CLINICAL AND MOLECULAR EPIDEMIOLOGY OF HUMAN RHINOVIRUSES IN LOW TO MIDDLE INCOME COUNTRIES

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DECLARATION

I, Vicky Lynne Baillie declare that this thesis is my own work. It is being submitted for the degree of Doctor of Philosophy in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

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14th day of June 2017

DEDICATION

I dedicate this work to my loving husband Chris, he has been my pillar of strength throughout the past four years. I could not have completed this thesis without your support, encouragement and understanding.

To my parents - my mom, dad and John - who have made this journey possible and who have given me everything I needed to succeed in both my studies and in life.

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PRESENTATIONS ARISING FROM THIS THESIS

1. Association between rhinovirus and *Streptococcus pneumoniae* among cases and controls in the Pneumonia Etiology for Child Health (PERCH) study: A preliminary analysis. *10th Pneumococci and Pneumococcal Disease Symposium,* Glasgow. July 2016. (Poster presentation: ISPPD-0540)

2. Rhinovirus and *Streptococcus pneumoniae* interactions in the Pneumonia Etiology for Child Health (PERCH) study: A case-control analysis. *Wits Research day,* Johannesburg. August 2016.

3. The impact of HIV-1 on the clinical epidemiology of Human rhinovirus in South African and Zambian Children: A case-control analysis in the PERCH study. 35th Annual Meeting of the European Society for Paediatric Infectious Diseases. Madrid, Spain, 23-27 May 2017. (Poster discussion: ESP17-0579)

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ABSTRACT

Introduction: Human rhinovirus (HRV) is the most prevalent virus detected in children with respiratory symptoms; however, its aetiological role during disease episodes remains unclear as detection of HRV is also ubiquitous among asymptomatic children. We evaluated the clinical epidemiology of HRV-associated disease among children hospitalised with severe and very severe pneumonia together with community control children living in Africa and Southeast Asia. In addition, we explored the associations between the molecular subtyping and nasopharyngeal viral loads of the HRV species and their ability to cause viraemia as potential markers for HRV disease.

Methods: Using a case-control study conducted in seven countries, we compared the clinical characteristics of children (1-59 months of age) hospitalised with HRV-associated pneumonia between August 2011 - January 2014 and age-frequency matched controls. Nasopharyngeal swabs from the cases and controls were tested for HRV, together with 27 other respiratory pathogens, with quantitative real-time PCR assays. The 5' NCR region of the HRV positive samples were sequenced to determine the species/strains of HRV and phylogenetic analysis was performed. Additionally, the blood samples from a limited number of cases (n=210) and controls (n=212) were tested for the presence of HRV viraemia and the 5' NCR sequence of positive blood samples were further characterised.

Results: Overall, HRV detection was 1.45-fold (aOR 95% CI: 1.29-1.62) higher among children hospitalised with pneumonia (24%) compared to controls (21%, $P<0.005$); including being 2.08-fold (28% vs 18%, aOR 95% CI: 1.75-2.47) more associated with case status among children 12-59 months of age. The HRV-associated cases were younger (13.1 months) than controls with HRV infection (15.4 months, $P=0.001$) and more likely to be malnourished (30% vs. 12%, $P<0.001$) and HIV-1 exposed (10% vs. 8%, $P=0.046$). HRV nasopharyngeal viral load was significantly higher among cases compared to controls (3.7 vs. 3.5 $\log_{10}$ copies/mL, $P<0.001$). Also, HRV viraemia was 7.02-fold (aOR 95% CI 1.70-28.94) more prevalent among cases (7%) compared to controls (2%, $P=0.007$). Moreover, HRV nasopharyngeal viral loads ≥ 4 $\log_{10}$ copies/mL differentiated between viraemia positive and negative cases. There was, however, no difference in the molecular subtyping of the HRV species prevalence among cases (HRV-A:48%; HRV-B:7%; HRV-C:45%) and controls (HRV-A:45%; HRV-B:10%; HRV-C:45%,

P=0.496); as well as no evidence of seasonal or temporal clustering of the HRV species over time.

Among cases, HRV detection was less likely to be associated with presence of radiographically confirmed pneumonia (40% vs 46%, *P*=0.001) or hospital stay >3 days (52% vs 61%, *P*=0.001). It was, however, positively associated with older age (13.1 months vs. 11.3 months, *P*<0.001) and presence of wheeze (46% vs. 31%, *P*<0.001) compared to the HRV uninfected cases. HRV was the sole virus detected in the 53% of cases and generally there were no differences in severity or clinical presentation among cases with HRV mono-infections compared to those with HRV-mixed infections. The HRV mono-infections, however, were associated with a 2.83-fold (aOR 95% CI: 1.44-5.53) higher case fatality ratio than cases with HRV and other viral mixed infections (10% vs. 5%, *P*=0.002). The HRV-associated case fatalities were more likely to have markers of bacterial co-infections compared to the HRV-associated cases that survived.

Among the HRV species, HRV-C compared to HRV-A cases were older (12.1 vs. 9.4 months, *P*=0.033), more likely to present with wheeze (35% vs. 25%, *P*=0.031) and 2.59-fold (aOR 95% CI: 1.23-5.95) more likely to be associated with viraemia (12% vs. 2%, *P*=0.025). Conversely, the HRV-A infected cases were more likely to have radiographically confirmed pneumonia (46%) compared to HRV-C infected cases (36%, *P*=0.040) and HRV-A mono-infected cases were more likely to have hospital stay of >3 days (72%) than HRV-C mono-infected cases (54%, *P*=0.039).

Conclusion: HRV detection, especially among children 1-5 years of age, was associated with severe lower respiratory tract infection; however, HRV detection was ubiquitous with a high degree of genetic diversity among both cases and controls. Thus the true etiologic role of HRV during childhood disease, especially among infants, remains uncertain. Nonetheless, HRV nasopharyngeal viral loads $\geq 4\log_{10}$ copies/mL in conjunction with HRV viraemia are potential markers for HRV-associated severe respiratory disease. Among cases, HRV-A was associated with radiographically confirmed pneumonia and generally more severe disease than HRV-C which was more associated with viraemia and wheezing disease.

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ABBREVIATIONS

AdV	Adenovirus
aOR	Adjusted odds ratio
ARI	Acute respiratory infection
ALRTI	Acute lower respiratory tract infection
CAP	Community acquired pneumonia
CDHR-3	Cadherin-related family member 3 receptors
CHBAH	Chris Hani Baragwanath Academic Hospital
CI	Confidence interval
CMV	Cytomegalovirus
CRP	C-reactive protein
CSF	Cerebrospinal Fluid
CXR	Chest X-ray
DNA	Deoxyribonucleic acid
FTD	Fast Track Diagnostics
H. inf	*Haemophilus influenza*
HiB	*Haemophilus influenzae* Type B
HBoV	Human Bocavirus
HCoV	Human Coronavirus
HDP	High Density Pneumococcus
HEU	HIV exposed but HIV-uninfected
HIV	Human Immunodeficiency virus
HMPV	Human Metapneumovirus
HREC	Human Research Ethics committee
HRV	Human rhinovirus
ICU	Intensive care unit
InFV	Influenza virus
ILI	Influenza-like-illness
IPD	Invasive pneumococcal disease
IRB	Institutional review board
IQR	Inter quartile range
JHSPH	Johns Hopkins Bloomberg School of Public Health
LRTI	Lower respiratory tract infection
M. cat	*Moraxella cattarhalis*
MCPP	Microbiologically confirmed pneumonia

NP	Nasopharyngeal
O_2	Oxygen
OP	Oropharyngeal
OR	Odds ratios
PCR	Polymerase Chain Reaction
PCV	Pneumococcal conjugate vaccine
PERCH	Pneumonia Etiology for Child Health
PIV	Parainfluenza virus
P. jiroveci	*Pneumocystis jiroveci*
PRISMA	Preferred Reporting Items for Systematic and Meta-Analyses
RSV	Respiratory syncytial virus
RMPRU	Respiratory and Meningeal Pathogens Research Unit
RNA	Ribonucleic acid
RTI	Respiratory tract infection
S. aureus	*Staphylococcus aureus*
SD	Standard deviation
SOWC	State of the World's children
S. pneu	*Streptococcus pneumoniae*
TB	*Tuberculosis*
URTI	Upper respiratory tract infection
UTM	Universal transport media
WHO	World Health Organisation

STUDENTS CONTRIBUTION

Protocol and Ethics: All protocols as well as South African HREC ethics application for the PhD were prepared and obtained by VB.

Recruitment of participants: All study participants were recruited and enrolled by study nurses and doctors at the respective sites.

Laboratory component: All processing of South African samples was performed or overseen by VB and all the South African laboratory assays - including the RT-PCR testing of the respiratory samples, blood samples and all the antibiotic activity assays as well as sequencing assays - were conducted by VB. The culturing of the sterile site samples, including the blood and CSF samples, were conducted by the National Health Laboratory Service. All South African laboratory related data were entered onto the PERCH database by VB. The laboratory component for the other site were conducted by the individual sites local laboratory staff; however, the rhinovirus positive samples from the Zambian and Mali sites were shipped to South Africa for molecular typing of the strain.

Statistical analysis: All statistical and phylogenetic analyses were conducted by VB

1.0 Introduction

1.1 Epidemiology of Childhood pneumonia

Pneumonia is the leading cause of childhood morbidity and mortality globally, including approximately 120 million cases annually [1]. In 2013, approximately 6.6 million children under 5 years of age died with 3.257 million deaths due to infectious causes, with pneumonia causing 0.935 million deaths. Approximately 60% of all under-5 childhood deaths occurred in sub-Saharan Africa [2]. Based on reporting to the Department of Home Affairs, in 2013, diseases of the respiratory system accounted for 10.4% of all deaths in South Africa. Influenza and pneumonia overall were the second leading natural cause of death, accounting for 23 727 deaths in 2013, down from 33 847 deaths in 2011 [3]. Furthermore, in 2013, there were 25 993 deaths in infants, 9 101 deaths in the 1-4 year old age group, 3 382 in children 5-9 years old and 3 261 in children 10-14 years old. In infants, influenza and pneumonia accounted for 2 343 (9%) of the deaths, making it the third most common cause of death in South African infants, following respiratory and cardiovascular disorders specific to the perinatal period (3 727 deaths, 14.3%) and intestinal infectious diseases (3 591, 13.8%) [3].

The burden and epidemiology of pneumonia in children has been significantly impacted by the Human Immunodeficiency Virus (HIV) epidemic, as HIV-infected children are at an increased risk for severe pneumonia, bacteraemia and recurrent infections, with respiratory diseases being the leading cause of hospitalisation and death. In South Africa, the number of pneumonia hospitalisations had increased in the 1990s, largely due to an increase in the number of pneumonia admissions in HIV-infected children [4]. Of all children admitted to hospital in South Africa with severe pneumonia, approximately 45% are HIV positive, and whom had a 3-6 times greater case fatality ratio [5, 6].

1.2 Community-acquired pneumonia

Community-acquired pneumonia (CAP) is defined as an inflammatory lung infection which can be caused by a number of pathogens including viruses, bacteria and parasites [7, 8]. Determining the aetiology of pneumonia is difficult and traditionally no causative pathogens were identified in 20-60% of pneumonia cases [8-10]. This is due to the limited sensitivity

and specificity of previous methods for identifying specifically bacterial pathogens [11], further aggravated by many commensal organisms commonly colonising the airways, which limits the utility of sampling of the nasopharynx for identifying the causative agent of CAP.

Nevertheless, respiratory viruses are associated with 14-80% of childhood CAP episode in younger children [8]. In a study conducted in Finland between January 1993 and November 1995, 80% of 254 children hospitalised for CAP under the age of 2 years had evidence of respiratory viral infections, compared to only 49% in older children. Of these respiratory viral infections, Human rhinovirus (HRV) was the second most common infection (24%) after Respiratory Syncytial Virus (RSV; 29%) [12]. In Oakland, CA in 2007, HRV was found to be the most prevalent virus (21%) followed by Parainfluenza viruses (PIVs) (17%) and RSV (15%) [13].

HRV was also found to be present in 50% of the children admitted to the intensive care unit in California with a lower respiratory tract infection (LRTI) in children of 1.4 years' median age [14]. In this study, it was also established that children with HRV infection more frequently required mechanical ventilation and had longer hospital stay compared to those whose LTRIs were associated with other viral pathogens, suggesting that HRV infection was associated with severe LRTI [14]. More recently in a study conducted in Italy over four consecutive winter and early spring periods (November to end April) starting in 2007 and ending in 2011, a total of 592 children (1 month to 14 years) were enrolled with signs and symptoms of CAP [15]. A total of 435 of the children were positive for a respiratory viral infection, RSV was found to be most prevalent (31.7%) followed by HRV (24.3%) [16].

1.3 Human rhinovirus (HRV)

HRV was first identified in 1956 [17] in patients presenting with mild upper respiratory tract infection (URTI), and has since been reported to be the most widespread cause of the common cold in both children and adults. HRV fall into the *Picornaviridae* family in the *Enterovirus* genus. They are single stranded positive-sense non-enveloped ribonucleic acid (RNA) viruses with a 7.2kb genome. The viral genome is composed of three main sections – a 5' untranslated region, an open reading frame of the polyprotein and a 3' untranslated region. The polyprotein open reading frame codes for the four capsid protein (VP1-4) and the non-structural genes [18]. Presently more than 100 serotypes have been classified into 3

species - species A (HRV-A: 74 known serotypes), species B (HRV-B: 25 serotypes) and most recently classified species C (HRV-C) [19-22]. HRV-C was only classified as a novel species in 2007, following studies which identified novel HRV genotypes [10, 23-26]. The HRV serotypes are classified according to their nucleotide sequence homologies; HRV-C shares a 53-57% amino acid homology with HRV-A and HRV-B and the different HRV species use different host surface protein receptors for viral entry, namely ICAM-1 and low density lipoprotein for HRV-A and HRV-B **[27, 28]** whereas HRV-C uses the human cadherin-related family member 3 receptors (CDHR-3) **[29]**. These differences in receptors could potentially indicate differences in mechanisms of infection and replication for HRV-C, which could indicate differences in pathogenicity between the three HRV species. Figure 1.1 is the phylogenetic tree showing the relationship between all HRV serotypes known to date based on full genome sequences.

Previously, HRV were detected using standard virus culture in susceptible cell lines of either HeLa or human embryo lung fibroblasts cultured in tubes that rotated slowly at 33^{o}C. Acid lability tests were then used to differentiate between HRV and enteroviruses (a closely related member of the *Picornaviridae* family). This process was very time consuming and laborious, sometimes taking up to 10 days to get a positive result, and was relatively insensitive which most likely lead to the underestimation of HRV as an important pathogen [30], especially as current HRV culture techniques cannot culture the HRV-C serotypes [19]. The inability to culture HRV-C using traditional methods is linked to the different host receptor proteins required for HRV-C binding and replication [29]. However, since the introduction of diagnostic polymerase chain reaction (PCR) assays, which has been shown to be at least three times more sensitive at detecting HRV infections [31], HRV's importance and role in severe illness has been re-evaluated. Furthermore, it is feasible that additional new serotypes of HRV may yet be discovered, with a newly diverging fourth HRV species (HRV-D) having been proposed recently [32].

