Severity of Respiratory Syncytial Virus Lower Respiratory Tract Infection with Viral Coinfection in HIV-uninfected Children

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Running title: RSV Viral Coinfection and Severity

Key Points: It is still not clear why some children have life-threatening RSV disease. We found that RSV and any viral coinfection compared to RSV monoinfection is not associated with more severe disease. However, increased life-threatening disease in RSV-ADV and RSV-Infl coinfection warrants further study.

Abstract

Background: Molecular diagnostics enable sensitive detection of respiratory viruses but their clinical significance remains unclear in pediatric lower respiratory tract infections (LRTI). We aimed to determine whether viral coinfections increased life-threatening disease in a large cohort.

Methods: Molecular testing was performed for respiratory viruses in nasopharyngeal aspirates collected from children aged <5 years within 24 hours of hospital admission during sentinel surveillance for Severe Acute Respiratory Illness (SARI) hospitalisation conducted in South Africa during February 2009−December 2013. The primary outcome was life-threatening disease defined as mechanical ventilation, intensive care unit admission or death.

Results: Of 2,322 HIV-uninfected children with respiratory syncytial virus (RSV)-associated lower respiratory tract infection (LRTI), 1330 (57.3%) had RSV monoinfection, 38 (1.6%) had life-threatening disease, 575 (24.8%) had rhinovirus (RV), 347 (14.9%) had adenovirus (ADV) and 30 (1.3%) had influenza virus (Infl). RSV and any other viral coinfection was not associated with severe disease (OR: 1.4; 95% 0.7–2.6), ADV coinfection had increased odds of life-threatening disease
(aOR: 3.4, 95%CI: 1.6 – 7.2, p=0.001), and Infl coinfection had increased odds of life-threatening disease and prolonged length of stay (aOR: 2.1,95%CI: 1.0–4.5, p=0.05) compared to RSV monoinfection.
Conclusion: RSV coinfection with any respiratory virus is not associated with more severe disease when compared to RSV alone in this study. However, increased life-threatening disease in RSV-ADV and RSV-Infl coinfection warrants further study.

Key words: respiratory syncytial virus, viral coinfection, lower respiratory tract infection

Introduction

Respiratory syncytial virus (RSV) is a global health problem, causing an estimated 66,000 to 199,000 deaths per year globally in children less than 5 years of age(1). The clinical manifestations of RSV infection range widely from a mild, self-limiting upper respiratory tract infection (URTI) to severe lower respiratory tract infection (LRTI) which may lead to death. Risk factors for severe disease include premature birth, low birth weight immunocompromised status, chronic lung disease, congenital heart disease, HIV-infection, and Down syndrome(2–8); however, the majority of infants hospitalized for RSV LRTI are previously healthy children(9).

Currently, management options for RSV-associated disease are limited, with supportive treatment as the cornerstone of clinical care(10). Therefore, it is essential to gain insight into factors contributing to disease severity in order to effectively direct future preventive and therapeutic interventions.

The development of sensitive molecular diagnostics for the detection of respiratory viruses has given insight into the viral respiratory dynamics during severe respiratory infection(11). There are conflicting data on whether viral coinfection results in more severe RSV-associated LRTI. Whereas some studies report an association for RSV-human metapneumovirus (HMPV) coinfection and less severe disease(12–14), others report more severe disease associated with RSV-HMPV, RSV-rhinovirus (RV), RSV-adenovirus (ADV), and any coinfection compared to identification of RSV alone (15–21). Furthermore, no association with disease severity for RSV-RV, RSV-HMPV, and any viral coinfection have been reported by others(13,22–25). The majority of these studies are limited by assessment over a single season(15,18,26), lack of adjustment for confounders, and small sample size (n=38–666 RSV cases), all of which could bias the interpretation of the results.

The aim of this study was to evaluate the effect of respiratory viral coinfection on disease severity among children hospitalized with RSV-associated LRTI.

