# Sapovirus prevalence in children less than five years of age hospitalised for diarrhoeal disease in South Africa, 2009 to 2013

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**Highlights:**

Sapovirus was detected in children hospitalised with acute diarrhoea and in deaths

Sapoviruses are common in males, in the second year of life during summer and autumn

Factors associated with SaV detection included overcrowding and norovirus infections

HIV-infected children with SaV had bloody stool and poor access to sanitation

**Abstract:**

*Background:* Although sapovirus (SaV) has been detected in 2.2% to 12.7% of gastroenteritis cases globally, there are limited data on SaV epidemiology.

*Objectives:* Describe the epidemiology, clinical characteristics and factors associated with SaV gastroenteritis in hospitalised children <5 years of age in South Africa.

*Study design:* Between 2009 and 2013 during prospective diarrhoeal surveillance, stool specimens were collected from four sites and screened for SaVs and associated enteric pathogens using ELISA, microscopy, conventional and real-time PCR. Epidemiological and clinical data were compared in patients with or without SaV. Odds ratios were assessed by bivariate and stepwise multivariable logistic regression analysis.

*Results:* Sapoviruses were detected in 7.7% (238/3103) of children admitted to hospital and 11.4% (9/79) of deaths. Sapovirus was detected more commonly in children 19-24 months compared to <6 months (aOR=2.3; p=0.018) and in males (aOR=2.0; p=0.001). Additional factors associated with SaV detection included residing with ≥ 7 inhabitants compared to ≤3 (aOR=2.2; p=0.011) and concomitant norovirus infections (aOR=3.0; p=0.003). HIV-infected children with SaV were more likely to have bloody stools (aOR=16.8; p<0.001), low birth weight (<2.5kg; aOR=5.8; p=0.007) and live in environments without flush toilets (aOR=8.1; p=0.003) compared to HIV-uninfected children.

*Conclusions:* Sapoviruses, which are perceived to cause mild diarrhoea, were detected in hospitalised children and diarrhoeal deaths in South Africa. Determinants increasing the odds of SaV included overcrowding and concomitant infections while HIV-infected children

with SaV displayed bloody stools, low birth weight and reduced access to proper sanitation. Mitigation strategies against SaV infections include improved sanitation.

**Abbreviations:**

Sapovirus (SaV), norovirus (NoV), norovirus genogroup I (NoV GI), norovirus genogroup II (NoV GII), less than five years of age (<5), 95% confidence interval (95% CI), rotavirus (RV), South Africa (SA), Chris Hani Baragwanath Hospital (CHBH), Mapulaneng Hospital (MPH), Matikwane Hospital (MKH), Edendale Hospital (EDH), World Health Organization (WHO), human immunodeficiency virus (HIV), weight/volume (w/v), odds ratio (OR), adjusted odds ratio (aOR), interquartile range (IQR)

## 1. Background

Sapoviruses (SaV) are associated with acute diarrhoea and outbreaks among all age groups [1]. Along with norovirus (NoV), they are classified in the *Caliciviridae* family [2]. Noroviruses are the second most common cause of virus-associated diarrhoea in children <5 years of age, responsible for an estimated 12% of gastroenteritis [3]. While NoVs have been extensively studied, research on SaV is less advanced [1].

A systematic review of SaVs revealed prevalence in sporadic gastroenteritis cases ranging from 2.2% in children ≤6 years in Kenya to 12.7% in Japan in all ages [1, 4, 5]. In these studies, SaVs were common during the winter months and in younger children [1]. Sapovirus prevalence has only been described in a few sub-Saharan African countries, including Kenya (5.7% in <14 years) [6], Tanzania (5.7-6.4% in <5 years) [7, 8], Malawi (8% in <5 years) [9], South Africa (SA; 4.1-8.4% in all ages) [10, 11], Gabon (9.5% in <5 years) [12] and Burkina Faso (18% in <5 years) [13]. These studies utilized molecular methods for SaV detection with an average prevalence of 7.5% (95% CI 3.4%-11.5%) among diarrhoeal cases.