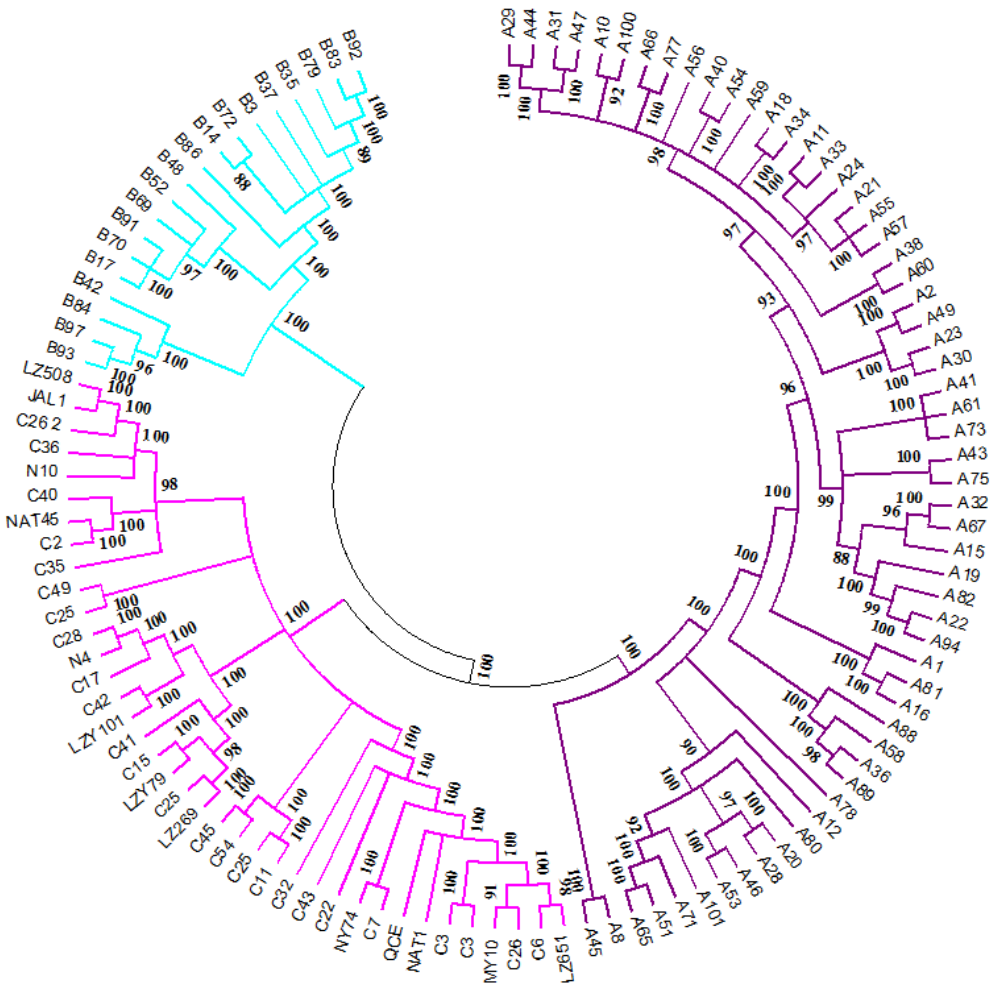

Figure 1.1: Phylogenetic tree of known HRV-A, HRV-B and HRV-C serotypes. The tree was generated with neighbour-joining methods from full length RNA genome sequences obtained from GenBank. The numbers at the branch nodes are bootstrapping values (percentages of 2 000 replicates) indicating the confidence of the branching.

HRV has been widely associated with URTI; however, they are now also considered as an important pathogen contributing to the pathogenesis of severe LRTIs in children requiring hospitalisation [10, 14, 15, 22, 33-35]. HRV has also been associated with otitis media, sinusitis, exacerbation of asthma, cystic fibrosis and bronchitis and is the leading cause of wheeze and chronic obstructive pulmonary disease [35-39]. HRV has also been shown to be one of the most significant viral pathogens in elderly people [40, 41], occasionally resulting in high mortality rate – for example, in one case series 12 (21.4%) of 56 patients died due to acute infection which was linked to the HRV serotype A82 [40]. It has also been hypothesised that HRV may be one of the most important LRTI pathogens in children during non-influenza and non-RSV seasons [14].

Studies from Africa, Asia, Europe, America and Australia suggest that HRV-C may cause more severe illness [26, 37, 42, 43], be more associated with acute asthma [44] and be more prevalent in LRTIs, in influenza-like-illnesses (ILI) [10] and that they may have different seasonal circulation patterns compared to the other HRV species [45]. However, more recently in a prospective household-based study conducted in Peru in children younger than 3 years of age in which weekly active surveillance was conducted, in comparison to HRV-A and -C, HRV-B species was more commonly associated with fever, reduced appetite and malaise in children with acute respiratory infections (ARI); although HRV-B species only represented 10% of the HRV population. Furthermore, children repeatedly positive for HRV infections were due to new HRV strain acquisitions, rather than prolonged HRV infection with a single strain [46].

Attributing causality of illness to HRV is complicated by the high prevalence of detection of HRV in asymptomatic individuals [47] and the fact that virus shedding can occur for between 10-14 days in some people [48]. The vast diversity of HRV serotypes (>100) may account for the differences in clinical phenotypes between sick and asymptomatic individuals as different serotypes may favour viral replication at different temperatures in the different regions of the respiratory tract, with certain serotypes favouring replication at the lower temperatures found in the upper respiratory tract and others favouring replication at the higher temperatures found in the lower respiratory tract [49].

In order to determine the clinical significance of HRV detection in both diseased and healthy individuals, several studies have examined HRV prevalence in both hospitalised children and healthy controls. In the majority of these studies, the cases had significantly higher prevalence of HRV detected than the controls [50-54]; however, these studies often did not investigate the HRV molecular epidemiology further [51, 54] or were not designed to specifically address clinical epidemiology of specific HRV species in relation to disease severity [50, 52].

1.3.1 HRV as a pathogen or an innocent coloniser?

The aetiological role of HRV during disease remains difficult to determine. Understanding the importance of HRV infection is critical when it comes to determining future strategies

for disease treatment and prevention. RSV is known to be one of the most important childhood respiratory pathogens and similar to HRV it also has a large variability in disease severity; however, during infection it has been shown that RSV nasopharyngeal viral load is closely correlated with disease severity in children [55-57]. Thus, studies have tried to determine whether HRV nasopharyngeal viral loads are also correlated to disease severity, with conflicting results between studies. In a study conducted in The Netherlands [58] on asthmatic children with LRTI, no association was observed between HRV nasopharyngeal viral load and disease severity. In a study from Japan, HRV nasopharyngeal viral load was not associated with disease severity in children less than 11 months age, but was associated with disease severity in older children, although overall there was no association [59]. Conversely, in a study conducted in Italian immunocompromised and immunocompetent children and adults, HRV nasopharyngeal viral load was correlated with disease severity for all age groups [60].

Alternative to using nasopharyngeal viral loads, some respiratory viruses - such as RSV, Influenza virus and Cytomegalovirus (CMV) - have also been shown to spread and cause more severe disease by causing a viraemic state [61-63]. Studies have now confirmed that HRV can also cause viraemia, [64-67] i.e. where viruses enter the bloodstream of an individual thus allowing it to have access to other areas of the body resulting in a systemic infection. The presence of a systemic infection caused by HRV, in particular HRV-C, was highlighted in a case report of a 14 month old child presenting with complicated acute pericardial disease following an acute LRTI [67]. In this patient HRV-C was detected in the child's bronchoalveolar lavage, pericardial fluid, plasma and stool samples. They reported the virological findings together with the childs' medical history which suggested that the acute respiratory tract infection resulted in a HRV-C blood viraemia, which then allowed the virus to extend to the pericardial space, which then contributed to the acute pericarditis [67]. Other case studies have identified HRV viraemia between 11.4% to 12.3% of children presenting with positive HRV respiratory samples [64-66]. In studies which looked at the HRV molecular epidemiology of viraemia positive patients, HRV-C was significantly more likely to cause viraemia than both HRV-A and -B, [64, 66] suggesting that HRV-C might have a different pathogenesis to HRV-A and -B. Furthermore, the prevalence of HRV viraemia differed based on the type of ARI episode, with viraemia rates being higher during episodes of asthma exacerbations (25%) compared to 7.7%, 4% and 0% during URTI, bronchiolitis and pneumonia respectively [65]. HRV viraemia positivity was also

significantly associated with more severe disease, with viraemic children having higher respiratory rates, white blood cell counts, C-reactive protein levels (CRP), lower blood oxygen saturation levels and thus more often requiring oxygen therapy [64]. In addition, viraemia was also associated with higher respiratory sample HRV viral loads, suggesting that higher viral loads might be a prerequisite for the development of HRV viraemia [64].

Thus, it has been hypothesised that the detection of HRV viraemia, as well as high viral loads could allow for the differentiation between respiratory infections in which HRV is the causal agent and respiratory infections in which HRV is merely a bystander [64]. Unfortunately, asymptomatic individuals were not enrolled as controls in any of these HRV viraemia studies to test this hypothesis.

HRV could also interact with other pathogens resulting in more severe disease. *Streptococcus pneumoniae* is one of the leading causes of bacterial invasive disease and respiratory tract infections (RTI) in children worldwide [68] and is estimated to cause approximately 18% of all severe pneumonia episodes and 33% of pneumonia related deaths in children in Africa and Southeast Asia [1]. Asymptomatic colonisation of the nasopharynx by *S. pneumoniae* is common during childhood and generally resolves without progressing to disease; however, it can also develop into invasive pneumococcal disease (IPD) and pneumonia. Many risk factors for the progression of *S. pneumoniae* colonisation to disease have been identified, including young age, day care attendance, passive household smoking, a history of asthma, a lack of breastfeeding, younger siblings, and underlying illness such as a respiratory virus infection [69, 70].

Respiratory viruses, including RSV, PIV and Influenza virus, have been shown to predispose individuals to secondary bacterial infections through the upregulation of certain receptors on respiratory epithelial cells thus promoting the adhesion of bacteria to the cells which could contribute to disease progression [71]. A similar upregulation of *S. pneumoniae* adherence to epithelial cells post HRV infection was observed in cultured human airway epithelial cells, suggesting that HRV infection might also predispose individuals to pneumococcal disease [72], leading to several studies which have tried to analyse the HRV and pneumococcal disease association further. Peltola et al. [73] reported that HRV was found in two out of nine children hospitalised with bacteraemic pneumococcal infection, and

suggested that the viral infection may have predisposed these patients to the IPD, as respiratory symptoms were observed prior to the onset of the more severe disease symptoms [73]. In a follow up study, they analysed the mean rates of IPD in young children compared to the rates of HRV activity by analysing the data from several surveillance studies and hospital databases in Finland, and reported that IPD rates correlated with HRV activity in the general community but not with RSV and influenza [74]. Additionally, in a study conducted in Peru from 2009-2011 on children with ARI, they found that HRV was associated with increased densities of pneumococcus in the nasopharynx [75]. They suggested that ARI episodes are most likely driven by complex virus-bacteria-host dynamics which require further work to fully elucidate. Subsequent to these studies there has been a paucity of data further characterising whether HRV infections are a risk factor for IPD.

1.3.2 Human HRV in low to middle income countries in Africa and Southeast Asia

HRV are increasingly attributed to be an important cause of respiratory illness in children [36, 38, 49, 76]. In high-income countries, HRV and enteroviruses (both from the *Picornaviridae* family of viruses) have been proposed as the leading cause of respiratory infections; however, there is a paucity of similar data from low to middle income countries where under-5 childhood respiratory morbidity and mortality is greatest [77]. Thus the greatest burden of HRV associated disease likely occurs in low to middle income settings, where there are the greatest gaps in knowledge on HRV prevalence, species distribution and seasonal patterns.

We undertook a systematic literature review to identify the prevalence and molecular epidemiology of HRV in Africa and Southeast Asia up until January 2016, according to the Preferred Reporting Items for Systematic and Meta-Analyses (PRISMA) guidelines [78]. We searched PubMed, Scopus, Cochrane, MEDLINE, Global Health library and the World Health Organisation (WHO) regional databases.

Searches for articles published in English, French and Portuguese were included in the analysis. The objective of this review was to elucidate the role of HRV associated lower respiratory tract infection (LRTI) prevalence and incidence among children living in low to middle income countries. Due to the limited sensitivity of culture techniques for HRV [31,

79], only studies which employed molecular diagnostic techniques were included. Furthermore, we screened the references in the reviewed manuscripts for any additional relevant manuscripts reporting on HRV in low to middle income countries in Africa and Southeast Asia. Also, Google Scholar searches were undertaken to search for country specific publications that reported on HRV associated LRTI from African or Southeast Asian countries.

Studies reporting on observational surveillance reports, cross-sectional, retrospective, cohort, and prospective data for prevalence rates of HRV were included in the review. Review articles were excluded from this review; however, we did assess references in these to identify any additional citations that were relevant to this review. We included all appropriate studies regardless of gender of study participant or case definition used for the study. Only studies which reported on HRV in children were included. Citations which detailed studies done outside of Africa and Southeast Asia were excluded. In cases where multiple reports were identified from the same authors with overlapping study periods, only the most recent or most comprehensive article was included for the review. Information on study region and population, study design, period of recruitment, recruitment of cases and controls, case definitions, age range of cases and controls, HRV prevalence and incidence rates in cases and controls, molecular epidemiology of HRV in cases and controls, the prevalence and co-infections with other respiratory viruses and bacteria were extracted from all relevant articles and included in the review.

In addition, an exploratory meta-analysis, using exact conditional logistical regression, was undertaken to compare the prevalence of HRV detection in the case-control studies in order to determine the pooled odds ratios and confidence intervals for cases versus controls. Also, meta-analysis was conducted on the case studies which reported HRV prevalence by age-categories (1-12months, 1-5 years and 5-10 years), so as to determine the odds ratio for HRV infection by age group. All data analyses were conducted using STATA version 13.0 (College Station, TX, USA) and SAS version 9.2 (Cary, NC, USA). Two tailed *P*-values <0.05 were considered to be statistically significant.

The research identified 712 citations from PubMed, Scopus, Cochrane, MEDLINE, Global Health library and the WHO regional databases (Figure 1.2). A total of 635 manuscripts

were removed through the elimination of duplicate articles and through the screening of the titles and abstracts, leaving 77 articles for assessment of the full text. Of these, 55 manuscripts did not pass the inclusion criteria screening and were eliminated from the review - the main reasons being that the studies were conducted in all age groups and HRV results were not reported separately for children (n=19) and duplication of study materials published by the same authors with overlapping study period and geography (n=11). An additional 8 manuscripts were identified through reviewing the references in the included manuscripts as well as through Google Scholar searches for country wide reporting on HRV prevalence [80-87]. Consequently, 31 articles were included in this review; Figure 1.2.

We identified 23 manuscripts reporting on HRV prevalence data from only 13 of the 54 African countries, six of which were from South Africa [35, 54, 88-91], four from Kenya [92-95], two each from Madagascar [81, 96] and Mozambique [80, 97] and one each from Angola [98], Botswana [99], Burkina Faso [100], Burundi [16], Morocco [101], Niger [102], Nigeria [103], Senegal [104] and Zambia [105]. From Southeast Asia, we identified 8 manuscripts reporting on HRV prevalence from 6 of the 11 Southeast Asian/Pacific countries, including two each from Vietnam [84, 85] and Thailand [86, 106] and one from Cambodia [83], Malaysia [87], Philippines [66], and Singapore [29, 107].