Methods

Study Site, Design, and Population

Children less than 5 years of age hospitalized with severe acute respiratory illness (SARI) were enrolled in a prospective, hospital-based, sentinel surveillance study conducted at six sites in four provinces in South Africa from February 2009 through December 2013 as described elsewhere(27). Four rural, peri-urban and urban hospital sites enrolled children in three provinces (Gauteng, Mpumalanga, KwaZulu-Natal) and two sites were added in a fourth province (North West) in June 2010. There were a total of 24 pediatric ICU beds available across all sites.

Data Collection and Case Definition

SARI was defined among hospitalized children as: physician-diagnosed sepsis or LRTI in children aged 2 days to 3 months; or physician-diagnosed LRTI in children aged 3 months to 5 years, presenting within 7 days of symptom onset. Exclusion criteria were transfer from another hospital, neonates who were never discharged after delivery, and children residing outside of the hospital catchment area. A nasopharyngeal aspirate (NPA) in 4 milliliters normal saline and a blood sample were collected from the child ideally within 24 hours of admission but up to 7 days after onset of symptoms. Specimens were transported within 72 hours of collection to the National Institute for Communicable Diseases (NICD) in Johannesburg for viral and bacterial analysis. Demographic and hospitalization data were collected by interview and record review and children were followed up to hospital discharge.

Laboratory Testing

RSV infection was confirmed via multiplex real-time reverse-transcription polymerase chain reaction (PCR) assay performed on collected NPAs. NPAs were also tested for 8 other viruses; ADV, parainfluenza virus 1, 2, and 3 (PIV 1-3), influenza A and B viruses (infl), HMPV, RV and enterovirus (EV) with the same molecular testing technique(28). RV clades A, B, and C were detected in the primer set utilized(29). ADV testing was not done from August-October 2009 due to limited availability of reagents(28). PCR data were semi-quantitative and specimens with a Ct value <37 were considered positive. To detect pneumococcal infection both blood culture for *Streptococcus pneumoniae* and whole blood *lytA* PCR were performed on blood specimens, although blood cultures were not systematically performed on all patients(30). HIV testing was performed on a whole blood specimen or dried blood spot using a HIV PCR assay for children <18 months of age and HIV enzyme-linked immunosorbent assay (ELISA) for children ≥18 months of age. QMCD external quality assessment for all viruses in the panel were performed as well as annual WHO panels for influenza alongside live and post hoc data quality checks.

Outcomes

The primary outcome of this study, life-threatening disease, was defined as a composite outcome of mechanical ventilation, ICU admission or death. The secondary outcome was life-threatening disease or prolonged length of hospital stay (LOS) ≥ 5 days.

Statistical Analyses

Continuous variables were described using mean (standard deviation) or median (interquartile range). Differences in mean/median of continuous variables were tested with the two-sided t-test or a non-parametric Mann-Whitney test when appropriate. Categorical variables were described with

frequencies and percentages and compared between groups using χ^2 test or Fisher's Exact test if there were less than 5 observations in one group.

Logistic regression was used to assess the association between any viral co-infection (at least one of the follwing viruses detected: HMPV, RV, ADV, EV, Infl, PIV1, PIV2, PIV3) and virus-specific co-infections on the study outcomes as described above among RSV-positive children. In addition, we compared ADV-RSV and Infl-RSV coinfections to ADV and Infl monoinfection as coinfection with these pathogens among RSV-positive children was found to be associated with increased risk of life-threatening disease. This analysis was implemented to assess whether ADV and Infl monoinfection were the driver of severe disease. Results were expressed as odds ratios (OR) with 95% confidence intervals (CI). Multivariate logistic regression was performed by use of the manual forward stepwise procedure including variables with a p-value <0.2 in univariate analyses. The analysis was adjusted for age using the following subgroups: <6 months and ≥6 months. The primary analysis was conducted on the HIV-uninfected population, subsequently, a separate analysis was performed for the HIV-infected population due to significantly elevated mortality rate and altered immune status of this subgroup.
We considered p <0.05 to be significant for all analyses. The statistical analysis was performed using STATA/SE 13.1 (StataCorp, College Station, TX).

