Rotavirus was first detected by electron microscopy in young children with diarrhoea in South Africa in 1976, and 25 years later rotavirus vaccine was introduced into the routine childhood immunisation schedule in the country. Prof Barry Schoub played a role in both these events and in many milestones along that journey, from discovery to introduction. Several seminal findings were identified by Schoub et al in the early years after rotavirus identification in South Africa, including the antigenic relatedness of rotavirus strains, and several important epidemiological and clinical findings. This laid the foundation for the role that South Africa has played in pioneering rotavirus research work in, and for, Africa. Early efforts at establishing a regional network for rotavirus epidemiology and surveillance studies have expanded into a continent-wide network using standardised protocols with reporting to the World Health Organization. In addition, clinical studies conducted in South Africa paved the way for the national introduction of the vaccine, while also addressing specific questions of relevance to the global community, including safety in HIV-infected children and efficacy in an African population.

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Introduction

Each year, an estimated 1.34 million children worldwide die from severe, dehydrating diarrhoea, and millions more are hospitalised. This constitutes approximately 16% of total childhood deaths. Rotavirus is recognised as the major enteric pathogen associated with this morbidity and mortality in developing countries, and this pathogen is associated with an estimated 527,000 (range 475,000-580,000) deaths annually in infants and young children. All children are infected with rotavirus in the first few years of life and the reported incidence of rotavirus disease does not vary significantly between industrialised countries and the developing countries of Africa and Asia, indicating that socio-economic improvements in water and sanitation may not reduce rotavirus diarrhoea. Nevertheless, the inequity in access to health care means that the vast majority of deaths are in children in the poorest countries of the world. This has prompted the international prioritisation of rotavirus vaccines as a primary strategy for the reduction of the mortality associated with this infection.

In the developing countries of Africa, Latin America and Asia, rotavirus infection is observed in 30-50% of infants and young children hospitalised with diarrhoeal illness, highlighting the enormous impact of rotavirus infection in these countries. In addition, rotavirus disease is aggravated in young children in developing countries for several reasons. First, the infection generally occurs at an earlier age in children in Africa, where approximately 75-80% of rotavirus infections occur before the first birthday, and they often have more frequent infections; between two and five rotavirus infections per year have been documented in Africa. Furthermore, rotavirus infection is often complicated by infection with multiple rotavirus strains and other enteric pathogens, and is more severe in nature. These infants are also beset with much other co-morbidity, including other infectious diseases and malnutrition. Thus, immunisation is considered the optimal strategy to deliver an impact on the burden and severity of rotavirus disease.

History of rotavirus in South Africa

Soon after Bishop in Australia discovered rotaviruses by negative staining electron microscopy (EM) in the duodenal biopsies of young children with diarrhoea, Schoub detected rotavirus particles in the stools of young South African children with diarrhoeal disease. In the following years, several important observations regarding rotavirus infection were recorded in South African studies conducted by this group.

First, several early studies conducted in and around Johannesburg identified epidemiological differences in rotavirus infection among different racial groups. In the white population, rotavirus infections had a seasonal peak in the colder months of the year, similar to that seen in temperate climates, while infections in the black population was non-seasonal with a low threshold of activity constant throughout the year. This observation led to a number of other studies, including a survey of seroprevalence that demonstrated that the black African population was exposed earlier and to a greater extent than their white counterparts.
The early epidemiological studies were conducted in small numbers of infants over a short period of time, and this might have confounded these early reports.

A larger epidemiological study was conducted at Ga-Rankuwa Hospital, in a township near Pretoria, to examine the prevalence of rotavirus-associated gastroenteritis in black infants and to investigate the seasonal parameters of rotavirus infection.\textsuperscript{18,19} The overall prevalence of rotavirus-associated illness was 32.8\% among children hospitalised with diarrhoea, but this varied considerably by season. In fact, the study identified a definite seasonal pattern, with a peak in autumn and a secondary peak occurring in late winter or early spring.\textsuperscript{18,19} This seasonal variation in rotavirus prevalence in South African infants has now been documented in several other studies in various areas of the country and in all population groups.

Nevertheless, the early perceived differences in the epidemiology of rotavirus infection in population groups separated through the practices of apartheid led to other studies. In a study comparing the molecular epidemiology of rotavirus strains in Ga-Rankuwa and Pretoria, two different pools of rotavirus existed in each population, despite the fact that they lived in close proximity. Strains were carried between the two groups and "emerged" within one group after a lag of one to two years from the other.\textsuperscript{20}

Second, neonatal rotavirus infection was found to confer protection against subsequent severe disease.\textsuperscript{21} Using EM of stool specimens for diagnosis (Figure 1), rotavirus infection was assessed in asymptomatic black and white newborns in different maternity hospitals: none of the 37 white neonates, but 30 (49\%) of the 61 black neonates, shed rotavirus (p <0.0005).\textsuperscript{21} The high incidence of neonatal infection observed in black infants, who had supposedly a low incidence numbers of infants over a short period of time, and this might have confounded these early reports.

