Respiratory Viruses in Young South African Children with Acute Lower Respiratory Infections and Interactions with HIV

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Highlights

• Human rhinovirus is the most common virus in cases with pneumonia or bronchiolitis and in controls.

• HIV-infected children were more likely to be diagnosed with pneumonia than bronchiolitis.

• RSV was not identified in any HIV-infected cases compared with one third of HIV-uninfected cases.

Abstract

Background: Human rhinovirus (RV) is the most common respiratory virus and has been associated with frequent and severe acute lower respiratory infections (ALRI). The prevalence of RV species among HIV-infected children in South Africa is unknown.

Objectives: To describe the prevalence of respiratory viruses, including RV species, associated with HIV status and other clinical symptoms in children less than two years of age with and without ALRI in Pretoria, South Africa.

Study Design: Nasopharyngeal aspirates were collected from 105 hospitalized ALRI cases and 53 non-ALRI controls less than two years of age. HIV status was determined. Common respiratory viruses were identified by PCR, and RV species and genotypes were identified by semi-nested PCR, sequencing and phylogenetic tree analyses.

Results: Respiratory viruses were more common among ALRI cases than controls (83.8% vs. 69.2%; p=0.041). RV was the most commonly identified virus in cases with pneumonia (45.6%) or bronchiolitis (52.1%), regardless of HIV status, as well as in controls (39.6%). RV-A was identified in 26.7% of cases and 15.1% of controls while RV-C was identified in 21.0% of cases and 18.9% of controls. HIV-infected children were more likely to be diagnosed with pneumonia than bronchiolitis (p<0.01). RSV was not identified in any HIV-infected cases (n=15) compared with 30.6% of HIV-uninfected cases (n=85, p=0.013), and was identified more frequently in bronchiolitis than in pneumonia cases (43.8% vs. 12.3%; p<0.01).

Conclusions: RV-A and RV-C are endemic in South African children and HIV infection may be protective against RSV and bronchiolitis.

Background

Acute lower respiratory infections (ALRI) account for an estimated 1.3 million deaths each year in children under 5 years of age, 43% of which occur in Africa.[1] Respiratory viruses are the most common cause of respiratory infections in children.[2] Advances in molecular methods including polymerase chain reaction (PCR) have led to increased sensitivity for viral detection and the identification of several new viruses and viral species. However, advanced

diagnostics are largely limited to the developed world and studies investigating a comprehensive range of pathogens are lacking in African countries. The majority of respiratory viral aetiological studies in Africa have utilized traditional cell culture and serological methods, and data for viruses other than respiratory syncytial virus (RSV) are scarce. Studies of ALRI in African children published as recently as 2013 did not screen for common respiratory viruses such as human rhinovirus (RV), coronavirus and bocavirus.[3]

RV is the most common virus identified in children with respiratory infections worldwide and is responsible for upper and lower respiratory tract infections including pneumonia and bronchiolitis. The identification of a third RV species, RV-C, first reported in 2006[4, 5] has led to several investigations of its prevalence, conducted predominantly in developed countries. The majority of these studies in hospitalized children found that RV-C was the most prevalent RV species and was often associated with wheezing, asthma and ALRI.[4, 6-12] Only three studies have investigated the prevalence of RV species in African children,[13-15] one of which was in South African children with acute wheezing illness. Our first hypothesis was that RV, and more specifically RV-C, is the most common and severe cause of ALRI in young South African children.

Much of our understanding on the viral aetiology of childhood ALRI in Africa is based on studies conducted prior to the HIV epidemic that has engulfed many African countries. Evidence suggests that HIV infection plays an important role in the frequency and outcome of ALRI.[16] Pneumonia is the leading cause of morbidity and mortality in HIV-infected children.[17] Few studies have investigated the viral aetiology of ALRI in HIV-infected children. A recent study from South Africa reported that a respiratory virus was identified in the majority of both HIV-infected and HIV-uninfected children, with RV being the most

frequently identified.[18] No studies have investigated RV species in HIV-infected children. Our second hypothesis was that all respiratory viruses, including RV-C, are more prevalent in HIV-infected children than in HIV-uninfected children.

Objectives

The objective of this study was to describe the prevalence of respiratory viruses, including RV species, associated with HIV status and other clinical symptoms in children less than two years of age with and without ALRI from Pretoria, South Africa.

Study Design

Study population

A prospective case-control study was conducted between July 2011 and November 2012 in Pretoria, South Africa. Children 0-2 years of age admitted to the Steve Biko Academic Hospital or Tshwane District Hospital and diagnosed with ALRI were enrolled as cases and there were no other exclusion criteria. A diagnosis of pneumonia or bronchiolitis was determined by the treating physician using standard diagnostic procedures. Pneumonia was diagnosed in children with respiratory distress and either chest X-ray changes (e.g. consolidation or effusion) or auscultatory findings (e.g. crepitations or bronchial breathing) while bronchiolitis was diagnosed in children with respiratory distress and at least one of the following; wheeze, chest X-ray changes (e.g. hyperinflation) or Hoover's sign (inward movement of the lower rib cage during inspiration). Age-matched children presenting to the same hospitals with a non-respiratory illness or injury over the same period were enrolled as controls. Control children were hospitalized for non-respiratory illnesses including epilepsy, febrile convulsions, gastroenteritis, elective cardiac surgery, cardiac catheterization and pre-

existing neurological problems. Exclusion criteria for controls included current signs or symptoms of respiratory illness.