Ethical Considerations

The study protocol was approved by the University of the Witwatersrand Ethics Committee and the KwaZulu-Natal Human Biomedical Research Ethics Committee (protocol M081042 and BF157/08). Details of consenting, which included written informed consent from the parent or primary caregiver of the child have been described.(30) This surveillance was deemed non-research by the US Centers for Disease Control and Prevention (NRD 2012 6197).

Results

Study Population

During February 2009 to December 2013, 10,128 children less than five years of age were enrolled, including 2,404 (23.7%) with RSV-associated LRTI. Our total HIV-uninfected population with RSV-associated LRTI was 2,322 children. We performed a sensitivity analysis to validate HIV status and found that the untested and HIV-negative population did not differ in baseline characteristics or underlying conditions and both had a similar mean RSV Ct value of 25.1 (SD: 5.1) and 25.7 (SD: 4.8; p=0.003) respectively [Supplementary Table 1].

Prevalence of Viral Coinfection

Table 1 details the prevalence of respiratory virus coinfections among children hospitalized for RSV-associated LRTI, including stratification by age groups <6 and ≥6 months of age. The prevalence of any respiratory viral coinfection was more common among children aged ≥6 months (529; 51.1%) compared to those aged <6 months (463; 36.0%; p<0.001). The prevalence of RSV-PIV1, RSV-PIV2 and RSV-PIV3 dual infection were <1% in both groups. Rhinovirus was the most prevalent co-infecting virus, found among 23.5% of children aged <6 months and 26.4% of children aged ≥6 months; followed by RSV-ADV coinfection (8.3% and 23.2% in children aged <6 months and ≥6 months; p<0.001) and RSV-EV coinfection (5.6% and 11.5% in children aged <6 months and ≥6 months; p<0.001). The different permutations of viral coinfections in the RSV-positive population are elucidated in a coinfection matrix [Supplementary Table 2].

We compared the prevalence of viruses in the presence (n=2,404) or absence of RSV (n=7,447) and found that the presence of RSV was associated with a lower prevalence of all other respiratory viruses during RSV season [Supplementary Appendix: Figure 1]. In the RSV-negative population 19.3% (1436/7447) of children hospitalized for LRTI had 2 or more viruses detected in the respiratory tract with the most prevalent viruses being RV and ADV respectively.

Demographic and Clinical Characteristics

We examined the prevalence of demographic and clinical characteristics amongst RSV monoinfection cases and those with any respiratory virus coinfection, stratified by age <6 months and 6 months or older. 1,287 children were aged less than 6 months and 1,035 were aged 6 months or older. The median age for RSV monoinfection was 4.2 months (IQR: 1.9 – 9.6 months) and 6.6 months for RSV with any viral coinfection (IQR: 3.0 – 14.7 months). Age was associated with RSV and any viral coinfection in both children less than 6 months of age (p<0.0001) and aged 6 months or older (p=0.05) [Table 2].

We described the demographics and underlying conditions of RSV monoinfection and coinfections in Table 2. Underlying conditions were not more prevalent in viral coinfection than in RSV monoinfection (p=0.29 children aged <6 months, p=0.38 children aged 6 months or older).

Respiratory Viral Coinfections and Disease Severity

Within the RSV-positive population <5 years old, 26 children (1.1%) were admitted to the ICU, 21 children (0.90%) needed mechanical ventilation, and 8 children died (0.34%). Seventeen of the twenty-one children (81%) who received mechanical ventilation were admitted to the ICU. Sixty-seven percent of children were hospitalized for fewer than 5 days. When comparing RSV with any respiratory viral coinfection to RSV monoinfection, we found no overall association between any viral infection and life-threatening disease (OR=1.4, 95%CI 0.71-2.6, p=0.36) [Table 3]. We found