In addition to clinical case surveillance, river water and sewerage can be monitored for SaV as undertaken in SA [14] and Kenya [15]. Water from three rivers in Gauteng was monitored over two years and SaVs were detected in 18% (3/17), 45% (29/64) and 89% (16/18) of specimens [14]. In Kenya, SaV was detected in 34% (10/29) of river water samples and in 31% (4/13) of urban sewerage samples [15], indicating the widespread distribution of SaVs.

The clinical presentation of SaV is thought to be milder than rotavirus (RV) or NoV [16, 17]. Sapoviruses have been associated with diarrhoea (95% of patients) and vomiting (60% of patients), with clinical symptoms lasting a median of six days in community-based studies in the Netherlands [18]. However, SaVs have also been associated with asymptomatic infections and were detected in 28.6% (16/56) of children in a day-care study in Brazil [19]. Another study in three sites in the United States detected SaVs in 5.4% (42/782) of diarrhoea cases and 4.2% (21/499) of controls [20].

Mortality associated with SaV is rare, but may occur especially among elderly patients [21]. There is currently limited data on SaV mortality and disease in other vulnerable groups including neonates, infants and immunocompromised individuals with HIV infection [1].

Since April 2009, a sentinel diarrhoea surveillance programme was established in SA to monitor diarrhoeal diseases after RV vaccine introduction into the national immunization program. In addition to monitoring changes in RV epidemiology and strain circulation, we undertook clinical and molecular studies on other enteric pathogens in hospitalised children <5 years of age.

## 2. Objectives

This study investigated the prevalence of SaVs between 2009 and 2013 and described the epidemiology and clinical characteristics of hospitalised children <5 years of age with acute diarrhoea testing positive for SaV.

## 3. Study design

### *3.1. Study sites, data and clinical specimen collection*

The prospective surveillance enrolled children <5 years of age who were admitted to sentinel hospitals for diarrhoea treatment. The sentinel sites were located in three provinces (Gauteng, Mpumalanga and Kwa-Zulu Natal) and included: Chris Hani Baragwanath Hospital (CHBH; 2009-2013), Mapulaneng Hospital (MPH; 2009-2013), Matikwane Hospital (MKH; 2009-2013) and Edendale Hospital (EDH; 2010-2013).

Acute diarrhoea was defined using the World Health Organization (WHO) definition of "three looser than normal stools within a 24 hour period", with ≤7 days duration. Children were enrolled daily and followed until discharge (Monday to Friday; 8am-5pm), after obtaining written consent from their parents, using systematic sampling. Surveillance officers collected demographic, socio-economic and risk factor data on standardised questionnaires from parent interviews and medical record reviews. Stool specimens were obtained within 48 hours of admission. Dried blood spots were also collected for anonymised human immunodeficiency virus (HIV) testing in sites where HIV results were not available.

### *3.2. Data management and statistical analysis*

Demographic and clinical characteristics as well as selected factors associated with diarrhoeal disease were compared in patients with or without SaV using STATA 12 (StataCorp LP, College Station TX). We also compared these characteristics in HIV-infected and HIV-uninfected children who tested SaV-positive. Chi-square tests were used to compare categorical data and Wilcoxon rank-sum test to compare medians, with a statistical significance of $p \leq 0.05$. Bivariate analysis and stepwise multivariable logistic regression analysis were performed to identify factors associated with detection of SaVs and for factors associated with HIV infection in SaV positive cases.

*3.3. Laboratory analysis*

Stool specimens (10% w/v suspension in nuclease-free water) were extracted using the QIAamp® Viral RNA Mini Kit (Qiagen Inc., Valencia, CA). Briefly, 10µl extracted RNA was reverse transcribed with random primers using the Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics GmbH, Mannheim, Germany). Specimens were screened for SaV in monoplex reactions using 5µl cDNA, LightCycler® 480 Probe Master Kit (Roche) and primers and probes from a published method [22]. Screening for other enteric viruses, bacteria and parasites has been described elsewhere [23, 24]. Dried blood spots were tested using the AmpliPrep® (Roche) for automated extraction; and COBAS® TaqMan® (Roche) for automated real-time detection of HIV-1.

