Public Health Bulletin
South Africa

Trends in enteric virus circulation in children <5 years of age at selected sentinel sites in South Africa from 2009 to 2023

Nicola Page[1,2,3], Sandrama Nadan[1], Siobhan Johnstone[4], Tersia Kruger[1], Rembuluwani Netshikweta[1], Nadia Strydom[1], Phuti Sekwadi[1], Michelle Groome[4]

[1] Centre for Enteric Diseases, National Institute for Communicable Diseases, a division of the National Health Laboratory Service, Johannesburg, South Africa

[2] Department of Medical Virology, School of Medicine, Faculty of Health Sciences, University of Pretoria, Pretoria, South Africa

[3] School of Health Systems and Public Health, Faculty of Health Sciences, University of Pretoria, Pretoria, South Africa

[4] South African Medical Research Council Vaccines and Infectious Diseases Analytics Research Unit, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

Summary

Diarrhoeal surveillance was established at sentinel hospital sites prior to the introduction of the oral rotavirus vaccine (Rotarix®) into the South African immunisation programme in 2009, making it possible to compare rotavirus prevalence before and after the vaccination campaign. In addition to monitoring trends in rotavirus hospitalisations and outpatient consultations, the surveillance programme has also investigated trends in diarrhoea associated with other enteric viruses (norovirus, sapovirus, adenovirus, and astrovirus) in children <5 years of age. The data show that rotavirus prevalence has declined since vaccine introduction as a result of increasing vaccine coverage, but rotavirus is still a common cause of childhood diarrhoea. In addition, norovirus GII and adenovirus type F should be considered for vaccine development.

Introduction

Prior to the rotavirus (RV) vaccine introduction, RV contributed an estimated total of 453 000 deaths worldwide in children <5 years of age in 2008, accounting for 37% of all diarrhoea–related deaths.[1] By 2017–2018, the estimates had declined to 208 009 RV deaths in children <5 years of age,[2] primarily due to the introduction of the vaccine. After introducing Rotarix® (RV1) into South Africa in late 2009, there were declines in RV–specific (54–58% reduction in children <5 years) and all–cause diarrhoeal hospitalisations (45–65% reduction in children ≤12 months and 40–50% reduction in children 13–24 months of age).[3,4] Acute diarrhoeal diseases were, however, reported to cause 15% of hospital admissions in children <5 years of age at a South African tertiary hospital in 2016[5], indicating that despite the introduction of RV vaccination, additional prevention measures for diarrhoeal diseases were required. The World Health Organization (WHO) recently estimated that diarrhoeal diseases still cause approximately 1.7 billion episodes and approximately 443 832 deaths annually in children <5 years of age.[6]

The Aetiology, Risk Factors, and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health and Development Project (MAL–ED) enrolled children within a few days of birth and followed them until two years of age, collecting monthly stool specimens, diarrhoeal episode specimens, as well as anthropometric and development variables.[7] The study was based at sites in eight countries—Pakistan, Nepal, India, Bangladesh, Peru, Brazil, South Africa (Thohoyandou, Limpopo Province), and Tanzania—with historically high incidences of diarrhoeal disease and undernutrition. Led by the Fogarty International Center of the National Institutes of Health and the Foundation for the National Institutes of Health, the study was conducted between November 2009 and February 2014.[7] Results showed that viral diarrhoea occurred in 36.4% (95% confidence interval (CI) 33.6–39.5) of participants[7], highlighting the importance of enteric viruses in diarrhoeal disease burden in infants and young children. The most common enteric viruses detected were RV, norovirus (NoV; genogroups I and II), adenovirus (AdV; type F), sapovirus (SaV), and astrovirus (AstV).

Diarrhoeal diseases still contribute to morbidity and mortality in South African children post–RV vaccine introduction.[5] Syndromic surveillance for diarrhoea has been co–ordinated by the Centre for Enteric Diseases (CED), National Institute for Communicable Diseases (NICD), a division of the National Health Laboratory Service,

since 2009. Between 2009 and 2020, the Rotavirus Sentinel Surveillance Programme (RSSP) monitored the effect of the rotavirus vaccine on the number of rotavirus and diarrhoea hospitalisations in children <5 years of age. In addition, rotavirus strain circulation, severity of rotavirus disease in HIV–infected children, and other enteric viruses and bacteria were examined.

The African network for improved diagnostics, epidemiology, and management of common infectious agents (ANDEMIA) was established in response to the disproportionately high morbidity and mortality caused by infectious diseases in Africa, and the limited funding dedicated to common infectious diseases, including acute respiratory tract (RTI) and gastrointestinal (GI) infections, and acute febrile disease of unknown cause (AFDUC).[8] Supported by German funders and collaborators, the ANDEMIA project involved enrolment of RTI, GI, and AFDUC cases and controls at selected hospital–based sentinel sites in Cote d'Ivoire, Burkina Faso, the Democratic Republic of the Congo, and South Africa. Surveillance was conducted between July 2018 and April 2022. In South Africa, participants were enrolled at the Kalafong, Mapulaneng and Matikwane hospitals.