the same to be true for our secondary outcome, including extended length of stay (aOR=0.83, 95%CI 0.69-1.0, p=0.05). After adjusting for confounders, RSV-ADV coinfection had a 3.4 increased odds of life-threatening disease when compared to RSV monoinfection (95%CI: 1.6 –7.2, p=0.001) [Table 3]. RSV-ADV coinfection was not associated with the secondary outcome (aOR: 1.0 95%CI: 0.80–1.4, p=0.76). When we compared RSV-ADV coinfection to ADV monoinfection we found no relation to life-threatening disease (aOR=0.78, 95%CI 0.37–1.6, p=0.51) and decreased life-threatening disease and extended length of stay (aOR=0.51, 95%CI 0.38–0.70, p<0.001). The median ADV Ct value was significantly lower in ADV monoinfection (29.7, IQR: 20.8 – 34.3) when compared to RSV-ADV infection (33.2, IQR: 30.1 –35.5), p<0.0001. Finally, RSV-Infl showed increased odds for our secondary outcome including prolonged length of stay (aOR=2.1, 95%CI 1.0–4.5, p<0.05). We identified an increased odds of our secondary outcome for RSV-Infl when compared to Infl alone (aOR=2.1, 95%CI 1.0–4.4, p=0.04). No other viral coinfections showed increased odds of severe disease compared to RSV monoinfection.

In the HIV-infected population, 6.3% (n=5) of children had life-threatening disease. Mean RSV Ct value was significantly lower in the HIV-infected population than the HIV-uninfected population (RSV Ct-value 27.1 (SD:5.1) vs 25.5 (SD: 4.8), p=0.003). Similarly, in this population, we found no association between any viral coinfection and more severe disease when compared to RSV monoinfection [Supplementary Table 4].

RSV Viral Load and Disease Severity

We found a mean Ct value of 25.1 (SD: 4.6) for children aged <6 months and 26.0 (SD: 5.0) for children aged 6 months or older (p<0.0001). RSV viral load was not associated with life-threatening disease in children with RSV monoinfection or children with RSV with any coinfection. When included in our multivariate model, RSV Ct values were not found to be associated with life-threatening disease (aOR 1.0; 95% CI: 0.94 – 1.1) or the secondary outcome including increased length of stay (aOR 1.0, 95% CI: 0.98 –1.0).

Discussion

In general, our study did not corroborate the findings from previous smaller studies, that children hospitalized with LRTI characterized by RSV coinfection with respiratory viruses had more severe disease compared to children with RSV monoinfection(15,31,32). We did, however, identify an association between RSV-ADV coinfection and life-threatening disease which may be indicative of synergistic pathogenesis leading to respiratory failure or that severe disease in these children was largely driven by coinfection with adenovirus. The association of RSV-ADV coinfection with severe disease was, however, not evident when we assessed prolonged hospitalization. Our data are supported by findings of another study in which RSV-ADV coinfection showed statistically

significant increases in hospital length of stay, days with supplemental oxygen use, ICU admission, and mechanical ventilation when compared to RSV monoinfection in children hospitalized for LRTI although no comparison was made with ADV monoinfection(33). In a study of mixed RSV-ADV infection, RSV-ADV coinfection was not found to be more severe than ADV alone when examining duration of fever, oxygen requirement and length of hospital stay(34). Another study of 9 RSV-confirmed infants found 75% (3/4) of children with RSV-ADV coinfection died despite mechanical ventilation(35). Even though ADV alone may be responsible for more severe disease, clinical features such as hospital stay were not found to differ between RSV and ADV hospitalized LRTI(36). However, increased pathogenicity may be explained by distinctly different immunological responses produced by RSV and ADV. ADV induces IFN-γ production activating the classical antiviral defence mechanism and heightened mononuclear cell activation compared to RSV, possibly leading to more severe disease with co-infection(37).

The increased odds of severe disease for RSV-ADV coinfection may warrant further exploration on a host and pathogen level. Virus-virus interactions can be classified into three categories: (1) viral genes or gene products interacting directly; (2) host environment changes that result in indirect interaction; or (3) immunological interactions(38). It is plausible that similar mechanisms that enhance bacterial superinfection may also enhance viral superinfection, namely depletion of host defences due to initial viral infection(39).