## 4. Results

The study included 3103 children with 100% screened for SaV, adenovirus, NoV genogroup I and II (NoV GI and GII), astrovirus and bocavirus. Additional enteric pathogens investigated included RV (99.7%; 3093/3103), bacteria (88.6%; 2748/3103) and parasites (48.5%; 1508/3103). Sapovirus was detected in 7.7% (238/3103) of specimens, ranging from 6.3% in 2012 to 8.7% in 2010 (Table 1). Similar SaV prevalence was observed at different sentinel sites (Table 1). Sapovirus was the sole pathogen detected in 2.4% (73/3103) of cases and 2.5% (2/79) of deaths.

Sapovirus seasonality was not pronounced (Fig. 1), although prevalence tended to be higher in summer and autumn (November to April; 10%) compared to winter and spring (May to October; 6%; $p<0.001$).

**Table 1 :**

Bivariate and multivariable analysis of demographic, clinical and risk factors associated with SaV infections. Only variables with p-values <0.2 in the bivariate analysis were reported and included in the multivariable model with the exception of child's HIV status.

| Parameter | Sapovirus prevalence n/N (%) | Bivariate analysis | | Multivariable analysis | |
|---|---|---|---|---|---|
| | | Odds Ratio (OR; 95% Confidence Interval (95% CI)) | p-value | Adjusted OR (aOR; 95% CI) | p-value |
| **Year** | | | | | |
| 2009 | 43/635 (6.8%) | Ref | | | |
| 2010 | 80/919 (8.7%) | 1.3 (0.9-1.9) | 0.166 | | |
| 2011 | 43/553 (7.8%) | 1.2 (0.7-1.8) | 0.506 | | |
| 2012 | 29/459 (6.3%) | 0.9 (0.6-1.5) | 0.765 | | |
| 2013 | 43/537 (8.0%) | 1.2 (0.8-1.9) | 0.419 | | |
| **Sentinel site** | | | | | |
| CHBH | 145/1764 (8.2%) | Ref | | | |
| EDH | 30/310 (9.7%) | 1.2 (0.8-1.8) | 0.395 | | |
| MKH | 44/670 (6.6%) | 0.8 (0.6-1.1) | 0.174 | | |
| MPH | 19/359 (5.3%) | 0.6 (0.4-1.0) | 0.060 | | |
| **Age in months** | | | | | |
| 0-6 | 55/1191 (4.6%) | Ref | | Ref | |
| 7-12 | 83/915 (9.1%) | 2.1 (1.4-2.9) | <0.001 | 1.7 (1.1-2.7) | 0.022 |
| 13-18 | 53/459 (11.6%) | 2.7 (1.8-4.0) | <0.001 | 2.0 (1.2-3.4) | 0.011 |
| 19-24 | 25/232 (10.8%) | 2.5 (1.5-4.1) | <0.001 | 2.3 (1.2-4.5) | 0.018 |
| >24 | 22/302 (7.3%) | 1.6 (1.0-2.7) | 0.064 | 1.7 (0.7-3.9) | 0.177 |
| Male | 156/1781 (8.8%) | 1.4 (1.1-1.9) | 0.009 | 2.0 (1.3-2.9) | 0.001 |
| **Clinical symptoms** | | | | | |
| **Maximum number of stools in 24 hours** | | | | | |
| 1-3 | 55/643 (8.6%) | Ref | | | |
| 4-5 | 124/1602 (7.7%) | 0.9 (0.6-1.2) | 0.520 | | |
| ≥6 | 48/752 (6.4%) | 0.7 (0.5-1.1) | 0.123 | | |
| **Vomiting** | 171/234 (8.2%) | 1.3 (1.0-1.8) | 0.081 | | |
| **Vomiting duration in days** | | | | | |
| 0 | 63/981 (6.4%) | Ref | | | |
| 1 | 36/437 (8.2%) | 1.3 (0.9-2.0) | 0.216 | | |
| 2 | 54/655 (8.2%) | 1.3 (0.9-1.9) | 0.162 | | |
| ≥3 | 78/971 (8.0%) | 1.3 (0.9-1.8) | 0.170 | | |
| **Maximum number of vomits in 24 hours** | | | | | |
| 0 | 63/981 (6.4%) | Ref | | | |
| 1 | 33/337 (9.8%) | 1.6 (1.0-2.5) | 0.041 | | |
| 2-4 | 113/1410 (8.0%) | 1.3 (0.9-1.7) | 0.143 | | |
| ≥5 | 18/233 (7.7%) | 1.2 (0.7-2.1) | 0.474 | | |
| **Admission temperature** | | | | | |
| ≤37°C | 67/942 (7.1%) | Ref | | Ref | |
| 37.1°C – 38.4°C | 44/588 (7.5%) | 1.1 (0.7-1.6) | 0.786 | 1.1 (0.7-1.6) | 0.657 |
| 38.5°C – 38.9°C | 13/82 (15.6%) | 2.5 (1.3-4.7) | 0.006 | 2.7 (1.3-5.3) | 0.005 |
| ≥39°C | 9/92 (9.8%) | 1.4 (0.7-2.9) | 0.351 | 1.3 (0.6-2.8) | 0.445 |
| **Risk factors** | | | | | |
| **Child's HIV status** | | | | | |
| Negative | 187/2467 (7.6%) | Ref | | | |
| Positive | 31/357 (8.7%) | 1.2 (0.8-1.7) | 0.466 | | |
| **Feeding (first 4 months)** | | | | | |
| Breast | 100/1046 (9.6%) | Ref | | | |
| Formula | 48/845 (5.7%) | 0.6 (0.4-0.8) | 0.002 | | |

