

## Temporal association of rotavirus vaccination and genotype circulation in South Africa: Observations from 2002 to 2014

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## Abstract

Background: Rotavirus vaccination has reduced diarrhoeal morbidity and mortality globally. The monovalent rotavirus vaccine was introduced into the public immunization program in South Africa (SA) in 2009 and led to approximately 50% reduction in rotavirus hospitalization in young children. The aim of this study was to investigate the rotavirus genotype distribution in SA before and after vaccine introduction.

Materials and methods: In addition to pre-vaccine era surveillance conducted from 2002-2008 at Dr George Mukhari Hospital (DGM), rotavirus surveillance among children <5 years hospitalized for acute diarrhoea was established at seven sentinel sites in SA from April 2009 to December 2014. Stool specimens were screened by enzyme immunoassay and rotavirus positive specimens genotyped using standardised methods.

Results: At DGM, there was a significant decrease in G1 strains from pre-vaccine introduction (34%; 479/1418; 2002-2009) compared to post-vaccine introduction (22%; 37/170; 2010-2014;  $p$  for trend <0.001). Similarly, there was a significant increase in non-G1P[8] strains at this site ( $p$  for trend <0.001). In expanded sentinel surveillance, when adjusted for age and site, the odds of rotavirus detection in hospitalized children with diarrhoea declined significantly from 2009 (46%; 423/917) to 2014 (22%; 205/939;  $p$ <0.001). The odds of G1 detection declined significantly from 2009 (53%; 224/421) to 2010-2011 (26%; 183/703; aOR=0.5;  $p$ <0.001) and 2012-2014 (9%; 80/905; aOR=0.1;  $p$ <0.001). Non-G1P[8] strains showed a significant increase from 2009 (33%; 139/421) to 2012-2014 (52%; 473/905; aOR=2.5;  $p$ <0.001).

Conclusions: Rotavirus vaccination of children was associated with temporal changes in circulating genotypes. Despite these temporal changes in circulating genotypes, the overall reduction in rotavirus disease in South Africa remains significant.

## Introduction

Globally, rotavirus mortality in children <5 years was estimated at 527 000 (465 000 – 591 000) in 2000 and reduced to 215 000 (197 000 – 233 000) in 2013 with more than half of the latter occurring in sub-Saharan Africa [1]. Two rotavirus vaccines (Rotarix, GlaxoSmithKline Biologicals and RotaTeq, Merck & Co) have been shown to be both safe and effective [2]. The delivery of rotavirus vaccines to children in sub-Saharan Africa should, therefore, provide an important tool in the fight against diarrhoeal diseases [3].

The icosahedral rotavirus particle has outer capsid proteins, VP7 and VP4, that are able to independently elicit an immune response and were important epitopes during vaccine development [4]. These proteins specify the G and P genotypes, respectively, and to date 35 G and 50 P genotypes have been described [5,6]. In human infections, five globally predominant (G1, G2, G3, G4 and G9), one recently emerged (G12) and one regionally predominant (G8) G types circulate while two globally predominant (P[8] and P[4]) and one regionally predominant (P[6]) P genotypes are detected [7,8].

In South Africa (SA) between 2003 and 2005, a prospective burden of disease study investigated children <5 years presenting to the Dr George Mukhari Hospital (DGM) for the treatment of diarrhoea. The study estimated that rotavirus was responsible for 17 644 – 25 630 hospitalizations in children <2 years of age annually [9]. The study also examined rotavirus genotypes prior to vaccine introduction [10]. The dominant rotavirus genotype varied year on year (G2P[4] in 2003, G1P[8]/G1P[6] in 2004, G3P[8]/G3P[6] in 2005 and G1P[8] in 2006) and a variety of unusual genotypes and G/P combinations were detected [10].

In August 2009, the rotavirus vaccine was introduced into the South African public immunization programme. Preliminary impact analysis estimated that rotavirus vaccine introduction reduced rotavirus hospitalizations by 54-58% in 2010 and 2011 in children <5 years [11]. Further, a case control

study from SA demonstrated rotavirus vaccine effectiveness against rotavirus diarrhoea hospitalization of 57% (95% CI 40–68) for two doses [12].

After the introduction of the monovalent rotavirus vaccine in Mexico and Brazil, reports of increases in the proportion of rotavirus genotypes not present in the vaccine formulation emerged raising concern around replacement disease due to non-vaccine genotypes [13,14]. In Malawi, post-monovalent vaccine introduction surveillance found higher vaccine effectiveness against fully or partially homotypic rotavirus strains, with lower estimates for heterotypic strains [15]. In Botswana, G2P[4] predominated after monovalent vaccine introduction with G2P[4] vaccine effectiveness of 59% [16]. However, subsequent analyses showed similar vaccine effectiveness against homotypic and heterotypic strains and, lack of dominance of any one rotavirus strain post-vaccine introduction [17].