Data and sample collection

A nasopharyngeal aspirate (NPA) was collected from each child on the day of recruitment. If HIV-infection was not already documented in hospital records, HIV was tested for using an enzyme-linked immunosorbent assay (ELISA). If antibodies to HIV were positive, HIV-infection was confirmed with by PCR (HIV-1 DNA Amplicor test (version 1.5, Roche Molecular Systems, Inc., Branchburg, NJ)). A demographic and clinical questionnaire was administered by the study doctor to parents or guardians of enrolled cases and controls.

Virus detection

Common respiratory viruses (adenovirus, RSV, bocavirus, coronavirus, parainfluenza viruses, influenza viruses and metapneumovirus) were identified using a tandem multiplex real-time PCR assay as previously described.[19] RV identification and genotyping was based on a molecular method to determine RV genotypes and to differentiate closely related enteroviruses from RV.[20] Viral RNA was extracted from a 240µl volume of NPAs using the QIAGEN QIAamp Viral RNA Mini Kit (Spin protocol), reverse transcribed to cDNA, and used for the PCR amplification of a 260-bp variable sequence in the 5′ non-coding region of the RV genome using in-house designed primers. The DNA sequence of the PCR products was determined by the Australian Genome Research Facility. Genotypes were assigned based on comparisons of the 5′ non-coding region sequences with those of 101 classical serotypes as well as 52 newly identified genotypes using ClustalX software (Conway Institute, University College Dublin, Dublin, Ireland). Representative samples of each

genotype have previously been sequenced at the VP4-VP2 coding region to confirm the species assignment.[21, 22]

Statistical analyses

Demographic and clinical symptoms associated with virus detection were examined using chi-squared (x^2) or Fisher's exact tests (categorical variables) and analysis of variance (ANOVA) models (continuous variables). Statistical analyses were performed using SPSS version 22.0 (SPSS Inc., Chicago, ILL, USA) and a p-value <0.05 was considered statistically significant.

Results

Population demographics

One hundred and five ALRI cases and 53 controls were included in the current analyses. HIV infection was more common among ALRI cases than among controls (Table 1). There

Table 1. Population demographics of ALRI cases and controls.

	ALRI Cases (n = 105)	**Pneumonia (n = 57)**	**Bronchiolitis (n = 48)**	**Non-ALRI Controls (n = 53)**	**p-value[a]**
Male, n (%)	71 (67.6%)	37 (64.9%)	34 (70.8%)	30 (56.6%)	0.173
Age at recruitment in months, mean (SD)	7.14 (5.75)	7.96 (6.26)	6.17 (4.98)	8.80 (7.08)	0.115
Ethnicity- "black", n (%)	83 (79.0%)	49 (86.0%)	34 (70.8%)	44 (83.0%)	0.554
HIV-infected,b n (%)	15 (15.0%)	14 (25.0%)	1 (2.3%)	2 (4.1%)	0.049

Bold values signify the two groups that were being compared and were significantly different as shown by the p-value.

[a] p-Value comparing ALRI cases and non-ALRI controls.

[b] HIV status for nine children was unknown.

were no other differences in the population demographics between the cases and controls. Of the 105 ALRI cases, 57 (51%) were diagnosed with pneumonia (64.9% male) and 48 (46%) were diagnosed with bronchiolitis (70.8% male). Of the 15 HIV-infected cases, only one was diagnosed with bronchiolitis (6.7%) and the rest with pneumonia (93.3%) (p=0.002).

Respiratory virus identification

Of the 158 NPAs from 105 cases and 53 controls, at least one respiratory virus was identified in 88 (83.8%) cases and 37 (69.8%) controls (p=0.041). Table 2 compares viral frequencies between cases and controls and between pneumonia and bronchiolitis cases. RV was the most common respiratory virus identified in both pneumonia (45.6%) and bronchiolitis cases (52.1%; Figure 1) and in controls (39.6%; Table 2). Among the pneumonia cases, adenovirus (31.6%) was the next most commonly identified virus, while among the bronchiolitis cases, RSV (43.8%) was the next most commonly identified virus. RSV was more common in bronchiolitis cases (43.8%) than in pneumonia cases (12.3%; p<0.001, Figure 1) and more common in bronchiolitis cases (43.8%) than controls (17.0%; p=0.003). RSV was also more common among HIV-uninfected cases than among HIV-infected cases (30.6% vs. 0%, p=0.013; Figure 2). Among HIV-uninfected children RSV was identified in 34.1%, 40.9% and 10.5% of children aged <6 months (n=44), 6-<12 months (n=22) and 12-<24 months (n=19), respectively. However, no RSV was identified in HIV-infected children <6 months of age (n=7), 6-<12 months of age (n=4) or 12-<24 months of age (n=4).

