010**

Infants/mothers	Group[a]		*P*
	1	2	
Infants			
No. evaluated in full	98	106	–
Males, no. (%)	55 (56.1)	57 (53.8)	0.736
Mean birth weight, kg (SD)	3.0 (0.5)	3.1 (0.5)	0.641
Median age, weeks (range)			
On enrolment	6.3 (5.7–7.7)	6.1 (5.9–7.3)	0.200
When investigated after first vaccine dose	15.7 (11.6–23.4)	15.3 (13.9–19.9)	0.418
When investigated after second vaccine dose	20.6 (17.4–27.4)	20.1 (18.0–24.1)	0.408
Serum anti-rotavirus IgG, GMT (95% CI)			
On enrolment[b]	1508.6 (1194.8–1904.9)	1524.7 (1220.4–1904.9)	0.948
Serum anti-rotavirus IgA, GMT (95% CI)			
On enrolment	14.2 (9.8–20.6)	14.6 (10.0–21.5)	0.910
After first dose of vaccine[c]	53.4 (36.7–77.7)	46.0 (29.7–71.3)	0.612
After second dose of vaccine	137.7 (90.2–210.2)	122.7 (84.6–177.9)	0.685
Mothers			
Serum anti-rotavirus IgA, GMT (95% CI)			
On enrolment[d]	26.2 (15.6–44.0)	27.4 (16.5–45.5)	0.908
After infant received first dose of vaccine[e]	164.0 (103.8–259.1)	186.8 (125.4–278.1)	0.670
Breast-milk anti-rotavirus IgA, GMT (95% CI)			
On enrolment[e]	39.3 (28.5–51.6)	42.4 (32.2–55.7)	0.624
After infant received first dose of vaccine[e]	52.2 (39.3–69.2)	56.4 (41.4–76.8)	0.715
Breast-milk neutralizing activity, GMT (95% CI)			
On enrolment	8.8 (6.0–13.0)	8.3 (6.0–11.5)	0.813
After infant received first dose of vaccine[f]	19.1 (12.9–28.3)	21.5 (14.9–31.1)	0.662

CI, confidence interval; GMT, geometric mean titre; SD, standard deviation.
[a] Each infant was randomly assigned to either Group 1 – in which there was an abstention from breastfeeding for at least 1 h before and after each dose of rotavirus vaccine – or Group 2 – in which unrestricted breastfeeding was encouraged.
[b] No data available for four infants (one in Group 1).
[c] No data available for 21 infants (11 in Group 1).
[d] No data available for one mother of an infant in Group 1.
[e] No data available for one mother of an infant in Group 2.
[f] No data available for two mothers (one of an infant in Group 1).

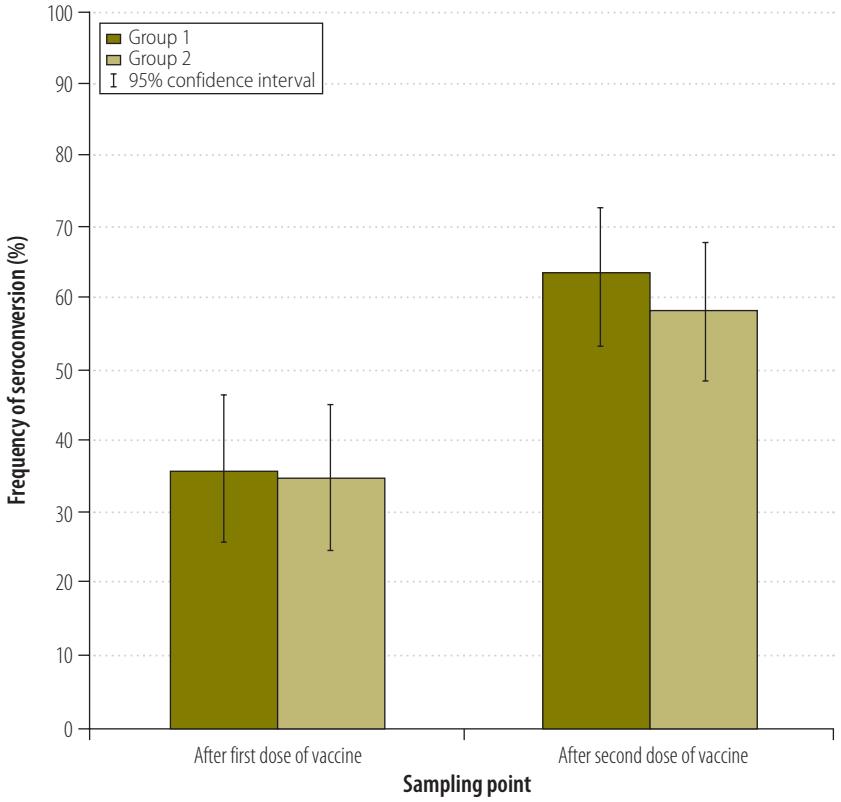
IgA, neutralizing activity or both – in both Group 1 and Group 2 (Table 1). At each time point that we investigated, the distribution of titres among Group 1 infants was similar to that seen in Group 2 (data not shown). There were also no significant between-group differences in the frequency of seroconversion after either one dose ($P = 0.859$) or two doses ($P = 0.485$) of Rotarix® (Fig. 2). The frequency of seroconversion in the whole study cohort was 35% (95% confidence interval, CI: 28–42) after one dose of Rotarix® and 61% (95% CI: 54–68) after two doses.

Discussion

There has been much speculation about the reasons for the relatively low efficacy and immunogenicity of rotavirus vaccines in low- and middle-income countries in Africa and Asia. One hypothesis is that rotavirus-specific antibodies and other neutralizing factors present in breast milk may diminish a breastfed infant's immune responses to a rotavirus vaccine – by lowering the effective titre of vaccine delivered to the infant's gut. This hypothesis was supported by the results of two recent in vitro studies in which levels of lactoferrin, anti-rotavirus IgA and neutralizing activity in breast milk samples from mothers who were breastfeeding their infants were evaluated. Breast milk with low titres of rotavirus-specific neutralizing activity did not seem to affect the vaccine virus but milk with high titres could neutralize the vaccine virus, even when diluted.[18,19] These observations indicated that a short abstention from breastfeeding at the time of each vaccination could potentially improve the immunogenicity of a rotavirus vaccine. However, the results of the present study indicate that abstaining from breastfeeding for at least an hour before and after each of two doses of Rotarix® had no significant effect on the frequency of seroconversion among the vaccinated infants or the titres of anti-rotavirus IgA in the sera of the same infants.

The results of earlier, related in vivo studies – in which the effects of breast milk on immunogenicity and seroconversion after the administration of candidate vaccines against rotavirus were investigated – were equivocal.[21–23] Although each of two meta-analyses led to the conclusion that breastfeeding did significantly reduce the immune response to a single dose of rhesus rotavirus vaccine, the data analysed came from studies that differed markedly in terms of the number of vaccine doses,

Fig. 2. **Frequency of seroconversion in infants assigned to two arms of a study on the effect of breastfeeding on the immunogenicity of rotavirus vaccine, South Africa, 2009–2010**



Note: Each enrolled infant was randomly assigned to either Group 1 – in which there was an abstention from breastfeeding for at least 1 h before and after each dose of rotavirus vaccine – or Group 2 – in which unrestricted breastfeeding was encouraged. The bars indicate the median frequencies of seroconversion observed after one and two doses of rotavirus vaccine.

the antibody assays and the criteria for seroconversion and breastfeeding that were used.[13,14] It appears that the effect of breast milk interference on the immunogenicity of rotavirus vaccines can be overcome by administering more than one dose of vaccine.[15]

Our results are consistent with those of a study on the effects of breastfeeding on antibody response to oral polio vaccine in infants. In this polio study, the frequency of post-vaccination seroconversion among infants on unrestricted breastfeeds was found to be similar to that seen among infants abstaining from breastfeeding for a period of time.[24]

The mothers of both groups of infants enrolled in the present study showed higher titres of anti-rotavirus IgA and rotavirus-specific neutralizing activity after their infants had received one dose of Rotarix® than before this dose. These increases may partly reflect the mothers' natural infection with rotavirus during the peak rotavirus season in South Africa, which generally runs from April to September each year.[4] However, the majority (71%) of the maternal samples collected after the corresponding infants had received one dose of Rotarix® were collected after the rotavirus season of 2009 and before the onset of the rotavirus season of 2010. No attempt was made to check the mothers of the enrolled infants for the signs or symptoms of rotavirus disease. An alternative explanation for the increasing immunogenicity of the mothers to the Rotarix® vaccine strain is that maternal antibodies were boosted by the vaccination of the infants of these women. The conferring of immunity to people in close contact with vaccinated children has been observed in studies on oral polio vaccine[25] and may also occur with rotavirus vaccine. The horizontal transmission of a human rotavirus vaccine strain – from vaccinated children to their unvaccinated twins – was demonstrated in a placebo-controlled study of twins who lived in close contact with each other in the Dominican Republic.[26]