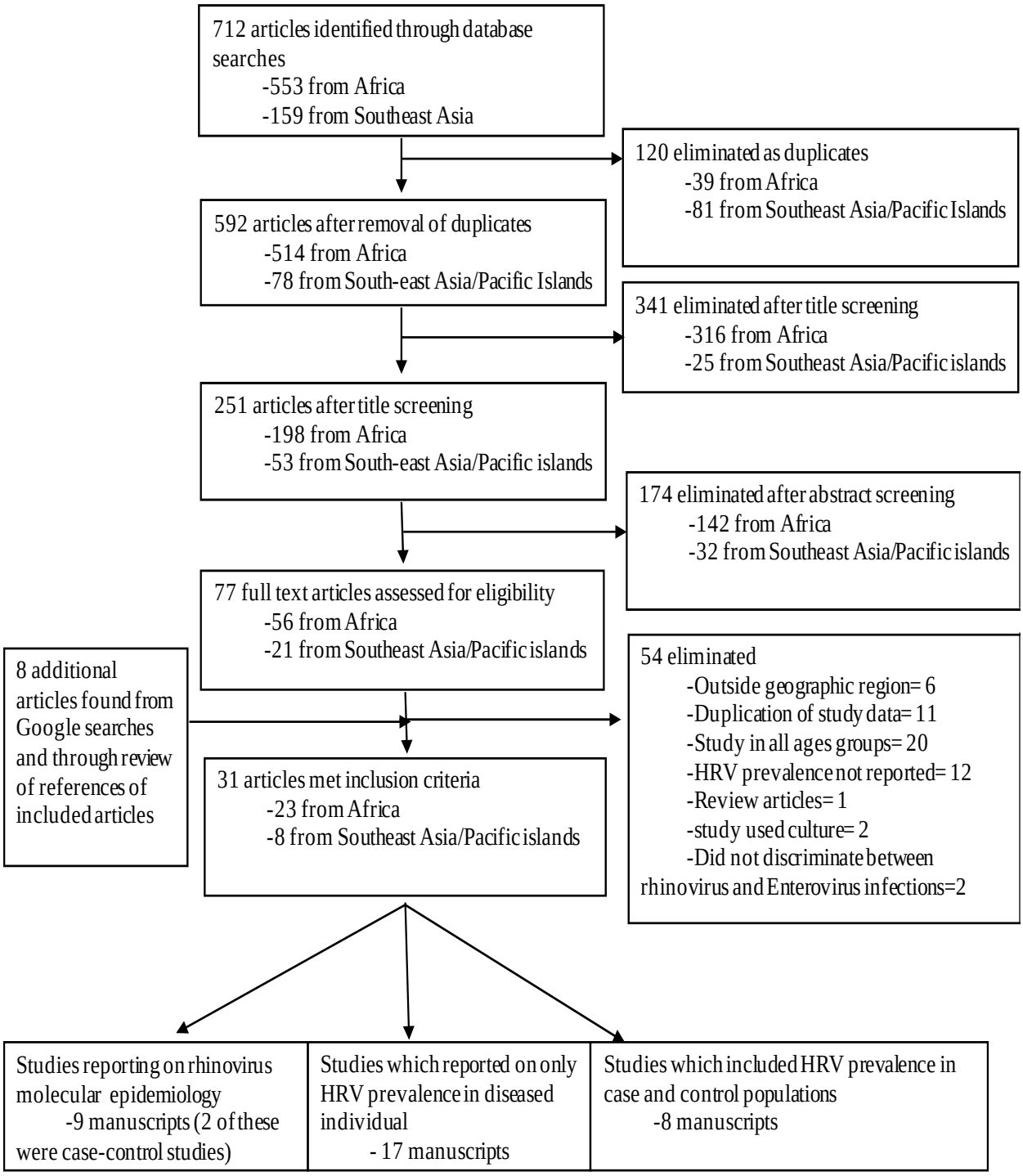

Figure 1.2: Flow diagram of article selection reporting on HRV in Africa and Southeast Asia

Of the 31 manuscripts included in the review, 9 reported on the molecular epidemiology of HRV detected within the population [16, 35, 66, 85-87, 92, 99, 107] and only two (Kenya and Botswana) of the 9 enrolled controls [92, 99]; Table 1.1.

In the Kenyan study [92], children less than 12 years of age presenting at the local hospital with any severity of LRTI were enrolled (n=1759), as well as children with or without an URTI as a control group (n=254). HRV was detected in 22% of the cases (n=380/1759; with HRV-A, HRV-B, HRV-C constituting 47%, 5% and 48%, respectively) and 24% (P=0.38) of the controls (n=61/254; HRV-A, HRV-B, HRV-C constituting 41%, 15% and 44% respectively). None of the HRV species were associated with more severe disease when comparing between the cases with LRTI; and similarly the prevalence of HRV detection and species distribution was similar between cases with severe disease compared to controls with URTI. Nevertheless, the authors cautioned that due to the study design, including imbalance between case and control enrolment, they were unable to make definitive conclusions on HRV epidemiology and disease severity. The controls were also enrolled from a convenient sample of children presenting at the hospital for routine immunisation, and might not have been representative of the population. Also, they experienced challenges to classify all the HRV positive samples, with 25% (n=109/441) of the samples failing to amplify during sequencing, which was significantly higher among samples from controls (44%, n=27/61) compared to cases (22%, n=82/280, P<0.001), which further limited the robustness of their findings. In addition, due to limited availability of clinical data, they were unable to analyse for any association between the HRV types and disease severity; and also did not investigate for other respiratory virus co-infections [92].

Similar findings as in Kenya were observed in Botswana [99] which enrolled 310 cases and 133 matched case-controls. This study too did not identify a difference in prevalence of HRV between cases aged 1-23 months hospitalised for pneumonia (31%; n=97/310) and controls (30%; n=40/133, P>0.99) who were matched for age and date of enrolment. In this study, 75% of cases had at least one respiratory virus detected, including RSV, which in contrast to HRV was detected in 35% (n=107/310) of the cases and only 2% (n=2/133) of the controls (P<0.001). Co-infections between HRV, RSV, Influenza A and B virus, PIVs (type 1-3), human Metapneumovirus (HMPV) and Adenovirus occurred in 8% (n=25/310) of the cases overall, of which 64% (n=16/25) were between RSV and HRV. Serotyping was conducted in 84 of the cases positive for HRV (HRV-A=44%, HRV-B=8%, HRV-C=48%); however, in the case-control matched participants only 34 cases (HRV-A=44%, HRV-B=15%, HRV-C=41%) and 31 controls (HRV-A=51%, HRV-B=10%, HRV-C=39%, P=0.99) were serotyped, with no difference in serotype distribution observed [99]. This study did not explore whether there was any association between specific HRV type and

disease severity. Both of these studies acknowledged that their sample size were not sufficient to fully elucidate the causal role of HRV in the aetiology of pneumonia.

The remaining seven studies which analysed the clinical and molecular epidemiology of HRV did not enrol controls. Two of the studies sequenced less than 100 HRV positive samples and only provided a descriptive analysis of the molecular characterisation of HRV without associating it with disease outcome [35, 107]. The South African [35] study focused on wheezing in children younger than 5 years and found that HRV was the most common virus detected (58%; n=128/220), with HRV-C being the most commonly detected species (HRV-A=37%, HRV-B=11% and HRV-C=52%), although only 55% (n=71/128) of the samples were serotyped. Smuts et al. [35] also investigated for RSV, influenza virus, adenovirus, PIVs, HMPV, human Coronavirus (HCoV) and human Bocavirus (HBoV); and found that co-infection occurred in 16% (n=20/128) of the HRV positive samples. The most common co-infecting virus being HMPV (n=8/128, 6.3%), followed by HBoV (n=6/128, 4.7%) and HCoV (n=1/128, 0.8%). Comparing the HRV positive and negative participants, cases with HRV were more likely to have vomiting and less likely to be febrile. In the Singaporean study (Tan et al. 2009), HRV was the most commonly detected respiratory virus (13%; n=64/500) in children hospitalised with ARI; which included 48% (n=31/64) with LRTI, 25% (n=16/64) with URTI and 27% (n=17/64) with undefined symptoms. The molecular epidemiology of HRV was determined in 90% (n=58/64) of the samples, with HRV-A dominating (HRV-A=73%, HRV-B=14% and HRV-C=3%). HRV co-infections were detected in 8% (n=10/128) of the participants, most commonly with HBoV (n=7/10) followed by RSV (n=2/10), and 60% of these co-infections were in children hospitalised with LRTI, suggesting that HRV associated co-infections may result in more severe disease episodes albeit not being statistically significant.

In the remaining five studies which examined the clinical and molecular epidemiology of HRV, there were suggestive associations between HRV infection and disease severity. In the Burundian study [16] which enrolled children aged 1 month to14 years of age hospitalised with LRTI, HRV was identified among 40% of cases, with a dominance of HRV-A (A=55%, HRV-B=12 and HRV-C=32%). HRV-A infections were the most frequently identified HRV species in children with pneumonia and bronchiolitis diagnosis, whereas HRV-C infections were most common in children presenting with wheezing. They did not,

however, investigate the association of HRV with any other respiratory pathogens. In the Malaysian study [87], 165 children younger than 5 years of age hospitalised with acute LRTIs were enrolled, among whom HRV was identified in 33% of cases. Although these cases were also investigated for RSV, HMPV, Influenza-A and Influenza-B, PIVs, HCoVs, HBoV and adenovirus, the majority (67%) of HRV cases were mono-infections, whilst HRV was most likely to form co-infections with RSV (n=11/18; 61%). Children with HRV mono-infections were significantly older than children with RSV mono-infections (11.8 versus 9 months), and younger than children with Influenza-A mono-infections (11.8 versus 12.7 months). They also reported that children with HRV mono-infections were hospitalised earlier during the course of disease, 1.9 days' post symptom onset compared to 4 and 4.8 days for RSV and Influenza mono-infections respectively, $P<0.001$. Furthermore, children with HRV infections were less likely to be febrile (67%) compared to RSV (92%, $P<0.003$) and Influenza (100%, $P=0.044$) associated LRTI cases. The molecular distribution of the HRV mono-infections were HRV-A=61%, HRV-B=0 and HRV-C=39%; with HRV-C being more commonly associated with wheezing, vomiting, rhonchi, higher neutrophil counts and generally more severe disease compared to HRV-A infections. Furthermore, HRV-A was detected more frequently in younger age groups (6-11 months) and HRV-C in older children (12-23 months) [87].

In the Thai study [86], of 289 children hospitalised with acute LRTI, 30% were positive for HRV, with HRV-C being most common (HRV-A=33%, HRV-B=9% and HRV-C=58%). HRV was a mono-infection in 62% of the positive cases, whilst co-infections in the others included RSV (36%), Influenza and adenovirus (18% each). Other viruses investigated for included: PIVs, HBoV, Influenza-A and Influenza-B, Adenovirus, RSV, HMPV and Polyomaviruses (WU/KI). The authors, however, concluded that the sample size was inadequate for statistical analysis between disease outcomes of HRV compared to other respiratory viruses, or by HRV species and disease severity. However, 50% and 72% of hospitalised participants infected with HRV-C presented with asthma and wheeze, respectively.

The Vietnamese [85] study enrolled 1082 children hospitalised with ARI, with HRV identified in 30% of cases, including 72% mono-infections. Of the 91 mixed HRV co-infections which included investigation for Influenza-A and Influenza-B, HMPV, PIVs,

HCoVs, adenovirus, HBoV and RSV which accounted for 53% (n=48) of the co-infections. Similarly to the Malaysian study [87], children with HRV mono-infections were older than children with RSV mono-infections (9 versus 7 months, P=0.02) and younger than children with influenza mono-infections (9 versus 22.5 months, P<0.001). Furthermore, similar to the South African [35] and Malaysian study [87], children with HRV mono-infections were less likely to present with febrile disease compared to the other virus mono-infections (P<0.001). HRV mono-infections were also more likely associated with hypoxia (12.4%) compared to RSV (3.8%, P=0.002) and PIVs mono-infections (0%, P=0.02); and presented more often with wheezing (63.2%) compared to Influenza mono-infections (42.3%, P=0.038). Finally, the blood eosinophil count was significantly elevated during HRV mono-infections in comparison to all the other virus mono-infections (P<0.001). As eosinophil levels are a predictor of reactive airway disease, this could been indicative of these children having underlying asthma, or that HRV might predispose to subsequent asthma [85]. On comparison of mono and mixed HRV infections, generally there were no differences in clinical features, except that HRV co-infections were more likely to be associated with chest retractions (70.3% versus 57.3%, P=0.032). Of the 18% of the HRV serotyped, HRV-A was the most dominant (HRV-A=76%, HRV-B=0, HRV-C=24%) [85].

Finally, in the Philippino study [66], HRV was identified in 33% of the 816 children hospitalised with severe pneumonia. The molecular epidemiology of these cases also indicated HRV-A as being the most dominant - HRV-A=56%, HRV=10% and HRV-C=34% - whilst 1% of samples failed to amplify and 10% were identified as enteroviruses. Viraemia for HRV was identified among 12% (n=30/243) of the HRV cases tested, with cases with HRV-C being 15.04-fold more likely to be viraemic (31%; n=26/83) compared to HRV-A (n=4/135, 3%, P<0.01) and HRV-B (n=0/25, P<0.01). Furthermore, cases with HRV viraemia had significantly lower oxygen saturation for both HRV-A and HRV-C compared to those without viraemia. Also, HRV-A cases with viraemia were significantly more likely to have wheezing (100%) compared to HRV-A cases without viraemia (45%, P<0.05); with a similar trend observed for HRV-C (69% vs. 48%, P=0.1). Case fatality ratio, however, did not differ by the presence (3%) or absence (9.5%, P=0.37) of viraemia. This study concluded that HRV-C is more likely to be associated with viraemia [66], which could explain that the association of increased disease severity reported in several studies with HRV-C compared to the other HRV species [26, 37, 42, 43]. This study did not investigate for any other respiratory virus infections [66].

