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Trends in enteric pathogen circulation in participants >5 years of age at selected sentinel sites in South Africa, 2018–2023

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Summary

Many studies have investigated diarrhoeal disease in children <5 years of age, but diarrhoeal disease in older children, adults and the elderly are often overlooked. Data gaps in disease burden and aetiology make designing targeted interventions for these populations difficult. This study examined trends in enteric pathogen prevalence in participants >5 years of age at selected sentinel sites in South Africa between 2018 and 2023. Among older children, adults and the elderly, our surveillance shows that those living with HIV (PLHIV; 53%; 331/621) constitute a large proportion of diarrhoeal cases. Enteric bacterial infections (31%) seemed to drive most of the disease burden, with EIEC/*Shigella* spp. detected frequently (20%). Enteric viruses were detected in 27% of cases >5 years of age presenting with diarrhoea but did not seem to be a major cause of disease in older individuals, likely due to acquired immunity. The exception was norovirus genogroup II in PLHIV, and continued monitoring is recommended. There was also a significant decline in human adenovirus prevalence between 2018 and 2023, decreasing from 28% in 2020 to 3% in 2023. These declines may be related to the use of the Janssen Ad26.COV2.S SARS-CoV-2 vaccine, although additional investigation will be required. This study begins to provide a better understanding of the aetiology of diarrhoeal disease in older populations in South Africa and may inform targeted interventions.

Introduction

Since 1990, global diarrhoeal disease mortality has decreased from 60.6 (46.7-79.6) age-standardised deaths per 100 000 population to 15.4 (10.9-20.9) in 2021.[1] Interventions to combat diarrhoeal diseases have led to a substantial increase of 1.1 years in global life expectancy, with sub-Saharan Africa reporting an increase of 0.8 to 2.4 years in life expectancy.[1] However, in sub-Saharan Africa, mortality estimates are still high, with 54.4 (33.9-76.7) age-standardised deaths per 100 000, equating to 434 000 (310 000 – 570 000) deaths across all ages in this region.[1]

While diarrhoeal disease burden and associated aetiology have been well studied in children <5 years of age with reliable estimates of morbidity and mortality, estimates in older children and adults are based on limited studies.[2] Diarrhoea in older children and adults often results in lost productivity and increased burden on healthcare systems and may contribute to substantial mortality and morbidity, specifically in ageing populations and populations with high HIV prevalence.

Without known aetiology or burden estimates, tailoring diarrhoeal disease interventions is difficult. Strategies focused on the delivery of safe water (piped and chlorinated water) to prevent water-borne pathogens,[3] infection control approaches in institutions and facilities, prompt outbreak investigations, and anti-microbial stewardship within animal husbandry and human disease management, as well as improved food and water safety policy,[4] could all be explored. By generating data to support and guide the design of interventions, these public health programmes could be effectively targeted to the populations that are most at risk for diarrhoeal disease.

In this study, we examined trends in enteric pathogen prevalence in participants >5 years of age at selected sentinel sites in South Africa between 2018 and 2023.

Materials and methods

Data from 2018 to 2023 were obtained from two sources:

Diarrhoeal Diseases Sentinel Surveillance (DDSS). Participants of any age presenting for the treatment of diarrhoea (as defined by the World Health Organization (WHO); any duration) at selected sentinel hospitals and clinics were approached for enrolment (Table 1). Enrolment was conducted systematically from Monday to Friday (08:00–17:00), after informed consent was obtained from the patient or a parent or guardian. Demographic, clinical and outcome data were collected in a structured questionnaire by dedicated surveillance officers from participants residing in the site catchment area for at least seven days prior to illness. Only diarrhoeal cases in participants >5 years of age who provided a stool specimen for testing were included in the analysis. Additional data on viral load (VL) and antiretroviral therapy (ART) were available for some participants of the DDSS.