| | | | | | |
|---|---|---|---|---|---|
| Breast and formula | 9/131 (6.9%) | 0.7 (0.3-1.4) | 0.319 | | |
| **Feeding (after 4 months)** | | | | | |
| Breast | 51/564 (9.0%) | Ref | | | |
| Formula | 92/1361 (6.8%) | 0.7 (0.5-1.0) | 0.083 | | |
| Breast and formula | 10/103 (9.7%) | 1.1 (0.5-2.2) | 0.829 | | |
| Solids | 32/291 (11.0%) | 1.2 (0.8-2.0) | 0.361 | | |
| **Mother's educational status** | | | | | |
| None | 2/43 (4.7%) | Ref | | | |
| Primary school | 13/180 (7.2%) | 1.6 (0.3-7.4) | 0.549 | | |
| Secondary school | 130/1671 (7.8%) | 1.7 (0.4-7.2) | 0.453 | | |
| Tertiary education | 13/108 (12.0%) | 2.8 (0.6-13.0) | 0.187 | | |
| **Housing material** | | | | | |
| Brick | 164/2316 (7.1%) | Ref | | | |
| Tin/wood | 46/529 (8.7%) | 1.2 (0.9-1.8) | 0.201 | | |
| Traditional | 23/207 (11.1%) | 1.6 (1.0-2.6) | 0.036 | | |
| **Number of inhabitants in house** | | | | | |
| ≤3 | 110/1474 (7.5%) | Ref | | Ref | |
| 4-6 | 32/434 (7.4%) | 1.0 (0.7-1.5) | 0.950 | 0.9 (0.6-1.5) | 0.746 |
| ≥7 | 16/116 (13.8%) | 2.0 (1.1-3.5) | 0.017 | 2.2 (1.2-4.2) | 0.011 |
| **Attend crèche** | 35/330 (10.6%) | 1.5 (1.0-2.2) | 0.043 | | |
| **Multiple pathogens** | | | | | |
| **Enteric pathogens present** | | | | | |
| Sapovirus only | 73/1358 (5.4%) | Ref | | Not included in model | |
| Sapovirus + one enteric pathogen | 88/695 (12.7%) | 2.6 (1.8-3.5) | <0.001 | | |
| Sapovirus + two or more enteric pathogens | 77/271 (28.4%) | 7.0 (4.9-10.0) | <0.001 | | |
| Rotavirus | 45/895 (5.0%) | 0.6 (0.4-0.8) | 0.001 | 0.3 (0.2-0.6) | <0.001 |
| Norovirus GI | 13/94 (13.8%) | 2.0 (1.1-3.6) | 0.025 | 3.0 (1.4-6.3) | 0.003 |
| Astrovirus | 23/214 (10.7%) | 1.5 (1.0-2.4) | 0.081 | | |
| Bocavirus | 20/175 (11.4%) | 1.6 (1.0-2.6) | 0.057 | | |
| Bacteria | 77/870 (8.9%) | 1.4 (1.0-1.8) | 0.041 | | |
| Parasites | 21/190 (11.0%) | 1.5 (0.9-2.4) | 0.131 | | |