Nevertheless, recent modelling utilizing genotype-specific hospitalization data from Belgium pre- and post-vaccine introduction, predicted that G1P[8] strains would be eliminated while G2P[4] strains would persist, which was suggested to be driven by differences in homotypic versus heterotypic immunity of second infections [18]. To date, there is limited characterisation of rotavirus genotypes post-vaccine introduction from African countries and continued surveillance is required to elucidate what effect (if any) rotavirus vaccination has on circulating rotavirus strains in resource poor settings.

The aim of this study was to evaluate the rotavirus genotype distribution before (2002-2009) and after (2010-2014) rotavirus vaccine introduction into the South African national immunization program.

## **Materials and Methods**

Hospital-based rotavirus surveillance in children <5 years presenting with acute diarrhoea has been conducted at DGM since early 1980s [19]. Data on rotavirus detection and genotype distribution from DGM between 2002 and 2008 were obtained from the Medical Research Council of South Africa-

Diarrhoeal Pathogens Research Unit (MRC-DPRU) based at Sefako Makgatho Health Sciences University. A dedicated surveillance officer approached parents/guardians of children <5 years admitted for the treatment of acute diarrhoea at DGM for enrolment in the study. Acute diarrhoea was defined according to the World Health Organization (WHO) definition of “three or more looser than normal stools within a 24-hour period” with duration of seven days or less. Children were enrolled from Monday to Friday and a stool specimen was collected within 48 hours of admission in 2002 and 2007-2008. Between 2003 and 2006, surveillance at DGM was improved with the existing enrolment expanded to include children presenting on weekends as well as outpatient visits. These data have been previously published as aggregated information [10,20].

An expanded prospective hospital-based sentinel surveillance system for acute diarrhoea was established in April 2009 prior to the introduction of the rotavirus vaccine. The surveillance sites included: Chris Hani Baragwanath Academic Hospital (CHBAH; 2009-2014; Gauteng Province), DGM 2009-2014; Gauteng/North West Province border), Mapulaneng Hospital (MPH; 2009-2014; Mpumalanga Province (MP)), Matikwane Hospital (MKH; 2009-2014; MP), Edendale Hospital (EDH; 2010-2014; Kwa-Zulu Natal Province), Ngwelezane Hospital (NGH; 2010-2013; Kwa-Zulu Natal Province) and Red Cross Children’s Hospital (RCCH; 2010-2014; Western Cape Province). All children <5 years, who were admitted to a sentinel hospital for the treatment of acute diarrhoea (WHO definition; seven days or less), were approached for enrolment. Enrolment was conducted systematically from Monday to Friday (8am-5pm) and demographic, clinical and outcome data were collected in a structured questionnaire by dedicated surveillance officers from consenting parents/guardians. Stool specimens were collected for rotavirus screening.

### **Laboratory procedures**

All specimens from DGM were tested at the MRC-DPRU. Between 2002 and 2008, stool specimens were screened using the IDEIA™ Rotavirus kit (DAKO Ltd., Cambridgeshire, UK) [10,20]. Surveillance

specimens from 2009 to 2014 were screened using the ProSpecT™ Rotavirus Microplate Assay (Oxoid, Basingstoke, UK). Stool specimens from the remaining expanded surveillance sites were tested at the Centre for Enteric Diseases (CED), National Institute for Communicable Diseases (NICD). The CED screened stools samples with the GastroVir-Strip (Coris Bioconcept, Gembloux, Belgium) in 2009 and the ProSpecT™ Rotavirus Assay between 2010 and 2014.

Rotavirus positive samples detected using enzyme immunoassays were further characterized to determine the G and P genotype. The dsRNA was extracted from the stool using TRI-Reagent LS (Molecular Research Centre, Cincinnati, OH) or the QIAamp Viral RNA Mini kit (Qiagen, Hilden, Germany) and genotyped using standardized RT-PCR methods and primers for G-specific (G1, G2, G3, G4, G8, G9, G10, G12) and P-specific (P[4], P[6], P[8], P[9], P[10], P[11], P[14]) genotypes [21]. Where the genotyping methods produced inconclusive results, VP7 and VP4 genes were amplified and sequenced. In 2003-2005 and 2009-2014, all rotavirus positive specimens were typed from DGM and the expanded surveillance while in 2002 and 2006-2008, only subsets of rotavirus strains were typed from DGM [10,20].

### **Statistical analyses**

The surveillance at DGM varied between 2002 and 2014 and, therefore, cannot be utilized to compare changes in rotavirus incidence over time. However, we did compare rotavirus and selected genotype (G1, P[8] and non-G1P[8]) proportions in diarrhoeal stools before (2002-2009) and after (2010-2014) rotavirus vaccine introduction, using the Royston chi-square test for trend. We also report Wilson confidence intervals around binomial proportions. 2009 was included in the pre-vaccine period because the rotavirus vaccine was introduced into the immunization programme after the typical rotavirus season and the vaccine coverage was low in this year.