Table 1.1: HRV prevalence and molecular epidemiology by Region (Africa and Southeast Asia) and Country

Country	Study population	Case definition	Median age	Study duration	# Cases enrolled	HRV prevalence*	Percentage species distribution in cases
Africa							
Botswana [99]	<2 years	Hospitalised with WHO defined pneumonia	6.1 months	April 2012-Aug 2014	310	31%	A=36% B=7% C=41%
Burundi [16]	<14 years	Hospitalised with LRTI	1.94 years	Nov 2010 – Oct 2011	462	40.3%	A=43.5% B=9.7% C=25.3%
Kenya [92]	<12 years	Hospitalised with LRTI	Not reported	Jan 2007-Dec 2009	1759	21%	A=47% B=4.4% C=48%
South Africa [35]	2-59 months	Presenting with acute wheezing	12.2 months	May 2004-Nov 2005	220	58.2%	A=37% B=11% C=52%
Southeast Asia							
Malaysia [87]	<5 years	Hospitalised with LRTI	11.8 months	June-Dec 2009	165	33%	A=61% B=0% C=39%
Philippines [66]	<15 years	Hospitalised with severe pneumonia	9 months	May 2008 – May 2009	816	29.8% in nasal swabs. 12.3% in serum samples	A=55% (3% in serum) B=10% (0% in serum) C=34% (32% in serum)
Singapore [107]	<12 years	Hospitalised with RTI	Not reported	Oct 2005-Mar 2007	Retrospective study of 500 samples	12.8%	A=73% B=14% C=3%
Thailand [86]	Paediatric patients	Hospitalised with LRTI	Not reported	Feb 2006-Feb 2008	289	30%	A=33% B=8% C=58%
Vietnam [85]	<15 years	Hospitalised with RTI	9 months	April 2010-May 2011	1082	30%	A=75.9% B=0% C=24.1%

HRV: Human rhinovirus; ALRTI: Acute lower respiratory tract infection; LRTI: Lower respiratory tract infections; URTI: Upper respiratory tract infections; RTI: Respiratory tract infection; WHO: World Health organisation. *All HRV prevalence's were detected in nasopharyngeal swabs unless stated otherwise

Seven studies were identified that enrolled both cases with severe respiratory disease and asymptomatic controls or controls with mild respiratory disease to determine the attributable role of HRV in the aetiology of LRTI [54, 83, 92, 93, 95, 99, 106]; Table 1.2. Of these seven studies, there was higher odds for HRV to be prevalent in LRTI cases than controls in only the South African study (33% vs. 18.8%, P=0.02); however, the lower bound of 95% confidence interval of these estimates approximated 1.0; Figure 1.3 [54]. The study from South Africa [54] enrolled children presenting at the hospital and were categorised as acute LRTI (ALRTI) presenting at the emergency room but not requiring hospitalisation, children hospitalised as well as patients requiring intensive care unit (ICU) treatment. They also enrolled a limited number of controls which were children presenting at a vaccine centre. They reported that HRV prevalence was higher among cases requiring hospitalisation (36%) compared to controls (19%, P=0.047) and there was a trend for all the ALRTI cases (regardless of hospitalisation) to have a higher HRV prevalence compared to the controls (32.5% versus 19%, P=0.072). HRV was detected as a mono-infection in 50% of all the ALRTI cases, and HRV was detected as a mono-infection in 88% of the hospitalised cases (n=88/109). A significantly greater proportion of the children with HRV mono-infections had to be hospitalised and required ICU treatment compared to children hospitalised with HRV co-infections (P<0.001). They also investigated for RSV, Influenza virus (A and B), PIV (Type 1-3), HCoV (OC43, NL63, 229E, HKU1), Adenovirus (AdV), HMPV and HBoV and found that HRV was the most prevalent virus detected (33%) followed by RSV (30.1%) and HBoV (6.1%) [54].

The Cambodian study [83] was the only other case-control study which analysed the detection of HRV further than only its prevalence in the cases and controls. They found that HRV (20%) was detected in similar prevalence to RSV (19%). They also investigated for Influenza (A and B), PIV (type 1-4), AdV, HMPV, HBoV, HCoV (OC43, NL63, 229E, HKU1) and enterovirus. HRV was the second most commonly detected mono-infection (n=169/204, 83%) following RSV (n=167/192, 87%); and HRV was most commonly detected together with RSV (n=10/35, 29%) and the HBoV (n=8/35, 23%). They found that infections with RSV and HRV could not be distinguished clinically for either infants or children 1-4 years of age. They also enrolled 50 controls but did not elaborate on the inclusion criteria for these controls or how they went about recruiting the controls. HRV was

detected in 12% (n=6/50) of the controls and RSV was detected in 8% (n=4/50) [83]. The number of controls was too small for any additional analysis.

The remaining five studies found no difference in HRV prevalence between cases (47.5%-18%) and the controls (19%-50%) [92, 93, 95, 99, 106] and hence, did not undertake any further analysis specific to HRV. The only studies which matched community controls to cases were from Botswana, with matching including for age, HIV status and period of enrolment [99] and Kenya [95] which age frequency matched the cases and controls as well as matched for month of the year of enrolment. The controls for the remainder of the studies were either a convenience sample of children presenting for routine immunisation or in vaccine trials. This could have biased their generalisability to the population. Furthermore, the number of cases enrolled greatly outnumbered the number of control participants, possibly further compromising statistical comparisons.

Table 1.2: HRV prevalence reported in case-control studies

Study area	Ages	Case definition	# cases enrolled (n)	HRV positives cases (n, %)	Control definition	# controls enrolled (n)	HRV positive controls (n, %)
RSA [54]	<5 years for case <2 years for controls	ALRTI	610	201 (33%)	Community controls without respiratory disease	48	9 (18.8%)
Botswana [99]	1-23 months	Hospitalised with Pneumonia	310	97 (31.3%)	Community based controls without ALRTI, age and enrolment date matched to cases	133	40 (30%)
Kenya [93]	<5 years	Hospitalised with severe or very severe pneumonia	204	97 (47.5%)	Presenting for immunisation or medicine refills. Non severe illness	48	24 (50%)
Kenya [92]	1 day-12 years	Hospitalised with LRTI	1759	380 (21.6%)	Presenting for routine immunisation- with or without URTI	254	61 (24%)
Kenya [95]	<5 years	Hospitalised with severe or very severe pneumonia	810	184(23%)	Convenient sample of children presenting to outpatient clinics - with or without URTI	369	82 (22%)
Cambodia [83]	<5 years	Hospitalised with LRTI	1006	204 (20%)	Not reported	50	6 (12%)
Thailand [106]	<5 years	Hospitalised with LRTI	903	169 (18%)	Convenient sample from outpatients without fever, cough, sore throat or diarrhoea	116	14 (12%)

Abbreviations – n: number; HRV: Human rhinovirus; ALRTI: Acute lower respiratory tract infection; LRTI: Lower respiratory tract infections; URTI: Upper respiratory tract infections

All HRV prevalence in the table are based on nasopharyngeal samples

Overall, there was no difference observed in the meta-analysis on prevalence of HRV identification between cases of ARI (23.9%; which varied in their severity and clinical presentation between studies) and controls (23.7%, P=0.197; which varied in their representivity to the general population); Figure 1.3. Also, this meta-analysis needs to be interpreted with caution, since in addition to differences in case definitions and control selection between the studies, the studies also varied in the age groups included; Table 1.2.

Study	Cases	Controls	OR (95% CI)	P-value
Kenya (Onyango et al. 2012)	380/1759 (21.6%)	61/254 (24.02%)	0.87 (0.64,1.21)	0.37
Botswana (Kelly et al. 2015)	97/310 (31.29%)	40/133 (30.08%)	1.06 (0.67,1.70)	0.82
RSA (Venter et al. 2011)	143/399 (35.84%)	9/48 (18.75%)	2.42 (1.11,5.84)	0.02
Kenya (Breiman et al. 2015)	97/204 (47.55%)	24/48 (50%)	0.91 (0.46,1.79)	0.87
Cambodia (Guerrier et al. 2013)	204/1006 (20.28%)	6/50 (12%)	1.86 (0.78,5.43)	0.2
Thailand (Hasan et al. 2014)	169/903 (18.72%)	14/116 (12.07%)	1.68 (0.93,3.26)	0.09
Combined	1090/4581 (23.79%)	154/649 (23.73%)	1.24 (0.89,1.72)	0.197

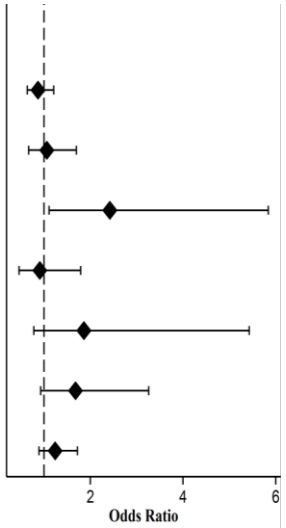

Figure 1.3: Studies reporting on the proportion of HRV-associated cases and controls. Using exact conditional logistic regression, odds ratios (OR) were used to compare the proportions of children with HRV-associated severe disease (cases) versus children with asymptomatic HRV infections (controls).

The remaining 17 studies were primarily surveillance studies conducted in children presenting with respiratory infections and mainly focused on other more established respiratory pathogens such as RSV and Influenza virus [81, 94, 96, 97, 101, 102, 104]; Table 1.3. HRV prevalence in these studies was reported as part of the general surveillance, and no further analyses of the prevalence or clinical presentation of HRV were undertaken in these studies. Furthermore, the studies varied in age groups of children enrolled, case definition with some focusing only on URTI [91, 94], others only on LRTI [80, 88, 89, 96, 101, 102, 105], others reported on both URTI and LRTI separately [81, 84] or some which enrolled all RTI and did not differentiate between URTI and LRTI cases [90, 97, 98, 100, 103, 104].

The majority of the surveillance studies, however, did focus on LRTI in children, including 7 which were hospital based [80, 89, 96, 101, 102, 105, 108] and two others which enrolled hospitalised children with either LRTI or URTI and reported the results separately [81, 84]; Table 1.3. In these studies, the prevalence of HRV ranged from 17%-53%, with the Madagascar study [96] reporting the lowest prevalence (17%). In this study, HRV was the third most common detected virus following RSV (n=130/295, 44%) and influenza-A virus (n=71/295, 24%). HRV was detected as a mono-infection in 14% of cases, and more commonly detected as a co-infection with RSV (n=12/49, 24%) and influenza-A virus (n=9/49, 18%). The study from Morocco [101] reported the highest HRV prevalence (53%), followed by RSV (n=125/684, 18%) and adenovirus (n=16/684, 17%). No further analysis was conducted on HRV in this study, as was the case in the majority of the other observational studies, with many authors suggesting that the pathogenic role of HRV during disease remained uncertain due to the high prevalence reported in asymptomatic individuals and prolonged duration of shedding of HRV [81, 84, 88, 102, 105].

Similarly, in the studies which did not distinguish between LRTI or URTI, the prevalence of HRV ranged from 10% to 42%. The study conducted in Senegal [104] had the lowest prevalence of HRV (10%) and was conducted in children <5 years age with fever and any other sign of respiratory infection. The inclusion of fever as a pre-requisite for screening, might explain why influenza virus was the most prevalent virus detected in this study (n=25/82, 31%), followed by RSV (n=13/82, 16%). Nevertheless, HRV was predominantly identified as a mono-infection in this study, with only a single case of HRV-RSV mixed infection observed. The prevalence of HRV in the other four studies ranged from 33%-42%.

The study in Burkina Faso [100] reported the highest prevalence of HRV (42%), 85% of which were mono-infections. In the Mozambican study [80] the overall prevalence of HRV in infants with ARI episodes was 26%. This tended to be higher among hospitalised cases (36%, n=10/28, P=0.22), making it the most prevalent virus identified among hospitalised ARI cases of which 67% were mono-infections, and adenovirus being the most common co-infection among those with a mixed-infection.

The other studies were conducted in children presenting with URTI, the first included children with acute otitis media, among whom HRV was identified on nasopharyngeal swabs in 38% of cases [91], and as the most prevalent virus identified irrespective of HIV status (33% in HIV-infected and 39% in HIV-uninfected children). In the study from Kenya [94] among older children (5-10 years) presenting with malaria like symptoms, HRV was detected in 26% of cases, including among 37% of the cases who tested positive for malaria.

Table 1.3: The prevalence of HRV in paediatric surveillance studies of respiratory disease

			LRTI			URTI			URTI AND LRTI combined		
	Median age	**Ages included**	**# cases (n)**	**Prevalence**	**Case definition**	**# cases (n)**	**HRV prevalence**	**Case definition**	**# cases (n)**	**HRV prevalence**	**Case definition**
Enrolled patients with both URTI and LRTI.											
Madagascar [81]	2 years	2-59 months	178	43 (24%)	Physician diagnosed LRTI	117	18 (15%)	URTI with or without cough	295	61 (21%)	URTI and LRTI combined
Vietnam [84]	1.4 years	<15 year	557	153 (28%)	Hospitalised with LRTI	391	113 (29%)	Hospitalised with URTI	948	266 (28%)	Hospitalised with ALRTI
Enrolled only patients with LRTI											
Madagascar [96]	1.1 years	<5 years	290	49 (17%)	Hospitalised with LRTI	URTI cases not included in this study					
Morocco [101]	21.5 months	2-59 months	684	360 (53%)	Hospitalised with Severe pneumonia	URTI cases not included in this study					
Mozambique [80]	not reported	<5 years	807	196 (24%)	Hospitalised with severe pneumonia	URTI cases not included in this study					
South Africa [88]	not reported	<5 years	8393	3115 (37%)	Hospitalised with LRTI	URTI cases not included in this study					
South Africa [89]	10 months	<2 years	1460	466 (32%)	Hospitalised with LRTI	URTI cases not included in this study					
Zambia [105]	9.6 months	<5 years	297	57 (19%)	Hospitalised with SARI	URTI cases not included in this study					
Niger [102]	9 months	<5 years	160	47 (29%)	Hospitalised with SARI	URTI cases not included in this study					
Enrolled only patients with URTI											
Kenya [94]	7 years	5-10 years	LRTI cases not enrolled in this study			197	52 (26%)	URTI with Malaria like symptoms	LRTI cases not enrolled in this study		
South Africa [91]	14 months	<5 years	LRTI cases not enrolled in this study			260	98 (38%)	Acute Otitis Medias	LRTI cases not enrolled in this study		
Enrolled all RTI and did not differentiate between URTI and LRTI											
Mozambique [97]	6 months	<12 months	LRTI and URTI not reported separately						333	86 (26%)	ARI – inpatients and outpatients
Angola [98]	36 months	1 month – 14 years	LRTI and URTI not reported separately						102	34 (33%)	RTI - outpatients
Burkina Faso [100]	Not	<3 years	LRTI and URTI not reported separately						209	88 (42%)	RTI – inpatients

	reported					and outpatients
Nigeria [103]	not reported	<14 years	LRTI and URTI not reported separately	246	87 (35%)	RTI – inpatients and outpatients
Senegal [104]	not reported	<5 years	LRTI and URTI not reported separately	82	8 (10%)	RTI - outpatients
South Africa [90]	4.7 months	2-13months	LRTI and URTI not reported separately	195	76 (39%)	Admission to ICU with positive respiratory virus sample

Abbreviations - ALRTI: Acute lower respiratory tract infection; LRTI: Lower respiratory tract infections; URTI: Upper respiratory tract infections
All shaded blocks indicate that these fields were not applicable to these studies

The differences in age groups and clinical definitions could account for the variability in HRV detection (16%-53%) between the studies; Table 1.3. Of the 17 surveillance studies, 10 provided the prevalence of HRV among LRTI cases by age groups. The majority of the studies stratified for infants and age 1-5 years (9/10). Furthermore, 3 studies included prevalence in children >5 years. Nevertheless, the meta-analysis on prevalence of HRV did not differ by age groups, including 39% (95% CI: 0.32-0.46) among the 1 to 12-month age group (Figure 1.4A) and 36% in the 1 to 5-year group (95% CI: 0.31-0.41) (Figure 1.4B) and 5 to 10-year age group (Figure 1.4C).