Table 2.Viruses and viral species identified in nasopharyngeal aspirates of cases compared with controls and pneumonia cases compared with bronchiolitis cases.

	Cases (n = 105)	**Controls (n = 53)**	**p-value**	**Pneumonia (n = 57)**	**Bronchiolitis (n = 48)**	**p-value**
Any virus	88 (83.8%)	37 (69.8%)	0.041	45 (78.9%)	43 (89.6%)	0.141
Rhinovirus (RV)a	51 (48.6%)	21 (39.6%)	0.286	26 (45.6%)	25 (52.1%)	0.509
RV-A	28 (26.7%)	8 (15.1%)	0.242	15 (26.3%)	13 (27.1%)	0.653
RV-B	0 (0%)	2 (3.8%)	0.117	0 (0%)	0 (0%)	0.356

	Cases (n = 105)	Controls (n = 53)	p-value	Pneumonia (n = 57)	Bronchiolitis (n = 48)	p-value
RV-C	22 (21.0%)	10 (18.9%)	0.850	10 (17.5%)	12 (25.0%)	0.440
Respiratory syncytial virus (RSV)	28 (26.7%)	9 (17.0%)	0.175	7 (12.3%)	21 (43.8%)	0.000
RSV-A	18 (17.1%)	8 (15.1%)	0.743	5 (8.8%)	13 (27.1%)	0.013
RSV-B	10 (9.5%)	1 (1.9%)	0.075	2 (3.5%)	8 (16.7%)	0.022
Adenovirus	33 (31.4%)	15 (28.3%)	0.687	18 (31.6%)	15 (31.3%)	0.971
Adenovirus B	16 (15.2%)	4 (7.5%)	0.170	9 (15.8%)	7 (14.6%)	0.864
Adenovirus C	27 (25.7%)	13 (24.5%)	0.871	15 (26.3%)	12 (25.0%)	0.878
Bocavirus	23 (21.9%)	14 (26.4%)	0.527	12 (21.1%)	11 (22.9%)	0.818
Coronavirus	13 (12.4%	6 (11.3%)	0.847	7 (12.3%)	6 (12.5%)	0.973
Coronavirus- 0C43	12 (11.4%)	6 (11.3%)	0.984	6 (10.5%)	6 (12.5%)	0.751
Coronavirus- 229E	0 (0%)	0 (0%)		0 (0%)	0 (0%)	
Coronavirus- HKU1	0 (0%)	1 (1.9%)	0.158	0 (0%)	0 (0%)	
Coronavirus- NL63	1 (1.0%)	0 (0.0%)	0.476	1 (1.8%)	0 (0%)	0.356
Metapneumovirus	7 (6.7%)	3 (5.7%)	0.806	5 (8.8%)	2 (4.2%)	0.346
Influenzab	8 (7.6%)	2 (3.8%)	0.349	6 (10.5%)	2 (4.2%)	0.221
Influenza A H1N1	0 (0%)	0 (0%)		0 (0%)	0 (0%)	
Influenza A H3HA	5 (4.8%)	1 (1.9%)	0.372	3 (5.3%)	2 (4.2%)	0.793
Influenza B	4 (3.8%)	1 (1.9%)	0.514	4 (7.0%)	0 (0%)	0.061
Influenza C	0 (0.0%)	1 (1.9%)	0.158	0 (0%)	0 (0%)	
Parainfluenza virus 1–4	10 (9.5%)	3 (5.7%)	0.404	6 (10.5%)	4 (8.3%)	0.703

Bold values signify the two groups that were being compared and were significantly different as shown by the p-value.

[a] RV overall and RV species numbers differ because two HRV-positive specimens were not genotyped.
[b] Some children with adenovirus, coronavirus or influenza virus had two sub-types (e.g. one influenza-positive specimen was positive for both Influenza A H3HA and Influenza B.