The diarrhoeal diseases sentinel surveillance (DDSS) began in late 2020 with the aim of determining the magnitude of outpatient consultations, hospitalisations and deaths in all ages at selected sentinel sites in South Africa, and to define the major aetiological agents and environmental factors associated with the disease. Continued monitoring of RV as well as other enteric viruses in children <5 years of age will allow tracking of RV vaccine coverage, inform the need for improved RV vaccine candidates or strategies to address residual disease burden, and identify other enteric pathogens contributing to diarrhoeal diseases.

Using data from these surveillance programmes, we examine the long–term trends in enteric virus prevalence in children <5 years of age seeking treatment for diarrhoea at selected sentinel sites in South Africa between 2009 and 2023.

Methods

Rotavirus sentinel surveillance programme (RSSP): Children <5 years of age admitted to sentinel hospitals for the treatment of acute diarrhoea (WHO definition: ≤7 days duration) were targeted for enrolment (Table 1). Enrolment was conducted systematically during office hours from Monday to Friday after obtaining informed consent from a parent or guardian. Using a structured questionnaire, dedicated surveillance officers collected demographic, clinical, and outcome data from medical records and the parents of participants who had been residing in the site catchment area for at least seven days prior to illness.

African network for improved diagnostics, epidemiology and management of common infectious agents (ANDEMIA): Patients of any age presenting to study hospitals or clinics for the treatment of diarrhoea (WHO definition: ≤30 days duration), respiratory tract infections, or acute febrile disease of unknown cause were approached for enrolment (Table 1).[8] Unmatched controls comprising individuals attending the hospital or clinic for reasons other than diarrhoea (vaccination clinic, orthopaedic, or surgical wards) and without gastrointestinal

symptoms (vomiting or diarrhoea) in the past three weeks were also enrolled. Only diarrhoeal cases and controls in children <5 years who provided a stool specimen were included in the analysis. Enrolment was done similarly to the RSSP with informed consent sought from the patient, a parent, or guardian.

Diarrhoeal diseases sentinel surveillance (DDSS): Participants of any age presenting for the treatment of diarrhoea (WHO definition: any duration) at selected sentinel hospitals and clinics were enrolled (Table 1). Enrolment was done similarly to the RSSP with informed consent sought from the patient, a parent or guardian. Only diarrhoeal cases in children <5 years who provided a stool specimen were included in the analysis.

Table 1. Sentinel sites by province and surveillance programs enrolling participants and collecting stool specimens, South Africa, 2009–2023.

Site	Province	Years surveillance conducted	Program
Chris Hani Baragwanath Academic Hospital	Gauteng	2009–2020	RSSP
Mapulaneng Hospital	Mpumalanga	2009–2017	RSSP
		2018–2022	ANDEMIA
Matikwane Hospital	Mpumalanga	2009–2018	RSSP
		2018–2022	ANDEMIA
Dr George Mukhari Hospital	Gauteng	2009–2013	RSSP
Edendale Hospital	KwaZulu–Natal	2010–2017	RSSP
Red Cross Children's Hospital	Western Cape	2010–2016	RSSP
		2021–2023	DDSS
Ngwelezane Hospital	KwaZulu–Natal	2010–2013	RSSP
Kimberley Hospital	Northern Cape	2014–2016	RSSP
Polokwane Hospital	Limpopo	2015–2016	RSSP
Pelonomi Hospital	Free State	2015–2020	RSSP
		2020–2023	DDSS
Dora Nginza Hospital	Eastern Cape	2016–2018	RSSP
Klerksdorp/Tshepong Hospital	North West	2016	RSSP
		2021–2023	DDSS
Kalafong Hospital	Gauteng	2018–2022	ANDEMIA
		2022–2023	DDSS
Mitchell's Plain Hospital	Western Cape	2021–2023	DDSS
Eastridge Clinic	Western Cape	2021–2023	DDSS
Kabokweni Clinic	Mpumalanga	2022–2023	DDSS

RSSP—Rotavirus sentinel surveillance programme
ANDEMIA—African network for improved diagnostics, epidemiology, and management of common infectious agents
DDSSP—Diarrhoeal diseases sentinel surveillance programme

Stool specimens were collected from participants within 48 hours of enrolment. Specimens were screened for RV (commercial enzyme immunoassay (EIA) and standardised characterisation protocols) and enteric pathogens (commercial molecular detection kits and in–house real–time detection assays) at the CED, NICD. Rotavirus detection and characterisation of specimens collected from Dr George Mukhari Hospital were done at the Diarrhoeal Pathogens Research Unit (DPRU), Sefako Makgatho Medical Sciences University, Ga–Rankuwa. Adenovirus–positive specimens from the RSSP were further characterised to establish species diversity by nucleotide sequence analysis of the hexon gene fragment or adenovirus species–specific PCR assays.[9,10] Controls enrolled as part of the ANDEMIA study between 2019 and 2022 were also screened for enteric viruses.