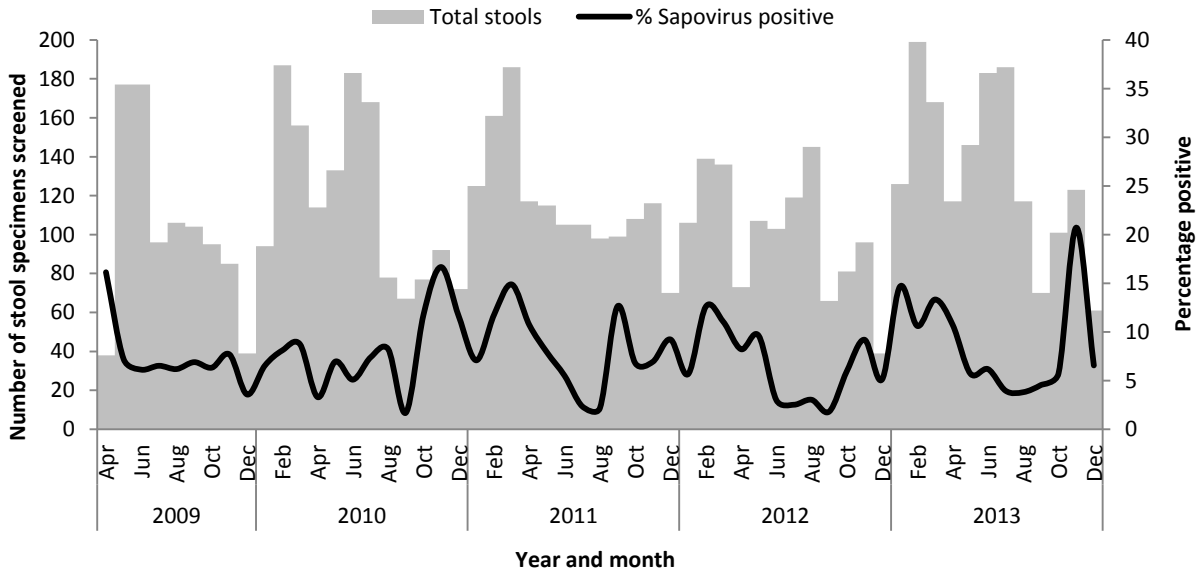


**Fig. 1.** Detection of SaVs in children <5 years in selected sites in South Africa by month between 2009 and 2013.

The median age of SaV detection in children was 11 months (IQR 7-16), two months later than SaV negative cases (8 months; IQR 4-15). Children between seven and 24 months of age displayed 1.7-2.3 times greater odds of having SaV detected compared to children <6 months (p=0.022-0.011; Table 1). Male children were also at greater odds of having SaV detected compared to female children (adjusted odds ratio (aOR) =2.0 (1.1-1.9); p=0.001; Table 1).