Using 2009 as the baseline year, the odds ratio and significance ( $p \leq 0.05$ ) of rotavirus detection at all sentinel sites (including DGM) were calculated using multivariable logistic regression analysis and a model adjusted for site and age. Rotavirus genotypes were compared by year and site using the Royston chi-square test for trend. In addition, selected genotype (G1, P[8] and non-G1P[8]) prevalence from the expanded surveillance sites was evaluated by year with Wilson confidence intervals calculated for binomial proportions. Genotype prevalence (G1, P[8] and non-G1P[8]) was evaluated for two time periods (2010-2011 and 2012-2014) with odds ratios and significance ( $p \leq 0.05$ ) calculated using multivariable logistic regression in a model adjusted for site and age. All analysis was implemented using STATA 14.1 (StataCorp LP, College Station TX).

## Results

### *Temporal changes in rotavirus prevalence and genotype distribution pre- and post-vaccine introduction at Dr George Mukhari Hospital*

Between January 2002 and December 2014, 6870 stool specimens from DGM were screened with 23% (95% confidence interval (95% CI) 20-25; 1588/6870) positive for rotavirus (Table 1). The annual detection rate ranged from 17% (95% CI 15-20) in 2003 and 2012 (95% CI 12-24) to 30% in 2006 (95% CI 27-37; Table 1) and the proportion of rotavirus positive specimens did not change significantly over the time period ( $p$  for trend = 0.08).

Among rotavirus positive specimens, the proportion of G1 strains varied from 10-52% in the pre-vaccine era (2002-2009) and ranged from 0-61% in the post-vaccine era with no cases detected in 2012 and 2013 (Table 1). Nevertheless, there was a decline in the proportion of G1 strains over the post-vaccine period ( $p$  for trend  $< 0.001$ ). A similar decreasing trend was not noted for P[8] strains at DGM ( $p$  for trend = 0.11). Conversely, there was an increase in the proportion of non-G1P[8] genotypes over time



Table 1. Rotavirus prevalence and G1, P[8] and non-G1P[8] strain prevalence at Dr George Mukhari Hospital between 2002 and 2014\*.

Year	Total stools	Rotavirus positive		G1 strains		P[8] strains		Non-G1P[8] strains	
		n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)
2002	927	203	22 (19-25)	97	48 (41-55)	123	61 (54-67)	34	17 (12-22)
2003	942	161	17 (15-20)	56	35 (28-42)	54	34 (27-41)	77	48 (40-56)
2004	623	134	22 (19-25)	69	51 (43-60)	77	57 (49-66)	38	28 (21-37)
2005	925	228	25 (22-28)	23	10 (7-15)	123	54 (47-60)	68	30 (24-36)
2006	674	200	30 (27-34)	103	52 (45-58)	142	71 (64-77)	38	19 (14-25)
2007	741	189	26 (23-29)	19	10 (7-15)	33	17 (13-24)	144	76 (70-82)
2008	680	162	24 (21-27)	38	23 (18-31)	49	30 (24-38)	56	35 (28-42)
2009	526	141	27 (23-31)	74	52 (44-61)	91	65 (56-72)	36	25 (19-33)
2010	231	46	20 (15-26)	11	24 (14-38)	16	35 (23-49)	28	61 (46-74)
2011	198	44	22 (17-29)	9	20 (11-35)	35	80 (65-89)	6	14 (6-27)
2012	157	27	17 (12-24)	0	0 (0-12)	19	70 (52-84)	8	30 (16-48)
2013	134	25	19 (13-26)	0	0 (0-13)	1	4 (1-20)	23	92 (75-98)
2014	112	28	25 (18-34)	17	61 (42-76)	16	57 (39-73)	9	32 (18-51)

\*The Wilson confidence intervals are indicated for binomial proportions.

(p for trend <0.001; Table 1). However, non-G1P[8] strain percentage fluctuated greatly over the study period, varying from 17-76% pre-vaccine and 14-92% post-vaccine introduction (Table 1).

*Temporal changes in rotavirus genotype distribution in the rotavirus vaccine era*

Between April 2009 and December 2014, 7474 stools were collected from children enrolled in the expanded surveillance program (including DGM) and 27% (2038/7474) tested positive for rotavirus (Table 2). Rotavirus detection rates decreased from 46% (423/917) in 2009 to 22% (205/939) in 2014 (Table 2). Documentation of vaccination status was available from 94% (7006/7474) of the children (Table 2). Rotavirus vaccination in children admitted for acute diarrhoea increased between 2009 (33% (297/905) and 2014 (92% (478/518); Table 2), with the odds of rotavirus detection in hospitalized children with diarrhoea decreasing significantly in 2010-2014 compared to 2009 (adjusted odds ratio (aOR)=0.3-0.4; p<0.001; Table 2).