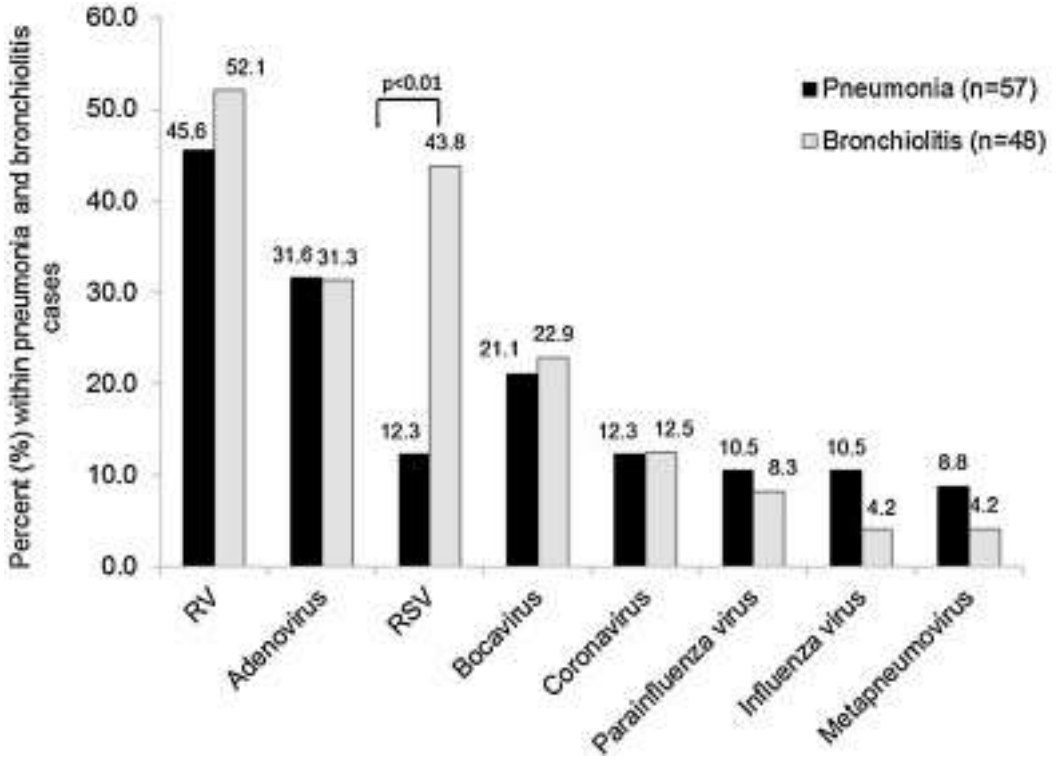

Figure 1 Respiratory viruses identified in nasopharyngeal aspirates of pneumonia (n=57) and bronchiolitis (n=48) cases.

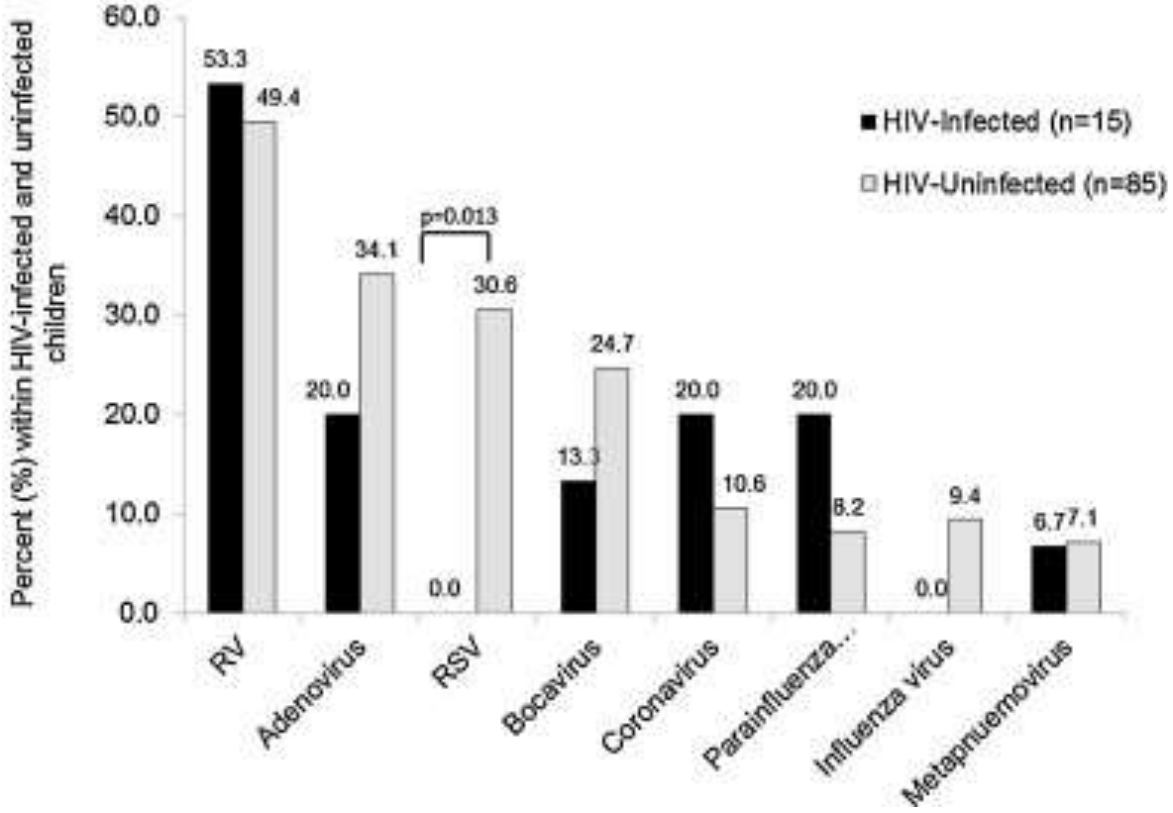

Figure 2 Respiratory viruses identified in nasopharyngeal aspirates of HIV-infected (n=15) and HIV-uninfected cases (n=85). Cases with unknown HIV status excluded.