The overall frequency of seroconversion observed in the present study following the second dose of Rotarix® – 61% – is reassuring, bearing in mind that the rotavirus vaccination schedule currently followed in South Africa, of doses at 6 and 14 weeks of age, has not yet been studied in clinical trials. This frequency of seroconversion is similar to the values observed in the South African trial of the clinical efficacy of Rotarix®, which were 57.1% (95% CI: 44.7–68.9) after two doses and 66.7% (95% CI: 54.0–77.8) after three doses.[8] However, comparisons between the efficacy trial and the present study must be made with caution since different assays to assess immunogenicity were used in the two investigations. In the efficacy trial, a commercial enzyme-linked immunosorbent assay with a cut-off point for seroconversion of 20 "units" per ml was used, whereas we used another immunosorbent assay and defined seroconversion as a fourfold or greater increase in an infant's titres of anti-rotavirus IgA. The dosing schedule used in the two investigations also differed: the two-dose group in the efficacy trial received Rotarix® at 6 and 10 weeks of age, while in the present study the same vaccine was given at approximately 6 and 14 weeks of age. The identification of a correlate of protection after rotavirus vaccination has proven difficult, mainly because of differences between the relevant studies in terms of vaccine type, dose schedule, regional pre-immunization titres, laboratory assays and the impact of natural exposure. However, serum titres of anti-rotavirus IgA have been identified as one important predictor of protection.[20]

There were some limitations to our study. The second dose of Rotarix® could only be given to some of the infants one month later than scheduled because the vaccine stocks became exhausted between April and May of 2010. This meant that not all infants received two doses before the onset of the rotavirus season in 2010. Towards the end of our study, therefore, there may have been natural rotavirus in circulation and this could have affected the titres of the antibodies that we investigated, in both the infants and the mothers. However, the level of natural exposure to rotavirus would have been similar in the two study groups and not likely to have affected our conclusions.

We only assessed whether abstention from breastfeeding for about 1 h before and after each vaccination had an effect on immunogenicity. Although it remains possible that a more prolonged abstention may have had a greater impact, leaving an infant unfed for more than 2 h may not be programmatically possible or acceptable. Although the mothers of Group 2 infants were encouraged to breastfeed their infants soon after the vaccines were given, the length of each first feed after the vaccination was not recorded. As suckling is often used as a comfort, a full feed may not have been given at this time. The interaction between vaccine and breast milk in the Group 2 infants may therefore have been less than the maximum possible.

The present study was designed to detect a 20% difference in the frequency of seroconversion between the two groups of infants. A larger sample size may have allowed detection of a significant difference of smaller magnitude. However, policy-makers are unlikely to promote changes in national breastfeeding habits if such changes only lead to small increases in the immunogenicity of rotavirus vaccines given to infants.

Abstention from breast-feeding for 1 h before and after vaccination does not appear to have any significant effect on the immune response to Rotarix® in a low- to middle-income African setting. The reason or reasons that oral rotavirus vaccines appear to have relatively low efficacy in low-income settings – which may include enteric co-infections and concurrent medical conditions – require further investigation. The potential for boosting the efficacy of such vaccines – for example, by zinc supplementation or the co-administration or probiotics – and other approaches to rotavirus vaccination – such as vaccines derived from neonatal strains or parenteral vaccines – should also be investigated. ■

Acknowledgements

We thank the mothers and children whom we investigated for participating in our study. We are also grateful to the staff of the University of the Witwatersrand's Respiratory and Meningeal Pathogens Research Unit – for their dedicated work; to the doctors who assisted with participant enrolment and follow-up; and to Elizabeth R Zell – for her valuable discussions. MJG, AK, NvN and SAM have dual appointments with the Medical Research Council: Respiratory and Meningeal Pathogens Research Unit, University of the Witwatersrand, Johannesburg, South Africa. SAM also has a dual appointment with the National Institute for Communicable Diseases, Centre for Vaccines and Immunology, Sandringham, South Africa.

Funding: This work was supported by the Respiratory and Meningeal Pathogens Research Unit, University of the Witwatersrand and the United States Centers for Disease Control and Prevention.

Competing interests: None declared.

ملخص

أثر الرضاعة الطبيعية على قابلية اكتساب المناعة للقاح الفيروس العجلي البشري الموهن الحي الفموي: تجربة عشوائية على الرضع غير المصابين بعدوى فيروس العوز المناعي البشري في سويتو، بجنوب أفريقيا

الغرض تحري أثر الامتناع عن الرضاعة الطبيعية لمدة ساعة، قبل كل تطعيم وبعده، على استجابات الرضع المناعية لجرعتي لقاح الفيروس العجلي.

الطريقة في سويتو، بجنوب أفريقيا، تم تسجيل أزواج الأمهات والرضع غير المصابين بفيروس العوز المناعي البشري عند الحضور لتلقي تطعيمات الرضع في سن "6 أسابيع". وتم تخصيص كل رضيع على نحو عشوائي للمجموعة 1 – التي تم تأجيل الرضاعة الطبيعية فيها لمدة ساعة واحدة على الأقل قبل أو بعد كل جرعة من لقاح الفيروس العجلي – أو المجموعة 2 – التي تم تشجيع الرضاعة الطبيعية غير المقيدة فيها. وتم استخدام مقايسات الممتز المناعي المرتبط بالإنزيمات لتقييم عيارات الغلوبولين المناعي A الخاص بالفيروس العجلي في عينات مصل الدم التم تم جمعها من كل رضيع قبل كل جرعة من اللقاح مباشرة وبعد الجرعة الثانية بشهر واحد. وتم اعتبار الزيادة بنسبة أربعة أضعاف أو أكثر في عيارات الغلوبولين المناعي A الخاص بالفيروس العجلي بعد التطعيم بين الرضع مؤشراً على انقلاب تفاعلية المصل.

النتائج تشابه الرضع القابلون للتقييم في المجموعة 1 (العدد=98) مع الرضع في المجموعة 2 (العدد=106) في الخصائص الديمغرافية الأساسية وعيارات الغلوبولين المناعي A المضاد للفيروس العجلي السابقة لتطعيمهم. وبعد جرعات اللقاح الثانية، تشابهت عيارات المتوسط الهندسي للغلوبولين المناعي A المضاد للفيروس العجلي في أمصال دم رضع المجموعة 1 مع العيارات في أمصال دم رضع المجموعة 2 (الاحتمال=0.685) وتشابه معدل تواتر انقلاب تفاعلية المصل في رضع المجموعة 1 مع المعدل في رضع المجموعة 2 (الاحتمال = 0.485).

الاستنتاج لم يكن للامتناع عن الرضاعة الطبيعية لمدة ساعة واحدة قبل كل جرعة تطعيم وبعدها، بين الرضع غير المصابين بفيروس العوز المناعي البشر، أثر كبير على استجابة الرضع المناعية للقاح الفيروس العجلي.

摘要

母乳喂养对人轮状病毒口服减毒活疫苗免疫原性的影响：南非索韦托艾滋病病毒未感染婴儿随机试验

目的 调查在每次疫苗接种之前和之后一个小时避免母乳喂养对婴儿对两剂量轮状病毒疫苗的免疫反应的影响。

方法 在南非索韦托，在提出"6周"婴儿免疫接种的母婴对中招募未受艾滋病毒（HIV）感染的母婴对。将每个婴儿随机指定到第1组（该组在每剂轮状病毒之前和之后至少隔开1小时母乳哺育）或者第2组（该组鼓励不受限制的母乳喂养）。使用酶联免疫吸附试验评估在每剂疫苗即将接种时和接种第二剂之后1个月所收集每个婴儿血清样本中的轮状病毒特异性IgA滴度。在这些婴儿当中，如果疫苗接种之后轮状病毒特异性IgA的滴度出现四倍或更多的增加，则表示出现血清转换。

结果 在基线人口特征和疫苗接种前抗轮状病毒IgA滴

度方面，第 1 组（n=98）和第 2 组（n=106）可评估婴儿的结果相似。在第二剂疫苗接种之后，第 1 组婴儿血清抗轮状病毒 IgA 的几何平均滴度与第 2 组婴儿血清中的滴度相似（P=0.685），第 1 组婴儿血清转化率和第 2 组第 2 组婴儿相似（P=0.485）。

结论 在南非未受艾滋病毒感染的婴儿中，在每剂疫苗接种之前和之后一个小时避免母乳喂养对婴儿对轮状病毒疫苗的免疫反应没有显著影响。

Résumé

Effet de l'allaitement sur l'immunogénicité du vaccin oral vivant atténué contre le rotavirus humain: un essai randomisé parmi les nourrissons séronégatifs à Soweto, en Afrique du Sud

Objectif Étudier l'effet de l'abstention d'allaitement, une heure avant et après chaque vaccination, sur les réactions immunitaires des nourrissons à deux doses de vaccin contre le rotavirus.

Méthodes À Soweto, en Afrique du Sud, des couples mère-enfant non infectés par le virus de l'immunodéficience humaine (VIH) ont été inscrits à cette étude, alors qu'ils se présentaient pour les vaccinations des nourrissons de 6 semaines. Chaque enfant a été placé au hasard dans le groupe 1 – au sein duquel l'allaitement était reporté au moins d'une heure avant et après chaque dose de vaccin contre le rotavirus - ou dans le groupe 2 - au sein duquel l'allaitement sans restriction a été encouragé. Des dosages immuno-enzymatiques ont été utilisés pour évaluer les titres d'IgA spécifiques du rotavirus dans des échantillons de sérum prélevés chez chaque enfant immédiatement avant chaque dose de vaccin et un mois après la deuxième dose. Chez les nourrissons, une augmentation par 4 ou plus des titres d'IgA spécifiques du rotavirus après la vaccination a été considérée comme une indication de séroconversion.