African network for improved diagnostics, epidemiology, and management of common infectious agents (ANDEMIA). Participants of any age presenting to a study hospital for the treatment of diarrhoea (WHO definition, ≤30 days' duration), respiratory tract infections or acute febrile disease of unknown cause (AFDUC) were approached for enrolment (Table 1).[5] Unmatched controls were also enrolled as part of the study and included individuals attending the hospital or clinic for reasons other than diarrhoea. Control participants were mainly enrolled from vaccination clinics and orthopaedic or surgical wards and did not report any gastrointestinal symptoms (vomiting or diarrhoea) in the past three weeks. Controls were not matched for age and HIV status due to operational constraints during the SARS-CoV-2 pandemic. Only diarrhoeal cases and controls in participants >5 years of age who provided a stool specimen for testing were included in the analysis. Enrolment was done similarly to the DDSS, with informed consent sought from the patient or a parent or guardian. Data on viral loads was not collected for participants of ANDEMIA. Analysis of CD4 counts and antiretroviral therapy (ART) in this dataset has been published separately and was not included in this analysis.[6]

Table 1. Sentinel sites and diarrhoeal surveillance programmes enrolling participants >5 years of age and collecting stool specimens, South Africa, 2018–2023.

Site	Province	Years surveillance conducted	Programme
Mapulaneng Hospital	Mpumalanga	2018–2022	ANDEMIA[a]
Matikwane Hospital	Mpumalanga	2018–2022	ANDEMIA[a]
Hluvukani Clinic	Mpumalanga	2019–2020	ANDEMIA[a]
Red Cross Children's Hospital	Western Cape	2021–2023	DDSS[b]
Pelonomi Hospital	Free State	2020–2023	DDSS[b]
Klerksdorp/Tshepong Hospital	North West	2021–2023	DDSS[b]
Kalafong Hospital	Gauteng	2018–2022	ANDEMIA[a]
		2022–2023	DDSS[b]
Michell's Plain Hospital	Western Cape	2021–2023	DDSS[b]
Eastridge Clinic	Western Cape	2021–2023	DDSS[b]
Kabokweni Clinic	Mpumalanga	2021–2022	ANDEMIA[a]
		2022–2023	DDSS[b]

[a]ANDEMIA - African Network for Improved Diagnostics, Epidemiology and Management of Common Infectious Agents

[b]DDSS – Diarrhoeal Diseases Sentinel Surveillance

Stool specimens were collected from participants within 48 hours of enrolment. Specimens were screened for rotavirus (RIDASCREEN® Rotavirus [R-Biopharm AG, Darmstadt, Germany] and standardised characterisation protocols) and enteric pathogens (FastTrack Diagnostics Viral and Bacterial Gastroenteritis and Stool Parasite Real-time PCR kits [Siemens Healthcare GmbH; Erlangen, Germany] and in-house real-time enteric virus detection assays) at the Centre for Enteric Diseases (CED), National Institute for Communicable Diseases, a division of the National Health Laboratory Service. The viral assays detect rotavirus (RV), norovirus genogroup II (NoVGII), norovirus genogroup I (NovGI), sapovirus (SaV), human adenovirus (HAdV) and human astrovirus (HAstV). The bacterial assays detect Shiga-toxin-producing *Escherichia coli* (STEC), *Campylobacter jejuni/coli/lari* (Campy), *Clostridioides difficile* (Cdiff), *Yersinia enterocolitica* (YE), *Salmonella* spp. (Salm), and enteroinvasive *E.coli/Shigella* spp (EIEC/Shig). The stool parasite kit detects *Entamoeba hystolytica* (EH), *Cryptosporidium* spp. (Crypt), and *Giardia lamblia* (Giard).

Descriptive analyses of patient data are presented as medians with interquartile ranges (IQRs) as well as enteric pathogen prevalence in cases and controls. The Wilcoxon rank sum test was used to evaluate differences in case and control age. The HIV status of participants with a known result was presented as a percentage. The two-sample test of proportions was used to evaluate differences in HIV status in cases and controls and in enteric pathogen prevalence by health facility. Enteric pathogen prevalence in diarrhoea cases was compared to controls, and an odds ratio, 95% confidence intervals and level of significance ($p < 0.05$) were calculated using the case-control odds calculator in STATA version 12 (StataCorp LP, College Station, TX). A similar sub-analysis was performed on people living with HIV (PLHIV). Trend analysis was performed using the ptrend programme (version 2.0.0 PR 27oct2014[7]), and enteric pathogen prevalence was described in cases by year and HIV status for each year. All analysis was performed using STATA.