Rhinovirus species and genotypes

Seventy of 72 (97%) RV-positive NPAs from cases and controls were successfully genotyped, of which 36 (51.4%) were RV-A, 2 (2.86%) were RV-B and 32 (45.7%) were RV-C (Figure 3). The frequencies of each genotype identified in cases and controls are listed in Supplementary Table 4. No single genotype was identified more than five times in this population, with only one RV-A genotype and one RV-C genotype being identified five times. There was no difference in the prevalence of RV species between ALRI cases and controls, pneumonia and bronchiolitis cases (Table 2) or HIV-infected and HIV-uninfected children.

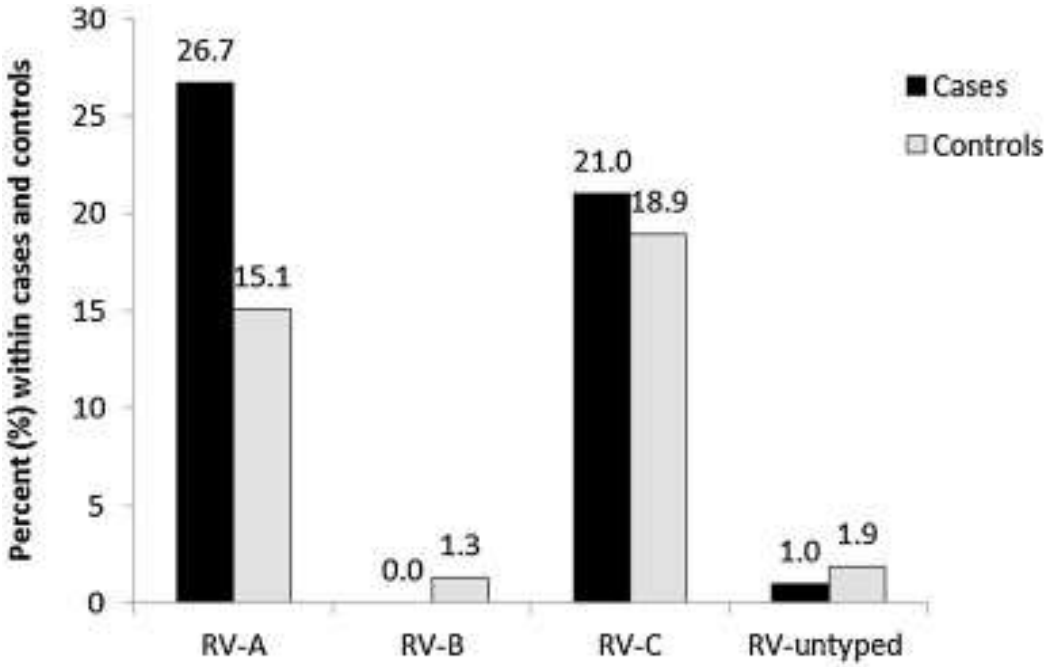

Figure 3 Human rhinovirus species identified in ALRI cases (n=105) and controls (n=53).

Respiratory viral co-infections

Of the 105 cases with at least one respiratory virus identified, 34 (32.4%) had a single virus infection, while co-infection of 2, 3, 4 or 5 viruses was identified in 32 (30.5%), 14 (13.3%), 7 (6.7%) and 1 (1.0%) cases respectively. Of the 53 controls with more than one respiratory

virus identified, 13 (25.0%) had a single virus infection, while co-infection of 2, 3, 4 or 5 viruses was identified in 15 (26.9%), 7 (13.5%), 1 (1.9%) and 1 (1.9%) controls respectively. RV-adenovirus was the most common viral co-infection in both cases (16.2%) and controls (9.4%).

Clinical symptoms

There were significantly more HIV-infected children among the pneumonia than bronchiolitis cases and wheeze was reported more frequently among bronchiolitis than pneumonia cases (Table 3). RSV was positively associated with wheeze ($p<0.01$) and negatively associated with HIV infection ($p=0.013$) and runny nose ($p<0.047$; Supplementary Table 1). No other clinical associations were observed for any other virus including RV species or number of co-infections (Supplementary Tables 1, 2 and 3).

Table 3. Associations between ALRI diagnosis and clinical symptoms among cases.

	Pneumonia (n = 57)	**Bronchiolitis (n = 48)**	**p-value**
HIV-infected	14 (24.6%)	1 (2.3%)	0.002
Cough	52 (91.2%)	46 (95.8%)	0.346
Wheeze	14 (24.6%)	27 (56.3%)	0.001
Shortness of breath	44 (77.2%)	38 (79.2%)	0.808
Fever	31 (54.4%)	29 (60.4%)	0.534
Weak and tired	36 (63.2%)	21 (43.8%)	0.047
Runny nose	22 (38.6%)	25 (52.1%)	0.166
Nasal Congestion	35 (61.4%)	31 (64.6%)	0.737
Sneeze	18 (31.6%)	18 (37.5%)	0.524

Bold values signify the two groups that were being compared and were significantly different as shown by the p-value.