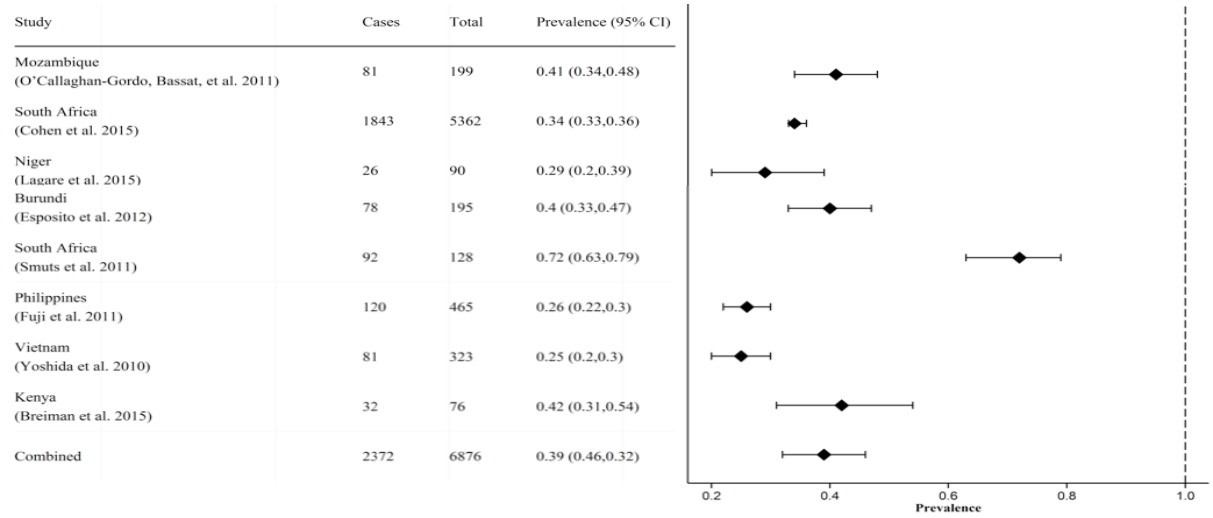

Figure 1.4A: The prevalence of HRV in children <12 months. Using exact conditional logistic regression, odds ratios (OR) were used to compare the proportions of children with HRV-associated disease in infants.

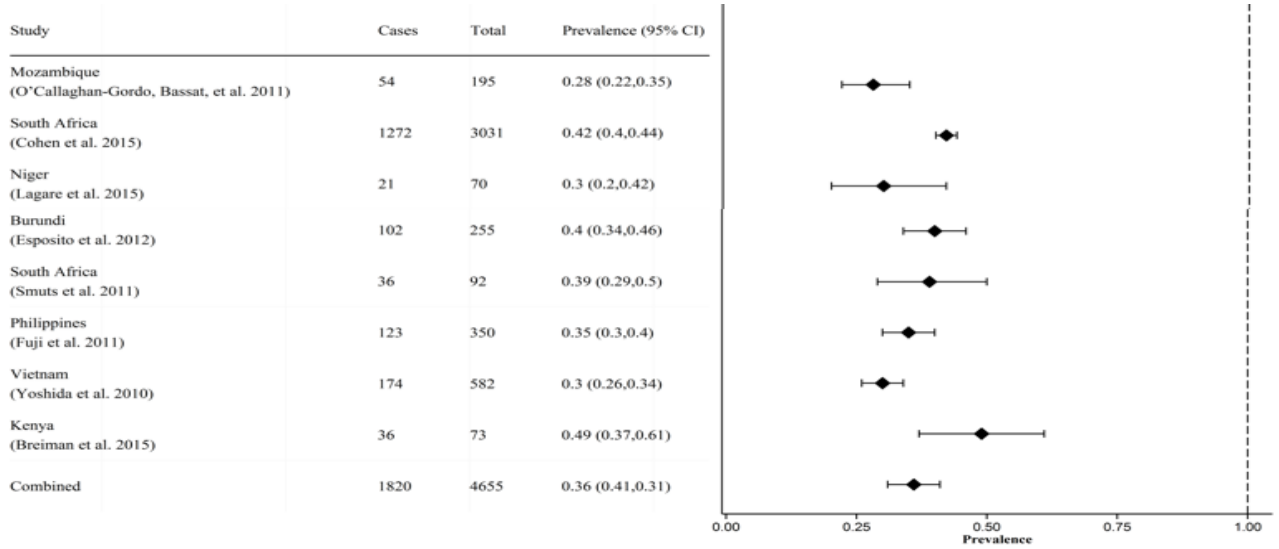

Figure 1.4B: The prevalence of HRV in children 1-5 years of age. Using exact conditional logistic regression, odds ratios (OR) were used to compare the proportions of children with HRV-associated disease in children 1-5 years of age.

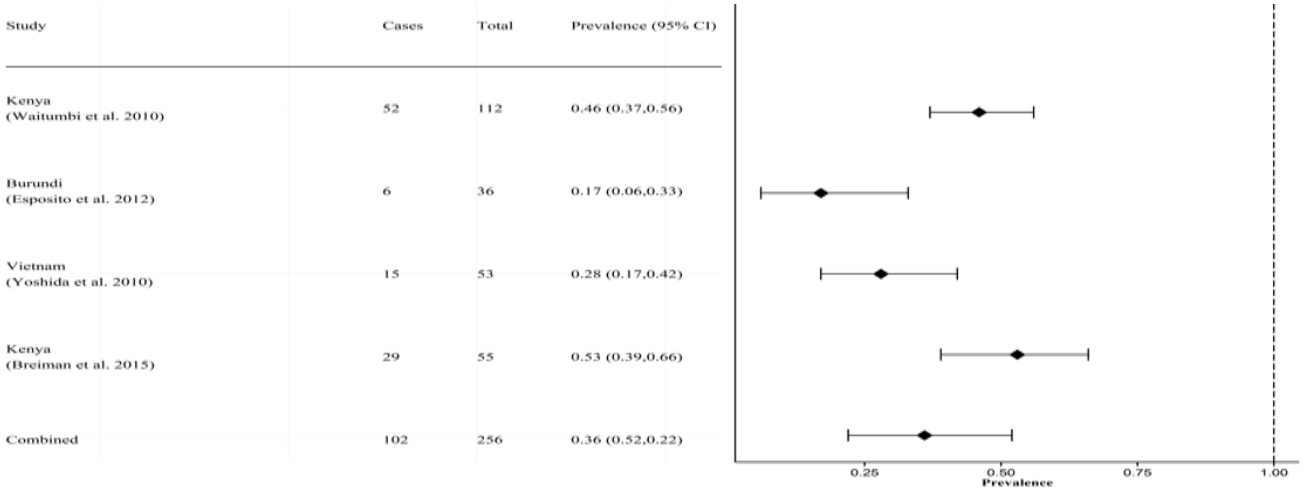

Figure 1.4C: The prevalence of HRV in children 5-10 years of age. Using exact conditional logistic regression, odds ratios (OR) were used to compare the proportions of children with HRV-associated disease in children 5-10 years of age.

Our systematic review on HRV in low to middle income countries in Africa and Southeast Asian/Pacific countries confirms the scarcity and limitations of the available clinical and molecular epidemiology data. This further emphasises the uncertainty on the role of HRV in the pathogenesis of childhood respiratory disease, even though in all of the studies HRV was one of the most if not the most prevalent virus detected. In the studies which reported on more than just the prevalence of HRV in the study population, it was evident that HRV was most commonly detected as the only respiratory virus. None of the studies, however, analysed for co-infections with bacteria even though HRV infections have been shown to enhance both subsequent infection with *Streptococcus pneumoniae* and *Staphylococcus aureus* through the upregulation of bacterial adhesion to the respiratory epithelial cells and through the modulation of the host immune responses [109, 110]. This is most likely because bacterial co-infection relationships are difficult to fully elucidate as the currently available diagnostic tools for diagnosing bacterial pneumonia are insensitive, with blood culture sensitivity ranging between 15-30%, whilst obtaining samples from the site of infection is challenging with direct aspiration of the lungs rarely performed [95, 111].

The need for a large sample size was highlighted by the two studies which investigated the HRV molecular epidemiology in both case and controls [92, 99], but failed to analyse the epidemiology in terms of clinical outcomes, disease severity or even basic comparison between case and control populations due to the limited statistical power of the study, particularly the limited number of control subjects. In the studies which did not enrol controls [16, 35, 66, 85-87, 107], the analysis of the different HRV species in terms of clinical outcomes and disease outcome showed that the HRV species could in fact have different clinical presentations; however, without the controls it is uncertain what the contribution of different HRV types is in the pathogenesis and aetiology of LRTI. Nevertheless, the studies provide some indication that HRV might be an important respiratory pathogen, especially in developing countries where HRV is found to be the most prevalent virus detected in RTI cases irrespective of age group and clinical syndromes.

A large scale surveillance project is needed which looks at both severe RTI and healthy controls to fully elucidate the attributable pathogenic role of the different HRV species in severe LRTI among children in low to middle income countries. This will need to include an in-depth clinical and socio-economic analysis from cases and healthy cohorts, to fully study

the clinical manifestation and risk factors for HRV infection; and also to evaluate the impact of HIV infection on HRV associated severe pneumonia hospitalisations.

1.4 The Pneumonia Etiology Research for Child Health (PERCH) project

The PERCH project is a case-control study aimed at fully characterising the aetiology of WHO-defined severe and very severe pneumonia in children aged 28 days to 59 months in nine sites across seven countries in Africa and Southeast Asia making it the biggest study looking at the aetiology of childhood pneumonia since the Board of Science and Technology for International Development studies which were conducted in the late 1980s [112]. In order to provide a comprehensive analysis of epidemiological data from a broad representation of regions where the highest incidences of pneumonia related morbidity and mortality currently occurs [113] the study included five sites from five African countries and four sites from two Southeast Asian countries: Basse, The Gambia; Kilifi, Kenya; Bamako, Mali; Lusaka, Zambia; Soweto, South Africa; Dhaka and Matlab, Bangladesh and Nakhom Phanom and Se Kaeo, Thailand. Table 1.4 provides a summary of the sites and the recruitment statistics for the countries involved in the PERCH project. Enrolment into the project started at the South African and Kenyan sites in mid-August 2011 and recruitment into the project was completed at all sites by February 2014. During the recruitment phase of PERCH, a combined total of 3648 Cases (239 HIV positive) and 4541 Controls (229 HIV positive) were enrolled in the project.

The main difference between PERCH and other pneumonia aetiology studies is the specially designed and highly sensitive molecular detection techniques employed at all the sites for determining the cause of pneumonia as well as the case-control study design. This design provides vital information on the controls which in turn allows for additional interpretation and extrapolation of the finding from the pneumonia cases. This is especially true for interpreting the results from the nasopharyngeal samples which are blurred by the presence of colonisation organisms and for identifying risk factors which are associated with severe and very severe pneumonia. Strict enrolment criteria for both the cases and the controls, sampling strategies and laboratory testing algorithms were employed and standardised across all the sites to allow for analysis of cross site data [114].

The PERCH cases were children hospitalised with WHO-defined severe and very severe pneumonia and the controls were enrolled from the community based on age-frequency matches to the cases, additionally the controls were also HIV status matched at the Kenya and South African sites where HIV prevalence was high. The controls could have signs and symptoms of ARI provided they did not have severe or very severe pneumonia requiring hospitalisation. Cases and controls were subjected to blood draws, nasopharyngeal (NP) swabs and urine collection. Additional sample collection amongst cases included induced sputum, gastric aspirates, tracheal aspirates, lung aspirates, pleural fluid taps and post-mortem lung biopsy if clinical relevance was indicated. Extensive information on participants' demographic, nutritional, co-morbidities, family environment and socio-economic status were obtained on enrolment to allow for risk factor analysis. Clinical assessments were performed on controls and cases at enrolment (i.e., on admission to the hospital for cases) and then again for the cases at 24 and 48 hours after admission, and on the day of discharge from hospital.

Standard culture diagnostic techniques were performed on the samples (blood, pleural fluids, lung aspirates, tracheal aspirates and induced sputum) collected from the cases; in addition, state-of-the-art molecular diagnostic techniques were performed on the respiratory samples collected from the cases and the controls in order to better understand the attributable role of analysed respiratory pathogens in severe and very severe pneumonia. The primary molecular diagnostic panel was designed by Fast Track Diagnostics (FTD) (Luxembourg) and were real-time multiplex PCR assays which detected 33 respiratory pathogens including viral, bacterial and fungal parasites. The specificity has been validated using positive plasmid controls and the sensitivity using a large number of respiratory samples. In all validation experiments the primer and probes for the different pathogens showed a 100% specificity and sensitivity.

Table 1.4. A summary of the countries involved with PERCH together with their recruitment information

Site	Country	HIV prevalence	Urban/ Rural	Date enrolment commenced	Date enrolment completed	# Cases enrolled	# Controls enrolled
Soweto	South Africa	Very High	Urban	Aug 2011	Aug 2013	919	962
Kilifi	Kenya	Medium	Rural	Aug 2011	Nov 2013	579	793
Basse	The Gambia	Low	Rural	Nov 2011	Oct 2013	539	580
Bamako	Mali	Low	Urban	Jan 2012	Jan 2014	557	583
Lusaka	Zambia	High	Urban	Oct 2011	Sep 2013	574	645
Sa Kaeo and Nakhon Phanom	Thailand	Low	Mixed	Jan 2012	Jan 2014	192	541
Dhaka and Matlab	Bangladesh	Low	Mixed	Jan 2012	Dec 2013	377	607

Abbreviations - HIV: Human Immunodeficiency virus

1.5 Justification and Objectives

Studies have identified HRV as one of the most common viruses associated with childhood CAP; however, many gaps in knowledge still exist in terms of the role of HRV infection in severe pneumonia cases and the impact of different HRV species on disease severity. The majority of the HRV studies have focused on the prevalence of HRV in hospitalised children only, and thus have been unable to attribute the causal role of HRV in disease as they have either collected limited clinical background and/or have not enrolled asymptomatic controls positive for HRV infection. In the studies which have analysed HRV prevalence and species distribution, the studies have lacked power to evaluate the distribution in relation to clinical manifestation in diseased children. Another limitation of studies on HRV include it being limited in the geographical distribution of the studies, with the majority conducted in single geographic locations over a limited time period, thus restricting the generalisability of the findings. This has contributed to the conflicting data on the role of HRV in the aetiology of childhood severe pneumonia. This is especially true for low to middle income countries where the burden of pneumonia disease is greatest.

The case-control PERCH project provides an attractive framework to examine the prevalence, as well as the molecular distribution of HRV species in both sick and asymptomatic children over a large geographical location over a period of two calendar years. In addition, HRV viraemia and viral load as well as pneumococcal disease and HRV infection associations and mixed virus co-infection interactions can be studied in a large study population with controls to help interpret the results. Thus allowing for an in-depth analysis of the causal role that HRV plays during severe respiratory disease in low to middle income countries. To our knowledge, a study such as the one proposed which leveraged on data and samples from all the PERCH sites, has not been undertaken at such an international level to delineate the clinical and molecular epidemiology of HRV associated with severe and very severe pneumonia in children.

1.6 Aims and Objectives

i. To determine the clinical epidemiology of HRV in 9 sites, across 7 countries in Africa and Southeast Asia in HIV-uninfected children under 5 years of age who are hospitalised for WHO-defined severe or very severe pneumonia, including seasonality and risk factors associated with severe illness.

ii. To determine the associations between HRV and pneumococcal disease as well as other commonly detected respiratory viruses, including Influenza virus (A, B and C), RSV (A and B), AdV, HMPV (A and B), HBoV, PIV (Type 1-4) and HCoV (OC43, NL63, 229E, HKU1).

iii. To determine the impact of HIV-infection on the clinical epidemiology of HRV associated severe pneumonia in two countries with a high prevalence of HIV.

iv. To determine the molecular subtyping of HRV in children younger than 5 years over a two-year period from three African PERCH sites, i.e. Bamako in Mali, Lusaka in Zambia and Soweto in South Africa. This would include evaluation on whether there is any difference in HRV species associated with asymptomatic colonisation, mild upper respiratory illness and severe or very severe childhood CAP.

v. To undertake an analysis of the clinical and molecular subtyping of HRV and its ability to cause viraemia in South African children hospitalised with WHO-defined severe or very severe pneumonia compared with asymptomatic controls.