Third, the role of maternal antibody transferred to the newborn via breast milk was assessed in both humans and an animal model.\textsuperscript{23} Rotavirus antibodies were not identified in the colostrum or the breast milk of either humans or mice, suggesting that passive immunity may not prevent transmission. However, more recent studies have indicated that the role of maternal antibodies in breast milk is likely to play a major role.\textsuperscript{24}

Finally, these early studies were made possible by the discovery of the antigenic relatedness of the simian rotavirus strain, SA-11, and human rotaviruses.\textsuperscript{25} Schoub described two different elements of antigenic relatedness. VP6, the inner capsid protein, is the major antigen of the viral particle in both animal and human strains and is now utilised in most immunoassays to detect the presence of human rotavirus particles in the stool. The VP7, the outer capsid protein, is the major neutralising antigen of the virus. The cross-reactivity between the SA-11 strain (serotype G3) was also present in some human strains of serotype G3 specificity. Finally, while at the National Institutes of Health (NIH), Bethesda, Schoub also examined the growth characteristics of human and animal rotaviruses and found that trypsin, which enhanced the infectivity of human strains, was not essential for the growth of animal isolates.\textsuperscript{26}

**Epidemiology of rotavirus in South Africa**

In virtually all studies investigating rotavirus as an aetiological agent of diarrhoeal disease in South Africa, the virus has been identified as the single most important pathogen associated with acute infantile gastroenteritis. A review of the epidemiology of rotavirus infection in South Africa was previously published\textsuperscript{27} and is only summarised here.

In studies that included age- and sex-matched controls, rotavirus was recovered significantly more often from patients with diarrhoea (median 20\%, range 13-26\%) than from controls (3.25\%). In a more recent and thorough analysis, Seheri found the rate of rotavirus detection in hospitalised children was consistent with this figure.\textsuperscript{28} In outpatient studies, children with diarrhoea had a lower level of rotavirus excretion (median 15\%), suggesting that rotavirus was more commonly identified in children with more severe disease.\textsuperscript{27}

**Age distribution of patients with rotavirus infection**

Most studies investigating the rate of rotavirus disease in young children have focused on children with diarrhoea under two or three years of age. However, rotavirus shedding is observed in over 75\% of the infants under 12 months of age with diarrhoeal disease in the studies where this information is detailed. Schoub and colleagues first noted this early age of acquisition of rotavirus infection in African populations in the late 1970s.\textsuperscript{12} Overall, more than 95\% of the rotavirus cases occurred in children under the age of 18 months in the inpatient studies, where they were hospitalised for diarrhoea. In outpatient studies, 72\% (range 51-85\%) of the rotavirus
excretion occurred in children under 12 months of age with diarrhoea.²⁷

**Seasonality of rotavirus infection**

Several early studies of the seasonality of rotavirus at various locations with different climatic conditions in South Africa identified two recurrent features of the disease. First, rotavirus infection occurred year-round in all locations studied, albeit at low levels in some areas. Secondly, in each region, rotavirus cases increased during the cooler and drier months.

Three early studies with small numbers of patients conducted for short periods of two to four months in Johannesburg reported local differences in the seasonality of rotavirus disease.¹²-¹⁴ These studies indicated that rotavirus diarrhoea was predominant in winter in white children,¹² but occurred year-round at low levels in black children.¹³,¹⁶ However, these studies were conducted only in summer in black children and only in the winter months in white children, which likely confounded the results obtained. Certainly more recent studies have indicated that rotavirus shedding in children of all racial groups in South Africa follows the seasonal pattern observed in other temperate climates.²⁹-³² In the longest study performed to date in South Africa, a clear autumn-winter peak in rotavirus excretion can be seen in black children in Ga-Rankuwa every year (our own unpublished data, Figure 2).

**Multiple microbial infections**

Several studies investigating the aetiology of childhood diarrhoea from stool samples have identified the presence of more than one pathogen in a single patient. Studies in South Africa have found multiple pathogens in 17% of the children surveyed (range 7.4-39%). The highest rate of mixed infections was observed in a study which included investigation for the enterotoxigenic *Escherichia coli*, adenoviruses and the small round-structured viruses⁸,²⁹ which are often not included in standard aetiological surveys. A similar pattern of mixed infection was noted in the children from outpatient studies (12%) and serves to highlight the high microbial load to which these children are exposed.