Other factors

Age

Comparing viral identification between four age groups (0-<6 months (n=54), 6-<12 months (n=28), 12-<18 months (n=16) and 18-<24 months (n=7)) for ALRI cases, identification of at least one respiratory virus increased with age ($p=0.001$, Figure 4). Adenovirus and bocavirus

were more common among children aged 6-<18 month than among children in the 0-<6 and 18-<24 month age groups (p<0.01 and p=0.014 respectively). Influenza and metapneumovirus increased with age (p=0.021 and p=0.040 respectively). Although not statistically significant, RV detection was highest at 18-<24 months and RSV detection was highest at 6-<12 months.

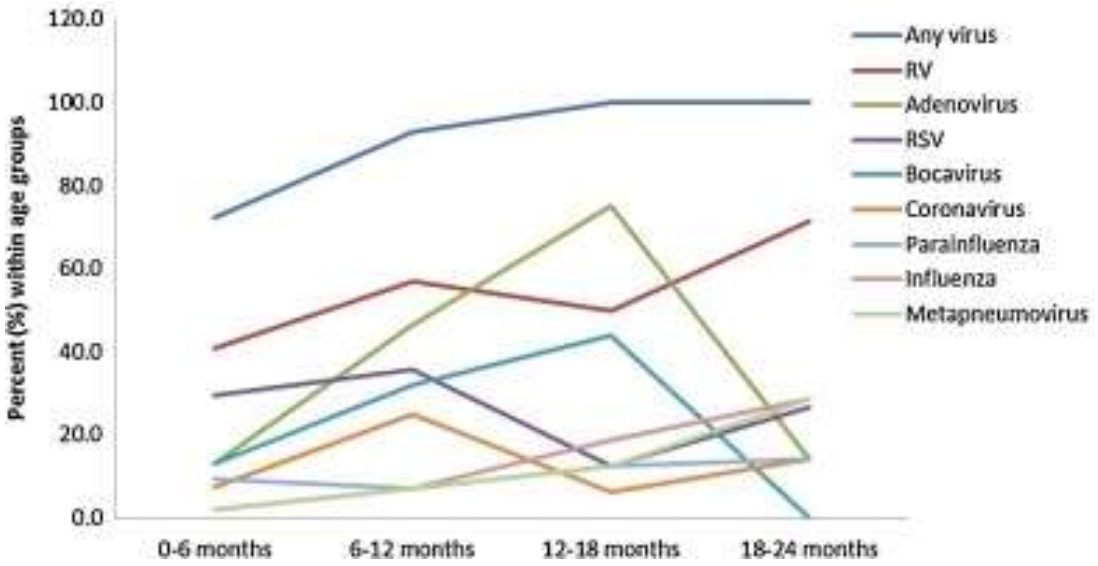

Figure 4 Identification of respiratory viruses by age group in ALRI cases.

Seasonality

More children were recruited during the winter (June to August, 33.8%) and spring (September to November, 33.8%) than autumn (March to May, 20.0%) and summer (December to February, 12.5%). Cases and controls were recruited equally throughout the four seasons (p=0.223). Comparing viral identification between the four seasons, overall among the cases and controls, we observed significant seasonality patterns for RSV, coronavirus, influenza and metapneumovirus. RSV was identified more often in autumn (43.2%) and winter (48.6%) than in spring (8.1%) and summer (0%, p <0.001), coronavirus was identified more often in winter (57.9%) and spring (36.8%) than in autumn (5.3%) and summer (0%, p=0.035), influenza was identified most in spring (80%, p=0.012) and

metapneumovirus was identified more often in autumn (50.0%) and winter (40.0%) than in spring (10.0%) and summer (0%, p=0.050). RV was equally prevalent throughout the seasons. In cases alone, the observed seasonality patterns were significant for RSV (p<0.01) and influenza (p<0.01). No seasonality patterns were observed for controls alone.

Discussion

Identification of at least one respiratory virus was more common among ALRI cases compared with controls (84% vs. 70%). We identified a higher prevalence of respiratory viruses as well as viral co-infections (51% in cases and 44% in controls) than previously reported in young children with or without ALRI in South Africa.[18, 23, 24] However, comparable rates have been reported in paediatric ALRI populations outside South Africa,[25] and a possible explanation for this may be environmental or genetic factors. The high viral identification rate among controls challenges the pathogenic role of some viruses. However, viral identification was more common among cases than controls, and therefore supports literature on the importance of viruses in ALRI. RSV was more common in bronchiolitis cases compared with pneumonia cases or with controls, reaffirming the role of RSV in bronchiolitis.