A total of 2038 rotavirus positive stool specimens were analysed for G and P genotypes (Figure 1). The six predominant strains included G2P[4] (466/2038; 23%); G1P[8] (421/2038; 21%), G12P[8] (384/2038; 19%), G9P[8] (254/2038; 12%); G2P[6] (179/2038; 9%) and G8P[4] (113/2038; 6%; Figure 1; Supplementary Table 1). These strains represented 89% (1817/2038) of all the rotavirus strains detected during the surveillance period.

Between 2009 and 2014, there were differences in the rotavirus genotypes circulating at sentinel sites in SA (Table 3; Supplementary Table 2). While G1P[8] strains predominated in all the sites under surveillance in 2009, the proportion positive varied substantially between sites (36-58%; p for trend=0.03). A similar observation was noted in 2013; G2P[4] strains predominated in 5 of 6 sites with detection between 70-88% (p for trend =0.01). In 2010, the first year of rotavirus vaccine introduction with vaccination levels around 50%, three genotypes were predominant at the sentinel sites under

Table 2. Rotavirus prevalence in children <5 years at sentinel sites and reported vaccination coverage (1 dose or more) of rotavirus between 2009 and 2014\*.

Year	Rotavirus prevalence n/N (%)	Rotavirus vaccination coverage (one dose or more) n/N (%)	Unadjusted odds ratio (95% confidence interval); p-value	Adjusted odds ratio (95% confidence interval); p-value
2009	423/917 (46%) <sup>§</sup>	297/905 (33%)	Reference	Reference
2010	351/1418 (25%)	754/1411 (53%)	0.4 (0.3-0.5); p<0.001	0.4 (0.3-0.5); p<0.001
2011	358/1398 (26%)	1061/1393 (76%)	0.4 (0.3-0.5); p<0.001	0.4 (0.3-0.5); p<0.001
2012	272/1209 (22%)	1081/1198 (90%)	0.3 (0.3-0.4); p<0.001	0.3 (0.3-0.4); p<0.001
2013	429/1593 (27%)	1486/1581 (94%)	0.4 (0.4-0.5); p<0.001	0.4 (0.4-0.5); p<0.001
2014	205/939 (22%)	478/518 (92%)	0.3 (0.3-0.4); p<0.001	0.3 (0.2-0.4); p<0.001

\*The model is adjusted for age and site; § - Surveillance started on 20 April 2009

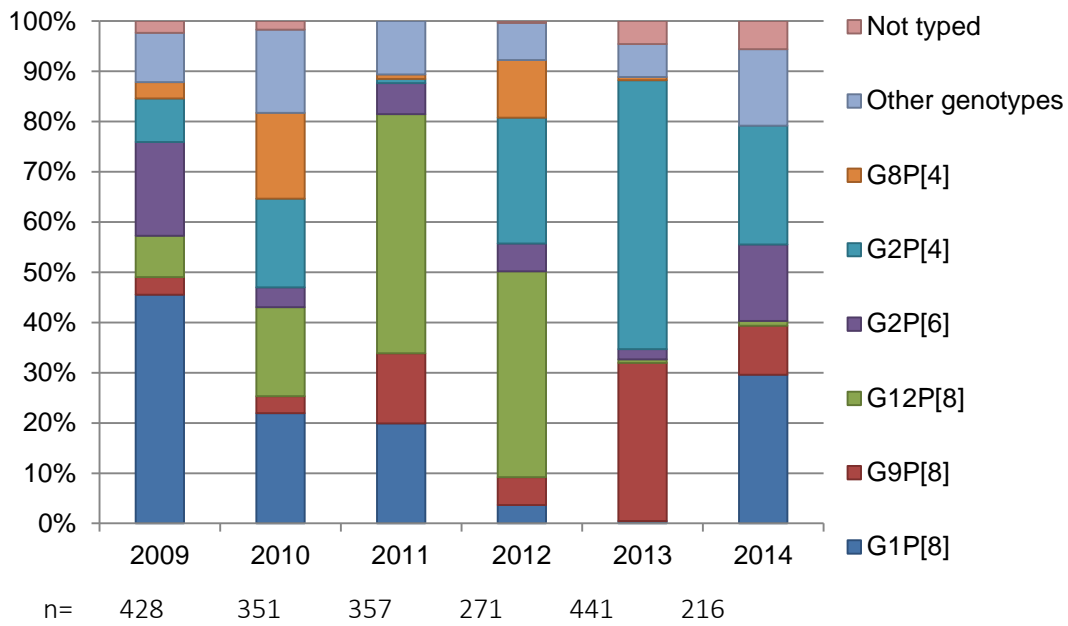


Figure 1. Summary of the six most common rotavirus genotypes detected by year at sentinel surveillance sites between 2009 and 2014. Other genotypes include G1P[6], G1P[4], G2P[8], G3P[8], G3P[4], G3P[14], G4P[8], G4P[6], G8P[8], G8P[6], G9P[6], G9P[4], G12P[6], G12P[4] and mixed types at detection rates of 1-2% per year.