Rotavirus vaccine coverage rates are described from vaccination status at time of disease rather than time of vaccination, and are thus reflective of earlier childhood. Continuous data are presented as medians with interquartile ranges (IQRs) and categorical variables as numbers and percentages. The odds ratios and significance ($p<0.05$) of RV detection in vaccinated and unvaccinated children were calculated using multivariable logistic regression analysis and a model adjusted for site and age. Trend analysis was performed using the ptrend (version 2.0.0 PR 27 Oct 2014) programme. Enteric virus prevalence was compared between cases and controls and between healthcare settings (odds ratios calculated at significance $p<0.05$). The Wilcoxon rank sum test was used to evaluate differences between cases and controls by age. All analyses were conducted in STATA version 12 (StataCorp LP, College Station, TX).

Results

Between 2009 and 2023, 11 250 cases (with specimens) were enrolled, of which 10 567 were children <5 years of age. A total of 330 controls was also recruited as part of the ANDEMIA study, of which 147 were children <5 years. The number of sentinel sites varied during the study period from four in 2009 to ten in 2016 (Table 1). Most of the cases were enrolled at hospitals (97%; 10274/10567).

The median age of diarrhoea cases varied from 8 months (2009–2011) to 11 months (2016, 2017, and 2019). The median age of diarrhoea cases decreased during and after the SARS–CoV–2 pandemic to 9 months (2020 and 2021; Table 2). Rotavirus vaccination coverage increased to 94% receiving one dose and 80% receiving two doses in 2019 (Table 2). A drop in vaccination coverage was noted during and after the SARS–CoV–2 pandemic (2020–2023; 80–94% in 2019 versus 70–89% in 2020 and 69–84% in 2021; Table 2).

Disease outcome was recorded for participants in the RSSP and DDSS (86%; 9085/10567). Of these, 170 deaths were recorded during the 15–year surveillance period (2%; 170/9085). Rotavirus was detected in 18% (30/170) of recorded deaths. Deaths in cases presenting for the treatment of diarrhoea as well as deaths where RV was detected in stool specimens were higher prior to extensive RV vaccination (2009; n=16 diarrhoea–related deaths and n=9 deaths with RV detected, Table 2). Since 2017, diarrhoea–related deaths in sentinel facilities fell below 2% and in 2023 fell below 1% (Table 2). In addition, no RV was detected in any diarrhoea–related deaths at the

surveillance sites in 2018–2021 and 2023. Two diarrhoea–related deaths with RV detected were identified in 2022 (Table 2).

Table 2. The median age in months, outcome, and rotavirus (RV) vaccination status by year of enrolment for children <5 years of age participating in sentinel surveillance studies, South Africa, 2009–2023.

Year	Sites (n)	Median age in months (IQR)	Outcome recorded (n)	Deaths % (n)	Deaths; RV detected % (n)	RV vaccination recorded (n)	~RV1; 2 doses % (n)	~RV1; 1 dose % (n)
2009	4	8 (4–12)	693	2% (16)	1% (9)	712	0 (2)	0 (1)
2010	7	8 (4–13)	1203	3% (34)	<1% (4)	1238	13 (155)	27 (335)
2011	7	8 (4–13)	1239	2% (28)	<1% (3)	1249	36 (454)	67 (843)
2012	7	10 (5–16)	1093	2% (17)	<1% (3)	1128	60 (672)	85 (954)
2013	7	9 (5–15)	1434	2% (31)	<1% (4)	1478	67 (993)	89 (1311)
2014	6	9 (4–17)	665	2% (10)	<1% (1)	838	51 (430)	62 (521)
2015	8	10 (6–16)	540	2% (13)	<1% (2)	683	50 (344)	59 (403)
2016	10	11 (6–18)	532	2% (9)	<1% (1)	615	65 (399)	76 (469)
2017	6	11 (6–17)	220	1% (3)	<1% (1)	237	68 (161)	80 (189)
2018	6	10 (6–18)	168§	0% (0)	0% (0)	201*	74 (148)	83 (166)
2019	6	11 (6–18)	153§	1% (1)	0% (0)	158*	80 (127)	94 (148)
2020	6	9 (5–16)	64§	0% (0)	0% (0)	66*	70 (46)	89 (59)
2021	9	9 (4–16)	240§	1% (2)	0% (0)	265*	69 (182)	84 (223)
2022	9	10 (5–18)	468§	1% (5)	<1% (2)	496*	73 (364)	87 (433)
2023	7	10 (5–17)	373	<1% (1)	0% (0)	384	74 (283)	83 (320)

§Outcome recorded in RSSP and DDSS surveillance only.
*Vaccination data captured in RSSP and DDSS surveillance only.
~Vaccine coverage rates are described from vaccination status at time of disease.