RV was the most common respiratory virus identified in ALRI cases as well as in controls. RV-A and RV-C were of similar prevalence, challenging our hypothesis that RV-C is the most common cause of ALRI in South African children. In the only other study of RV species in South African children, RV-C was the most prevalent.[13] However, Smuts *et al* investigated young children with acute wheezing illness rather than ALRI. Only two studies investigated the prevalence of RV species in children with ALRI in Africa with one reporting RV-A as the most common species in children with ALRI[15] and the other finding no

difference in the prevalence between RV-A and RV-C between ALRI cases and controls or between ALRI severity groups.[14] Further investigations including different ALRI clinical groups may help ascertain the role of RV species in ALRI.

This is the first study to describe the prevalence of RV species among HIV-infected and HIV-uninfected young South African children with ALRI. Contrary to our hypothesis, respiratory viruses were not more common in HIV-infected children than in HIV-uninfected children. Surprisingly, RSV was more common in HIV-uninfected children than in HIV-infected children. Consistent with our findings, a recent study from South Africa also found that RSV and metapneumovirus were identified less frequently in HIV-infected children than in HIV-uninfected children.[26] However, Moyes *et al* found that HIV-infected children in South Africa had a higher risk of hospitalization with RSV-associated ALRI than HIV-uninfected children.[27] In another South African study, HIV-infected children had a higher prevalence of KI polyomaviruses and coronavirus-OC43 and a lower prevalence of human bocavirus and WU polyomaviruses than in HIV-uninfected children.[18] There are limited viral data on HIV-infected children with ALRI[24, 28] and further studies including a larger HIV-infected cohort are needed.

Of the 15 HIV-infected ALRI cases, only one had bronchiolitis. An under-representation of HIV-infected children among bronchiolitis cases has been reported previously in South Africa.[16, 29] It was suggested that altered host responses result in differing clinical presentations between HIV-infected and uninfected children.[29] The role of HIV-infection on respiratory outcome requires further investigation.

Multiple viral identifications were common in both cases and controls. There were no associations between the number of viruses and clinical symptoms among ALRI cases. There is conflicting evidence regarding the association between multiple viral identifications and disease severity with some studies reporting an association[30-36] and others reporting no differences.[37, 38] Using molecular methods, we are unable to differentiate sub-clinical infection from pathogen-specific disease. Further investigations including viral load may contribute to understanding the role of co-infections.

Identification of at least one respiratory virus was more common in children 6-18 months of age compared with younger children (0-6 months of age) or older children (18-24 months of age). One explanation is increased protection by maternal antibodies during the first 6 months of life which decreases in the following year as infants begin to produce more of their own antibodies which reach protective levels after 1-2 years of age. Our findings confirm that age is an important risk factor for viral respiratory infection.

A limitation of the study is the small sample size, particularly among the HIV-infected group. Secondly, the use of hospital controls rather than community controls may contribute to the high viral identification rate among controls. Thirdly, although nasopharyngeal identification of viruses has been associated with lower respiratory tract infections, it also occurs among asymptomatic individuals and hence, may not be entirely representative of the aetiological agents of the lower airway. Since we cannot differentiate asymptomatic from symptomatic infection, a virus-positive NPA suggests, but does not prove causation. Studies in larger cohorts including HIV-infected children are needed to better understand the role of respiratory viruses in ALRI and HIV. Nonetheless, this study has contributed to our

understanding of the epidemiology and aetiology of respiratory viruses and interactions with HIV in children hospitalized with ALRI in South Africa.

Funding

This study was supported by grants from the National Health and Medical Research Council and the National Research Foundation South Africa.

Competing Interests

The authors have no competing interests to declare.

Ethical Approval

This study was approved by the University of Western Australia Human Research Ethics Committee and University of Pretoria Ethics Committees prior to commencement. Written informed consent was obtained from parents or guardians prior to participation.

Acknowledgments

The authors thank all the children and families who participated in the study. This study resulted from the collaborative work of groups from the School of Paediatrics and Child Health, Faculty of Medicine, Dentistry and Health Sciences, University of Western Australia and the University of Pretoria, South Africa.

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Supplementary Table 1 Associations between viral identification and clinical symptoms among cases.