Results

Between 2018 and 2023, 677 cases in participants >5 years of age with stool specimens were enrolled into surveillance. A total of 183 controls of participants >5 years of age with stool specimens were also recruited as part of the ANDEMIA study. The number of sentinel sites varied during the study period from three in 2018 to 12 in 2022 (Table 2). Diarrhoea cases were enrolled at hospitals (60%; 404/677) and clinics (40%; 273/677).

The median age of cases was 34 years (IQR 23–49) overall and ranged from 39 years in 2019 and 2021 to 30 years in 2022 and 2023 (Table 2). HIV status was available for 92% (621/677) of cases, of which 53% (331/621) were PLHIV. Between 2018 and 2023, there was a significant decline in the number of PLHIV enrolled into diarrhoeal surveillance (p for trend <0.001; Table 2).

Table 2. Enteric virus prevalence by year for diarrhoea cases >5 years of age and enrolled in sentinel surveillance studies between 2018 and 2023, South Africa. The number of sites, median age and % HIV-infected by year are also included.

Year	Sites (n)	Median age in years (IQR)	HIV % (n/N)	Total tested (n)	HAdV %(n)	NoVGII %(n)	RV % (n)	HAstV %(n)	SaV %(n)	NoVGI %(n)
2018	3	36 (27–51)	76 (39/51)	55	20 (11)	20 (11)	11 (6)	4 (2)	0 (0)	0 (0)
2019	4	39 (30–56)	67 (85/127)	138	19 (26)	5 (7)	4 (6)	6 (8)	3 (4)	1 (2)
2020	4	35 (28–53)	69 (36/52)	61	28 (17)	13 (8)	7 (4)	5 (3)	7 (4)	0 (0)
2021	10	39 (25–55)	62 (56/91)	106	13 (14)	7 (7)	10 (11)	2 (2)	5 (5)	3 (3)
2021	4	41 (26–59)	61 (44/72)	85	14 (12)	7 (6)	13 (11)	2 (2)	6 (5)	4 (3)
2022	12	30 (20–42)	42 (62/146)	156	7 (14)	8 (13)	4 (6)	4 (6)	3 (4)	3 (5)
2022	4	39 (26–54)	52 (13/25)	28	4 (1)	14 (4)	0 (0)	0 (0)	4 (1)	0 (0)
2023	7	30 (20–44)	34 (53/154)	161	3 (5)	4 (7)	5 (8)	1 (2)	0 (0)	2 (4)
Total				677	13 (87)	8 (53)	6 (41)	3 (23)	3 (17)	2 (14)

Lines highlighted include results for ANDEMIA sites only. HAdV = human adenovirus; NoVGII = norovirus genogroup II; RV = rotavirus; HAstV = human astrovirus; SaV = sapovirus; NoVGI = norovirus genogroup I

Of the 113 PLHIV enrolled in the DDSS, viral loads were recorded for 57% (64/113; 75% (48/64) with VL <400; 9% (6/64) with VL 400–10000; and 16% (10/64) with VL >10000). Of the 113 PLHIV, information on ART was recorded for 103 cases. Of the 103 cases, 23% (24/103) were not on ART, and 77% (79/103) reported being on ART. Of these, 77% (61/79) were on first-line treatment, 13% (10/79) were on second-line treatment, and 4% (3/79) were on third-line treatment. The remaining 6% (5/79) were defaulters, newly initiated to treatment, or the ART regimen was not recorded.

Between 2018 and 2023, enteric viruses were detected in 27% (186/677) of cases >5 years of age enrolled, with individual enteric virus prevalence ranging from 2% to 13% (Table 2). During the study, NoVGII prevalence peaked at 20% (11/55) in 2018 and 13% (8/61) in 2020 (Table 2). However, there was an overall decrease in NoVGII cases between 2018 and 2023 (p for trend = 0.03). Trend analysis indicated a significant decline in HAdV detection between 2018 and 2023 (p for trend < 0.001) from a high of 28% (11/55) in 2020 to 3% (5/161) in 2023 (Table 2), albeit an inconsistent decline over time. No significant trends in the circulation of RV (p for trend = 0.31), HAstV

(p for trend = 0.07), SaV (p for trend = 0.28) and NoVGI (p for trend = 0.13) were noted between 2018 and 2023 (Table 2).