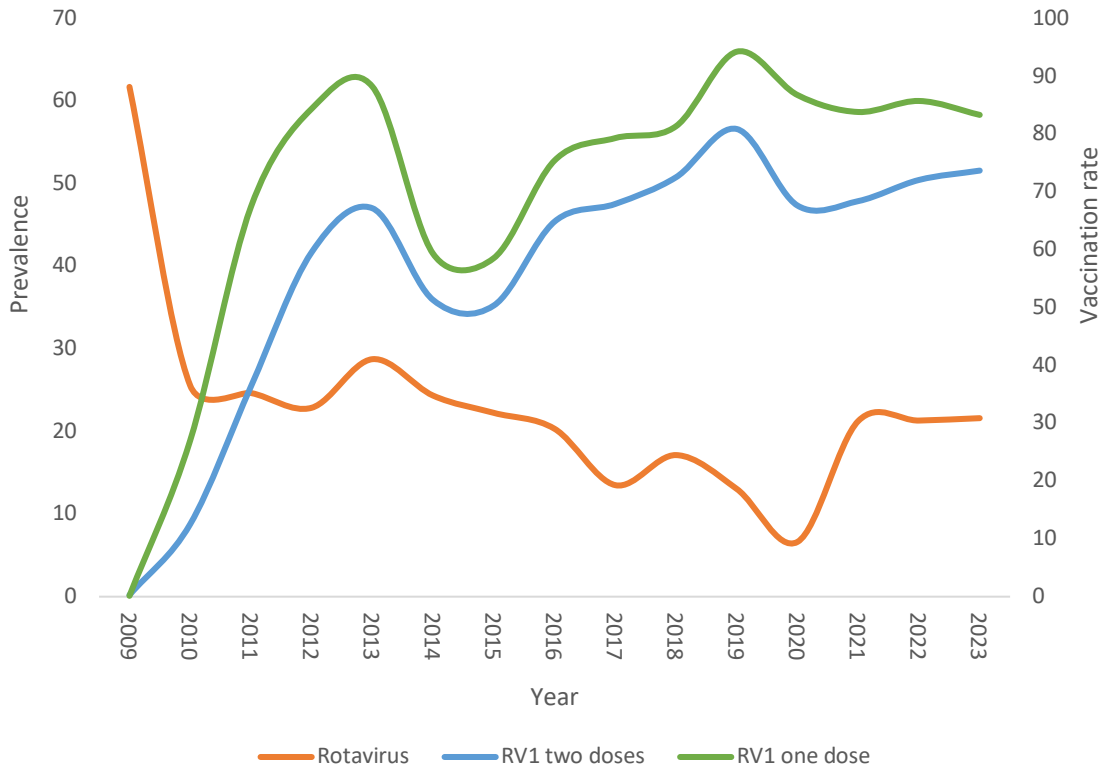

Figure 1. Rotavirus prevalence and vaccination (RV1) coverage by dosage and year at participating sentinel sites, South Africa 2009–2023.

Rotavirus prevalence declined annually from 62% in 2009 to 14% in 2019, with an exception noted in 2013, where prevalence was 29% (Figure 1, Table 3). Rotavirus vaccination status in participants enrolled in 2014 and 2015 surveillance was 59–62% receiving one dose and 50–51% receiving two doses (Table 2, Figure 1), reflective of lower vaccination rates in 2013 and early 2014. Rotavirus prevalence was very low in 2020 (7%) due to the SARS-CoV-2 pandemic, and post-pandemic RV prevalence increased to levels seen just after RV vaccine introduction (22–23%). Receiving at least one dose of RV vaccine was protective against RV diarrhoea (adjusted odds ratio (aOR) 0.77 (95% confidence interval (CI) 0.69–0.85; p <0.001). The median age for RV infections was 8 months (IQR 5–13), and RV was detected more frequently in hospitals compared to clinic settings (26% vs. 20%; p=0.05, Table 3).

Table 3. Enteric virus prevalence by year for children <5 years* of age participating in sentinel surveillance studies, South Africa 2009–2023.