We found that coinfection of RSV and any other virus was not related to disease severity. This is in line with a retrospective study which found that clinical severity did not differ between RSV monoinfection and viral coinfection with 17 different respiratory viruses (40). A recent meta-analysis of clinical disease severity and viral coinfection versus monoinfection found no clinical difference in severity between these two groups even when constrained to more pathogenic respiratory viruses (influenza, RSV, HMPV, PIV)(19). Another meta-analysis of single and multiple virus respiratory infections (influenza, RV, ADV, HMPV, coronavirus, bocavirus, PIV1-3) and severity of disease concluded that the influence of coinfection on disease severity remains unclear due to the heterogeneity of results(41).

In our study, the highest prevalence of viral coinfection was detected in HIV-uninfected children older than 6 months hospitalized for LRTI. This is in accordance with findings from a number of studies which found multiple viral respiratory infection to be associated with older age(21,33,42,43) when compared to RSV monoinfection. Increased rates of virus infection with increasing age have been described previously in this surveillance population(30). The increased prevalence of respiratory viral coinfections among children aged 6 months or older may be explained by increased exposure to respiratory viruses, an increased immune response during primary infection which discourages viral coinfection, or increased susceptibility due to waning maternal antibodies(44).

In the presence of RSV, our data show lower prevalence of non-RSV viruses in children hospitalized for viral respiratory illnesses during the RSV season [Supplementary Figure 1]. This could be indicative of viral interference in which the presence of RSV in the community inhibits infection by or circulation of other viruses. Evidence of viral interference has been found in studies of children who received influenza vaccine(45,46) and among children receiving immunoprophylaxis for RSV(47). In both groups the prevalence of non-preventatively targeted viruses was higher than among comparison groups that did not receive vaccination or immunoprophylaxis. However, these speculations and the clinical relevance of some of these identified viruses need further exploration with a more suitable study design.

The strength of this study lies in the large sample size which allowed us to look at different permutations of coinfection within the RSV population and compare them to RSV monoinfection only and to draw conclusions about an infrequent, yet important, outcome. Furthermore, we were able to control for important confounders of disease severity including age and prematurity. Finally, we did not limit our assessment of respiratory viral coinfection to a single season.

There were some limitations to our study. Given that viral data were collected at one timepoint after disease onset it is difficult to link viral detection to etiology of LRTI. Some respiratory viruses are frequently detected in asymptomatic children and infants. RSV, HMPV, influenza and ADV are significantly more prevalent in symptomatic children while RV is commonly found in asymptomatic individuals(48). Another study of infants up to 12 months of age found that detection of RSV, RV, Infl, ADV, HMPV are highly associated with symptoms with an odds ratio >4 for presence of symptoms while for EV detection is not significantly associated with symptoms(49). In South Africa, ADV was only moderately associated with severe disease as it was commonly identified in controls – the attributable fraction of ADV detection was 10.1%(50). Viral detection may also be an artefact of prolonged viral shedding: adenovirus, for example, is known to exhibit longer low-level shedding(51). In the RSV-ADV coinfection population we found more frequent low level virus than in the population with ADV monoinfection, which may be indicative of prolonged viral shedding and acute infection respectively [Figure 1]. The multiplex PCR used was limited in its ability to discriminate between hRV and EV due to cross-reactivity and therefore these coinfections are not optimally characterized within this population. Furthermore, the definition of any viral coinfection is limited by the respiratory viruses we did not test for although the clinical significance of many of those (e.g. human coronavirus and human bocavirus) as LRTI etiological agents also remain to be fully elucidated. Another limitation was that we only had semi-quantitative data for viral load.

Conclusion

In conclusion, the present study contributes to a better understanding of the role of viral coinfection in children hospitalized for RSV-associated LRTI. Molecular diagnostics for respiratory viruses may

serve as an important diagnostic tool in pediatric LRTI, but the possible synergy of multiple viruses in the respiratory tract is an area with no clear consensus. In our study, we found RSV and any respiratory viral coinfection was not associated with more severe disease. The association between RSV-ADV coinfection and life-threatening disease in hospitalized children less than 5 years of age warrants further exploration and may be explained by enhanced ADV disease alone.