Bacteria were detected in 31% (209/677), parasites in 8% (56/677) and mixed pathogen infections (defined as detection of two or more enteric pathogens) in 17% (114/677) of cases >5 years of age enroled in the study. Individual bacterial species were detected at levels from <1% to 20%, and parasite prevalence ranged from <1% to 6% (Table 3). Significant declines in prevalence over the study period were noted for *Cryptosporidium* spp., declining from 10% (7/55) in 2018 to 4% (6/161) in 2023 (p for trend p=0.05); *C. difficile*, declining from 7% in 2018 (4/55) and 2019 (9/138) to 0% in 2022 and 2% (3/161) in 2023 (p for trend = 0.003); and STEC, declining from 7% (9/138) in 2019 to 1% (1/161) in 2023 (p for trend <0.001). No significant trends in the circulation of EIEC/*Shigella* spp. (p for trend = 0.30), *Campylobacter jejuni/coli/lari* (p for trend = 0.40), *Salmonella* spp. (p for trend = 0.92), and *Giardia lamblia* (p for trend = 0.23) were noted between 2018 and 2023 (Table 3).

Table 3. Enteric bacteria and parasite detection by year for diarrhoea cases >5 years of age and enrolled in sentinel surveillance studies between 2018 and 2023, South Africa.

Year	Total screened (n)	EIEC/ Shig % (n)	Crypt %(n)	Campy %(n)	C.diff %(n)	Salm %(n)	Giard %(n)	STEC %(n)	YE %(n)	EH %(n)
2018	55	27 (15)	13 (7)	4 (2)	7 (4)	5 (3)	0 (0)	2 (1)	0 (0)	0 (0)
2019	138	23 (32)	9 (13)	5 (7)	7 (9)	2 (3)	4 (6)	7 (9)	1 (2)	0 (0)
2020	61	16 (10)	8 (5)	10 (6)	5 (3)	0 (0)	5 (3)	0 (0)	0 (0)	0 (0)
2021	106	14 (15)	5 (5)	5 (5)	6 (6)	4 (4)	1 (1)	0 (0)	0 (0)	0 (0)
2021	85	13 (11)	5 (4)	6 (5)	7 (6)	2 (2)	1 (1)	0 (0)	0 (0)	0 (0)
2022	156	19 (30)	5 (8)	4 (7)	1 (1)	3 (5)	1 (1)	0 (0)	0 (0)	0 (0)
2022	28	14 (4)	7 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
2023	161	20 (33)	4 (6)	3 (5)	2 (3)	3 (5)	2 (3)	1 (1)	0 (0)	1 (1)
Total	677	20 (135)	6 (44)	5 (32)	4 (26)	3 (20)	2 (14)	2 (11)	<1 (2)	<1 (1)

Lines highlighted include results for ANDEMIA sites only. EIEC = enteroinvasive *Escherichia coli*; Shig = *Shigella* spp.; Cryp = *Cryptosporidium* spp.; Campy = *Campylobacter jejuni/coli/lari*; C.diff = *Clostridioides difficile*; Salm = *Salmonella* spp.; Giard = *Giardia lamblia*; STEC = Shiga-toxin producing *E. coli*; YE = *Yersinia enterocolitica*; EH = *Entamoeba hystolytica*

There was a significant difference in the detection of NoVGII among hospitalised cases (10%; 40/404) compared to cases in outpatient settings (5%; 13/273; p=0.01) as well as for detection of HAdV in hospitalised cases (18%; 73/404) compared to those in outpatient settings (5%; 14/273; p<0.001) (Table 4). Significant differences were also noted in *Cryptosporidium* spp. (9%; 38/404 in hospital cases versus 2%; 6/273 in outpatient cases; p<0.001), *Campylobacter jejuni/coli/lari* (6%; 25/404 in hospital cases versus 3%; 7/273 in outpatient cases; p=0.03), *C. difficile* (6%; 24/404 in hospital cases versus 1%; 2/273 in outpatient cases; p<0.001), *Giardia lamblia* (3%; 14/404 in hospital cases versus 0% in outpatient cases; p=0.002) and STEC cases (2%; 11/404 in hospital cases versus <1%; 1/273 in outpatient cases; p=0.03; Table 5). There were no significant differences in the detection of the remaining enteric pathogens in hospitalised cases compared to those in outpatient settings (Tables 4 and 5).