Year	Total specimens screened	RV %(n)	AdV %(n)	NoVGII %(n)	SaV %(n)	AdV F %(n)	AstV %(n)	NoVGI %(n)
2009	708	62 (437)	20 (144)	8 (58)	7 (49)	7 (48)	5 (36)	2 (11)
2010	1236	26 (316)	18 (221)	17 (213)	8 (97)	7 (88)	11 (134)	3 (43)
2011	1249	25 (308)	13 (166)	13 (160)	9 (111)	5 (59)	5 (64)	4 (52)
2012	1128	23 (258)	14 (157)	12 (132)	7 (77)	7 (77)	5 (62)	3 (30)
2013	1478	29 (425)	16 (233)	8 (122)	9 (134)	8 (125)	6 (93)	2 (35)
2014	838	24 (204)	14 (121)	8 (67)	9 (75)	7 (57)	7 (55)	4 (31)
2015	683	22 (152)	22 (152)	5 (31)	7 (51)	10 (70)	5 (36)	2 (11)
2016	615	20 (125)	37 (225)	11 (67)	10 (62)	15 (92)	5 (33)	3 (19)
2017	237	14 (32)	12 (28)	9 (21)	3 (7)	7 (16)	4 (9)	<1 (1)
2018	315	17 (54)	16 (51)	19 (59)	4 (14)	4* (8/201)	5 (15)	2 (7)
2019	437	13 (57)	26 (112)	13 (57)	3 (14)	4* (7/158)	7 (32)	3 (11)
2020	226	7 (15)	26 (59)	8 (18)	6 (13)	8* (5/66)	7 (15)	2 (4)
2021	489	21 (104)	29 (144)	16 (77)	6 (30)	ND	3 (16)	2 (9)
2022	544	21 (116)	25 (136)	11 (62)	6 (32)	ND	11 (60)	4 (21)
2023	384	22 (83)	22 (84)	13 (49)	4 (16)	ND	7 (26)	3 (12)
Total	10567	25 (2686)	19 (2033)	11 (1193)	7 (782)	8* (652/ 8601)	6 (686)	3 (297)
Median age (IQR)		8 (5–13)	10 (6–16)	8 (5–13)	11 (7–16)	8 (5–14)	9 (5–15)	10 (5–17)
Facility								
Hosp	10274	26 (2626)	19 (1913)	11 (1161)	7 (764)		6 (667)	3 (291)
Clin	293	20 (60)	26 (78)	11 (32)	6 (18)		6 (19)	2 (6)

*Co-detection of >1 pathogen was noted in children <5 years; % prevalence may sum to >100%.
RV=rotavirus; AdV= adenovirus; NoVGII=norovirus genogroup II; SaV=sapovirus; AdV F=adenovirus type F; AstV=astrovirus; NoVGI=norovirus genogroup I

Norovirus genogroup II (NovGII) prevalence was lower than RV between 2009 and 2017; it thereafter circulated at similar levels between 2018 and 2020 (Figure 2). The median age for NoVGII infections was eight months (IQR 5–13, Table 3). Rotavirus levels were higher than NoVGII during the post-SARS-CoV-2 period (Figure 2). While RV declined significantly between 2009 and 2023 (p for trend <0.001), no significant trends in NoVGII circulation by year were noted (p for trend = 0.80).

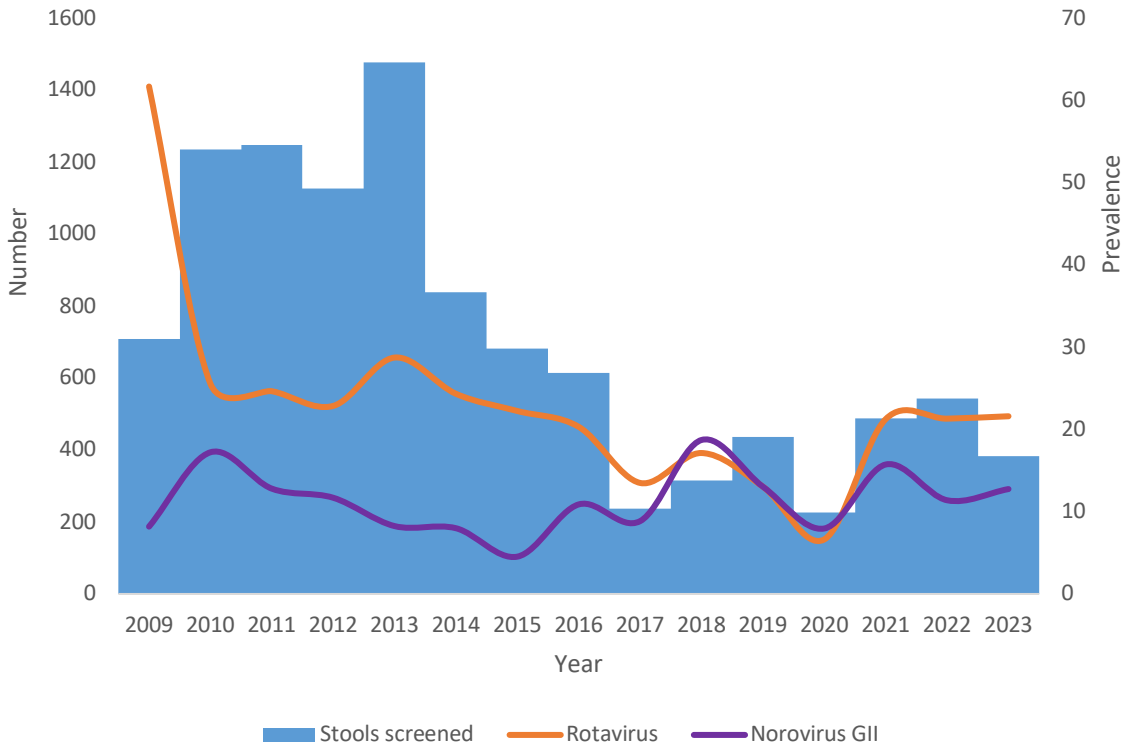

Figure 2. Rotavirus and norovirus GII prevalence by year in children <5 years of age participating in sentinel surveillance studies, South Africa 2009–2023.