Résultats Les nourrissons évaluables du groupe 1 (*n* = 98) étaient similaires à ceux du groupe 2 (*n* = 106) en ce qui concernaient leurs caractéristiques démographiques de base et leurs titres d'IgA anti-rotavirus avant la vaccination. Après les deuxièmes doses de vaccin, les titres moyens géométriques d'IgA anti-rotavirus du sérum des nourrissons du groupe 1 étaient semblables à ceux du sérum des nourrissons du groupe 2 (*P* = 0,685), et la fréquence de séroconversion des nourrissons du groupe 1 était semblable à celle des nourrissons du groupe 2 (*P* = 0,485).

Conclusion Chez les nourrissons non infectés par le VIH en Afrique du Sud, l'abstention d'allaitement pendant au moins une heure avant et après chaque dose de vaccination n'a eu aucun effet significatif sur la réaction immunitaire des nourrissons à un vaccin contre le rotavirus.

Резюме

Влияние грудного вскармливания на иммуногенность пероральной живой вакцины ослабленного ротавируса человека: рандомизированное исследование среди ВИЧ-неинфицированных младенцев в Соуэто (Южная Африка)

Цель Исследовать эффект воздержания от грудного вскармливания в течение часа до и после каждой вакцинации двумя дозами ротавирусной вакцины на иммунные реакции младенцев.

Методы В исследовании, проводимом в Соуэто (Южная Африка), принимали участие пары мать-ребенок, не зараженные вирусом иммунодефицита человека (ВИЧ) и проходящие «6-недельную» иммунизацию младенцев. Каждый ребенок был случайным образом распределен в одну из групп: группу 1, в которой грудное вскармливание не проводилось, по крайней мере, в течение 1 часа до и после введения каждой дозы ротавирусной вакцины, или группу 2, в которой матерям было предложено неограниченное грудное вскармливание. Для оценки титра антител IgA к ротавирусу в образцах сыворотки использовался иммуноферментный анализ сыворотки, отбираемой у каждого младенца непосредственно перед введением каждой дозы вакцины и через один месяц после введения второй дозы. Увеличение в титрах ротавирусных антител IgA в четыре и более раз после вакцинации считалось показателем сероконверсии у младенцев.

Результаты Дети в группе 1 (*n* = 98) были похожи на детей в группе 2 (*n* = 106) по своим базовым демографическим характеристикам и титрам антиротавирусных антител класса IgA, полученным в ходе предварительной вакцинации. После введения второй дозы вакцины средние геометрические величины титров анти-ротавирусных IgA в сыворотке детей из группы 1 были аналогичны данным величинам у детей из группы 2 (*P* = 0,685), а частота сероконверсии среди детей в группе 1 была такой же, как и у детей в группе 2 (*P* = 0,485).

Вывод Среди ВИЧ-неинфицированных южноафриканских младенцев воздержание от грудного вскармливания, по крайней мере, в течение 1 часа до и после каждой дозы вакцины не оказало существенного влияния на иммунный ответ младенцев на вакцину против ротавируса.

Resumen

Efecto de la lactancia materna en la inmunogenicidad de la vacuna oral contra el rotavirus humano vivo atenuado: un estudio aleatorizado en lactantes infectados por el VIH en Soweto, Sudáfrica

Objetivo Investigar el efecto de la abstención de la lactancia materna, una hora antes y después de cada vacuna, en las respuestas inmunes de los lactantes a dos dosis de la vacuna contra el rotavirus.

Métodos En Soweto, Sudáfrica, se inscribieron parejas de madres-bebés que no estaban infectados por el virus de la inmunodeficiencia humana (VIH), ya que se sometieron a las inmunizaciones de «seis semanas» de los lactantes. Se asignó al azar a cada niño al Grupo 1 – en el cual se aplazó la lactancia materna al menos 1 hora antes y después de cada dosis de la vacuna contra el rotavirus – o al Grupo 2 – en el cual se fomentó la lactancia sin restricciones. Se utilizaron ensayos de inmunoadsorción enzimática para evaluar los títulos de IgA específica de rotavirus en muestras de suero recogidas de cada lactante inmediatamente antes de cada dosis de vacuna y 1 mes después de la segunda dosis. Entre los lactantes, un incremento cuádruple o mayor en

los títulos de IgA específica de rotavirus tras la vacunación se consideró indicativo de seroconversión.

Resultados Las características demográficas de base y los títulos de vacunación previa de la IgA antirrotavirus de los lactantes evaluables del Grupo 1 ($n = 98$) fueron similares a los del Grupo 2 ($n = 106$). Tras la segunda dosis de la vacuna, los títulos de la media geométrica de la IgA antirrotavirus en los sueros de los lactantes del Grupo-1 eran similares a los de los sueros de los lactantes del Grupo-2 ($P = 0,685$) y la frecuencia de la seroconversión en los lactantes del Grupo-1 fue similar a la de los lactantes del Grupo-2 ($P = 0,485$).

Conclusión Entre los lactantes sudafricanos infectados por el VIH, la abstención a la lactancia materna durante al menos 1 hora antes y después de cada dosis de la vacuna no tuvo efectos importantes en la respuesta inmune de los lactantes a la vacuna contra el rotavirus.