There was little difference in the clinical characteristics based on individual and combined Vesikari score variables [25, 26] between SaV positive and negative groups although on multivariable analysis, fever (38.5°C – 38.9°C) was more common in SaV positive patients compared to SaV negative patients (aOR=2.7 (1.3-5.3); p=0.005). In addition, SaV positive cases were admitted for a shorter duration (median of two days (IQR 1-5)) compared to SaV negative cases (median of three days (IQR 1-6); p<0.001).

Factors associated with SaV detection in children that remained statistically significant in multivariable analysis included the numbers of household inhabitants and mixed pathogen infections (Table 1). Children living with seven or more people were at 2.2 times greater odds of having a SaV detected than children living with three people or less (1.2-4.2; p=0.011). In addition, SaV were mostly detected as mixed infections, with children at 2.6 times greater odds of having SaV and one other enteric pathogen (1.8-3.5; p<0.001) detected and at 7.0 times greater odds of having SaV with two or more pathogens (4.9-10.0; p<0.001) detected compared to single SaV infections. However, only RV (aOR=0.3 (0.2-0.6); p<0.001) and NoV GI (aOR=3.0 (1.4-6.3); p=0.003) were significant co-pathogens in multivariable analysis (Table 1). The association between SaV and NoV GI mixed infections remained significant after adjustment for month of collection (aOR=2.9 (1.4-6.1); p=0.006).

The HIV status was determined for 91% (2824/3103) of children. The median age of SaV detection in HIV-infected children was 13 months (IQR 9-19) compared to 11 months (IQR 7-

**Table 2**

Bivariate and multivariable analysis of demographic, clinical and risk factors associated with HIV status in children with SaV detected. Only variables with p-values <0.2 in the bivariate analysis were reported and included in the multivariable model.

| Parameter | HIV-infected prevalence n/N (%) | Bivariate analysis | | Multivariable analysis | |
|---|---|---|---|---|---|
| | | Odds Ratio (OR; 95% Confidence Interval (95% CI)) | p-value | Adjusted OR (aOR; 95% CI) | p-value |
| **Age in months** | | | | | |
| 0-6 | 3/49 (6.1%) | Ref | | | |
| 7-12 | 11/77 (14.3%) | 2.6 (0.7-9.7) | 0.167 | | |
| 13-18 | 9/47 (19.2%) | 3.6 (0.9-14.4) | 0.066 | | |
| 19-24 | 4/25 (16.0%) | 2.9 (0.6-14.2) | 0.185 | | |
| >24 | 4/20 (20.0%) | 3.8 (0.8-19.0) | 0.100 | | |
| **Year** | | | | | |
| 2009 | 3/35 (8.6%) | Ref | | | |
| 2010 | 15/70 (21.4%) | 2.9 (0.8-10.8) | 0.111 | | |
| 2011 | 7/42 (16.7%) | 2.1 (0.5-9.0) | 0.301 | | |
| 2012 | 3/28 (10.7%) | 1.3 (0.2-6.9) | 0.774 | | |
| 2013 | 3/43 (7.0%) | 0.8 (0.2-4.2) | 0.793 | | |
| **Sentinel site** | | | | | |
| CHBH | 11/128 (8.6%) | Ref | | | |
| EDH | 7/28 (25.0%) | 3.5 (1.2-10.2) | 0.019 | | |
| MKH | 8/43 (18.6%) | 2.4 (0.9-6.5) | 0.077 | | |
| MPH | 5/19 (26.3%) | 3.8 (1.2-12.5) | 0.028 | | |
| **Clinical symptoms** | | | | | |
| **Vomiting duration in days** | | | | | |
| ≤2 | 17/142 (12.0%) | Ref | | | |
| >3 | 14/72 (19.4%) | 1.8 (0.8-3.8) | 0.146 | | |
| **Blood in stool** | 7/23 (30.4%) | 3.1 (1.1-8.2) | 0.026 | 16.8 (3.5-79.7) | <0.001 |
| **Risk factors** | | | | | |
| **Birth term** | | | | | |
| Full term | 9/62 (14.5%) | Ref | | | |
| Pre-term (<37 weeks) | 9/24 (37.5%) | 3.5 (1.2-10.5) | 0.023 | | |
| **Birth weight** | | | | | |
| Normal (≥2.5kg) | 15/124 (12.1%) | Ref | | Ref | |
| Low (<2.5kg) | 7/20 (35.0%) | 3.9 (1.3-11.4) | 0.012 | 5.8 (1.6-20.7) | 0.007 |
| **Feeding (after 4 months)** | | | | | |
| Any breast milk | 6/61 (9.8%) | Ref | | | |
| Other | 20/112 (17.9%) | 2.0 (0.8-5.3) | 0.164 | | |
| **Water source** | | | | | |
| Indoor | 6/89 (6.7%) | Ref | | | |
| Outdoor | 25/128 (19.5%) | 3.4 (1.3-8.6) | 0.011 | | |
| **Sanitation** | | | | | |
| Flush | 8/107 (7.5%) | Ref | | Ref | |
| Other | 23/108 (21.3%) | 3.3 (1.4-7.9) | 0.006 | 8.1 (2.0-31.9) | 0.003 |
| **Attend crèche** | 1/34 (2.9%) | 0.1 (0.02-1.1) | 0.057 | | |
| **Outcome** | | | | | |
| Discharged | 27/206 (13.1%) | Ref | | | |
| Died | 3/8 (37.5%) | 4.0 (0.9-17.6) | 0.069 | | |