Table 3. Summary of the annual predominant genotype by site compared to the predominant genotype circulating each year\*.

Year	2009	2010	2011	2012	2013	2014
Predominant genotype <sup>§</sup>	G1P[8] 47%	G1P[8] 22%	G12P[8] 47%	G12P[8] 41%	G2P[4] 56%	G1P[8] 29%
Site						
DGM	G1P[8] 58%	G2P[4] 30%	G9P[8] 36%	G12P[8] 67%	G2P[4] 88%	G1P[8] 44%
CHBAH	G1P[8] 36%	G1P[8] 35%	G12P[8] 42%	G12P[8] 27%	G2P[4] 70%	G1P[8] 39%
MP	G1P[8] 55%	G2P[4] 28%	G12P[8] 47%	G12P[8] 47%	G2P[4] 84%	G1P[8] 40%
EDH	ND	G12P[8] 32%	G12P[8] 65%	G8P[4] 50%	G2P[4] 83%	G2P[4] 42%
RCCH	ND	G12P[8] 40%	G12P[8] 54%	G2P[4] 62%	G9P[8] 77%	G2P[4] 75%
NGH	ND	G12P[8] 52%	G12P[8] G1P[8] 33%	G12P[8] 71%	G2P[4] 86%	ND
p value for trend	0.03	<0.001	0.4	0.4	0.01	<0.001

\*Where the genotype at the site differed from the annual genotype, the boxes have been shaded. § - predominant genotype was calculated by summing genotypes detected at all sentinel sites and dividing by total rotavirus positives detected for a specified year.

surveillance (G1P[8], G2P[4] and G12P[8];  $p$  for trend  $< 0.001$ ; Table 3). In 2014, two genotypes circulated with G1P[8] strains predominant in 3 of 5 sites (39-44%) and G2P[4] predominant in 2 of 5 geographically diverse sites (42-75%;  $p$  for trend  $< 0.001$ ). In years where uncommon rotavirus strains (G12P[8] in 2011) circulated, the diversity of genotypes seemed to diminish (Table 3). Genotype G12P[8] emerged in 2010 in 3 of 6 sites and in 2011 and 2012 was detected at similar prevalence at all sites ( $p$  for trend=0.4).

Using 2009 as the baseline year, G1 strains decreased significantly in sentinel surveillance sites between 2010 and 2014 (2010-2011: aOR=0.5;  $p < 0.001$  and 2012-2014: aOR=0.1;  $p < 0.001$ ; Table 4). However, P[8] strains only decreased during the second analyses period (2012-2014: aOR=0.5;  $p < 0.001$ ; Table 4), mainly due to a G12P[8] epidemic in 2011. Rotavirus strains not present in the vaccine formulation (non-G1P[8]) showed a significant increase in proportion from 2012-2014 (aOR=2.5;  $p < 0.001$ ; Table 4), primarily due to the G2P[4] season in 2013.

## Discussion

The current study suggests that the monovalent rotavirus vaccine introduction into the national immunization program was temporally associated with changes in genotype circulation in SA. The decrease in G1 strains was similar at DGM, a site with genotyping data from 2002, as well as geographically diverse sentinel sites, using 2009 as a baseline. In Malawi, G1P[8] prevalence showed a non-significant decline of 54% post-vaccine introduction compared to the pre-vaccine era [15]. In Morocco, G1P[8] strains were not detected the third year after vaccine introduction [22]. These results seem to support in part the model predicted by Pitzer and colleagues that G1P[8] would decline [18].

However, reviews of genotypes after vaccine introduction have shown that efficacy in developing countries is diminished against both heterotypic and homotypic rotaviruses [17]. In SA, G1P[8] strains re-emerged in 2014 and were responsible for 29% of rotavirus cases. These results suggest that while

Table 4. Rotavirus G1, P[8] and non-G1P[8] genotype prevalence in children <5 years at sentinel sites between 2009 and 2014\*.

Period	Prevalence	Unadjusted odds ratio (95% confidence interval); p-value	Adjusted odds ratio (95% confidence interval); p-value
487			
489			
490			
491			
Genotype G1 strains			
2009	224/421 (53%)	Reference	Reference
2010 - 2011	183/708 (26%)	0.3 (0.2-0.4); p<0.001	0.5 (0.4-0.6); p<0.001
2012 - 2014	80/905 (9%)	0.09 (0.06-0.1); p<0.001	0.1 (0.1-0.2); p<0.001
Genotype P[8] strains			
2009	259/422 (61%)	Reference	Reference
2010 - 2011	499/708 (70%)	1.5 (1.2-2.0); p=0.002	1.3 (1.0-1.7); p=0.06
2012 - 2014	427/905 (47%)	0.6 (0.4-0.7); p<0.001	0.5 (0.4-0.6); p<0.001
Genotype non-G1P[8] strains			
2009	139/421 (33%)	Reference	Reference
2010 - 2011	193/708 (27%)	0.8 (0.6-1.0); p=0.04	0.9 (0.6-1.1); p=0.287
2012 - 2014	473/905 (52%)	2.2 (1.7-2.8); p<0.001	2.5 (1.9-3.3); p<0.001

\*The model is adjusted for age and site.

some strain selection related to vaccine pressure may exist, other host and virus factors may also be involved.