References

1. Tate JE, Burton AH, Boschi-Pinto C, Steele AD, Duque J, Parashar UD; WHO-coordinated Global Rotavirus Surveillance Network. 2008 estimate of worldwide rotavirus-associated mortality in children younger than 5 years before the introduction of universal rotavirus vaccination programmes: a systematic review and meta-analysis. *Lancet Infect Dis* 2012;12:136–41. doi: http://dx.doi.org/10.1016/S1473-3099(11)70253-5 PMID:22030330
2. Levels and trends of morbidity and mortality among children aged under-five years in South Africa, 2006–2010 [Internet]. Pretoria: Statistics South Africa; 2010. Available from: http://www.statssa.gov.za/publications/Report-03-09-10/Report-03-09-102010.pdf [accessed 31 July 2013].
3. Steele AD, Peenze I, de Beer MC, Pager CT, Yeats J, Potgieter N et al. Anticipating rotavirus vaccines: epidemiology and surveillance of rotavirus in South Africa. *Vaccine* 2003;21:354–60. doi: http://dx.doi.org/10.1016/S0264-410X(02)00615-1 PMID:12531632
4. Mapaseka SL, Dewar JB, van der Merwe L, Geyer A, Tumbo J, Zweygarth M et al. Prospective hospital-based surveillance to estimate rotavirus disease burden in the Gauteng and North West province of South Africa during 2003–2005. *J Infect Dis* 2010;202(Suppl):S131–8. doi: http://dx.doi.org/10.1086/653558 PMID:20684693
5. Rotavirus vaccines: an update. *Wkly Epidemiol Rec* 2009;84:533–40. PMID:20034143
6. Ruiz-Palacios GM, Pérez-Schael I, Velázquez FR, Abate H, Breuer T, Clemens SC et al.; Human Rotavirus Vaccine Study Group. Safety and efficacy of an attenuated vaccine against severe rotavirus gastroenteritis. *N Engl J Med* 2006;354:11–22. doi: http://dx.doi.org/10.1056/NEJMoa052434 PMID:16394298
7. Vesikari T, Matson DO, Dennehy P, Van Damme P, Santosham M, Rodriguez Z et al.; Rotavirus Efficacy and Safety Trial (REST) Study Team. Safety and efficacy of a pentavalent human-bovine (WC3) reassortant rotavirus vaccine. *N Engl J Med* 2006;354:23–33. doi: http://dx.doi.org/10.1056/NEJMoa052664 PMID:16394299
8. Madhi SA, Cunliffe NA, Steele D, Witte D, Kirsten M, Louw C et al. Effect of human rotavirus vaccine on severe diarrhea in African infants. *N Engl J Med* 2010;362:289–98. doi: http://dx.doi.org/10.1056/NEJMoa0904797 PMID:20107214
9. Armah GE, Sow SO, Breiman RF, Dallas MJ, Tapia MD, Feikin DR et al. Efficacy of pentavalent rotavirus vaccine against severe rotavirus gastroenteritis in infants in developing countries in sub-Saharan Africa: a randomised, double-blind, placebo-controlled trial. *Lancet* 2010;376:606–14. doi: http://dx.doi.org/10.1016/S0140-6736(10)60889-6 PMID:20692030
10. Zaman K, Dang DA, Victor JC, Shin S, Yunus M, Dallas MJ et al. Efficacy of pentavalent rotavirus vaccine against severe rotavirus gastroenteritis in infants in developing countries in Asia: a randomised, double-blind, placebo-controlled trial. *Lancet* 2010;376:615–23. doi: http://dx.doi.org/10.1016/S0140-6736(10)60755-6 PMID:20692031
11. Patel M, Shane AL, Parashar UD, Jiang B, Gentsch JR, Glass RI. Oral rotavirus vaccines: how well will they work where they are needed most? *J Infect Dis* 2009;200(Suppl 1):S39–48. doi: http://dx.doi.org/10.1086/605035 PMID:19817613
12. Levine MM. Immunogenicity and efficacy of oral vaccines in developing countries: lessons from a live cholera vaccine. *BMC Biol* 2010;8:129. doi: http://dx.doi.org/10.1186/1741-7007-8-129 PMID:20920375
13. Glass RI, Ing DJ, Stoll BJ, Ing RT. Immune response to rotavirus vaccines among breast-fed and nonbreast-fed children. *Adv Exp Med Biol* 1991;310:249–54. doi: http://dx.doi.org/10.1007/978-1-4615-3838-7_33 PMID:1667065
14. Pichichero ME. Effect of breast-feeding on oral rhesus rotavirus vaccine seroconversion: a metaanalysis. *J Infect Dis* 1990;162:753–5. doi: http://dx.doi.org/10.1093/infdis/162.3.753 PMID:2167344
15. Rennels MB. Influence of breast-feeding and oral poliovirus vaccine on the immunogenicity and efficacy of rotavirus vaccines. *J Infect Dis* 1996;174(Suppl 1):S107–11. doi: http://dx.doi.org/10.1093/infdis/174.Supplement_1.S107 PMID:8752299
16. Vesikari T, Prymula R, Schuster V, Tejedor JC, Cohen R, Bouckenooghe A et al. Efficacy and immunogenicity of live-attenuated human rotavirus vaccine in breast-fed and formula-fed European infants. *Pediatr Infect Dis J* 2012;31:509–13. doi: http://dx.doi.org/10.1097/INF.0b013e3182489cac PMID:22228235
17. Goveia MG, DiNubile MJ, Dallas MJ, Heaton PM, Kuter BJ; REST Study Team. Efficacy of pentavalent human-bovine (WC3) reassortant rotavirus vaccine based on breastfeeding frequency. *Pediatr Infect Dis J* 2008;27:656–8. doi: http://dx.doi.org/10.1097/INF.0b013e318168d29e PMID:18520448
18. Moon SS, Wang Y, Shane AL, Nguyen T, Ray P, Dennehy P et al. Inhibitory effect of breast milk on infectivity of live oral rotavirus vaccines. *Pediatr Infect Dis J* 2010;29:919–23. doi: http://dx.doi.org/10.1097/INF.0b013e3181e232ea PMID:20442687
19. Moon SS, Tate JE, Ray P, Dennehy PH, Archary D, Coutsoudis A et al. Differential profiles and inhibitory effect on rotavirus vaccines of nonantibody components in breast milk from mothers in developing and developed countries. *Pediatr Infect Dis J* 2013;32:863–70. PMID:23584581
20. Patel M, Glass RI, Jiang B, Santosham M, Lopman B, Parashar U. A systematic review of anti-rotavirus serum IgA antibody titer as a potential correlate of rotavirus vaccine efficacy. *J Infect Dis* 2013;208:284–94. doi: http://dx.doi.org/10.1093/infdis/jit166 PMID:23596320
21. Brüssow H, Benitez O, Uribe F, Sidoti J, Rosa K, Cravioto A. Rotavirus-inhibitory activity in serial milk samples from Mexican women and rotavirus infections in their children during their first year of life. *J Clin Microbiol* 1993;31:593–7. PMID:8384626
22. Rimer HC, Wasserman SS, Flores J, Pichichero ME, Losonsky GA. Rotavirus-specific breast milk antibody in two populations and possible correlates of interference with rhesus rotavirus vaccine seroconversion. *J Infect Dis* 1992;165:826–30. doi: http://dx.doi.org/10.1093/infdis/165.5.826 PMID:1373753
23. Rennels MB, Wasserman SS, Glass RI, Keane VA; US Rotavirus Vaccine Efficacy Group. Comparison of immunogenicity and efficacy of rhesus rotavirus reassortant vaccines in breastfed and nonbreastfed children. *Pediatrics* 1995;96:1132–6. PMID:7491235
24. John TJ, Devarajan LV, Luther L, Vijayarathnam P. Effect of breast-feeding on seroresponse of infants to oral poliovirus vaccination. *Pediatrics* 1976;57:47–53. PMID:174056
25. Dagan R, Fraser D, Handsher R. Acquisition of immunity in mothers of infants administered trivalent oral poliovirus vaccine. *Eur J Clin Microbiol Infect Dis* 1994;13:1029–32. doi: http://dx.doi.org/10.1007/BF02111822 PMID:7889964
26. Rivera L, Peña LM, Stainier I, Gillard P, Cheuvart B, Smolenov I et al. Horizontal transmission of a human rotavirus vaccine strain–a randomized, placebo-controlled study in twins. *Vaccine* 2011;29:9508–13. doi: http://dx.doi.org/10.1016/j.vaccine.2011.10.015 PMID:22008819

PAPER IV

Clinical Infectious Diseases

MAJOR ARTICLE

Prevaccination Rotavirus Serum IgG and IgA Are Associated With Lower Immunogenicity of Live, Oral Human Rotavirus Vaccine in South African Infants

Sung-Sil Moon,[1] Michelle J. Groome,[2,3] Daniel E. Velasquez,[1] Umesh D. Parashar,[1] Stephanie Jones,[2,3] Antoinette Koen,[2,3] Nadia van Niekerk,[2,3] Baoming Jiang,[1] and Shabir A. Madhi[2,3,4]

[1]Division of Viral Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia; [2]Department of Science and Technology/National Research Foundation: Vaccine Preventable Diseases, [3]Medical Research Council: Respiratory and Meningeal Pathogens Research Unit, University of the Witwatersrand, Johannesburg, and [4]National Institute for Communicable Diseases: National Health Laboratory Service, Centre for Vaccines and Immunology, Sandringham, South Africa

(See the Major Article by Emperador et al on pages 150–6.)

Background. Live oral rotavirus (RV) vaccines have shown modest efficacy among children in African countries for reasons that are not completely understood. We examined the possible inhibitory effect of preexisting antirotavirus antibodies on immunogenicity of monovalent RV vaccine (RV1).

Methods. Mother–infant pairs were enrolled at presentation for their routine immunization visit in Soweto, South Africa, when infants were aged 5–8 weeks. Infant serum samples were obtained before the first and second doses of RV1 and 1 month after the second dose. Maternal serum and breast milk samples were obtained prior to administration of each dose of RV1 to infants. RV-specific immunoglobulin G (IgG), IgA, and neutralizing activity in sera of infants and serum or breast milk samples of mothers were measured using enzyme-linked immunosorbent assays or a microneutralization test.

Results. Of the 107 serum pairs from infants who were seronegative for RV IgA at enrollment, we observed a strong positive association between IgG titers in pre-dose 1 sera of infants and mothers and significant negative associations between IgG titers in pre-dose 1 sera of infants and seroconversion to RV1 post-dose 1. Similarly, mothers whose infants' IgA seroconverted after RV1 had significantly lower pre-dose 1 IgG titers in sera than those whose infants did not seroconvert.

Conclusions. High levels of preexisting serum IgG, including transplacentally acquired maternal IgG, appeared to have an inhibitory effect on the immunogenicity of RV1 among infants and may, in part, contribute to lower efficacy of RV vaccines in this and other low-income settings.

Keywords. rotavirus; Rotarix; breast milk; transplacental antibody.

Rotavirus (RV) is the leading cause of severe gastroenteritis in young children and is responsible for 275 000–450 000 deaths, approximately 85% of which occur in low-income countries of Africa and Asia [1]. In South Africa, diarrhea accounts for approximately 18% of deaths among children aged <5 years [2], and RV infection is estimated to cause 17 644 to 25 630 hospitalizations annually among children aged <2 years [3]. Two live oral vaccines (Rotarix [RV1], GlaxoSmithKline Biologicals, and RotaTeq [RV5], Merck) are commercially available and recommended by the World Health Organization for all children worldwide and, to date, have been used for routine immunization programs in more than 60 countries [4]. Clinical trials of both vaccines showed that efficacy in middle- and high-income countries of North America, Europe, and Latin America ranged from 85% to 98% [5, 6]. However, the 2 vaccines are considerably less efficacious in low- to middle-income countries of Africa, Asia, and Latin America, with an efficacy ranging from approximately 40% to approximately 70% [7–11]. The reasons for this lower efficacy have not been fully explained but mimic the lower efficacy of other live oral vaccines such as oral polio vaccine (OPV) and typhoid and cholera vaccines in similar settings [8].

It has been reported that levels of RV immunoglobulin A (IgA), secretory IgA (sIgA), lactoferrin, and neutralizing activity in mothers' breast milk vary by setting, with higher titers found among women in low-income countries compared with those in high-income countries [12–14]. These high levels of neutralizing activity in breast milk, together with maternal antibody transferred to the infant through the placenta, may have an inhibitory effect on the infectivity of live-attenuated vaccine viruses in the gut and thus the ability of vaccines to induce robust immune response among infants [7, 9, 11, 15]. To test this hypothesis, we recently investigated the effect of a transient delay of breastfeeding before and after the first and second vaccination with RV1 on the immune response

Received 26 March 2015; accepted 1 September 2015; published online 23 September 2015.
Correspondence: B. Jiang, Division of Viral Diseases, National Center for Immunization and Respiratory Disease, MS G04, 1600 Clifton Rd, Atlanta, GA 30329 (bxj4@cdc.gov).