Table 4. Enteric virus detected in hospitalised and clinic cases >5 years of age and enrolled in sentinel surveillance between 2018 and 2023, South Africa.

Site	Total screened	HAdV % (n)	NoVGII % (n)	RV % (n)	HAstV % (n)	SaV % (n)	NoVGI % (n)
Clinic	273	5% (14)	5% (13)	6% (16)	2% (5)	2% (6)	2% (6)
Hospital	404	18% (73)	10% (40)	6% (25)	4% (18)	3% (11)	2% (8)
	677	13% (87)	8% (53)	6% (41)	3% (23)	3% (17)	2% (14)

HAdV = human adenovirus; NoVGII = norovirus genogroup II; RV = rotavirus; HAstV = human astrovirus; SaV = sapovirus; NoVGI = norovirus genogroup I.

Table 5. Enteric bacteria and parasites detected in hospitalised and clinic cases >5 years of age and enrolled in sentinel surveillance between 2018 and 2023, South Africa.

Site	Total (n)	EIEC/ Shig % (n)	Crypt %(n)	Campy %(n)	C.diff %(n)	Salm %(n)	Giard %(n)	STEC %(n)	YE %(n)	EH %(n)
Clinic	273	17% (47)	2% (6)	3% (7)	1% (2)	4% (10)	0% (0)	<1% (1)	<1% (1)	<1% (1)
Hosp.	404	22% (88)	9% (38)	6 % (25)	6% (24)	3% (10)	3% (14)	2% (10)	<1% (1)	0% (0)
Total	677	20% (135)	6% (44)	5% (32)	4% (26)	3% (20)	2% (14)	2% (11)	<1% (2)	<1% (1)

EIEC = enteroinvasive *Escherichia coli*; Shig = *Shigella* spp.; Cryp = *Cryptosporidium* spp.; Campy = *Campylobacter jejuni/coli/lari*; C.diff = *Clostridioides difficile*; Salm = *Salmonella* spp.; Giard = *Giardia lamblia*; STEC= Shiga-toxin producing *E. coli*; YE = *Yersinia enterocolitica*; EH = *Entamoeba hystolytica.*

Two deaths were recorded during the study period; both individuals were PLHIV. One individual had a VL of 400–10000, was on first-line ART and had NoVGII detected, while the other had a VL of <400, was on second-line ART and had no viruses detected, although *C. difficile* was identified.

Controls were significantly younger than cases (median age of cases 34 years, median age of controls 25 years; IQR (14–35); p<0.001). HIV status was available for 58% (107/183) of controls, with the proportion of PLHIV in the controls (27%; 29/107) lower than the proportion in cases (53%; 331/621; p<0.001). There were very few enteric pathogens detected in control participants, with most enteric viruses, bacteria and parasites detected at levels ≤4% (Tables 6 and 7). Two exceptions were noted: HAdV was detected at 9% (17/183) of control specimens collected over six years, with a peak detection of 14% (10/74) in 2021 (Table 6); *Cryptosporidium* spp. were detected at 6% (11/183) of control specimens, with peak detection in 2020 (11%; 3/27) and 2022 (11%; 7/61; Table 7).

Table 6. Enteric virus detection by year in individuals >5 years of age without diarrhoea participating in ANDEMIA studies between 2019 and 2022, South Africa.