16) in HIV-uninfected children. HIV-infected children with SaV detected spent a median of four days (IQR 1.7) in hospital compared to two days for HIV-uninfected children (IQR 1.4; p=0.007). Multivariable analysis revealed that HIV-infected children with SaV were 16.8 times more likely to have blood in the stool compared to HIV-uninfected children (3.5-79.7; p<0.001; Table 2). Other factors associated with SaV detection in HIV-infected children included low weight at birth (<2.5kg; aOR=5.8 (1.6-20.7); p=0.007) compared to normal weight at birth (>2.5kg) and using pit latrines, buckets or other sanitation (aOR=8.1 (2.0-31.9); p=0.003) compared to flush toilets (Table 2).

Sapoviruses were detected in 11.4% (9/79) of deaths in children <5, with three of the children HIV-infected. In two of the nine deaths, SaV was the sole pathogen detected with one child HIV-infected. Most of the children (7/9; 78%) who died resided in Mpumalanga Province and did not have access to flush toilets. In addition, although not statistically significant, mortality was higher in the HIV-infected children with SaV (37.5% versus 13.1%) compared to the HIV-uninfected children with SaV detected (Table 2).

## 5. Discussion

The five year SA study in a high HIV prevalence setting showed SaV in 7.7% of children <5 years hospitalized for diarrhoea with the majority infected during their second year of life. The SaV prevalence was similar to the 8.4% in individuals of all ages in Limpopo Province (2007-2008) and double the 4% in hospitalised children in Pretoria in 2008 [10, 11]. The median age of infection in SA was similar to the mean age of infection in Kenya (11 months) [4].

Sapoviruses were detected year round with slight increases during summer and autumn. These results are similar to a Malawian study which demonstrated an autumn peak (March-April) [9] and a Kenyan study where SaVs were detected all year round [4]. These results

are in contrast to studies from the northern hemisphere (Canada, United Kingdom, Denmark and Japan) where SaVs are more frequent during the cold winter season [27].

The severity of SaV infections is considered milder compared to NoV or RV [1]. These observations are supported by this study as SaV positive children were admitted for shorter hospital stays (median of two days) compared to SaV negative cases. However, our surveillance system, which was established to identify hospitalised diarrhoea cases, still detected the “milder” SaV cases. This may be partially due to the predominance of mixed pathogen infections and/or partially due to the presence of fever in patients with SaV (38.5°C-38.9°C; aOR=2.7 (1.3-5.3); p=0.005). A study in the Netherlands has previously demonstrated higher fever prevalence (≥37.5°C; 43%) in SaV positive patients compared to NoV positive patients [18].