Clinical Infectious Diseases® 2016;62(2):157–65
Published by Oxford University Press for the Infectious Diseases Society of America 2015. This work is written by (a) US Government employee(s) and is in the public domain in the US.
DOI: 10.1093/cid/civ828

Downloaded from http://cid.oxfordjournals.org/ at University of Witwatersrand on March 7, 2016

among human immunodeficiency virus (HIV)–unexposed infants in Soweto, South Africa [16]. We found that infants with delayed and unrestricted breastfeeding had similar RV IgA response, suggesting that a transient delay of breastfeeding at the time of vaccination did not appear to improve the immune response to RV1. In the present study, we performed more detailed analyses to examine associations between maternal antibodies, particularly preexisting RV IgG, and the immunogenicity of 1 or 2 doses of RV1 among infants in South Africa. We also assessed the potential impact of IgA from natural infection prior to vaccination on subsequent immune response to RV1.

METHODS

Participants and Specimen Collection

A previously described mother–infant pair population from a prospective, randomized, longitudinal cohort study was used in this analysis [16]. These healthy HIV-unexposed infants were enrolled at presentation for their routine immunization visit at Diepkloof Primary Health Clinic, Soweto, South Africa, between 2 December 2009 and 9 April 2010. Infants who were aged 5–8 weeks at enrollment, breast fed, and did not receive any previous vaccines except for OPV and bacille Calmette-Guérin at birth were included in the study. Mothers who tested seronegative for HIV at beyond 24 weeks gestational age were also enrolled. Infants with known underlying immunosuppressive conditions and failure to thrive (<3rd percentile weight-for-age or decrease of more than 2 major centiles since birth) were excluded.

All vaccines were provided according to the standard schedule of the expanded program on immunization in South Africa. RV vaccine RV1 was administered at 6 and 14 weeks of age, concurrently with heptavalent pneumococcal conjugate vaccine (Prevenar; Wyeth, Madison, Wisconsin). All infants received trivalent OPV (Merieux, Sanofi Pasteur, Lyon, France) at 6 weeks and Pentaxim (diphtheria toxoid, tetanus toxoid, acellular pertussis adsorbed–inactivated poliovirus–Haemophilus influenzae type b conjugate vaccine [DTap-IPV-Hib]; Sanofi Pasteur) and Heberbiovac-HB (hepatitis B vaccine; Heber Biotec, Havana, Cuba) at 6, 10, and 14 weeks. Serum samples were obtained from the infants before the first and second doses of RV vaccine and 1 month after the second RV dose. Maternal serum and breast milk samples were obtained prior to administration of each dose of RV in their infants. All specimens were kept frozen at −70°C until shipment on dry ice for analysis at the US Centers for Disease Control and Prevention (CDC). The Human Research Ethics Committee (Medical) of the University of the Witwatersrand (M090824) reviewed and approved the protocol. Written informed consent was obtained from the parent or guardian on behalf of the infant. Because the CDC tested only anonymized specimens, this research did not require review by the CDC Institutional Review Board. The trial was registered on the South African National Clinical Trial Register (DOH-27-0511-2991).

Specimen Testing

RV-specific IgA in breast milk and serum samples was determined using enzyme-linked immunosorbent assay as previously described [12]. Briefly, microplate wells were coated with rabbit hyperimmune serum to rhesus rotavirus (RRV) and incubated with diluted RV1 strain or blotto (5% skim milk in phosphate-buffered saline [PBS]). After washing, breast milk samples (1:5–1:5120) and serum samples (1:10–1:10 240) that were serially diluted in diluent buffer (1% skim milk and 0.5% [v/v] of 10% polyoxyethylene ether W1 in PBS) were added to the wells, followed by biotin-conjugated goat anti-human IgA antibodies (KPL, Gaithersburg, Maryland). After incubation and washing, extravidin (Sigma, St. Louis, Missouri) was added to the wells and incubated, and then the reactions were developed with 3,3′,5,5′-tetramethylbenzidine (TMB; Sigma) and stopped with 1*N* hydrogen chloride. Optical density (OD) was determined at 450 nm with an enzyme immunoassay reader (MRX Revelation; Dynex Technologies, Chantilly, Virginia). IgA titers in breast milk and serum were calculated as the reciprocal of the highest dilution that gave a mean OD greater than the cutoff value (3 standard deviations above the mean OD of the negative-control serum wells). RV-specific IgG in serum samples was tested and analyzed in the same manner as IgA except that 0.5% normal rabbit serum was added to the biotin-conjugate solution.

RV-specific neutralizing activity in breast milk was measured using a microneutralization assay as previously described [12]. Briefly, breast milk samples (50 µL) in 2-fold dilutions were mixed with an equal volume of trypsin-activated RV1 vaccine virus to yield a concentration of 4000 FFU/well and incubated at 37°C for 1 hour. Monolayers of MA104 cells grown in 96-well plates were washed with PBS and incubated with diluted breast milk and virus mixture. Following incubation at 37°C for 1 hour, the plates were washed with PBS and incubated with 100 µL of Iscove's modified Dulbecco's medium (Invitrogen, Carlsbad, California) containing 5 µg/mL trypsin. After 20 hours of incubation at 37°C, the plates were fixed with 15 µL of 37% formaldehyde at 4°C for 30 minutes. RV antigen in MA104 cells was detected by incubating plates with a rabbit anti-RRV hyperimmune serum, horseradish peroxidase-labeled anti-rabbit IgG, and then TMB. Neutralizing titer in a breast milk specimen was determined as the reciprocal of the highest dilution that showed a >70% reduction in the absorbance value compared with that in virus-only controls.

Statistical Analyses

Data analyses were performed using SPSS Statistics, version 20 (IBM Corp., Armonk, New York). The Wilcoxon signed-rank test was used to compare titers within or between groups. Comparisons between categorical variables were done using the χ^2 and relative risk tests. IgA titers ≥40 in serum were

Downloaded from http://cid.oxfordjournals.org/ at University of Witwatersrand on March 7, 2016

Table 1. Comparison of Mother–Infant Pairs (n = 181) Assigned to Analysis of Rotavirus (RV) Antibodies in Sera and RV Antibodies and Neutralizing Activity in Breast Milk by Infant Immunoglobulin A Seroconversion Post-Dose 1 and Post-Dose 2 of Rotarix

		Seroconversion Post-Dose 1 Status			Seroconversion Post-Dose 2 Status		
Subjects	Total (N = 181)	Yes (n = 64)	No (n = 117)	*P* Value[a]	Yes (n = 108)	No (n = 73)	*P* Value[a]
Infants							
Percentage (95% CI), n/N		35 (28–42), 64/181			60 (52–67), 108/181		**<.001**[b]
Age, in weeks, at dose 1 median (range)	6 (6–8)	6 (6–7)	6 (6–8)	.233	6 (6–8)	6.1 (6–7)	.799
Age, in weeks, at dose 2 median (range)	16 (12–24)	16 (14–23)	16 (12–20)	.981	16 (14–24)	15 (12–19)	**.006**
IgG pre-dose 1, median (range)	1280 (20–20 480)	1280 (20–10 240)	1280 (80–20 480)	**.010**	1280 (20–10 240)	1280 (80–20 480)	.072
IgA pre-dose 1, Positivity, % (95% CI), n/N	41 (34–48), 74/181	34 (22–46), 22/64	44 (35–54), 52/117	.188	32 (23–41), 35/108	53 (42–65), 39/73	**.005**
IgA pre-dose 1, median (range)	20 (1–1280)	20 (1–320)	20 (1–1280)	.151	20 (1–320)	40 (1–1280)	**.005**
IgA post-dose 1, median (range)	40 (1–10 240)	160 (40–10 240)	40 (1–1280)	**<.001**	80 (1–10 240)	40 (1–1280)	**.001**
IgA post-dose 2, median (range)	160 (1–20 480)	320 (40–20 480)	80 (1–5120)	**<.001**	320 (40–20 480)	40 (1–640)	**<.001**
Mothers							
IgG sera pre-dose 1, median (range)	10 240 (80–163, 840)	5120 (80–81 920)	10 240 (640–163 840)	**.031**	10 240 (80–163 840)	10 240 (640–163 840)	.235
IgA sera pre-dose 1, median (range)	40 (1–10, 240)	20 (1–5120)	40 (1–10 240)	.053	40 (1–10 240)	40 (1–5120)	.123
IgA sera post-dose 1, median (range)	160 (1–10 240)	160 (1–10 240)	160 (1–10 240)	.395	160 (1–10, 240)	320 (1–10 240)	.331
IgA BM, pre-dose 1, median (range)	40 (1–2560)	30 (1–1280)	40 (1–2560)	.499	20 (1–1280)	40 (1–2560)	.615
IgA BM post-dose 1, median (range)	40 (1–10 240)	40 (1–2560)	40 (1–10 240)	.520	40 (5–2560)	40 (1–10, 240)	.781
NA BM pre-dose 1, median (range)	8 (1–2048)	8 (1–2048)	8 (1–2048)	.445	8 (1–2048)	8 (1–2048)	.848
NA BM post-dose 1, median (range)	16 (1–2048)	16 (1–2048)	16 (1–2048)	.629	16 (1–2048)	16 (1–2048)	.737

Serum and breast milk specimens from 181 infant–mother pairs were analyzed as described in the text. Seroconversion was defined as a ≥4-fold rise in IgA titer from baseline to post-dose 2. Seropositivity of IgA was defined as titer ≥1:40.