Evaluation of G1P[8] strains before and after monovalent rotavirus vaccine introduction in Belgium showed a reduction in the proportion of strains within the vaccine strain cluster [23]. In addition, a novel double-gene reassortant with G1 and P[8] genes on a DS-1 backbone was detected in Japan, Thailand and Vietnam [24,25,26]. The re-emergence of G1P[8] strains in SA requires additional attention to determine whether natural strain fluctuation or gene reassortment contributed to the increased circulation in 2014. The G1P[8] strains did not persist in 2015 and were replaced by G9P[8] and G3P[4] rotaviruses in 2016 [27].

Increased rotavirus cases were noted in 2013 despite universal rotavirus vaccination being available. In the US, a biennial increase in rotavirus has been seen but “high” seasons remained below pre-vaccine levels [28]. One explanation for the larger season in 2013 as well as the re-emergence of G1P[8] strains in 2014 may be due to low rotavirus vaccine coverage in 2013; officially reported at 89% by the South African National Department of Health and estimated by WHO and UNICEF at 64% [29]. An alternative explanation could be an increase in rotavirus-specific antibodies in women who had children after the large rotavirus season in 2013. High levels of transplacental rotavirus antibodies have been shown to reduce vaccine seroconversion [30]. Children receiving rotavirus vaccine in 2014 may have had lower vaccine seroconversion due to maternal antibodies thereby allowing the temporary re-emergence of G1P[8] strains.

Genotype P[8] strains did not show a significant decrease at DGM between 2002 and 2014 ( $p$  for trend = 0.11) but did show a significant decline in sentinel surveillance sites in 2012-2014 compared to 2009 ( $p < 0.001$ ). These results highlight a study limitation as only one site had rotavirus genotyping data for more than one year before vaccine introduction. In addition, genotype varied between sentinel sites so



that data from one site may not be generalizable to the rest of the country. Genotype variation in different geographic sites in one country is not uncommon and was seen in SA before vaccine introduction [19] and in Morocco after vaccine introduction [22].

In addition to a decrease in G1, the study showed an increase in the proportion of non-G1P[8] strains in both DGM and the expanded surveillance sites. Although in the expanded surveillance sites, non-G1P[8] strains only significantly increased between 2012 and 2014 compared to 2009. Since the introduction of the monovalent rotavirus vaccine in SA in 2009, G2P[4] strains were the most common type detected, driven mostly by the rotavirus season in 2013. A similar increase in G2P[4] strains (37%) was noted in neighbouring Botswana in 2013 after monovalent rotavirus vaccine introduction in July 2012 [16].

Genotype G2P[4] has been frequently identified in Brazil since the introduction of the monovalent rotavirus vaccine in March 2006, accounting for >50% of infections in 2007, 2008, 2010 and 2011 [14]. Between 2001-2005, these strains were rarely detected [14]. Similarly, in Columbia where the monovalent vaccine was introduced in 2009, G2P[4] strains were predominant in 2010, 2011 and 2012 at levels between 32-86% [31]. However, whether the emergence of G2P[4] in South America was linked to natural fluctuations in rotavirus genotype distribution or the introduction of the monovalent rotavirus vaccine is unknown [14, 31, 32].

Unlike South America, G2s are relatively common in SA and re-emerge every few years [33]. Between 2003-2010, G2P[4] strains were responsible for 12% of infections at DGM [20]. While G2P[4] strains may have caused a greater proportion of rotavirus cases in 2013, these strains have not persisted. Fluctuations of other rotavirus genotypes (G12P[8] and G9P[8]) have also been seen in SA. Similarly, analyses of global genotype distribution showed that these strains do not persist [8,17].

Rotavirus genotype circulation cannot be predicted and genotypes are known to fluctuate in predominance year-on-year. Bishop and colleagues established the longest running rotavirus surveillance globally and have genotyped rotavirus strains dating back to 1977 in Australia [34,35,36]. Mathematical analysis of rotavirus strain circulation demonstrated annual (or seasonal), biannual (1-3 years) and quinquennial (3-5 years) epidemics that were influenced by different epidemic dynamics [37]. In addition, there was not an inherently more virulent rotavirus strain causing severe diarrhoea [37]. Rotavirus genotype data from SA supports these observations and continuous monitoring of rotaviruses may aid the identification of different genotype-specific patterns [10,20,33].