Astrovirus prevalence showed peaks in 2010 and 2022, while norovirus GI (NoVGI) prevalence ranged from 2–4% over the 15-year period (Figure 3). The median age for AstV was 9 months (IQR 5–15) and for NoVGI was 10 months (IQR 5–17, Table 3). No significant trends in the circulation of AstV (p for trend = 0.63) and NoVGI (p for trend = 0.29) were noted. The median age for SaV infections was 11 months (IQR 7–16; Table 3). Trend analysis indicated a significant decline in SaV detection between 2009 and 2023 (p for trend <0.001).

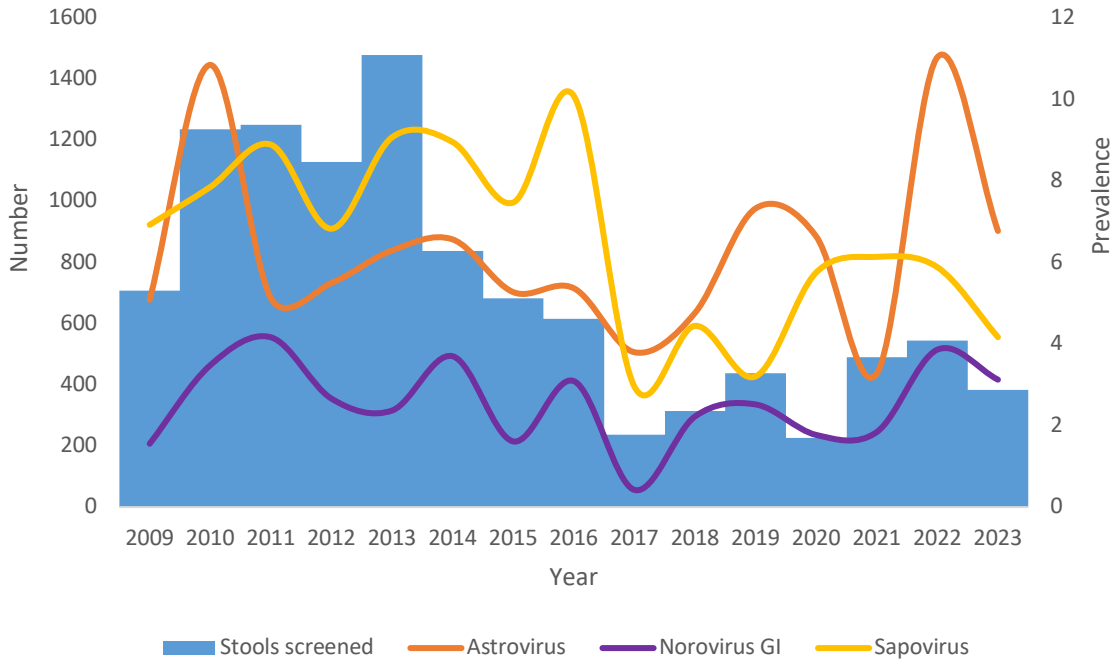

Figure 3. Astrovirus, norovirus GI, and sapovirus prevalence by year in children <5 years of age participating in sentinel surveillance studies, South Africa 2009–2023.

Adenovirus type F prevalence followed the AdV prevalence trend (Figure 4), with type F constituting 42% (651/1518) of circulating AdV strains in RSSP diarrhoea cases between 2009 and 2020. Adenovirus prevalence peaked in 2013 (36%, n=233) and 2016 (29%, n=225) with type F peaking in 2015 (10%, n=70) and 2016 (15%, n=92). Adenovirus was detected more commonly in clinic compared to hospital settings (p<0.001), and trend analysis showed a significant increase in both AdV and AdV type F detection between 2009 and 2023 (p for trend <0.001).