Significant *P* values are highlighted in bold ($P < .05$).

Abbreviations: BM, breast milk; CI, confidence interval; Ig, immunoglobulin; NA, neutralizing activity.

[a] Statistical difference was established by Wilcoxon rank-sum test.

[b] Statistical difference was established by χ^2 test.

Downloaded from http://cid.oxfordjournals.org/ at University of Witwatersrand on March 7, 2016

Table 2. Comparison of Mother and Rotavirus (RV)-Unexposed Infant Pairs (n = 107) Assigned to Analysis of RV Antibodies in Sera and RV Antibodies and Neutralizing Activity in Breast Milk, by Infant Immunoglobulin A Seroconversion Post-Dose 1 and Post-Dose 2 of Rotarix

		Seroconversion 1 Status			Seroconversion 2 Status		
Subjects	Total (N = 107)	Yes (n = 42)	No (n = 65)	*P* Value[a]	Yes (n = 73)	No (n = 34)	*P* Value[a]
Infants							
Percentage % (n/N)		39 (42/107)			68 (73/107)		**<.001**[b]
Age, in weeks, at dose 1 median (range)	6 (6–8)	6 (6–7)	6 (6–8)	.219	6 (6–8)	6 (6–7)	.756
Age, in weeks, at dose 2 median (range)	16 (14–23)	16 (14–23)	16 (14–19)	.767	16 (14–23)	15 (14–19)	.053
IgG pre-dose 1, median (range)	1280 (20–20 480)	1280 (20–10 240)	2560 (160–20 480)	**.004**	1280 (20–10 240)	2560 (320–20 480)	.051
IgA post-dose 1, median (range)	40 (1–10 240)	80 (40–10 240)	20 (1–60)	**<.001**	40 (1–10 240)	20 (1–60)	**<.001**
IgA post dose 2, median (range)	80 (1–20 480)	160 (40–20 480)	40 (1–2560)	**<.001**	160 (40–20 480)	20 (1–40)	**<.001**
Mother							
IgG sera pre-dose 1, median (range)	10 240 (80–163 840)	5120 (80–81 920)	10 240 (640–163 840)	**.040**	10 240 (80–163 840)	20 480 (640–163 840)	.166
IgA sera pre-dose 1, median (range)	40 (1–5120)	20 (1–5120)	40 (1–5120)	.203	40 (1–5120)	80 (1–2560)	.102
IgA sera post-dose 1, median (range)	160 (1–10 240)	160 (1–10 240)	160 (1–10 240)	.632	160 (1–10 240)	320 (1–10 240)	.255
IgA BM, pre-dose 1, median (range)	40 (1–2560)	40 (1–1280)	40 (1–2560)	.420	40 (1–1280)	40 (1–2560)	.534
IgA BM post-dose 1, median (range)	40 (5–10 240)	40 (10–2560)	40 (5–10 240)	.620	40 (5–2560)	40 (5–10 240)	.801
NA BM pre-dose 1, median (range)	8 (1–2048)	4 (1–2048)	8 (1–2048)	.849	8 (1–2048)	8 (1–2048)	.606
NA BM post-dose 1, median (range)	16 (1–2048)	16 (1–2048)	16 (1–2048)	.817	16 (1–2048)	12 (1–2048)	.616

Serum and breast milk specimens from 107 infant–mother pairs were analyzed as described in the text. Seroconversion was defined as a ≥4-fold rise in IgA titer from baseline to post-dose 2. Seropositivity of IgA was defined as titer ≥1:40.

Significant *P* values are highlighted in bold ($P < .05$).

Abbreviations: BM, breast milk; Ig, immunoglobulin; NA, neutralizing activity.

[a] Statistical difference was established by Wilcoxon rank-sum test.

[b] Statistical difference was established by χ^2 test.

Downloaded from http://cid.oxfordjournals.org/ at University of Witwatersrand on March 7, 2016

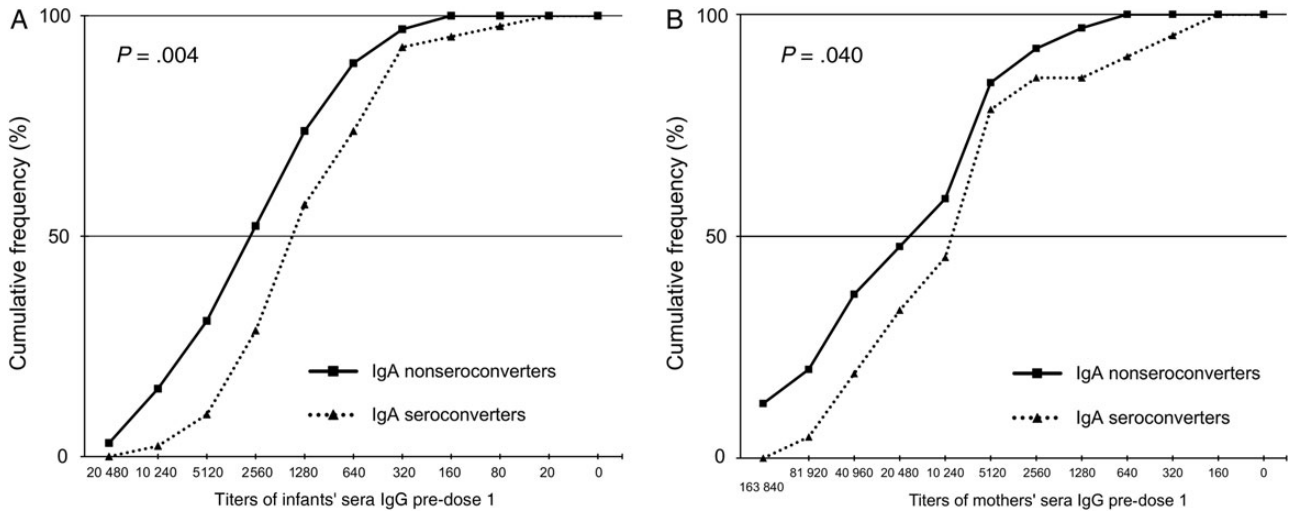


Figure 1. Cumulative frequency profiles of rotavirus immunoglobulin G (RV IgG) titers in pre-dose 1 sera of 107 RV-unexposed infants (*A*) and their mothers (*B*), by infant IgA seroconversion post-dose 1 of RV vaccine. Seroconversion was defined as a 4-fold increase in RV-specific IgA titers in post-dose 1 sera when compared with the corresponding preimmunization sera. Pre-dose 1 sera of infants and their mothers were tested for RV-specific IgG as described in the text.

considered positive. Seroconversion was defined as greater than 4-fold increase in RV-specific IgA titers following the first or second RV vaccine doses compared with titers prior to the first dose.

Univariate linear regression analyses were performed to examine the relationships between infants' IgG and mothers' IgG pre-dose 1 in sera and between neutralizing activity and IgA pre-dose 1 and 2 in breast milk specimens. Log transformed (log_2) titers of IgG, IgA, and neutralizing activity were used in the linear regression models. Linear regression models were assessed for violations of assumptions. Spearman rank correlations were used in univariate analyses to examine relationships between continuous variables when the assumptions of the regression model were not met. Univariate and multivariate logistic regression analyses were performed to examine the relationships between continuous variables and seroconversion post-dose 1 and 2. Finally, log_2 titers of IgA, IgG, and neutralizing activity were used in the logistic regression models. An alpha level of 0.05 ($P < .05$) was considered significant for all tests.

RESULTS

We enrolled 250 infants with a median age of 6.1 weeks (range 6–8) at the time of their first RV1 immunization, given concomitantly with OPV and other scheduled pediatric vaccines [16]. Because some infants were not fully adherent to the protocol and were excluded from the study, or some specimens were not collected, we analyzed available matched serum and breast milk samples from 181 mother–infant pairs. RV IgA seroconversion rate at post-dose 1 was 35% (64/181) and increased significantly to 60% (108/181) at post-dose 2 ($P < .001$). Then, we generated 2 groups based on the status of IgA seroconversion, that is, infants who did or did not seroconvert at post-dose 1 or post-dose 2, and evaluated biological predictors of the IgA seroconversion (Table 1). Infant pre-dose 1 serum RV IgG titers were significantly higher in the group of infants who did not seroconvert than those of infants who seroconverted at post-dose 1 ($P = .010$). While seroconverted and nonseroconverted post-dose 1 infants had similar pre-dose 1 serum IgA titers, we found that infants who did not seroconvert at post-dose 2 were more likely to be IgA positive at pre-dose 1 than those who seroconverted (53% vs 32%, $P = .005$).

To examine whether maternal antibodies influenced immune response to RV1 in infants, we compared titers of IgG, IgA in serum, and IgA and neutralizing activity in breast milk specimens of mothers with infants who seroconverted or did not seroconvert (Table 1). Mothers whose infants seroconverted post-dose 1 had significantly lower titers of IgG ($P = .031$) and IgA ($P = .053$) in pre-dose 1 sera than those whose infants did not seroconvert, but these differences were not significant in mothers at post-dose 2. There were no differences observed for titers of post-dose 1 IgA in serum and pre-dose 1 and post-dose 1 IgA and neutralizing activity in breast milk. For mothers whose infants seroconverted or did not seroconvert post-dose 2, none of the IgG, IgA, and neutralizing activity titers in serum or breast milk specimens were significantly different.