Notes

Disclaimer. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the US Centers for Disease Control and Prevention, USA or the National Institute for Communicable Diseases, South Africa.

Potential Conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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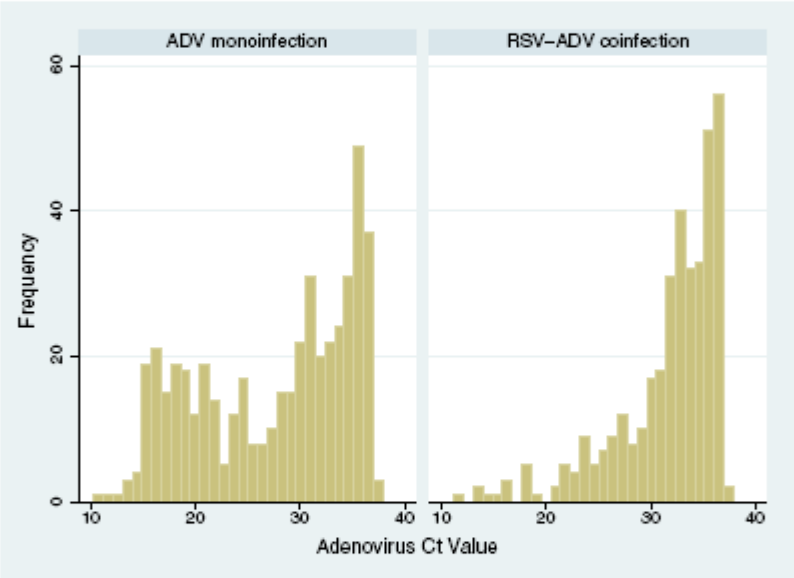

Figure 1

Histogram showing the frequency of adenovirus Ct values for children with ADV monoinfection and ADV-RSV coinfection. Abbreviations: ADV: adenovirus, RSV: respiratory syncytial virus.

Table 1: Respiratory viral coinfections, stratified by age group, in HIV-uninfected children aged <5 years with RSV-associated lower respiratory tract infections at six sentinel sites in South Africa, 2009 –2013

	All ages (N=2,322)		<6 months (N=1,287)		≥6 months (N=1,035)		
	Frequency	Percent	Frequency	Percent	Frequency	Percent	p-value
RSV monoinfection	1330	57.3	824	64.0	506	48.9	<0.0001
RSV any coinfection[a]	992	42.7	463	36.0	529	51.1	<0.0001
RSV-HMPV	26	1.1	16	1.2	10	0.97	0.53
RSV-RV	575	24.8	302	23.5	273	26.4	0.11
RSV-ADV	347	14.9	107	8.3	240	23.2	<0.0001
RSV-EV	191	8.2	72	5.6	119	11.5	<0.0001
RSV-Infl	30	1.3	11	0.85	19	1.8	0.04
RSV-PIV1	12	0.52	2	0.16	10	0.97	0.01
RSV-PIV2	12	0.52	4	0.31	8	0.77	0.12
RSV-PIV3	20	0.86	12	0.93	8	0.77	0.70

Analyses show frequency and percent of RSV monoinfection and different coinfection groups in the HIV-uninfected children <5 years of age as well as analysis stratified by age groups <6 months and ≥6months. The p-value is given for a comparison of the prevalence of a certain coinfection in the age group <6 months and ≥6 months. RSV: Respiratory Syncytial Virus, HMPV: human metapneumovirus; RV: rhinovirus; ADV: adenovirus; EV: enterovirus; Infl: influenza; PIV1: parainfluenza type 1; PIV-2: parainfluenza 2; PIV-3: parainfluenza 3. a: Any viral respiratory coinfection with HMPV, RV, ADV, EV, Infl, PIV1, PIV2, or PIV3.