2.0 Materials and Methods

2.1 The Study Sites

The nine PERCH sites were chosen so as to represent as broad an epidemiological region as possible and which would mirror the expected pneumonia epidemiological setting from 2015 and forwards. The sites were chosen taking vaccine schedules such as *Haemophilus influenza* (HiB) and pneumococcal conjugate vaccine (PCV) as well as HIV and malaria incidences into account. Thus the chosen sites had either already implemented or had plans in place to start implementing the HiB and PCV vaccine schedules, except for Thailand where the vaccines are freely available but too expensive for the majority of the population; in addition, they had diverse epidemiological settings including both Africa and Southeast Asia where the majority of the severe and deadly pneumonia incidences occurs. Furthermore, they had economic backgrounds ranging from low to upper middle income settings; as well as rural and urban sites and factors like varying altitudes and cooking materials [113].

Figure 2.1 shows the geographic locations of the seven countries involved in the PERCH study as well as Baltimore where the PERCH executive committee is based at the Johns Hopkins Bloomberg School of Public Health (JHSPH) and where the project was co-ordinated from.

Figure 2.1: The geographical locations of the seven PERCH countries are marked in blue and the PERCH co-ordination centre in Baltimore is marked with a blue asterix

2.1.1 Soweto, South Africa

The South Africa site was based at the Chris Hani Baragwanath Academic Hospital (CHBAH) in Soweto, which is the only public hospital available to the surrounding population of approximately 1.5 million people living in a low to middle income urban setting. In 2014, the estimated HIV-1 prevalence in South Africa was approximately 10.2% of the total population with approximately 5.51 million people living with HIV-1. The highest prevalence was in the 15-49 age category with an estimated 16.8% of the population having HIV-1 and it is estimated that approximately 20% of South African women in their reproductive ages are HIV-1 positive [3]. Thus the South Africa site had the highest HIV-1 prevalence out of all the sites. According to the United Nations Children's fund, State of the World's children (SOWC) for 2014, South Africa is rated number 58/195 on the under 5 mortality rating (1 being the worst affected and 195 having the lowest mortality rate) with an estimated under 5 mortality rate of 44 deaths per 1000 live births and 65% of children under 5 years seeking medical care for signs and symptoms of pneumonia [115]. The HiB vaccine was introduced into the national vaccine schedule in January 1999 and PCV in November 2009 and by 2014 it was estimated that the vaccine coverage for HiB was 65% and for PCV was 62% [115]. Enrolment into the PERCH project commenced on the 11 August 2011 and was completed at the end of August 2013 with a total of 919 cases and 962 controls enrolled into the study.

2.1.2 Bamako, Mali site

The Mali site was located at the Hospital Gabriel Toure' in Bamako and is responsible for treating all severe childhood disease episodes. In 2000 a review of all hospital admissions showed that 71% of all children under the age of 16 were admitted for infections, and approximately 21% of the children admitted died within 3 days of admission with pneumonia accounting for 12% of these deaths [116]. The area is classified as having a very high malaria incidence resulting in 11% of paediatric deaths [116]; however, the HIV-1 incidence is low with an estimated 0.9% adult HIV-1 prevalence in 2014 [115]. According to the SOWC for 2014, the under 5 mortality rate for Mali was 123 deaths per 1000 live births making it number 7/195 on the Under 5 mortality rating index with a mortality rate of 78 per 1000 live births during infancy and 40 per 1000 live births for children; additionally, approximately 42% of children under 5 years were seeking medical care for signs and symptoms of pneumonia [115]. The HiB vaccine was introduced into the national vaccine schedule in

January 2008 and PCV in March 2011 prior to the commencement of PERCH and in 2014 it was estimated that both vaccines had an immunisation coverage rate of roughly 74% of Mali's children. Enrolment into PERCH commenced in January 2012 and was completed in January 2014 with a total of 557 cases and 583 controls enrolled.

2.1.3 Lusaka, Zambia site

The Zambia site was located at the University Teaching hospital in Lusaka which serves approximately 1.3 million people living in a 70km radius of the hospital. Roughly 30% of their admissions are children under 5 years and 23% of admissions are due to severe respiratory illness with a pneumonia case fatality rate of up to 25.8%. The estimated under 5 mortality rate in Zambia for 2014 was 87 per 1000 live births, making it number 21/195 on the under 5 mortality index rating, the infant mortality rate was 56 per 1000 live births and the neonatal mortality rate was 29 per 1000 live births [115]. In Zambia it is estimated that the HIV-1 prevalence is approximately 12.5% with 1.1 million living with HIV-1, 150 000 of whom are children. The Zambian site was characterised as a poor urban area with a high HIV-1 infection and malaria prevalence rate [113]. The HiB vaccine is readily available and widely utilised since February 2004 and in 2014 it was estimated that 79% of Zambian children had received the full dosing schedule [115]; however, the PCV vaccine was only introduced to Lusaka in July 2012 approximately 1 year after enrolment into the PERCH project commenced. Enrolment into PERCH commenced in October 2011 and was completed in September 2013 with 574 cases and 645 controls enrolled.

The HRV molecular subtyping analysis was conducted on case and control samples from the sites in South Africa, Mali and Zambia as these three sites combined give a very good representation of Sub-Saharan Africa as a whole and allows for a case population of 2050 children hospitalised with pneumonia and a control population of 2190 asymptomatic or healthy controls. Additionally, the molecular subtyping of HRV in Mali and the Zambia has not been previous characterised. Furthermore, at the South African site the ability of HRV to cause viraemic infection among cases and controls was analysed.

2.1.4 Kilifi, Kenya site

The Kenyan site was conducted in the rural, coastal community in the Kilifi district at the Kilifi District hospital. They have approximately 4 500 paediatric admissions per year mainly due to pneumonia and neonatal illness. Malaria used to be a problem in the area; however, after extensive treatment and prevention strategies were employed the rates have greatly decreased. The under 5 mortality rate in 2014 was 99 per 1000 live births making it number 33/195 on the under 5 mortality rank with an infant mortality rate of 48 per 1000 live births and a neonate mortality rate of 26 per 1000 live births [115]. The HiB vaccine was introduced in January 2001 and the PCV vaccine in February 2011 and in 2014 the immunisation coverage for HiB was estimated to be 83% and 75% for PCV [115]. Large scale routine surveillance projects are being conducted at this site looking at the impacts of PCV on pneumococcal disease. The HIV-prevalence for 2014 was estimated to be 6% with approximately 1,6 million people living with HIV, 190 000 of which are children [115]. Enrolment into the PERCH project began in August 2011 and was completed in Nov 2013, a total of 579 cases and 793 controls were enrolled.

2.1.5 Basse, The Gambia

The Gambian site was conducted in the Basse region through the UK Medical Research Council. The Gambia was the first African country to introduce both the HiB vaccine (January 1997) and PCV (August 2009) into their national immunisation schedules with routine surveillance studies on the impact of both vaccines still being conducted. In 2014, the immunisation coverage for HiB was 97% and 96% for PCV [115]. HIV infection rates in The Gambia remains low [113] with roughly only 1.2% (n=13 000) people living with HIV, 2000 of which are children [115]. The under 5 mortality rate in 2014 was 74 per 1000 live births making it number 31/195 on the under 5 mortality rating index, the infant mortality was roughly 49 per 1000 live births and the neonate mortality rate was 28 per 1000 live births [115]. Enrolment into the study commenced in November 2011 and was complete in October 2013 with a total of 539 cases and 580 controls being enrolled.

2.1.6 Sa Kaeo and Nakhon Phanom, The Thailand sites

The Thailand enrolments were conducted at two rural sites in Thailand; the first was in the Sa Kaeo province which is located near to the Cambodian border in the East of Thailand, and the

second is in the Nakhon Phanom province in north eastern region bordering Laos. Both sites are classified as rural/periurban communities with very low HIV infection rates and no malaria. In 2014, it was estimated that approximately 1.1% (n=440 000) of people were living with HIV-1, 8 000 of which were children [115]. The HiB vaccine and PCV are not part of the national immunisation schedule and thus usage in the general population is very low with the vaccines only being available on the private market. The under 5 mortality rate in 2014 was 37 per 1000 live births making it 122/195 on the under 5 mortality ranking index, with an infant mortality rate of 11 per 1000 live births and a neonate mortality rate of 8 per 1000 live births [115]. Enrolment into the PERCH study commenced in January 2012 and was completed in January 2014 with a total of 192 cases and 541 controls enrolled over the two sites.

2.1.7 Matlab and Dhaka, The Bangladesh sites

Two Bangladesh sites were chosen for the PERCH study, one was a rural site in Chittagong serviced by the Matlab hospital and the other an urban site in Dhaka serviced by the Dhaka hospital. The HIV prevalence in Bangladesh is extremely low (<0.1%) with approximately 10 000 people living with HIV, less than 500 of which are children. The under 5 mortality rate in Bangladesh was estimated to be 41 per 1000 live births in 2014 making it number 60/195 on the under 5 mortality rank index, with a 33 per 1000 live births mortality rate during infancy and a neonatal mortality rate of 24 per 1000 live births [115]. The HiB vaccine was introduced in July 2009 and by 2014 the immunisation coverage was estimated to be 97% [115]. PCV was not in routine use at any point during the PERCH project at either of the Bangladesh sites. Enrolment into the project began in January 2012 and was completed in December 2013, with a total of 377 cases and 607 controls enrolled over the two sites.

The clinical epidemiology of HRV during severe and very severe pneumonia will be analysed in all cases and controls testing positive for HRV infection at all nine PERCH sites.

2.2 Patient enrolment

PERCH cases were children aged 28 days to 59 months hospitalised with WHO-defined severe or very severe pneumonia. Severe pneumonia was defined as signs and symptoms of cough or breathing difficulty and lower chest wall indrawing. Very severe pneumonia was

signs and symptoms of cough or difficulty breathing as well as at least one of the following: difficulty breastfeeding or drinking, vomiting, convulsions, central cyanosis, lethargy, unconsciousness or head nodding. Exclusion criteria for cases were children presenting with wheeze which resolved following bronchodilator therapy for lower chest wall indrawing, hospital admission within the previous 2 weeks or enrolment as a PERCH case within the previous 30 days or residing outside of the study catchment area.

Controls were community recruited children living within the same study catchment area as the cases without signs and symptoms of severe or very severe pneumonia. The controls were age frequency matched to the cases using the following age groups: 28 days to less than 6 months, 6 to less than 12 months, 12 to less than 24 months and 24 to 59 months. At the Zambia and South African site the controls were also HIV status matched to the cases. On enrolment into the study detailed information was obtained relating to patient demographics, history and clinical manifestations of the disease. The controls were also broken up into two groups: The ARI controls which had signs or symptoms of cough, runny nose, ear discharge, wheezing or difficulty breathing together with either a fever (temperature greater or equal to 38^{o}C in the past 48 hours) or a sore throat and the non-ARI controls which appeared healthy or asymptomatic at the time of sampling.

Cases and controls were enrolled throughout the year so as to obtain seasonal data as well as throughout the week including weekends and nights so as not to introduce any bias in case severity. The rationale for the inclusion and exclusion criteria of the cases and controls are detailed in Deloria-Knoll et al. [114].

2.3 Specimen collection and laboratory testing

Flocked nasopharyngeal (NP) swab (Flexible minitip, Copan ®) and a rayon oropharyngeal (OP) swab specimens were collected from all cases and controls on enrolment into the study. The swabs were placed in vials containing 3mL of Universal Transport Media (UTM) (Copan ®). The NP specimens were maintained at 4-8^{o}C for a maximum of 24 hours and then archived at -70^{o}C until testing occurred.

4.3.1 Nucleic Acid extraction

All sample testing was conducted in-country using standardised operating procedures and re-training was conducted periodically throughout the study period. Total nucleic acids were extracted from 400uL of the NP swabs in UTM using the NucleiSens EasyMag extraction system as per manufactures instructions (BioMerieux, Marcy l'Etoile, France) and eluted into a final volume of 110uL.

2.3.2 Real time PCR detection for HRV and other common respiratory pathogens

The total nucleic acid specimens from the NP swab were evaluated with the Fast-track Diagnostics multiplexed real-time PCR assays which tests for 33 respiratory pathogens according to manufactures instructions (FTD Respiratory Pathogens, Fast-Track Diagnostics, Sliema, Malta). The FTD-33 respiratory panel detected 19 viruses with 5 different multiplex Real Time (RT)-PCR assays – HRV (A, B and C not differentiated), Influenza virus (A, B and C separately), PIV (1, 2, 3 and 4 separately), Coronavirus (HKU1, OC43, NL63, 229E separately), Bocavirus, Human Metapneumoviruses (A and B not differentiated), RSV (A and B not differentiated), Cytomegalovirus (CMV), Adenovirus, Enterovirus and Parechovirus. The FTD-33 respiratory panel also detects nine different bacteria species and one fungal parasite using three multiplex RT-PCR assays - *Streptococcus pneumoniae, Staphylococcus aureus, Haemophilus influenzae* and *Haemophilus influenzae* type B, *Moraxella cattarahalis, Legionella* spp., *Salmonella* spp., *Chlamydia pneumoniae, Mycoplasma pneumoniae, Klebsiella pneumoniae* and *Bordetella pertussis* and the fungus *Pneumocystis jiroveci.* RT-PCR was performed using the Applied Biosystems 7500® instruments (Applied Biosystems, Foster City, CA) using the following cycling conditions: 50^{o}C for 15 minutes, 95^{o}C for 10 minutes followed by 40 cycles of 95^{o}C for 8 seconds and then 60^{o}C for 34 seconds.

Pathogen densities (copies/mL) were calculated with standard curves which were generated using ten-fold dilutions of plasmid standards provided by Fast Track Diagnostics together with the FTD-33 Respiratory panels. The standard curves were run in duplicate every three months and the cycle threshold values were used to determine a regression line from which the copy numbers present in each clinical sample was derived. The detection limits for the assays were from 10^4 up to 10^8 copies per mL. The resulting copy numbers were analysed and the appropriate dilution factors taken into account in order to provide quantitative viral or

bacterial load results for each sample which were expressed as $\log_{10}$ RNA copies per mL for viruses or $\log_{10}$ colony forming units per mL for the bacterium and fungus parasites. Each assay included template and non-template controls as well as an external internal controls supplied with the FTD-33 respiratory panels. In addition, external quality assessment programs which were monitored and set up by FTD, were conducted throughout the study period and reports and practical feedback were given, allowing sites to identify and resolve any potential problems.