We then performed a second analyses by excluding 74 infants who might have had an early neonatal RV infection, as evidenced by a pre-dose 1 RV IgA titer of ≥40 in serum (Table 2 and Figure 1). In this new subset of mother–infant pairs (n = 107), 39% of infants seroconverted post-dose 1 and 68% seroconverted post-dose 2. Infants who failed to seroconvert post-dose 1 had significantly higher pre-dose 1 RV IgG titers than those who seroconverted ($P = .004$). This trend continued

Downloaded from http://cid.oxfordjournals.org/ at University of Witwatersrand on March 7, 2016

Table 3. Predictors for Serum Immunoglobulin A Seroconversion to Rotarix in Rotavirus-Unexposed Infants (n = 107), South Africa, 2009–2010

	IgA Seroconversion Post-Dose 1						IgA Seroconversion Post-Dose 2					
	Univariate Analysis			Multivariate Analysis			Univariate Analysis			Multivariate Analysis		
Explanatory Variables	Coefficient	OR (95% CI)	*P* Value	Coefficient	Adjusted OR (95% CI)	*P* Value	Coefficient	OR (95% CI)	*P* Value	Coefficient	Adjusted OR (95% CI)	*P* Value
Infants												
IgG pre-dose 1	−0.38	0.69 (.53–.88)	**.004**	−0.30	0.74 (0.55–.99)	**.044**	−0.28	0.76 (.59–.98)	**.034**	−0.19	0.83 (0.63–1.08)	.166
IgA post-dose 1							0.36	1.43 (1.19–1.71)	**<.001**	0.34	1.40 (1.17–1.68)	**<.001**
Age at dose 1	−1.0188	0.36 (.10–1.36)	.133				0.15	1.16 (.33–4.05)	.817			
Age at dose 2	0.1043	1.11 (.85–1.44)	.436				0.32	1.38 (.99–1.91)	.055			
Gender (male)	−0.16	0.85 (.39–1.86)	.686				−0.14	0.87 (.39–1.97)	.741			
Weight at birth, kg	0.26	1.29 (.56–2.98)	.547				0.41	1.51 (.63–3.60)	.357			
Mothers												
IgG sera pre-dose 1	−0.23	0.80 (.66–.96)	**.015**	−0.11	0.90 (.72–1.11)	.311	−0.14	0.87 (.72–1.05)	.138			
IgA sera pre-dose 1	−0.1	0.90 (.81–1.01)	.066				−0.08	0.93 (.83–1.04)	.177			
IgA sera pre-dose 2							−0.09	0.91 (.80–1.05)	.19			
IgA BM, pre-dose 1	−0.07	0.93 (.78–1.11)	.411				−0.10	0.90 (.75–1.08)	.275			
IgA BM pre-dose 2							0.00	1.00 (.83–1.20)	.998			
NA BM pre-dose 1	−0.02	0.98 (.85–1.13)	.774				0.03	1.03 (.89–1.20)	.672			
NA BM pre-dose 2							0.04	1.04 (.90–1.20)	.559			

Log-2 transformed antirotavirus titers were used in the analysis as described in the text.

Significant *P* values are highlighted in bold ($P < .05$).

Abbreviations: BM, breast milk; CI, confidence interval; Ig, immunoglobulin; NA, neutralizing activity; OR, odds ratio.

Downloaded from http://cid.oxfordjournals.org/ at University of Witwatersrand on March 7, 2016

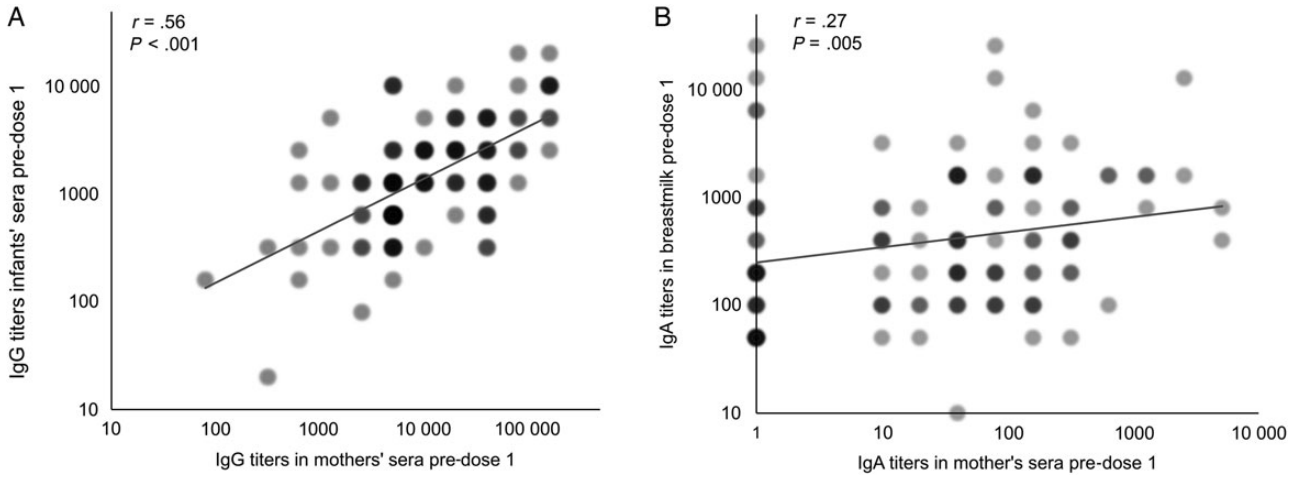


Figure 2. Association of rotavirus immunoglobulin G (RV IgG) titers in pre-dose 1 sera of 107 RV-unexposed infants and their mothers (*A*) and association of RV IgA titers in pre-dose 1 sera and breast milk from mothers (*B*). Log-transformed (log_2) IgG titers in paired mother and infant sera were analyzed by univariate linear regression analysis. The log_2 IgA titers were analyzed using Spearman correlation analysis. Regression models were assessed for violations of assumptions. Color intensity and circle size at each point were proportional to the number of samples.

until post-dose 2 ($P = .051$). Mothers whose infants seroconverted post-dose 1 had significantly lower median titers of IgG in pre-dose 1 sera than those whose infants did not seroconvert post-dose 1, but this difference was not seen post-dose 2. Similarly, infants who did not seroconvert and their mothers had higher cumulative IgG titers in pre-dose 1 sera than those who seroconverted at post-dose 1 (Figure 1). None of the titers for IgA or neutralizing activity in serum or breast milk specimens of mothers were significantly different regardless of seroconversion status post-dose 1 and post-dose 2 in infants.

We also performed univariate and multivariate analysis to identify variables that might influence IgA seroconversion (Table 3). The levels of pre-dose 1 IgG in infant sera were negatively associated with seroconversion post-dose 1 by both univariate and multivariate analyses and seroconversion post-dose 2 by univariate analysis. IgG titers in mothers' sera were negatively associated with seroconversion post-dose 1 in the univariate analysis only. We also analyzed the relationship between the pre-dose 1 IgG titers in infants and IgA titers in response to RV1 at post-dose 1 and post-dose 2. We found a relatively strong negative association between preexisting IgG titers in pre-dose 1 sera and IgA titers in post-dose 1 sera ($r = -0.28$, $P = .003$). Similarly, we found a weaker but significant negative association between preexisting IgG titers in pre-dose 1 sera and IgA titers in post-dose 2 sera ($r = -0.23$, $P = .016$; data not shown).

We further observed a strong significant association between IgG titers in pre-dose 1 sera of paired infants and mothers using univariate regression analyses ($r = 0.56$, $P < .001$; Figure 2*A*). We also found a weak but significant correlation between IgA titers in breast milk and sera from mothers at pre-dose 1 ($r = 0.27$, $P = .005$; Figure 2*B*). In addition, we found significant positive associations between RV IgA and neutralizing activity in breast milk at pre-dose 1 ($r = 0.41$, $P < .001$) and post-dose 1 ($r = .40$, $P < .001$; data not shown).

DISCUSSION

In this cohort of mothers and their HIV-unexposed infants in Soweto, South Africa, we demonstrated a strong association between levels of IgG in sera of mother–infant pairs at the time of first RV immunization, suggesting direct transplacental transmission of this antibody from mothers to infants. Infants who failed to seroconvert to RV1 had significantly higher RV IgG titers in pre-dose 1 sera than those who seroconverted. The second dose of RV1 somewhat overcame the interference of preexisting RV IgG since levels of this antibody in infants with a median age of 16 weeks (range 12–24) had waned. These findings indicate that maternal IgG may have interfered with the immune response to RV1 in infants.