Table 2: Demographic and clinical characteristics, stratified by age group, for any coinfection or RSV monoinfection in HIV-uninfected children < 5 years of age at 6 sentinel sites in South Africa, 2009 - 2013

	<6 MONTHS			≥6 MONTHS		
Characteristic	RSV Monoinfection (n=824)	Any Coinfection[a] (n=463)	p-value	RSV Monoinfection (n=506)	Any Coinfection[a] (n=529)	p-value
Demographics						
Age, months; median (IQR)	2.4 (1.3 – 3.8)	2.8 (1.9 – 4.0)	<0.0001	12.8 (8.1 – 21.0)	14.0 (9.0 – 22.7)	0.05
Female sex	354/824 (43.0)	199/463 (43.0)	0.99	223/506 (44.1)	227/529 (42.9)	0.71
Race, black (%)	810/823 (98.4)	454/463 (98.1)	0.63	493/504 (97.8)	519/528 (98.3)	0.58
Duration of Symptoms median (days, IQR)	2 (1 – 3)	2 (1 – 3)	0.44	2 (1 – 3)	2 (1 – 3)	0.16
Premature[b]	16/822 (2.0)	12/463 (2.6)	0.45	4/503 (0.80)	5/528 (0.95)	0.99
DOB within 10 weeks of start of RSV season (%)	524/824 (63.6)	295/463 (63.7)	0.97	182/506 (36.0)	210/529 (39.7)	0.22
RSV CT Value; mean (SD)	24.8 (4.5)	25.6 (4.6)	0.002	25.8 (4.6)	26.3 (5.3)	0.15
Crowding (5+ people in the household)	82/813 (10.1)	57/455 (12.5)	0.18	31/499 (6.2)	56/525 (10.7)	0.01
Underlying Conditions						
Underlying illness	20/823 (2.4)	16/463 (3.5)	0.29	15/505 (3.0)	21/528 (4.0)	0.38
Whole blood PCR+ S. Pneumo (%)	29/453 (6.4)	6/254 (2.4)	0.02	13/297 (4.4)	18/277 (6.5)	0.26
Outcomes						
Primary Outcome (%)	17/810 (2.1)	12/460 (2.6)	0.56	2/496 (0.4)	7/511 (1.4)	0.10
Secondary Outcome (%)	363/811 (44.8)	189/458 (41.3)	0.23	115/499 (23.1)	100/523 (19.1)	0.12

We compared demographic and clinical characteristics of any viral RSV coinfection compared to RSV monoinfection for HIV-uninfected children under 6 months and 6 months and older. S. Pneumo: *Streptococcus Pneumoniae*. Underlying conditions included asthma, chronic renal failure, splenectomy/asplenia, autoimmune disease, seizure disorders, malignancy, chronic lung disease, heart failure, organ transplant, diabetes, kwashiorkor/marasmus, prematurity, valvular heart disease, immunosupressive therapy, burns, nephrotic

syndrome, obesity, cirrhosis/liver failure, coronary artery disease, sickle cell, immunoglobulin deficiency, spinal cord injuries, COPD/emphysema or other as specified by parents. a: Any viral respiratory coinfection with HMPV, RV, ADV, EV, Infl, PIV1, PIV2, or PIV3. b: born at less than 37 weeks gestation. DOB: Date of birth.

Table 3: Primary outcome, univariate and multivariate analyses of RSV viral coinfection and life-threatening disease in HIV-uninfected children < 5 years of age at 6 sentinel sites in South Africa, 2009 - 2013

Co-infection	Life-Threatening Disease	MV, ICU, Death n/N (%)	OR (95% CI)	p-value OR	aOR (95% CI)	p-value aOR
Any[a]	No	19/1306 (1.5)	1.4 (0.71 – 2.6)	0.36		
	Yes	19/971 (2.0)				
HMPV	No	38/2251 (1.7)	-	-		
	Yes	0/26 (0.0)				
RV	No	31/1715 (1.8)	0.69 (0.30 – 1.6)	0.37		
	Yes	7/562 (1.3)				
ADV	No	27/1937 (1.4)	2.4 (1.2-4.8)	0.02	3.4 (1.6– 7.2)	0.001
	Yes	11/340 (3.2)				
EV	No	35/2091 (1.7)	0.96 (0.29 – 3.2)	0.95		
	Yes	3/186 (1.6)				
Infl	No	38/2248 (1.7)	-	-		
	Yes	0/29 (0.0)				
PIV1	No	37/2266 (1.6)	6.0 (0.75–48.3)	0.09		
	Yes	1/11 (9.1)				
PIV2	No	38/2265 (1.7)	-	-		
	Yes	0/12 (0.0)				
PIV3	No	37/2257 (1.6)	3.2 (0.41 – 24.2)	0.27		
	Yes	1/20 (5.0)				