2.3.3 Routine standard of care testing of case samples

Standard diagnostic techniques were also employed; blood cultures were conducted on all cases using Bactec bottles and processed through a Bact/Alert microbial system (Organon Teknika, Durham, NC). Positive blood cultures were identified via standard culture and biochemical tests. Where clinically indicated pleural fluids and gastric aspirates were collected and cultured using standard culture and biochemical tests. Additionally, at the South Africa, The Gambia, Mali and Bangladesh sites lung aspirates were collected and cultured if consolidations were visible on the chest X-rays. The pleural fluids and lung aspirates were also tested using the FTD-33 respiratory panels as discussed above as well as tested for pneumococcal antigens with the BinaxNow® antigen detection system as per manufacturers' instructions (Alere, Orlando, Florida). Standard diagnostics were also conducted on all case blood samples to determine white blood cell and neutrophil counts as well as C-reactive protein (CRP) levels, a marker of infection and inflammation. At the South African site, a randomly selected group of control participants' blood samples were also tested for CRP levels.

Microbiologically confirmed pneumococcal pneumonia (MCPP) was defined as having *Streptococcus pneumoniae* cultured from a normally sterile fluid – culture positive blood, pleural fluid or lung aspirate. In addition, a case was considered to have MCPP if the pleural fluid or lung aspirate was FTD33 PCR positive for pneumococcus or pneumococcus antigen positive on the BinaxNow® assay. In addition, in other PERCH analyses it has been determined that there is a strong association between high pneumococcal densities, in the nasopharynx or the whole blood (WB) samples, and the presence of MCPP in the pneumonia cases [117]. It was determined that the optimal nasopharynx colonisation density threshold for differentiating between MCPP cases and controls was >6.9 $\log_{10}$ copies/mL with a sensitivity

of 64% and specificity of 92%. The optimal WB density, measured through the *LytA* RT-PCR assay on all case and control WB samples, for discriminating MCPP cases from controls was determined to be >2.2 $\log_{10}$ copies/mL. These high density pneumococcal (HDP) thresholds together with MCPP were used as markers for likely MCPP in order to analyse the relationship between HRV and pneumococcus disease.

All the laboratory testing and clinical data up until this point were used for the clinical epidemiology chapters (Chapter 3 and 4).

2.4 Determination of HRV molecular subtyping

The Fast track respiratory 33 panels were used to identify cases and controls testing positive for HRV infection as well as to calculate the HRV viral load in the NP swabs. All FTD-33 testing was conducted in-country; however, once the samples, both case and controls, testing positive for HRV infection at the South Africa, Mali and Zambia sites were identified the aliquots of archived NP samples were transferred to South Africa for further testing with results given in Chapter 5 of this thesis.

The HRV positive samples were re-extracted as described previously and then further analysed using single round PCR assays targeting a 390bp area in the 5' non-coding region (NCR) region of the HRV genome [66, 118]. This assay targets 2/3 of the NCR region and has been proven to differentiate between all 101 HRV serotypes as well as all currently available HRV-C serotypes, in addition it was also able to identify previously unknown HRV-C strains straight from clinical samples [118]. The primer sequences were DK001 forward (5'- CAAGCACTTCTGTTTCC - 3') and reverse primer DK004 (5' – CACGGACACCCAAAGTAGT – 3'). Amplification was conducted using Promega Access RT according to manufacturers' instructions (Promega, Belgium) using the following cycling conditions: denaturation for 95^{o}C for 5 minutes, followed by 40 cycles of 95^{o}C for 15 seconds, annealing at 55^{o}C for 15 seconds and polymerisation at 72^{o}C for 60 seconds. Negative and positive controls were included in each run. The presence of a PCR product was confirmed and quantified through visualisation of the PCR product on an ethidium bromide stained 2% Agarose gel. The GeneRuler 100 base pairs DNA ladder (Thermo Scientific, Carlsbad, CA) was used to determine the nucleotide length of the PCR product, approximately 400 base pairs, as well as to quantify the DNA concentration prior to sequencing as per manufactures instructions. The PCR products were purified using the

Nucleofast 96 well plates (Macherey Nagel) and sequenced using the BigDye Terminator V3.1 sequencing kit (Applied BioSystem, Foster City, CA) in both the forward and reverse orientation using the same primers as used in the PCR (DK001 and DK004). The forward and reverse sequences were analysed and aligned using the ClustalW algorithm implemented in Geneious 4.7.6 [119], and the resultant consensus sequence was compared with reference HRV sequences using the nucleotide-nucleotide BLAST algorithm (http://www.ncbi.nlm.nih.gov) from GenBank in order to identify the HRV species (A, B and C). The typing of the isolates was confirmed through phylogenetic analysis against reference HRV strains (Discussed in detail in section 2.7.2).

2.5 Detection of HRV viraemia

The HRV viraemia analysis was only conducted on the cases and controls enrolled at the South Africa site. The Fast Track respiratory 33 panels were used to identify the cases and controls positive for HRV infection and to calculate the HRV viral loads. The testing of the respiratory samples was completed prior to the testing of the blood samples in order to prevent cross contamination. In addition, HRV viraemia was also tested for in 10% of cases and controls testing negative for HRV infection with results given in Chapter 6 of this thesis

Total nucleic acids were extracted from 200uL of archived blood samples using the NucliSens EasyMag automated extraction robot (Biomerieux, France) using the blood specific extraction protocol and eluted in 60uL. The presence of HRV viraemia was tested for using the same primers (DK001 and DK004) and methods as those used for determining the HRV molecular subtyping in the respiratory samples. These primers have been used in previous studies looking for HRV viraemia and were proven to be highly sensitive in blood samples [66].

2.6 The PERCH electronic database

There were over 30 case/control report forms completed per participant - 3 737 cases and 4 712 controls – with multiple samples collected from each participant. Thus in order to track and analyse such a large database an external company, EMMES incorporated (Rockville, MD), was contracted to oversee the development and maintenance of the electronic database from each site. Regular data management, quality indicators of study performance and reports were generated in order to monitor the progression of the project and to ensure the integrity of

the data from each site. This database was utilised for the clinical and molecular analysis of the HRV subtyping over the full PERCH study period.

2.7 Statistical analysis

All statistical analysis and reverse cumulative plots were performed using STATA Version 12.1 (College Station, TX, USA) and a two-sided *P*-value <0.05 was considered statistically significant.

2.7.1 Clinical Epidemiology analysis

Parametric tests (Student's t-test) were used on normally distributed continuous variables and Wilcoxon and chi-squared tests were used on the distribution of categorical variables where applicable. Binary, multinomial logistic regression and odds ratio analysis were used to model the prevalence of HRV within the study population. We initially performed univariate analysis which introduced site of enrolment, age categories, gender, HIV status, socio-economic status, severity of pneumonia diagnosis, presence of fever and hypoxia, chest X-ray findings and case fatality ratios as independent variables. Additionally, we also performed univariate analysis for markers of potential bacterial co-infection as well as the different respiratory viruses detected for by the FTD33 assays. If these independent variables were <0.2 in the univariate analysis, then they were introduced into the different multivariate models where applicable in order to remove the prospect of mutually co-founding variates.

In the clinical epidemiology chapters (3.0 and 4.0), Chapter 3.0 focused on HIV-uninfected children thus in addition to the variables mentioned above, all models were also adjusted for HIV-exposure whereby the mothers could have been HIV positive but the children were uninfected. Additionally, reverse cumulative dot plots, generated in STATA Version 12.1, were used to visually analyse the relationship between HRV NP viral load among cases compared to controls as well as to analyse whether HRV viral load is associated with severity of disease.

Chapter 4.0 focused on HIV-1-infected children from the sites with high HIV burdens - South Africa and Zambia. In this chapter the models were also adjusted for HIV infection and HIV exposure status.

2.7.2 Molecular epidemiology analysis

Chapter 5.0 focused on the clinical and molecular subtyping of HRV in Sub Saharan Africa. Thus the case-control and clinical analyses of the three HRV species were analysed and adjusted as above. The multivariate models were adjusted for HIV-1-infection.

For the phylogenetic analysis, the sequences were aligned with each other as well as with the published HRV-A, HRV-B and HRV-C sequences using the nucleotide-nucleotide BLAST algorithm available in GenBank (http://www.ncbi.nlm.nih.gov) in order to determine the HRV species present in the participants respiratory samples. Subsequent phylogenetic analysis was conducted using the ClustalW algorithm implemented in Geneious 4.7.6 [119]. The Phylogenetic trees were constructed using the neighbour-joining methods using Kimura's 2-parameter technique with bootstrap values estimated with 1000 bootstrap replications [120] with evolutionary analysis conducted in MEGA-6 [121]. HRV subtypes with bootstrap values >90% will be considered to form monophyletic groups.

Chapter 6.0 focused on the clinical and molecular subtyping of HRV in South Africa and focused on the nasopharyngeal HRV viral load using regression analysis adjusted for the variables mentioned above as well as HIV infection status. Reverse cumulative plots were used to determine thresholds capable of differentiation between viraemia positive and negative children.

2.8 Ethical considerations

The PERCH study was reviewed by 10 different institution review boards (IRB) – the initial overall clinical and laboratory testing protocols were reviewed by the JHSPH's IRB after which the protocols were customised for each of the sites and reviewed by their local boards. The revised protocols and approvals for each site were then submitted to the JHSPH's IRB as amendments. This allowed for the collective ownership of the project by the PERCH executive committee as well across the sites. The ethical approvals for each of the sites are listed below:

1. Study Site: South Africa conducted by the Respiratory and Meningeal Pathogens Research Unit, Wits Health Consortium, based at Chris Hani Baragwanath Academic Hospital
Wits HREC Approval number: M10M101129
Principal investigator: Prof Shabir A. Madhi

2. Study Site: Kilifi, Kenya conducted by the Kilifi-KEMRI Wellcome Trust Institute
Approval number from the Kenya Medical Research Institute: KEMRI/RES/7/3/1
Oxtrec Approval number: 60-09
Principal investigator: Dr Laura Hammitt

3. Study site: Basse in The Gambia at the MRC insitution
Approval number from The Gambia Government/MRC joint Ethics Committee: L2010.105
Principal investigators: Dr Stephen Howie

4. Study Site: Bamako, Mali at the University of Maryland Insitution
Approval number from FMPOS Ethics committee: 2011/07/FMPOS
Approval number from The University of Maryland Institutional Review Board (IRB): HP00048100
Principal Investigator*:* Dr Karen Kotloff

5. Study Site: Lusaka, Zambia at the Boston University Institution
Approval number from the Blue Panel IRB: H*P*-29860
Approval from the ERES Converge IRB: 2010-Dec-001
Principal Investigator: Dr Donald Thea

6. Study site: Dhaka, Bangladesh at the ICDDR and JHSPH institution
Approval number from ICDDR,B Ethics Review Committee: PR-11012
Principal Investigators: Dr Abdullah Brooks

7. Study site: Sa Kaeo and Nakhon Phanom in Thailand at the CDC-Thailand
Approval number from the Ethical Review Committee for Research in Human Subjects-Ministry of Public Health, Thailand: 17/2554
Principal Investigators: Dr Pasakorn Akarasewi

Approval to conduct the clinical and molecular subtyping analysis of HRV in the PERCH project was obtained from the PERCH executive committee on the 18th of June 2014 and ethical approval was granted by the University of the Witwatersrand Human Rights and Ethics Board (HREC number: M140906). The additional HRV viraemia analysis on the South African case and control samples was approved by the PERCH executive committee on the 14th of September 2015 and ethical approval was granted by the Wits HREC board (HREC number: M151042). The HREC clearance certificates are available in Appendix 1 and 2.

3.0 Clinical epidemiology of the Human rhinovirus (HRV) in African and Southeast Asian children: A case-control pneumonia aetiology study

The role of HRV in severe lower respiratory tract infection (LRTI) in children has yet to be fully elucidated, since HRV identification is ubiquitous in both diseased and asymptomatic children [47]. Additionally, advances in molecular diagnostic tools have enhanced the detection of HRV, as well as other viruses, in children with respiratory illness, including in the PERCH case-control study on aetiology of severe and very severe pneumonia in children among whom two or more viruses were often detected in the nasopharynx. Thus, detection of a virus during a disease episode does not necessarily imply causation.

In this chapter, the clinical epidemiology of HRV infections in children under 5 years of age hospitalised with WHO-defined severe or very severe pneumonia and community controls with mild upper respiratory tract infection or who were asymptomatic was characterised.

3.1 RESULTS

3.1.1 The study population

Between August 2011 and January 2014, 3 876 HIV-uninfected children under the age of five with WHO-defined severe or very severe pneumonia; and 4 997 community controls including asymptomatic children or children with URTI were enrolled into the PERCH study.

3.1.2 Characteristics of community controls by HRV status

Of the 4 977 control participants, 76% (n=3 796) were asymptomatic and 24% (n=1 181) had signs or symptoms of RTI. HRV was detected in 21% (n=1 056) of the controls and after multivariate logistic regression, HRV was found to be 1.56-fold (aOR 95% CI: 1.32-1.84) more likely to be present in children with URTI (25%, n=300/1 181) than asymptomatic controls (20%, n=756/3 796, $P<0.001$). There were no differences in the HRV viral load between the HRV-associated controls with URTI (3.5 $\log_{10}$ copies/mL) compared to the asymptomatic children (3.4 $\log_{10}$ copies/mL, $P=0.233$).

The all-site demographics and clinical characteristics of community controls showed that the HRV-associated community controls were younger (mean 13.2 months vs. 16 months,

P<0.001) than controls without HRV infections; but did not differ in other common risk factors for infections including day care attendance (16% vs. 19%, *P*=0.234), exposure to a smoker in the household (37% vs. 39%, *P*=0.202) and premature birth (12% vs. 10%, *P*=0.754). Conversely, the HRV-associated controls were 1.72-fold (aOR 95% CI: 1.43-2.07) more likely to have symptoms of rhinorrhoea (21% vs. 16%, *P*<0.001) and 1.62-fold (aOR 95% CI: 1.28-2.04) more likely to have a cough (11% vs. 8%, *P*=0<0.001) than controls without HRV infection. Furthermore, HRV infected controls were also more likely to have higher mean *S. pneumoniae* (*LytA*) density in the nasopharynx; including a higher percentage with densities >6.9 $\log_{10}$ copies/mL (11% vs. 7%, aOR1.54, 95% CI: 1.21-1.95, *P*<0.001) which in PERCH was associated with pneumonia, including microbiologically confirmed pneumococcal pneumonia among pneumonia cases [117]; Table 3.1.