**Rotavirus strain characterisation**

Finally, many studies have characterised rotavirus strains in circulation in South Africa. These have demonstrated the enormous diversity of strains circulating at any one time,³³-³⁵ the emergence of new strains³⁶ and the presence of strains bearing zoonotic reassortments.³⁷,³⁸ This will not be reviewed in this report.

**Regional rotavirus surveillance networks**

In 1997, an international consultative meeting held at the World Health Organization (WHO) in Geneva, Switzerland, entitled “Rotavirus Vaccines for Immunization of Children in Developing Countries”, made two substantive recommendations to advance the rotavirus agenda. First, the delegates noted a paucity of good data concerning the burden of rotavirus diarrhoea and strain characterisation of the VP7 and VP4 rotavirus types circulating in Africa and Asia. This recommendation led to the establishment of two regional networks for the surveillance of rotavirus epidemiology and strain characterisation in

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**Figure 2: Seasonality of rotavirus infection in South Africa**
Africa and Asia. These two networks were the forerunners of the current WHO Global Regional Rotavirus Surveillance Networks that were funded by the WHO, United States Centers for Disease Control and the Children's Vaccine Program, PATH (formerly Program for Appropriate Technology in Health). Since 2001, the Global Regional Rotavirus Surveillance Networks have contributed significantly to address the global burden of rotavirus disease, using standardised protocols and working in close collaboration with the national Ministries of Health and the WHO Regional Offices. This impressive array of surveillance data has provided the impetus for countries to consider the introduction of rotavirus vaccines. These data have also provided evidence for the huge burden of rotavirus disease that has led the international community and GAVI (Global Alliance for Vaccines and Immunisation) to target rotavirus vaccines as a priority for introduction in low-income countries.

The second identified gap in knowledge was the lack of data on the immunogenicity and efficacy of the current rotavirus vaccine candidates in African and Asian children, where limited trials with various candidates had not been particularly promising (see below). This led to the initiation and preparation of clinical trials with rotavirus vaccines in three countries in Africa in 1998 and 1999. In Ghana, a Phase II immunogenicity study with the rhesus reassortant rotavirus vaccine (RotaShield®), Wyeth Lederle) was initiated and over 100 infants were immunised with one or more doses of the vaccine by October 1999, when the study was halted (Fred Binka, personal communication). In South Africa, a small Phase I study with the bovine reassortant vaccine (UK strain, Wyeth Lederle) was completed and plans for the start of a Phase II immunogenicity study were well advanced by October 1999. In Guinea Bissau, a RotaShield® vaccine trial was ready to start enrolment, funded by the WHO (Kare Molbak, personal communication). At this time, all these clinical trials were halted owing to the reports of intussusception associated with the vaccine by October 1999, when the study was halted (Fred Binka, personal communication). In South Africa, a small Phase I study with the bovine reassortant vaccine (UK strain, Wyeth Lederle) was completed and plans for the start of a Phase II immunogenicity study were well advanced by October 1999. In Guinea Bissau, a RotaShield® vaccine trial was ready to start enrolment, funded by the WHO (Kare Molbak, personal communication). At this time, all these clinical trials were halted owing to the reports of intussusception associated with the use of the commercial vaccine in the USA. They were never re-initiated and the opportunity to assess the immunogenicity and efficacy of this vaccine in populations with high rotavirus burden and mortality was lost.

Rotavirus vaccine trials in South Africa

South Africa has played a central role in evaluating the next generation of rotavirus vaccines.

Early rotavirus vaccines in Africa

Although the new rotavirus vaccine candidates have demonstrated high efficacy in trials in developed countries, earlier rotavirus vaccine candidates tested in Africa had lower or negligible efficacy to prevent severe disease. Many factors likely played a role in these failures, including problems in the design and interpretation of the earlier trials. Nevertheless, other issues which may be relevant specifically to developing country populations in Africa and Asia are also indicated. These may include vaccine-related issues, such as the antigenic make-up of the bovine rotavirus vaccine strains used (VP7 serotype G6), which is not found in humans. Secondly, the vaccine dosages were at a lower range and may not have been sufficiently immunogenic to demonstrate a protective effect. This may be specifically relevant in sub-Saharan Africa, where other issues may influence the vaccine immunogenicity in young children, such as malnutrition and microbial load of other enteric pathogens and maternal antibodies, as described by Patel et al.