Information on SaV disease in immunocompromised individuals is limited [1]. Previous studies indicated that human caliciviruses were detected more frequently in HIV-infected children compared to HIV-uninfected children [28]. In a Kenyan study, SaV was detected in 5.7% (6/105) of HIV-infected children with five different genotypes detected [6]. The current study provides additional information revealing that HIV-infected children with SaV were at greater odds of have being born underweight, having blood in the stool and being admitted to hospital for longer periods compared to HIV-uninfected children with SaV.

Sapovirus mortality has only been reported from outbreaks in elderly long-term-care facilities [1]. Therefore, data on mortality in young children and immunocompromised individuals is lacking. The current study detected SaV in 11.4% (9/79) of diarrhoeal deaths in children <5 years and in two cases were the sole pathogen detected. In addition, SaV was detected in a higher proportion of deaths in HIV-infected compared to HIV-uninfected children (37.5% versus 13.1%) although this result was not statistically significant. Due to the abundance of mixed aetiologies, the low numbers of deaths recorded and the study design, no additional

conclusions could be made regarding mortality attributable to SaV and further study will be required to determine the role of SaV in diarrhoea mortality and HIV-infected children.

Person-to-person transmission of SaV has been documented in a long-term study in a home for infants in Japan [29] and similarly in this study, SaV transmission increased when close contact exposure intensified. Our findings support person-to-person spread as children living in homes with more than seven people were at greater odds of SaV detection than children living under less crowded conditions (aOR=2.2; p=0.011). In addition, poor access to sanitation was associated with increased odds in HIV-infected children with SaV (aOR=8.1 (2.0-31.9); p=0.003) and may indicate another potential source of transmission.

In this study, SaV was detected as the sole pathogen in only a third of cases (73/238). However, only concomitant rotavirus and norovirus GI infections remained statistically significant after adjusting for age, admission temperature and number of household inhabitants. The data suggests an interaction between SaV and NoV GI in mixed infections (aOR=3.0; p=0.003) although additional study will be required to elucidate this relationship. In contrast, SaV detection was associated with decreased odds (aOR=0.3; p<0.001) of a concomitant RV infection, supporting the discrete role of SaV in the overall burden of diarrhoeal disease.

The study has several limitations that should be considered. Missing data were dealt with by pairwise deletion and associations may be overestimated or underestimated. The assessment of the role of SaV in the overall diarrhoea burden and factors associated with SaV detection were limited by the comparison group selection i.e. children without SaV rather than children without diarrhoea. Factors associated with diarrhoea may lead to infections with multiple enteric pathogens and a corresponding inability to discern differences between comparison groups. The study enrolled hospitalised children in public facilities so findings are restricted to moderate to severe diarrhoea and should be extrapolated to less

severe outcomes or other socio-economic strata with care. No additional information was obtained from people who refused to participate or children who did not provide a stool sample, so there may be non-participation biases unaccounted for in the analysis. Parasite screening was only conducted in 48.5% of specimens due to availability which may have resulted in an underestimation of mixed parasite-SaV infections.

This study highlights the role SaV may play in diarrhoeal disease in hospitalised children and the associated deaths. There are currently no vaccines against SaV and intervention strategies to prevent and control SaV include improving sanitation amenities, educating communities on diarrhoeal diseases and hand hygiene and effective management of diarrhoeal diseases at facilities using the WHO Integrated Management of Childhood Illnesses. While the implementation of the Prevention of Mother to Child Transmission of HIV programme in SA has resulted in a decline in babies born with HIV [30], HIV-infected babies experience numerous diarrhoea episodes and require additional care. Continued surveillance will be required to monitor the impact of SaV on the overall diarrhoeal disease burden in SA.

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**Ethical approval**

Human Research Ethics Committee (Medical), University of Witwatersrand (M091018); Biomedical Research Ethics Committee, University of Kwa Zulu-Natal (BF074/09); Faculty of Health Sciences Research Ethics Committee, University of Pretoria (278/2015).

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