	RV (n=51)	Adenovirus (n=33)	RSV (n=28)	Bocavirus (n=23)	Coronavirus (n=13)	Parainfluenza virus (n=10)	Influenza virus (n=8)	Metapneumovirus (n=7)
HIV-infected	8 (16.0%)	3 (9.4%)	**0 (0.0%)***	2 (8.7%)	3 (25.0%)	3 (30.0%)	0 (0.0%)	1 (14.3%)
Cough	50 (98.0%)	30 (90.0%)	27 (96.4%)	22 (95.7%)	12 (92.3%)	10 (100.0%)	8 (100.0%)	7 (100.0%)
Wheeze	22 (43.1%)	14 (42.4%)	**18 (64.3%)****	10 (43.5%)	2 (15.4%)	3 (30.0%)	1 (12.5%)	1 (14.3%)
Shortness of breath	41 (80.4%)	27 (81.8%)	21 (75.0%)	16 (69.6%)	10 (76.9%)	7 (70.0%)	7 (87.5%)	5 (71.4%)
Fever	29 (56.9%)	22 (66.7%)	15 (53.6%)	17 (73.9%)	8 (61.5%)	5 (50.0%)	5 (62.5%)	5 (85.7%)
Weak and tired	25 (49.0%)	19 (57.6%)	12 (42.9%)	13 (56.5%)	8 (61.5%)	5 (50.0%)	6 (75.0%)	5 (71.4%)
Runny nose	24 (47.1%)	17 (51.5%)	**17 (60.7%)*****	10 (43.5%)	5 (38.5%)	3 (30.0%)	5 (62.5%)	5 (71.4%)
Congestion	34 (66.7%)	20 (60.6%)	14 (50.0%)	16 (69.6%)	9 (69.2%)	5 (50.0%)	5 (62.5%)	6 (85.7%)
Sneeze	18 (35.3%)	8 (24.2%)	13 (46.4%)	7 (30.4%)	2 (15.4%)	2 (20.0%)	3 (37.5%)	3 (42.9%)

p-value comparing clinical symptoms between virus-positive and virus-negative for each virus
* RSV HIV $p = 0.013$ (negative association)
** RSV wheeze $p<0.01$
*** RSV runny nose $p = 0.047$

Supplementary Table 2 Associations between human rhinovirus species identification and clinical symptoms (cases).

	RV-A n=28	RV-C n=22	RV-negative n=54	p-value
HIV-infected	6 (21.4%)	2 (9.09%)	7 (13.0%)	0.490
Cough	27 (96.4%)	22 (100.0%)	48 (88.9%)	0.159
Wheeze	10 (35.7%)	12 (54.5%)	19 (35.2%)	0.263
Shortness of breath	21 (75.0%)	19 (86.4%)	41 (75.9%)	0.556
Fever	12 (42.9%)	16 (72.7%)	31 (57.4%)	0.105
Weak and tired	12 (42.9%)	12 (54.5%)	32 (59.3%)	0.368
Runny nose	10 (35.7%)	13 (59.1%)	23 (42.6%)	0.240
Congestion	17 (60.7%)	17 (77.3%)	32 (59.3%)	0.315
Sneeze	11 (39.3%)	6 (27.3%)	18 (33.3%)	0.670

*No significant differences for RV-A or RV-C vs. all other viruses

Supplementary Table 3 Associations between number of viruses identified and clinical symptoms (cases).

	No virus n=17	1 virus n=34	2 viruses n=32	≥3 viruses n=22	p-value
HIV-infected	3 (17.6%)	5 (14.7%)	6 (19.2%)	1 (5.4%)	0.466
Cough	14(82.4%)	33 (97.1%)	29 (90.6%)	22 (100%)	0.112
Wheeze	4 (23.5%)	13 (38.2%)	16 (50.0%)	8 (36.4%)	0.333
Shortness of breath	15 (88.2%)	27 (79.4%)	20 (64.5%)	19 (86.4%)	0.184
Fever	10 (58.8%)	16 (47.1%)	19 (59.4%)	15 (68.2%)	0.459
Weak and tired	12 (70.6%)	17 (50.0%)	14 (43.8%)	14 (63.6%)	0.233
Runny nose	6 (35.3%)	13 (38.2%)	17 (53.1%)	11 (50.0%)	0.503
Congestion	10 (58.8%)	23 (67.6%)	20 (64.5%)	13 (59.1%)	0.899
Sneeze	8 (47.1%)	11 (32.4%)	10 (31.3%)	7 (31.8%)	0.687

Supplementary Table 4 Human rhinovirus genotypes (and frequency) identified in cases and controls

RV-A Genotypes	Frequency (Cases, Controls)	RV-B Genotypes	Frequency (Cases, Controls)	RV-C Genotypes	Frequency (Cases, Controls)
R01B	(2,0)	R03	(0,1)	W03 (C14)	(2,0)
R02	(2,1)	R06	(0,1)	W05 (C25)	(1,2)
R12	(3,0)			W12 (C02)	(4,1)
R13	(0,1)			W16 (C42)	(1,2)
R18	(1,1)			W19 (C46)	(0,1)
R25	(1,0)			W20 (C28)	(1,1)
R28	(4,1)			W23 (C11)	(1,0)
R34	(1,0)			W24 (C1/C3/C6)	(3,1)
R36	(1,0)			W26 (Cpat10)	(3,0)
R43	(2,0)			W30 (C31)	(1,0)
R44	(0,1)			W31 (C09)	(0,1)
R45	(1,0)			W37 (C26)	(2,0)
R49	(1,0)			W40 (C35)	(0,1)
R51	(1,0)			W41 (C19)	(1,0)
R54	(1,1)			W50 (C34)	(2,0)
R59	(1,0)				
R71	(2,0)				
R75	(1,0)				
R80	(2,0)				
R81	(0,1)				
R89	(0,1)				
Undetermined	(1,0)				