Year	Sites	Total screened	HAdV	NoVGII	RV	HAstV	SaV	NoVGI
			% (n)	% (n)	% (n)	% (n)	% (n)	% (n)
2019	3	21	5% (1)	10% (2)	0% (0)	10% (2)	5% (1)	0% (0)
2020	3	27	7% (2)	7% (2)	0% (0)	4% (1)	0% (0)	4% (1)
2021	3	74	14% (10)	4% (3)	1% (1)	0% (0)	0% (0)	4% (3)
2022	3	61	7% (4)	0% (0)	2% (1)	5% (3)	2% (1)	2% (1)
Total		183	6% (17)	4% (7)	1% (2)	3% (6)	1% (2)	3% (5)

HAdV = human adenovirus; NoVGII = norovirus genogroup II; RV = rotavirus; HAstV = human astrovirus; SaV = sapovirus; NoVGI = norovirus genogroup I.

Table 7. Enteric bacteria and parasites detection by year in individuals >5 years of age without diarrhoea participating in ANDEMIA studies between 2019 and 2022.

Year	Sites	Total (n)	EIEC/ Shig % (n)	Crypt %(n)	Campy %(n)	C.diff %(n)	Salm %(n)	Giard %(n)	STEC %(n)	YE %(n)	EH %(n)
2019	3	21	5% (1)	0% (0)	5% (1)	5% (1)	0% (0)	5% (1)	5% (1)	0% (0)	0% (0)
2020	3	27	4% (1)	11% (3)	4% (1)	4% (1)	4% (1)	7% (2)	0% (0)	0% (0)	0% (0)
2021	3	74	1% (1)	1% (1)	3% (2)	5% (4)	1% (1)	1% (1)	1% (1)	0% (0)	0% (0)
2022	3	61	0% (0)	11% (7)	2% (1)	2% (1)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)
Total		183	2% (3)	6% (11)	3% (5)	4% (7)	1% (2)	2% (4)	1% (2)	0% (0)	0% (0)

EIEC = enteroinvasive *Escherichia coli*; Shig = *Shigella* spp.; Cryp = *Cryptosporidium* spp.; Campy = *Campylobacter jejuni/coli/lari*; C.diff = *Clostridioides difficile*; Salm = *Salmonella* spp.; Giard = *Giardia lamblia*; STEC = Shiga-toxin producing *E. coli*; YE = *Yersinia enterocolitica*; EH = *Entamoeba hystolytica*.

The odds of any enteric viruses being detected in cases (27%; 186/677) compared to controls (17%; 31/183) was 1.86 (95% CI 1.20–2.93), and the result was statistically significant (p=0.004). Furthermore, the odds of bacteria being detected in cases (31%; 209/677) compared to controls (9.3%; 17/183) was 4.36, and the result was also statistically significant (p<0.001). While parasite detection was not associated with increased odds of diarrhoea (OR = 1.00 (95% CI 0.55-1.97); p=0.97) in study participants, there was a statistically significant increased odds of mixed pathogen infections in cases (17%; 114/677) compared to controls (9%; 16/183; OR = 2.11; 95% CI 1.20-3.93; p=0.007).

Only RV (OR = 5.83; 95% CI 1.49-50.19; p=0.006) and EIEC/*Shigella* spp. (OR=14.94; 95% CI 4.9-74.1; p<0.001) were significantly associated with diarrhoea cases compared to controls in older participants. Adenovirus (OR = 1.44; 95% CI 0.82-2.66; p=0.19), NoVGII (OR = 2.14; 95% CI 0.94-5.66; p=0.06), SaV (OR = 2.33; 95% CI 0.54-20.97; p=0.25),

HAstV (OR = 1.04; 95% CI 0.40-3.16; p=0.94) and NoVGI (OR = 0.75; 95% CI 0.25-2.70; p=0.59) were not significantly associated with diarrhoea cases compared to controls. In addition, *Cryptosporidium* spp. (OR = 1.09; 95% CI 0.29-3.99; p=0.92), *Campylobacter jejuni/coli/lari* (OR = 1.77; 95% CI 0.67-5.88; p=0.24), *C. difficile* (OR = 1.00; 95% CI 0.42-2.79; p=0.99), *Salmonella* spp. (OR = 2.75; 95% CI 0.66-24.5; p=0.16), *Giardia lamblia* (OR = 0.94; 95% CI 0.29-3.99; p=0.92) and STEC (OR = 1.49; 95% CI 0.32-14.0; p=0.60) were also not significantly associated with diarrhoea cases compared to controls.