This study has several limitations that should be considered. Rotavirus strains from DGM were partially typed in 2007 and 2008. However, most strains from the 2007-2008 were G2P[4] so any bias will be in trends for non-G1P[8] strains and not G1 or P[8] strains. Expanded diarrhoeal sentinel surveillance began on 20 April 2009 and did not include a full year for 2009; this may have resulted in an overestimation of rotavirus prevalence for 2009 and rotavirus vaccine impact. However, surveillance began at the start of the 2009 rotavirus season and information on the circulating 2009 rotavirus genotypes was captured. Hospitalized diarrhoea cases were only enrolled from Monday to Friday and the health seeking behaviour of the communities served by the sentinel hospitals was not assessed. Missing data was dealt with by pairwise deletion and information selection bias may be present affecting the estimates and associations observed.

The introduction of the monovalent rotavirus vaccine into the South African national immunization program coincided with a temporal decline in all-cause diarrhoeal hospitalizations [38]. Despite fluctuations in genotype distribution over the surveillance period, the overall reduction in both all-cause diarrhoea and rotavirus hospitalizations remains significant [11,12,38]. These results support the continued use of rotavirus vaccines in Africa and continued monitoring of rotavirus strain diversity is encouraged.

## **Ethical standards**

Ethical approval for the study was obtained from the Human Research Ethics Committee (Medical), University of Witwatersrand (M091018), the Biomedical Research Ethics Committee, University of Kwa-Zulu Natal (BF074/09), the Medunsa Research Ethics Committee, University of Limpopo (MREC/P/10/2009) and the Human Research Ethics Committee, University of Cape Town (068/2010).

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## **Declaration of interests**

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Supplementary Table 1. Summary of rotavirus genotypes detected by year at sentinel surveillance sites between 2009 and 2014.

Genotype	Prevalence (%)						Total
	2009	2010	2011	2012	2013	2014	
No of sites	4	7	7	7	7	6	
Rotavirus genotypes contained in the monovalent vaccine formulation							
G1P[8]	199 (47%)	78 (22%)	72 (20%)	10 (4%)	2 (<1%)	60 (29%)	421 (21%)
G1P[6]	14 (3%)	2 (1%)	3 (1%)	0 (0%)	0 (0%)	3 (1%)	22 (1%)
G1P[4]	8 (2%)	6 (1%)	1 (<1%)	0 (0%)	1 (<1%)	0 (0%)	16 (1%)
G2P[8]	0 (0%)	3 (1%)	1 (<1%)	2 (1%)	0 (0%)	0 (0%)	6 (<1%)
G3P[8]	1 (<1%)	0 (0%)	1 (<1%)	6 (2%)	13 (3%)	20 (10%)	41 (2%)
G4P[8]	0 (0%)	0 (0%)	1 (<1%)	0 (0%)	0 (0%)	0 (0%)	1 (<1%)
G8P[8]	6 (1%)	18 (5%)	4 (1%)	8 (3%)	2 (<1%)	0 (0%)	38 (2%)
G9P[8]	16 (4%)	12 (3%)	50 (14%)	15 (6%)	141 (33%)	20 (10%)	254 (12%)
G12P[8]	35 (8%)	63 (18%)	170 (47%)	111 (41%)	3 (1%)	2 (1%)	384 (19%)
<b>Total</b>	<b>279 (66%)</b>	<b>182 (52%)</b>	<b>303 (85%)</b>	<b>152 (56%)</b>	<b>162 (38%)</b>	<b>105 (51%)</b>	<b>1183 (58%)</b>
Rotavirus genotypes not contained in the monovalent vaccine formulation							
G2P[6]	81 (19%)	15 (4%)	24 (7%)	15 (6%)	9 (2%)	35 (17%)	179 (9%)
G2P[4]	38 (9%)	64 (18%)	3 (1%)	70 (26%)	240 (56%)	51 (25%)	466 (23%)
G3P[14]	0 (0%)	0 (0%)	0 (0%)	1 (<1%)	0 (0%)	0 (0%)	1 (<1%)
G3P[4]	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (<1%)	0 (0%)	2 (<1%)
G4P[6]	0 (0%)	0 (0%)	1 (<1%)	0 (0%)	0 (0%)	0 (0%)	1 (<1%)
G8P[6]	1 (<1%)	0 (0%)	0 (0%)	1 (<1%)	0 (0%)	0 (0%)	2 (<1%)
G8P[4]	14 (3%)	62 (18%)	3 (1%)	31 (11%)	3 (1%)	0 (0%)	113 (6%)
G9P[6]	1 (<1%)	0 (0%)	3 (1%)	0 (0%)	4 (1%)	2 (1%)	10 (1%)
G9P[4]	0 (0%)	2 (1%)	0 (0%)	0 (0%)	4 (1%)	1 (<1%)	7 (<1%)
G12P[6]	0 (0%)	2 (1%)	7 (2%)	0 (0%)	2 (<1%)	1 (<1%)	12 (1%)
G12P[4]	4 (1%)	7 (2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	11 (1%)
<b>Total</b>	<b>139 (33%)</b>	<b>152 (43%)</b>	<b>41 (11%)</b>	<b>118 (43%)</b>	<b>264 (62%)</b>	<b>90 (44%)</b>	<b>804 (39%)</b>
Mixed and non-typeable rotavirus strains							
Mixed	3 (1%)	16 (5%)	14 (4%)	2 (<1%)	2 (<1%)	10 (5%)	47 (2%)
Not typed	2 (<1%)	1 (<1%)	0 (0%)	0 (0%)	1 (<1%)	0 (0%)	4 (2%)
<b>Total</b>	<b>5 (1%)</b>	<b>17 (5%)</b>	<b>14 (4%)</b>	<b>2 (&lt;1%)</b>	<b>3 (1%)</b>	<b>10 (5%)</b>	<b>51 (4%)</b>
<b>Grand total</b>	<b>423/917 (46%)</b>	<b>351/1418 (25%)</b>	<b>358/1398 (26%)</b>	<b>272/1209 (22%)</b>	<b>429/1593 (27%)</b>	<b>205/939 (22%)</b>	<b>2038/7474 (27%)</b>