Finally, the differences in the epidemiology of rotavirus infection in these African countries may account for the failure of the vaccine candidates. Rotavirus is known to infect African infants at a younger age than their American or European counterparts. As more data are generated on the circulating rotavirus serotypes in Africa, a great diversity of antigenic types and combinations of strains has been recorded. The G6-specific vaccine candidates evaluated earlier may not have offered protection against these diverse types.

Rotavirus vaccine trials in South Africa

After the withdrawal of the RotaShield® vaccine in 1999, as a result of the association with intussusception, the WHO convened a second meeting on "Future Directions for Rotavirus Research in Developing Countries" (2000). The meeting recommended the need for establishing regional networks to assess the rotavirus burden of disease and strongly encouraged the "parallel development" of new rotavirus vaccine candidates in the developing world, especially in Africa and Asia, where this vaccine would save lives. The recommendation for a "parallel clinical development" of the new rotavirus vaccines led to the formation of the Rotavirus Action Partnership for Immunization and Development (RAPID), a coalition that came together to design clinical studies needed in developing countries in Africa and Asia, and to fund the undertaking of these studies. Under the RAPID programme, three safety and immunogenicity studies were completed in South African infants with the GSK monovalent rotavirus vaccine (Rotarix®, Rixensart, Belgium), and these have contributed to the global body of knowledge. The final Phase III efficacy study eventually provided the evidence critical to the national government making a decision to introduce rotavirus vaccine in South Africa. It also contributed to the global recommendation for the introduction of the vaccine in all populations.

Immunogenicity with concomitant administration oral polio vaccine

The first of these was a Phase II, double-blind, randomised, placebo-controlled study to assess the reactogenicity, safety and immunogenicity of two doses of the human rotavirus vaccine (10^5.2 ffu) in healthy infants following the Expanded Programme on Immunisation (EPI) vaccination schedule at 6, 10 and 14 weeks of age. The specific aim of this study was to identify whether the concomitant administration of two live, oral human virus vaccines [oral polio vaccine (OPV) and rotavirus vaccine] would result in interference of the immune
response to either vaccine, but most notably OPV given the importance of the Global Polio Programme. Healthy infants were randomised into three groups, to receive two oral doses of RIX4414, or placebo with OPV, or inactivated polio vaccine IPV (RIX4414 and OPV, RIX4414 and IPV, or placebo and OPV). Serum antirotavirus immunoglobulin A (IgA) antibodies (enzyme-linked immunosorbent assay, ELISA) and neutralising antibodies (microneutralisation assay) to poliovirus serotypes 1, 2 and 3 were measured. Serendipitously, infants were enrolled in two vaccination schedules (6-10 weeks and 10-14 weeks), as described in full elsewhere. Co-administration of RIX4414 with OPV did not result in a decrease in the high seroprotection rates observed against poliovirus serotypes 1, 2 and 3 detected after the third OPV dose (98-100%). However, the rotavirus IgA antibody seroconversion rates were significantly higher for the 10-14 weeks’ schedule (55-61%) compared to the 6-10 weeks’ schedule (36-43%). This study provided the first evidence that RIX4414 could be co-administered with routine EPI immunisations including OPV, showed that two doses of RIX4414 were well tolerated and immunogenic in South African infants, and suggested that maternal antibody might play a role in the diminishing efficacy of rotavirus vaccines in some low-income settings.

Seven deaths occurred during the first part of the study in which 271 subjects, approximately 5-10 weeks at first dose, were enrolled before the rotavirus season of 2002 (until February 2002). Among these fatal cases, at least six were attributed to human immunodeficiency virus (HIV) infection and disease. The mortality observed in this vaccine trial, although lower than the national and provincial figures for South Africa, led to two major developments. First, the study stopped recruitment to implement an assessment of the deaths and to establish a strategy of screening for HIV before further recruitment was allowed. One result of this delay in recruitment was that the second cohort of infants was enrolled at 10 and 14 weeks of age for the two-dose vaccine, which enabled the evaluation of the age of administration on the immune response to the vaccine. The second was that the Independent Data Monitoring Committee recommended that the vaccine of GSK Biologicals should be evaluated in HIV-infected infants in South Africa (see below).