In PLHIV, there was a significant difference in the detection of bacteria in cases (31%; 103/331) compared to controls (10%; 3/29; OR = 3.92; 95% CI 1.16-20.6; p= 0.02) with the association driven mainly by EIEC/*Shigella* spp. detection (19%; 62/331 of cases and 0 controls; OR = 1.73; p=0.01). There were no statistically significant differences in the detection of enteric viruses in cases (28%; 92/331) compared to controls (21%; 6/29; OR=1.48; 95%; CI 0.56-4.57; p=0.52) or in parasite detection in cases (11%; 35/331) compared to controls (0%; OR = 0.88; p=0.07) in PLHIV. Furthermore, there was also no association of mixed pathogen infections with cases compared to controls in PLHIV (OR = 1.44; 95% CI 0.47-5.89; p=0.51). In PLHIV, including cases (n=331) and controls (n=29), the enteric pathogens detected most frequently included EIEC/*Shigella* spp. (17%; 62/360), adenovirus (14%; 49/360), NoVGII (9%; 32/360), *Cryptosporidium* spp. (8%; 30/360), *C. difficile* (6%; 21/360) and rotavirus (6%; 21/360).

Discussion

In this study, the ANDEMIA surveillance case participants tended to be older (median age 39 years; IQR 27–56), while participants of the DDSS were younger (median age 29 years; IQR 20–42). This may be partially due to site selection and participant enrolment numbers at the various sites. A limitation of the study was that surveillance was not conducted at all sites for the full six years—some sites (RCCH) only enrolled paediatric cases, and the surveillance infrastructure was not nationally representative due to funding constraints. In addition, we only enrolled patients that visited the surveillance facilities, which may have influenced conclusions on disease severity, burden of disease within the community and socioeconomic factors. Despite these limitations, the surveillance has provided a snapshot into diarrhoeal diseases in adult populations in South Africa.

Diarrhoea is a commonly presenting symptom in PLHIV, especially those who are not on ART, with affected individuals experiencing severe and/or prolonged symptoms and at a greater risk for hospitalisation and death.[8] Previously, our diarrhoeal case surveillance (ANDEMIA; 2018–2021) detected a large proportion (70/99, 70.7%) of PLHIV with CD4+ cell counts <200 cells/µl.[6] No CD4+ counts were collected as part of diarrhoeal surveillance, but 16% (10/64) of the PLHIV enrolled in the study with a VL result available had VL >10 000. The significant decline in the proportion of PLHIV enrolled as cases during the current study suggests improved access to treatment for HIV disease in South Africa.

Most of the diarrhoeal disease burden in older children, adults and the elderly seemed to be driven by bacteria, detected in roughly a third of cases in the study and significantly associated with diarrhoea. In addition, EIEC/*Shigella* spp. detection was also significantly associated with diarrhoea and contributed to disease in both the clinic (17%) and hospital (22%) settings. In PLHIV, a similar picture emerged with the detection of bacteria associated with diarrhoea, with most of the burden (19%) driven by EIEC/*Shigella* spp. Any efforts to reduce *Shigella* spp. Infections, including provision of safe water and improved sanitation and hygiene measures, should

be promoted, as well as considering adult vaccination for vulnerable individuals should a *Shigella* vaccine become available.

Increased detection of *Cryptosporidium* spp., Campylobacter *jejuni/coli/lari*, *C. difficile*, STEC and *Giardia lamblia* in hospital cases suggests either that these pathogens may cause slightly more severe diarrhoeal symptoms, especially in PLHIV, or their involvement in mixed pathogen infections may result in more severe disease. Furthermore, *Cryptosporidium* spp., *C. difficile* and STEC prevalence declined over the six-year study period and may be related to corresponding declines in PLHIV enrolled in the study. However, only declines in *Cryptosporidium* spp. strongly correlated with declines in PLHIV (correlation coefficient = 0.84; p=0.04) and additional work should be done to elucidate these findings.