Maternal IgG is transported across the placenta by an active, receptor-mediated process during pregnancy, thereby protecting term infants against infection [17]. In newborns and young infants, RV IgG comes primarily from the mother in the first or second month of life, depending on settings. Since the median age of this infant cohort was 6.1 weeks at the first dose of vaccination, levels of RV IgG were expected to be high, as reported in Indian, Mexican, and Nicaraguan infants of similar age [18–20]. Our observations of high IgG levels in sera of these South African infants at age 6.1 weeks may explain higher seroconversion rates to RV1 when administered at a 3-dose (6, 10, and 14 weeks) or a 2-dose (10 and 14 weeks) schedule than at the 6- and 10-week schedule recommended by the manufacturer in the same setting [21]. Since transplacental RV

Downloaded from http://cid.oxfordjournals.org/ at University of Witwatersrand on March 7, 2016

IgG decreases with a half-life of 3–4 weeks, this 4-week delay in vaccine administration might explain the improved immunogenicity of the vaccine. Of note, since RV immunity is believed to be polygenic and cross-reactive, we think the preexisting serum IgG is a mixture of nonneutralizing RV antibody directed primarily against VP6 group antigen and, to a lesser degree, VP4 and VP7 serotype antigens. Thus, this inhibitory effect would apply for both RV1 and RV5 vaccines. Maternal antibody interference has also been reported in children who received polio and measles vaccines [22, 23].

We previously reported positive associations between levels of IgA and neutralizing activity against RV1, RV5, and 116E strains in breast milk of mothers in India and South Africa, but not in specimens from mothers in the United States [13]. In the present study, we again found significant correlations between RV IgA titers and neutralizing titers against RV1 strain in breast milk collected at the time of the first and second RV vaccinations from South African mothers. We also observed a significant association between IgA titers in pre-dose 1 sera and breast milk from mothers, suggesting some transmission of IgA between systemic and mucosal systems [24]. This association could also be explained by dual natural infection with RV in both mother and infant. Additionally, a correlation between IgA in sera and sIgA in breast milk has been reported [14, 25]. Of note, we observed similar levels of IgA and neutralizing activity in breast milk of mothers whose infants seroconverted or did not seroconvert, supporting the recent report of no effect from a short abstention of breastfeeding before and after each vaccination on immune response to RV1 in South African infants. However, we could not evaluate the effect of long-term breastfeeding on vaccine immunogenicity since all infants were breastfed. Long-term breastfeeding practices have been reported to be associated with lower immune response to RV1 or RV5 among infants in both high- and low-income countries [26–28].

The apparent inhibitory effect of maternal antibody could represent one of the complicated mechanisms to explain why live oral RV vaccines have been less immunogenic and effective in low-income settings such as Africa [11, 15] where infants typically acquire high titer RV IgG transplacentally from their mothers. In addition, preexisting serum IgA appeared to have some damping effect on vaccine immunogenicity as well. Future studies should investigate other potential confounders such as malnutrition, coinfections, comorbidities, and gut microbiota. Given that multiple factors are likely involved in gut enteropathy and reduced efficacy of oral vaccines and that intervention strategies to date have not shown any major improvements, alternative approaches, such as parenteral immunization with inactivated RV or subunit vaccine, should be assessed.

Notes

Disclaimer. The finding and conclusions in this report are those of the authors and do not necessarily represent the official positions of Centers for Disease Control and Prevention (CDC).

Financial support. The clinical aspect of this study was funded through core funding from the South African Medical Research Council (Respiratory and Meningeal Pathogens Research Unit) and Department of Science and Technology/National Research Foundation South African Research Chair Initiative on Vaccine Preventable Diseases. The funders had no role in the conduct of the study or in the write-up of the manuscript. The immunology work was funded by the CDC.

Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Global networks for surveillance of rotavirus gastroenteritis, 2001–2008. Wkly Epidemiol Rec **2008**; 83:421–5.
2. Statistics-South-Africa. Levels and trends of morbidity and mortality among children aged under-five years in South Africa, 2006–2010. Available at: http://www.statssa.gov.za/publications/Report-03-09-10/Report-03-09-102010.pdf. Accessed 28 September 2015.
3. Mapaseka SL, Dewar JB, van der Merwe L, et al. Prospective hospital-based surveillance to estimate rotavirus disease burden in the Gauteng and North West Province of South Africa during 2003–2005. J Infect Dis **2010**; 202(suppl): S131–8.
4. Glass RI, Parashar UD. Rotavirus vaccines—balancing intussusception risks and health benefits. N Engl J Med **2014**; 370:568–70.
5. 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**; 354:11–22.
6. 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**; 354:23–33.
7. Armah GE, Sow SO, Breiman RF, et al. Efficacy of pentavalent rotavirus vaccine against severe rotavirus gastroenteritis in infants in developing countries in sub-Saharan Africa: a randomised, double-blind, placebo-controlled trial. Lancet **2010**; 376:606–14.
8. Nelson EA, Glass RI. Rotavirus: realising the potential of a promising vaccine. Lancet **2010**; 376:568–70.
9. Zaman K, Dang DA, Victor JC, et al. Efficacy of pentavalent rotavirus vaccine against severe rotavirus gastroenteritis in infants in developing countries in Asia: a randomised, double-blind, placebo-controlled trial. Lancet **2010**; 376: 615–23.
10. Patel MM, Glass R, Desai R, Tate JE, Parashar UD. Fulfilling the promise of rotavirus vaccines: how far have we come since licensure? Lancet Infect Dis **2012**; 12:561–70.
11. Madhi SA, Cunliffe NA, Steele D, et al. Effect of human rotavirus vaccine on severe diarrhea in African infants. N Engl J Med **2010**; 362:289–98.
12. Moon SS, Wang Y, Shane AL, et al. Inhibitory effect of breast milk on infectivity of live oral rotavirus vaccines. Pediatr Infect Dis J **2010**; 29:919–23.
13. Moon SS, Tate JE, Ray P, et al. Differential profiles and inhibitory effect on rotavirus vaccines of nonantibody components in breast milk from mothers in developing and developed countries. Pediatr Infect Dis J **2013**; 32:863–70.
14. Novak D, Svennerholm AM. A comparison of seasonal variations in rotavirus antibodies in the breast milk of Swedish and Bangladeshi mothers. Acta Paediatr **2015**; 104:247–51.
15. Patel M, Shane AL, Parashar UD, Jiang B, Gentsch JR, Glass RI. Oral rotavirus vaccines: how well will they work where they are needed most? J Infect Dis **2009**; 200(suppl 1):S39–48.
16. Groome MJ, Moon SS, Velasquez D, et al. Effect of breastfeeding on immunogenicity of oral live-attenuated human rotavirus vaccine: a randomized trial in HIV-uninfected infants in Soweto, South Africa. Bull World Health Organ **2014**; 92:238–45.
17. Malek A, Sager R, Kuhn P, Nicolaides KH, Schneider H. Evolution of maternofetal transport of immunoglobulins during human pregnancy. Am J Reprod Immunol **1996**; 36:248–55.
18. Velazquez FR, Matson DO, Guerrero ML, et al. Serum antibody as a marker of protection against natural rotavirus infection and disease. J Infect Dis **2000**; 182:1602–9.
19. Becker-Dreps S, Samuel V, Velasquez Daniel, et al. Rotavirus-specific IgG antibodies from mothers' serum may inhibit infant immune responses to the pentavalent rotavirus vaccine. Pediatr Infect Dis J **2015**; 34:115–6.
20. Appaiahgari MB, Glass R, Singh S, et al. Transplacental rotavirus IgG interferes with immune response to live oral rotavirus vaccine ORV-116E in Indian infants. Vaccine **2014**; 32:651–6.

Downloaded from http://cid.oxfordjournals.org/ at University of Witwatersrand on March 7, 2016

21. Steele AD, Reynders J, Scholtz F, et al. Comparison of 2 different regimens for reactogenicity, safety, and immunogenicity of the live attenuated oral rotavirus vaccine RIX4414 coadministered with oral polio vaccine in South African infants. J Infect Dis **2010**; 202(suppl):S93–100.
22. Perkins FT, Yetts R, Gaisford W. A comparison of the responses of 100 infants to primary poliomyelitis immunization with two and with three doses of vaccine. Br Med J **1959**; 1:1083–6.
23. Albrecht P, Ennis FA, Saltzman EJ, Krugman S. Persistence of maternal antibody in infants beyond 12 months: mechanism of measles vaccine failure. J Pediatr **1977**; 91:715–8.
24. Gustafson CE, Higbee D, Yeckes AR, et al. Limited expression of APRIL and its receptors prior to intestinal IgA plasma cell development during human infancy. Mucosal Immunol **2014**; 7:467–77.
25. Hjelt K, Grauballe PC, Nielsen OH, Schiotz PO, Krasilnikoff PA. Rotavirus antibodies in the mother and her breast-fed infant. J Pediatr Gastroenterol Nutr **1985**; 4:414–20.
26. Adlhoch C, Hoehne M, Littmann M, et al. Rotavirus vaccine effectiveness and case-control study on risk factors for breakthrough infections in Germany, 2010–2011. Pediatr Infect Dis J **2013**; 32:e82–9.
27. Vesikari T, Prymula R, Schuster V, et al. Efficacy and immunogenicity of live-attenuated human rotavirus vaccine in breast-fed and formula-fed European infants. Pediatr Infect Dis J **2012**; 31:509–13.
28. Velasquez D, Bautista A, Esparza M, et al. Association between breastfeeding and immunogenicity of Rotarix in Mexican infants [abstract W12-8]. In: Scientific program and abstracts of the 33rd Annual Meeting of the American Society for Virology (Fort Collins, CO). Toledo, OH: American Society for Virology, **2014**:110.

Downloaded from http://cid.oxfordjournals.org/ at University of Witwatersrand on March 7, 2016