Immunogenicity of two vs. three doses of the vaccine

The second Phase II, double-blind, randomised, placebo-controlled study assessed the immunogenicity, reactogenicity and safety of two regimens of the human rotavirus vaccine given in two doses (at 10 and 14 weeks) or three doses (at 6, 10 and 14 weeks) at a virus concentration ($10^{6.5}$ CCID50) given to HIV-infected infants at 6, 10 and 14 weeks of age. Routine DTPw-HBV/Hib and OPV vaccines were administered concomitantly with the study vaccine. In brief, 100 HIV-positive infants aged 6 to 10 weeks were enrolled in this double-blind, 1:1 randomised, placebo-controlled study and were allocated to two groups to receive three doses of RIX4414 vaccine or placebo at approximately 6, 10 and 14 weeks of age, with a four-week interval between doses. Routine vaccines were concomitantly administered to all infants. Solicited and unsolicited symptoms were recorded at 15 and 31 days after each dose, respectively, and serious adverse events (SAEs) were recorded throughout the study period. The serum antirotavirus IgA concentrations and the immunodeficiency status of the infants were determined at screening and two months post-dose 3.

All solicited and unsolicited symptoms occurred at a similar frequency in both groups. Six fatal SAEs were observed in the RIX4414 group, and nine in placebo recipients were reported. At two months post-dose 3, the seroconversion rates were 57.1% (95% confidence interval [CI]: 34.8-78.2) in the RIX4414 and 18.2% (95% CI: 5.2-40.3) in the placebo groups, which were comparable with the earlier immunogenicity studies in South Africa. In addition, the
mean absolute CD4+ cell count, CD4+ percentage and HIV-1 viral load were comparable in both groups at screening and two months post-dose 3, indicating no adverse effect of the vaccine on these infants. 49

Phase III clinical efficacy study

A randomised, placebo-controlled, multicentre Phase III efficacy trial was conducted in South Africa and Malawi to evaluate the safety and efficacy of the human rotavirus vaccine.50 Healthy infants were randomised (1:1:1) to receive either two doses of Rotarix® (given at 10 and 14 weeks of age after placebo at 6 weeks), or three doses of Rotarix®, or three doses of placebo, respectively, at the recommended 6, 10, and 14 weeks of routine childhood vaccinations, including OPV. The episodes of gastroenteritis caused by circulating wild-type rotavirus during the first year of life were assessed through active follow-up and graded for severity using the Vesikari scale. Gastroenteritis was defined as diarrhoea (at least three looser-than-normal stools over a 24-hour period) with or without vomiting. Vaccine efficacy was calculated from two weeks post-last dose until one year of age.

Of 4,417 infants included in the per-protocol analysis, severe rotavirus gastroenteritis occurred in 4.9% of those followed in the placebo group and 1.9% of those in the pooled vaccine group, giving a combined vaccine efficacy of 61.2% (95% CI, 44.0% to 73.2%).50 The vaccine efficacy was lower in Malawi compared to that observed in South Africa, (49.4% vs. 76.9%, respectively). In the South African cohort, 3,168 healthy infants aged 5–10 weeks at dose one were enrolled and randomised to receive the vaccine or placebo.

Stool samples were analysed for the presence of rotavirus using ELISA, and then typed by real-time polymerase chain reaction (RT-PCR), followed by confirmatory reverse hybridisation techniques. Interestingly, the diversity of rotavirus strains observed in this study was enormous compared to other regions: G1, G2, G3, G8, G9 and G12 and P[4], P[6] and P[8] strains were circulating in the two countries during the follow-up. The study provided a good opportunity to assess the cross-protection of the monovalent human vaccine G1P[8] against these diverse serotypes. In South Africa, the G1P[8] strain was predominant (57.0%) and G2P[4] was second most common (16.7%). The Rotarix® vaccine provided a high level of protection against severe rotavirus gastroenteritis in South Africa, despite the heterogeneity of circulating strains, and provided good heterotypic protection against fully heterotypic G2P[4] and all P[4] types.51

Rotavirus vaccine introduction in South Africa

In early 2009, the WHO made a global recommendation for the introduction of rotavirus vaccines in all children,45 based at least in part on the vaccine efficacy study in South Africa and Malawi, and preliminary data from another vaccine trial in African and Asian infants.52 In May 2009, the Minister of Health of South Africa decided to introduce rotavirus vaccine into the routine immunisation schedule of all infants in South Africa after recommendation from the National Technical Advisory Group for Immunisation (NTAGI) chaired by Prof Barry Schoub.

The national launch of the vaccine occurred in September 2009 in three subdistricts of the Eastern Cape, making South Africa the first African country to introduce rotavirus vaccine. National rollout of the vaccine ramped up in April 2010. Currently, South African researchers, with funding from PATH, are conducting a case-control analysis to assess the impact of routine rotavirus vaccination on the burden of diarrhoeal hospitalisations, and the impact in HIV-infected infants. Preliminary results look promising, although the full analysis should be available in 2012.

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