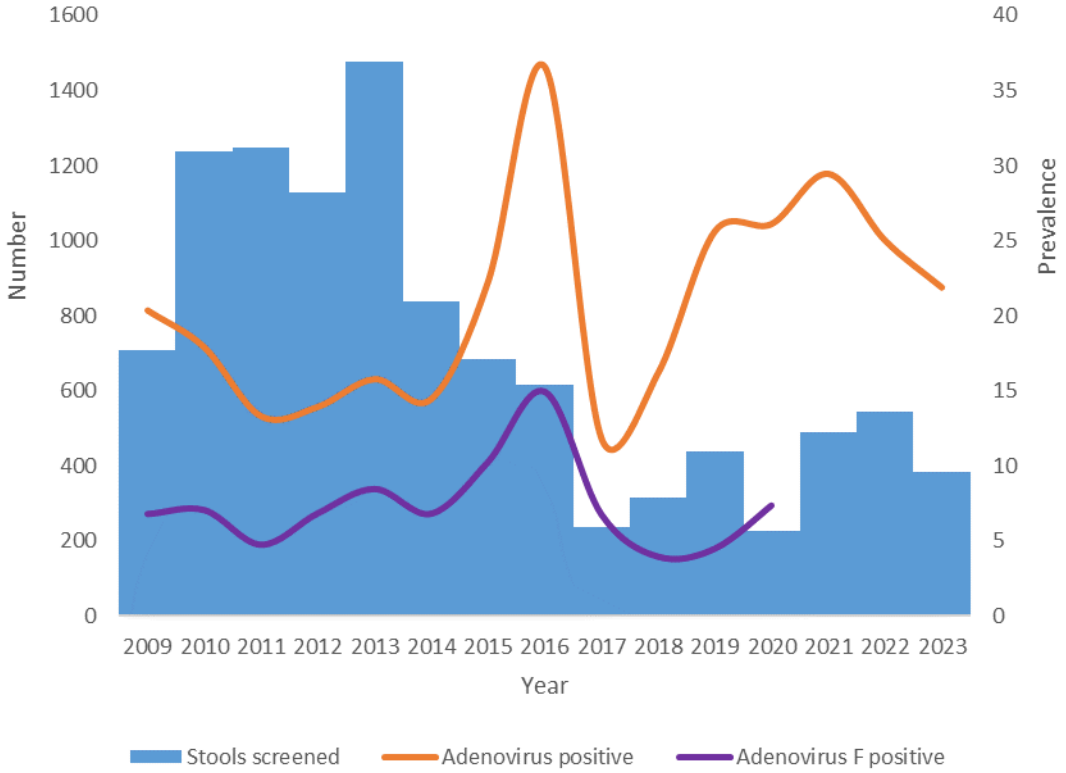

Figure 4. Adenovirus and adenovirus type F prevalence by year in children <5 years of age participating in sentinel surveillance studies, South Africa 2009–2023. Adenovirus type F characterisation was only done on Rotavirus sentinel surveillance program (RSSP) specimens (2009–2020).

Controls were unmatched for age due to operational constraints during the SARS-CoV-2 pandemic but were not significantly different between groups (median age of cases = 9 months, median age of controls = 10 months, p=0.25). Rotavirus (OR = 9.68 (95% CI 4.05–30.30); p<0.001), AdV (OR = 4.64 (95% CI 2.19–11.78); p<0.001), NoVGII (OR = 3.61 (95% CI 1.51–11.32); p=0.002), and SaV (OR = 2.86 (95% CI 1.09–10.66; p=0.03) were significantly associated with diarrhoea compared to controls in children <5 years. Astrovirus (OR = 2.48 (95% CI 0.94–9.26); p=0.06) and NoVGI (OR = 1.39 (95% CI 0.46–6.85); p=0.57) were not significantly associated with diarrhoea compared to controls in children <5 years. There were no significant differences in NoVGII, AstV, NoVGI, and SaV detection in clinics compared to hospital settings (Table 3).

Discussion

Rotavirus prevalence appeared to be influenced by vaccine coverage rates, and the increase in 2013 might have been due to low vaccine coverage during that year, as evident in lower vaccination levels in 2014 and 2015 participants. In 2020, RV prevalence dropped to 7%, and the regular RV season was attenuated and occurred later than previous years, likely due to non-pharmaceutical interventions (NPIs) to control the spread of SARS-CoV-2 such as hand washing, social distancing, regular cleaning of frequently touched surfaces, and closing of nursery schools. Following the initial SARS-CoV-2 pandemic, RV prevalence increased, likely due to reduced circulation during the early pandemic and lower vaccination rates, i.e., below 90% for one dose and

below 75% for two doses.

When RV prevalence declined, NoVGII became prominent in diarrhoea cases in children <5 years of age and was detected in all health settings. In addition to NoVGII in children <5 years, an increase in AdV and AdV type F prevalence was noted between 2009 and 2023. Characterisation of AdV strains should be conducted on ANDEMIA and DDSS specimens. Children <5 years of age could benefit from vaccination against NoVGII and AdV type F, and the development of vaccine candidates should therefore be considered.

Diarrhoea was significantly associated with RV, AdV, NoVGII, and SaV cases compared to controls in children <5 years of age in the ANDEMIA study and could reflect disease severity and propensity to seek care. Controls with AstV and NoVGI may be missed due to small sample size, mild presentation, and surveillance being mostly conducted in healthcare settings. Declining detection of SaV requires additional work to elucidate SaV trends or investigate the possibility of the emergence of SaV strains (genogroup V) not detected by the current molecular assays.

Conclusion

Owing to limited resources, the current diarrhoeal surveillance systems are not nationally representative and do not generate incidence data. The available data nevertheless show that although RV remains a common cause of childhood diarrhoea, prevalence in South Africa in children <5 years of age has evidently decreased with sustained use of the RV vaccine, and that receiving at least one vaccine dose is protective against RV diarrhoea.