Univariate analysis: all factors with P<0.20 were entered into the multivariate model. Multivariate anlysis: only factors with P<0.05 are shown; rhinovirus, human metapneumovirus, enterovirus, influenza, parainfluenza 2 and parainfluenza 3 were excluded because P≥ 0.20 in univariate analysis. a: Multivariate analysis also adjusted for covariates prematurity and age which were also found to be significant in univariate analysis and subsequently in multivariate anlaysis using the manual forward stepwise procedure. Primary outcome data was missing for 45 RSV-infected children. Abbreviations: MV: mechanical ventilation, ICU: Intensive Care Unit, RSV: Respiratory Syncytial Virus, OR: odds ratio, aOR: adjusted odds ratio. a: Any viral respiratory coinfection with HMPV, RV, ADV, EV, Infl, PIV1, PIV2, or PIV3.

Table 4: Secondary outcome, univariate and multivariate analyses of RSV viral coinfection and life-threatening disease or length of stay of 5+ days in HIV-uninfected children < 5 years of age at 6 sentinel sites in South Africa, 2009 - 2013

Coinfection	Secondary Outcome	MV, ICU, death, or LOS 5+ days n/N (%)	OR (95% CI)	p-value OR	aOR[a] (95% CI)	p-value aOR
Any[a]	No	289/981 (29.5)	0.73 (0.61-0.87)	<0.0001		
	Yes	478/1310 (36.5)				
HMPV	No	758/2265 (33.5)	1.1 (0.47 – 2.4)	0.90		
	Yes	9/26 (34.6)				
RV	No	599/1724 (34.7)	0.79 (0.64 – 0.97)	0.03		
	Yes	168/567 (29.6)				
ADV	No	671/1946 (34.5)	0.73 (0.57 – 0.94)	0.02		
	Yes	96/345 (27.8)				
EV	No	721/2101 (34.3)	0.61 (0.43 –0.86)	0.005		
	Yes	46/190 (24.2)				
Infl	No	753/2261 (33.3)	1.8 (0.85 – 3.6)	0.13	2.1 (1.0 – 4.5)	0.05
	Yes	14/30 (46.7)				
PIV1	No	765/2280 (33.6)	0.44 (0.09 – 2.0)	0.30		
	Yes	2/11 (18.2)				
PIV2	No	765/2279 (33.6)	0.40 (0.09 – 1.8)	0.23		
	Yes	2/12 (16.7)				
PIV3	No	761/2271 (33.5)	0.85 (0.33 – 2.2)	0.74		
	Yes	6/20 (30.0)				

Univariate analysis: all factors with P<0.20 were entered into the multivariate model. Multivariate analysis: only factors with P<0.05 are shown; rhinovirus, human metapneumovirus, parainfluenza 2 and parainfluenza 3 were excluded because P≥ 0.20 in univariate analysis. a: Multivariate analysis also adjusted for covariates prematurity and age which were also found to be significant in univariate analysis and subsequently in multivariate analysis using the manual forward stepwise procedure. Secondary Outcome was missing for 12 RSV-infected children. Abbreviations: MV: mechanical ventilation, ICU: Intensive Care Unit, LOS: length of stay, RSV: Respiratory Syncytial Virus, OR: odds ratio, aOR: adjusted odds ratio. a: Any viral respiratory coinfection with HMPV, RV, ADV, EV, Infl, PIV1, PIV2, or PIV3.