Supplementary Table 2. Rotavirus prevalence and G1, P[8] and non-G1P[8] strain prevalence at Chris Hani Baragwanath Academic Hospital, Mpumalanga sites (Mapulaneng and Matikwane Hospitals), Edendale Hospital, Red Cross Children's Hospital and Ngwelezane Hospital between 2009 and 2014\*.

Year	Total stools	Rotavirus positive		G1 strains		P[8] strains		Non-G1P[8] strains	
		n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)
Chris Hani Baragwanath Academic Hospital									
2009	429	196	46 (41-50)	85	43 (37-50)	96	49 (42-56)	88	45 (38-52)
2010	560	136	24 (21-28)	57	42 (34-50)	77	57 (48-65)	55	40 (33-49)
2011	305	67	22 (18-27)	17	25 (16-37)	58	87 (76-93)	7	10 (5-20)
2012	201	41	20 (15-26)	2	5 (1-16)	22	54 (39-68)	19	46 (32-61)
2013	271	88	32 (27-38)	1	1 (0-6)	17	19 (12-29)	70	80 (70-87)
2014	335	90	27 (22-32)	37	41 (32-51)	70	78 (68-85)	20	22 (15-32)
Mpumalanga Sites (Mapulaneng and Matikwane Hospitals)									
2009	208	104	50 (43-57)	66	63 (54-72)	74	71 (62-79)	21	20 (14-29)
2010	276	53	19 (15-24)	15	28 (18-42)	24	45 (33-59)	27	51 (38-64)
2011	152	38	25 (19-32)	8	21 (11-36)	35	92 (79-97)	3	8 (3-21)
2012	204	45	22 (17-28)	1	2 (0-12)	26	58 (43-71)	19	42 (29-57)
2013	192	44	23 (18-29)	1	2 (0-12)	7	16 (8-29)	37	84 (71-92)
2014	118	20	17 (11-25)	8	40 (22-61)	11	55 (34-74)	9	45 (26-66)
Edendale Hospital									
2010	84	19	23 (15-33)	5	26 (12-49)	15	79 (57-91)	3	16 (6-38)
2011	96	31	32 (24-42)	5	16 (7-33)	26	84 (67-93)	5	16 (7-33)
2012	56	14	25 (16-38)	0	0 (0-22)	6	43 (21-67)	8	57 (33-79)
2013	74	24	32 (23-44)	0	0 (0-14)	3	13 (4-31)	21	88 (69-96)
2014	55	19	35 (23-48)	2	11 (3-31)	5	26 (12-49)	14	74 (51-88)
Red Cross Children's Hospital									
2010	167	70	42 (35-49)	6	9 (4-17)	36	51 (40-63)	32	46 (35-57)
2011	496	160	32 (28-36)	41	26 (19-33)	145	90 (85-94)	14	9 (5-14)

2012	351	76	22 (18-26)	6	8 (4-16)	25	33 (23-44)	51	67 (56-77)
2013	434	147	34 (30-38)	1	1 (0-4)	125	85 (78-90)	22	15 (10-22)
2014	319	49	15 (12-20)	3	6 (2-17)	9	18 (10-31)	40	82 (69-90)
Ngwelezane Hospital									
2010	100	27	27 (19-36)	2	7 (2-23)	20	74 (55-87)	7	26 (13-45)
2011	151	18	12 (8-18)	6	33 (16-56)	12	67 (44-84)	6	33 (16-56)
2012	241	69	29 (23-35)	1	1 (3-8)	56	81 (70-89)	13	19 (11-30)
2013	488	100	20 (17-24)	0	0 (0-4)	10	10 (6-17)	90	90 (83-95)

\*The Wilson confidence intervals are indicated for binomial proportions.