While enteric viruses contribute to diarrhoea in older children, adults and the elderly (27%), they do not appear to be a major driver of disease in older populations, as only RV was significantly associated with diarrhoea. This may be due to acquired immunity against the remaining enteric viruses or biases associated with the study design. However, surveillance focused on NoVGII should be continued, as previous studies showed that NoVGII was more prevalent in PLHIV compared to HIV-uninfected individuals.[8,9] Moreover, this study showed that NoVGII prevalence among cases was doubled in hospitals compared to outpatient settings. The 2018 increase in NoVGII prevalence in older participants at levels close to 20% was also seen in children ≤5 years (19%; 59/315) and could be related to the emergence of the GII.4 Sydney 2012 variant in South Africa.[10,11]

Detection of most enteric pathogens in older participants was not significantly associated with diarrhoea compared to controls. This may be due to the sub-optimal enrolment of controls and inability to match on key confounders, including age and HIV status. Other factors that may also have contributed include the small sample size of both cases and controls, mild presentation of enteric viruses, bacteria or parasites in older participants and surveillance being conducted in healthcare settings.

An interesting finding from this study was the significant decline in HAdV between 2018 and 2023, with most of the decline occurring between 2020 and 2023. Some of the decline in older participants may be partially related to the administration of the Janssen Ad26.COV2.S vaccine against SARS-CoV-2, which contained a recombinant, replication-incompetent adenovirus serotype 26 (HAdV type D) vector encoding a full-length and stabilised SARS-CoV-2 spike protein.[12] The vaccine was administered to healthcare workers between February and May 2021 and then offered to the broader South African population ≥18 years of age as one of the vaccination options for the prevention of severe COVID-19 disease.[13] By the end of December 2023, 9.37 million doses had been administered to the South African population ≥18 years of age.[14] Human adenovirus detection was similar in cases and controls in 2021 (14% in cases and controls) and 2022 (7% in cases and controls). Unfortunately, control enrolment stopped in April 2022, and no controls were enrolled in 2023, so information on HAdV prevalence in healthy controls was suboptimal. Unlike the decline in HAdV prevalence in participants >5 years of age, an increase was noted in children ≤5 years and may be related to the age restriction of the Janssen Ad26.COV2.S vaccine administration.[10] Continued monitoring of HAdV prevalence in older participants should be done to investigate these trends.

Conclusion

Diarrhoeal surveillance in older children, adults and the elderly provides insight into disease in older populations in South Africa and could assist in guiding intervention strategies. Enteric bacteria were detected in 31% (209/677), viruses in 27% (186/677), mixed pathogen infections in 17% (114/667) and parasites in 8% (56/677) of cases >5 years of age. With the exception of RV and EIEC/*Shigella* spp., detection of most enteric pathogens was not associated with disease, likely due to acquired immunity. People living with HIV were identified as a group vulnerable to diarrhoeal disease, and efforts to elucidate disease in this population and provide better diagnostic and treatment guidelines should be continued.

Recommendations

- Enteric pathogen surveillance should be continued by CED in older participants to investigate the role of bacteria in diarrhoeal disease, levels of antimicrobial resistance in enteric pathogens in South Africa, and how mixed aetiology infections impact disease severity.
- Norovirus GII strains should continue to be monitored in participants >5 years of age, particularly in PLHIV.
- Typing of HAdV strains from ANDEMIA and DDSS should be done by CED to monitor HAdV strain circulation during and post SARS-CoV-2 vaccination using the AdV 26 vector vaccine and to monitor enteric AdV type F circulation.
- A larger cohort of matched adult control participants could be considered by CED in surveillance studies to investigate enteric pathogens and diarrhoea as well as the role of asymptomatic and mild infections in enteric pathogen transmission.
- Additional studies on diarrhoeal disease in PLHIV should be undertaken by CED and partners so that improved diagnostic and treatment guidelines can be developed for this vulnerable population.
- Vaccine developers could also consider NoV and *Shigella* spp. vaccine administration in PLHIV or vulnerable adults once candidates become available.

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Ethical approvals

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Conflict of interest

NP has received contractual fees from GlaxoSmithKline, has received 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 in the GSK Rotavirus Advisory Board. MG has received grants from the Bill & Melinda Gates Foundation and the South African Medical Research Council.

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