Recommendations

- Paediatric RV vaccine use should be encouraged among parents and healthcare workers, and vaccines should be administered to children at 6 and 14 weeks through EPI, public or private clinics.
- The South African Department of Health and the National Advisory Group for Immunisation (NAGI) should consider vaccines against NoVGII and enteric AdV for the South African paediatric population when they become available.
- Additional work should be done on SaV by CED/NICD to identify reasons for the decline in prevalence after 2016 and ensure that genogroup V or other SaV strains are not circulating undetected.
- Typing of AdV strains from ANDEMIA and DDSS should be done by CED/NICD to continue to monitor AdV type F impact on diarrhoeal disease.

Funding

The RSSP program was supported by GlaxoSmithKline [E–Track 200238]. The ANDEMIA programme was supported by the German Federal Ministry of Education and Research [grant number: 81203616].

Acknowledgements

Rotavirus Sentinel Surveillance Programme: Cheryl Cohen, Linda Erasmus, Kathleen Kahn, Constant Kapongo, Shabir Madhi, Jeffery Mphahlele, Jocelyn Moyes, Veerle Msimang, Ina Peenze, Mapaseka Seheri, Juno Thomas, Ute Hallbauer, Sibongile Walaza, and Heather Zar.

Diarrhoeal Diseases Sentinel Surveillance: Kate Bishop, Linda Erasmus, Mokupi Manaka, Cecilia Miller, Sunnieboy Njikho, Vanessa Quan, Juno Thomas, and Heather Zar.

ANDEMIA: Theunis Avenant, Nicolette du Plessis, Maryke de Villiers, and Marietjie Venter.

Ethical considerations

RSSP: Ethical approval for the study was obtained from the Human Research Ethics Committee (Medical), University of the Witwatersrand (M091018), the Biomedical Research Ethics Committee, University of KwaZulu–Natal (BF074/09), the Medunsa Research Ethics Committee, University of Limpopo (MREC/P/10/2009), the Human Research Ethics Committee, University of Cape Town (068/2010), and the Human Research Ethics Committee, University of the Free State (86/2014).

DDSS: Ethical approval for the study was obtained from the Human Research Ethics Committee (Medical), University of the Witwatersrand (M190664, M1809107, and M160667), and the Human Research Ethics Committee, University of Cape Town (165/2020).

ANDEMIA: Ethical approval for the study was obtained from the Human Research Ethics Committee (Medical), University of the Witwatersrand (M170403), and the Research Ethics Committee, University of Pretoria (101/2017).

Conflict of interest

NP has received contractual fees by GlaxoSmithKline, grants or contracts from the German Federal Ministry of Education and Research (BMBF) and the PATH Center for Vaccine Innovation and Access, and has participated on the GSK Rotavirus Advisory Board.

MG has received grants from the Bill & Melinda Gates Foundation and the South African Medical Research Council (SAMRC).

The remaining authors declare no conflict of interest. The funders had no role in the design of the study, in the collection, analysis, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

References

1. 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):136–141

2. Cohen AL, Platts–Mills JA, Nakamura T, et al. Aetiology and incidence of diarrhoea requiring hospitalisation in children under 5 years of age in 28 low–income and middle–income countries: findings from the Global Pediatric Diarrhea Surveillance network. *BMJ Glob Health*. 2022;7(9):e009548.

3. Msimang VMY, Page N, Groome MJ, et al. Impact of rotavirus vaccine on diarrhoeal hospitalization following introduction into the South African public immunization program. *Pediatr Infect Dis J* 2013; 32:1359–1364

4. 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; 14:1096–1104.

5. Makgatho E, Patel F, Solomon F, et al. The burden of acute diarrheal disease in young hospitalized urban South African children five years after rotavirus vaccine introduction: A retrospective descriptive study. *Pediatr Infect Dis J* 2019; 38:752–756

6. Diarrhoeal disease. World Health Organization. Updated March 7, 2024. Accessed June 11, 2024. https://www.who.int/news–room/fact–sheets/detail/diarrhoeal–disease

7. Platts–Mills JA, Liu J, Rogawski ET, et al. Use of quantitative molecular diagnostic methods to assess the aetiology, burden, and clinical characteristics of diarrhoea in children in low–resource settings: a reanalysis of the MAL–ED cohort study. *Lancet Glob Health*. 2018;6(12):e1309–e1318

8. Schubert G, Achi V, Ahuka S, et al. The African Network for Improved Diagnostics, Epidemiology and Management of common infectious Agents. *BMC Infect Dis.* 2021;21(1):539

9. Casas I, Avellon A, Mosquera M, et al. Molecular identification of adenoviruses in clinical samples by analyzing a partial hexon genomic region. *J Clin Microbiol* 2005;43:6176–6182

10. Xu W, McDonough ME, Erdman DD. Species–species identification of human adenoviruses by a multiplex PCR assay. *J Clin Microbiol* 2000;38:4114–4120