Table 3.1: The demographical, clinical and markers for bacterial co-infection of the Community controls by HRV infection status for all sites data combined

	HRV+ (n=1056)	HRV- (n= 3921)	Unadjusted *P*-value	Unadjusted OR (95% CI)	Adjusted *P*-value	Adjusted aOR (95% CI)
Age in months, mean (SD)	13.2 (12.9)	16.0 (14.7)	*P*<0.001		*P*<0.001	
Female, n(%)	515 (49%)	1957 (50%)	0.505	0.95 (0.83-1.09)	0.473	0.95 (0.83-1.09)
Never breast fed, n(%)	277 (7%)	312 (8%)	0.475	0.91 (0.70-1.28)	0.440	1.12 (0.84-1.51)
Under weight, n (%)[a]	127 (12%)	471 (12%)	0.990	1.0 (0.81-1.23)	0.890	1.02 (0.82-1.26)
HEU, n(%)[b]	177 (9%)	625 (9%)	0.714	1.05 (0.81-1.35)	0.817	1.02 (0.84-1.25)
Day Care attendance, n(%)	163 (16%)	739 (19%)	0.012	0.79 (0.65-0.95)	0.706	0.95 (0.74-1.23)
Smoker in household, n(%)	392 (37%)	1518 (39%)	0.334	0.94 (0.81-1.08)	0.202	0.91 (0.78-1.05)
Premature birth, n(%)[c]	130 (12%)	376 (10%)	0.009	1.32 (1.07-1.64)	0.234	1.01 (0.95-1.08)
Birth weight, mean (SD)	3.0 (0.6)	3.0 (0.5)	0.617		0.886	
Clinical features:						
Tachypnea, n(%)[d]	103 (10%)	457 (12%)	0.090	0.82 (0.66-1.03)	0.046	0.79 (0.63-0.99)
Cough, n(%)	120 (11%)	301 (8%)	*P*<0.001	1.55 (1.23-1.93)	*P*<0.001	1.62 (1.28-2.04)
Fever, n(%)[e]	56 (5%)	212 (5%)	0.898	0.98 (0.72-1.33)	0.789	0.96 (0.70-1.31)
Diarrhoea, n(%)	13 (1%)	82 (2%)	0.072	0.58 (0.32-1.05)	0.051	0.55 (0.30-1.00)
Rhinorrhoea, n(%)	223 (21%)	629 (16%)	*P*<0.001	1.40 (1.28-1.66)	*P*<0.001	1.72 (1.43-2.07)
Markers for Bacterial co-infection:						
***LytA* positive, n(%)[f]**	60 (6%)	193 (5%)	0.327	1.16 (0.86-1.57)	0.605	1.08 (0.80-1.47)
***S. pneu* load, mean (SD)[g]**	5.89 (1.17)	5.5 (2.1)	*P*<0.001		*P*<0.001	
HDP, n(%)[h]						
-Blood	36 (4%)	104 (3%)	0.192	1.29 (0.88-1.90)	0.334	1.21 (0.82-1.79)
-NP	113 (11%)	266 (7%)	*P*<0.001	1.65 (1.31-2.07)	*P*<0.001	1.54 (1.21-1.95)
Viral infections in the nasopharynx:						
-RSV, n(%)	24 (2%)	116 (3%)	0.233	0.76 (0.49-1.19)	0.277	0.78 (0.50-1.22)
-AdV, n(%)	134 (13%)	459 (12%)	0.381	1.09 (0.89-1.35)	0.121	1.23 (0.99-1.82)
-HMPV, n(%)	59 (6%)	147 (4%)	0.008	1.52 (1.11-2.07)	0.155	1.26 (0.92-1.75)
-HBoV, n(%)	139 (13%)	521 (13%)	0.916	0.99 (0.81-1.21)	0.727	1.04 (0.85-1.27)
-InFV A-C, n(%)	9 (1%)	104 (3%)	0.001	0.32 (0.16-0.63)	*P*<0.001	0.29 (0.15-0.58)
-PIV, n(%)	68 (6%)	246 (6%)	0.844	1.03 (0.78-1.36)	0.621	0.93 (0.70-1.23)
-HCoV, n(%)	93 (9%)	408 (11%)	0.126	0.83 (0.66-1.05)	0.049	0.79 (0.62-0.99)

Abbreviations - OR: Odds ratio; aOR: Adjusted odds ratio; CI: Confidence interval; SD: Standard deviation; NP: Nasopharyngeal; HRV: Human rhinovirus; HIV: Human immunodeficiency virus; HEU: HIV exposed but uninfected; HDP: High density pneumococcus; RSV: Respiratory Syncytial Virus, HMPV: Human Metapneumovirus; AdV: Adenovirus; PIV: Parainfluenza type 1-4; HBoV: Human Bocavirus; HCoV: Human Coronavirus (OC43, NL63, 229E and HKU1) and InFV: Influenza Virus (A, B and C); *S. pneu: Streptococcus pneumoniae*.

P-values from Chi-squared and Wilcoxon tests, logistic regression models adjusted for confounding variates (<0.2 in univariate analysis) where applicable, Odds ratio could not be calculated for continuous variables or variables with 0 values, thus cells left blank.

[a] - Underweight defined as weight for age <-2SD of the median age-sex specific WHO reference; [b] - HEU defined as HIV-uninfected but HIV-exposed. Undetectable viral load, HIV seronegative in the child with a positive maternal history. Positive maternal status based on self-report was accepted, except for seronegative children < 7 months of age where documented positive maternal status was required; [c] - Premature birth defined as gestational age <37 weeks; [d] - Tachypnea defined as respiratory rate >60 breaths/minute if aged <2 months, respiratory rate >50 breaths/minute if aged 2-12 months, respiration rate >40 breaths/minute if aged >12 months; [e] - Fever defined as temperature ≥38^{o}C; [f] - Blood sample positive for *S. pneumoniae* colonisation by *LytA* PCR; [g] - Bacterial load of *S. pneu* in the nasopharynx, expressed as $\log_{10}$ copies/mL; [h] - HDP defined as *S. pneumoniae* density in nasopharynx >6.9 and/or density in whole blood sample >2.2 $\log_{10}$ copies/mL.

Individual site control evaluations are available in Table 3.2A and B, with similar trends as seen in the all-site data for younger age, more cough, rhinorrhoea and high *S. pneumoniae* colonisation densities of the HRV infected controls compared to the HRV negative controls. In addition, prior to adjustment for potentially confounding variates (<0.2 in univariate

analysis) in the all-site data the HRV positive controls were associated with premature birth (12% vs. 10%, P=0.009 prior to adjustment); Table 3.1, with this association also observed specifically in Kenya (20% vs 11%, P=0.002), Mali (4% vs. 1%, P=0.050) and Thailand (11% vs. 7%, P=0.023).

Table 3.2A: The demographical and clinical characteristics of controls by HRV infection status for each African site individually

	Kenya (n=855)			Gambia (n=624)			Mali (n=724)			Zambia (n=533)			South Africa (n=823)		
	HRV+ (n=162)	**HRV- (n=693)**	***P*-value**	**HRV+ (n=177)**	**HRV- (n=447)**	***P*-value**	**HRV+ (n=143)**	**HRV- (n=581)**	***P*-value**	**HRV+ (n=93)**	**HRV- (n=440)**	***P*-value**	**HRV+ (n=194)**	**HRV- (n=629)**	***P*-value**
Age in months, mean (SD)	14.8 (14.3)	17.3 (14.7)	0.052	14.1 (13.5)	16.9 (14.7)	0.031	10.0 (9.6)	14.7 (13.5)	*P*<0.001	6.9 (6.2)	10.5 (11.3)	0.004	11.3 (11.3)	12.6 (12.6)	0.207
Female, n(%)	85 (52%)	319 (46%)	0.158	75 (42%)	220 (49%)	0.118	62 (43%)	304 (52%)	0.052	36 (39%)	226 (51%)	0.028	106 (55%)	317 (50%)	0.261
Never breast fed, n(%)	1 (1%)	6 (1%)	0.863	1 (1%)	1 (0%)	0.483	0	3 (1%)	0.836	2 (2%)	13 (3%)	0.646	66 (34%)	222 (35%)	0.793
Under weight, n (%)[a]	26 (16%)	99 (14%)	0.287	23 (13%)	79 (18%)	0.242	18 (13%)	61 (11%)	0.259	9 (10%)	56 (13%)	0.735	$_{10}$ (5%)	27 (4%)	0.603
HEU, n(%)[b]	0	2 (0%)	0.494	0	0	-	0	2 (0%)	0.482	31 (33%)	119 (27%)	0.221	52 (27%)	172 (27%)	0.882
Day Care attendance, n(%)	5 (3%)	42 (6%)	0.135	0	11 (2%)	0.053	107 (75%)	445 (77%)	0.657	0	15 (3%)	0.174	28 (14%)	93 (15%)	0.984
Smoker in household, n(%)	43 (27%)	214 (31%)	0.274	99 (58%)	248 (56%)	0.702	26 (18%)	122 (21%)	0.663	28 (30%)	122 (27%)	0.643	48 (30%)	198 (32%)	0.464
Premature birth, n(%)[c]	32 (20%)	75 (11%)	0.002	11 (6%)	22 (5%)	0.648	5 (4%)	7 (1%)	0.050	3 (3%)	28 (6%)	0.230	66 (34%)	184 (29%)	0.171
Birth weight, mean (SD)	3.1 (0.6)	3.1 (0.6)	0.502	3.1 (0.6)	2.9 (0.5)	0.165	3.3 (0.6)	3.3 (0.7)	0.859	2.9 (0.4)	2.9 (0.5)	0.955	3.0 (0.6)	3.0 (0.5)	0.188
Clinical Features:															
Tachypnea, n(%)[d]	31 (19%)	103 (15%)	0.248	23 (13%)	58 (13%)	0.942	20 (14%)	98 (17%)	0.443	6 (6%)	58 (13%)	0.145	11 (6%)	66 (11%)	0.055
Cough, n(%)	16 (10%)	26 (4%)	0.003	21 (12%)	42 (9%)	0.350	12 (8%)	48 (8%)	0.948	7 (8%)	29 (7%)	0.470	5 (3%)	$_{10}$ (2%)	0.401
Fever, n(%)[e]	8 (5%)	25 (4%)	0.370	25 (14%)	59 (13%)	0.725	2 (1%)	16 (3%)	0.363	0	4 (1%)	0.661	0	5 (1%)	0.407
Diarrhoea, n(%)	2 (1%)	14 (2%)	0.514	6 (3%)	18 (4%)	0.748	15 (3%)	1 (1%)	0.213	1 (1%)	7 (2%)	0.849	0	2 (0%)	0.566
Rhinorrhoea, n(%)	39 (24%)	119 (17%)	0.040	25 (14%)	45 (10%)	0.156	52 (36%)	196 (34%)	0.215	3 (3%)	11 (3%)	0.753	2 (1%)	12 (2%)	0.364
Laboratory markers:															
***LytA* positive, n(%)[f]**	9 (6%)	39 (6%)	0.930	15 (9%)	32 (8%)	0.563	12 (8%)	26 (5%)	0.068	1 (1%)	23 (6%)	0.109	20 (10%)	65 (10%)	0.930
HDP, n(%)[g]	5 (3%)	42 (6%)	0.150	26 (15%)	53 (12%)	0.427	39 (27%)	95 (16%)	0.002	8 (9%)	30 (7%)	0.482	37 (19%)	77 (12%)	0.022

Abbreviations - SD: Standard deviation; NP: Nasopharyngeal; HRV: Human rhinovirus; HIV: Human immunodeficiency virus; HEU: HIV exposed but uninfected; HDP: High density pneumococcus.

P-values from Chi-squared and Wilcoxon tests, logistic regression models adjusted for confounding variates (<0.2 in univariate analysis) where applicable, *P*-values could not be calculated for variables where both values are 0, thus cells left blank.

[a] - Underweight defined as weight for age <-2SD of the median age-sex specific WHO reference; [b] - HEU defined as HIV-uninfected but HIV-exposed. Undetectable viral load, HIV seronegative in the child with a positive maternal history. Positive maternal status based on self-report was accepted, except for seronegative children < 7 months of age where documented positive maternal status was required; [c] - Premature birth defined as gestational age <37 weeks; [d] - Tachypnea defined as respiratory rate >60 breaths/minute if aged <2 months, respiratory rate >50 breaths/minute if aged 2-12 months, respiration rate >40 breaths/minute if aged >12 months; [e] - Fever defined as temperature ≥38^{o}C; [f] - Blood sample positive for *S. pneumoniae* colonisation by *LytA* PCR; [g] - HDP defined as *S. pneumoniae* density in nasopharynx >6.9 and/or density in whole blood sample >2.2 $\log_{10}$ copies/mL.

Table 3.2B: The demographical and clinical characteristics of controls by HRV infection status for each Southeast Asian site individually

	Thailand (n=650)			Bangladesh (n=768)		
	HRV+ (n=92)	**HRV- (n=558)**	***P*-value**	**HRV+ (n=195)**	**HRV- (n=573)**	***P*-value**
Age in months, mean (SD)	20.7 (15.5)	21.1 (15.9)	0.815	14.9 (13.6)	18.5 (16.3)	0.006
Female, n(%)	49 (53%)	271 (49%)	0.391	102 (53%)	300 (52%)	0.915
Never breast fed, n(%)	5 (5%)	65 (12%)	0.084	2 (1%)	2 (0%)	0.228
Under weight, n (%)[a]	4 (4%)	33 (6%)	0.520	37 (19%)	116 (20%)	1.00
HEU, n(%)[b]	0	0		0	0	
Day Care attendance, n(%)	22 (24%)	132 (24%)	0.957	1 (1%)	1 (0%)	0.613
Smoker in household, n(%)	57 (62%)	327 (59%)	0.544	81 (42%)	287 (50%)	0.051
Premature birth, n(%)[c]	10 (11%)	40 (7%)	0.023	3 (2%)	20 (3%)	0.359
Birth weight, mean (SD)	3.1 (0.4)	3.0 (0.5)	0.463	2.9 (0.6)	2.9 (0.5)	0.956
Clinical Features:						
Tachypnea, n(%)[d]	2 (2%)	46 (8%)	0.067	10 (5%)	28 (5%)	0.892
Cough, n(%)	27 (29%)	81 (15%)	0.001	32 (16%)	63 (11%)	0.062
Fever, n(%)[e]	13 (14%)	73 (13%)	0.827	8 (4%)	30 (5%)	0.497
Diarrhoea, n(%)	2 (2%)	16 (3%)	0.783	1 (1%)	10 (2%)	0.200
Rhinorrhoea, n(%)	59 (64%)	175 (31%)	*P*<0.001	43 (22%)	71 (12%)	0.002
Laboratory markers:						
***LytA* positive, n(%)[f]**	1 (1%)	4 (1%)	0.708	2 (1%)	4 (1%)	0.511
HDP, n(%)[g]	2 (2%)	9 (2%)	0.700	27 (14%)	55 (10%)	0.094

Abbreviations - SD: Standard deviation; NP: Nasopharyngeal; HRV: Human rhinovirus; HIV: Human immunodeficiency virus; HEU: HIV exposed but uninfected; HDP: High density pneumococcus.

P-values from Chi-squared and Wilcoxon tests, logistic regression models adjusted for confounding variates (<0.2 in univariate analysis) where applicable, *P*-values could not be calculated for variables where both values are 0, thus cells left blank.

[a] - Underweight defined as weight for age <-2SD of the median age-sex specific WHO reference; [b] - HEU defined as HIV-uninfected but HIV-exposed. Undetectable viral load, HIV seronegative in the child with a positive maternal history. Positive maternal status based on self-report was accepted, except for seronegative children < 7 months of age where documented positive maternal status was required; [c] - Premature birth defined as gestational age <37 weeks; [d] - Tachypnea defined as respiratory rate >60 breaths/minute if aged <2 months, respiratory rate >50 breaths/minute if aged 2-12 months, respiration rate >40 breaths/minute if aged >12 months; [e] - Fever defined as temperature ≥38^{o}C; [f] - Blood sample positive for *S. pneumoniae* colonisation by *LytA* PCR; [g] - HDP defined as *S. pneumoniae* density in nasopharynx >6.9 and/or density in whole blood sample >2.2 $\log_{10}$ copies/mL.

In order to evaluate the interactions between HRV and the other respiratory viruses further we compared controls with HRV mono-infections (HRV was the only respiratory virus detected in the nasopharynx) to controls with viral co-infections; Table 3.3. HRV was most commonly detected as co-infecting viruses with HBoV (13%, n=139/400), AdV (13%, n=134/400) and then HCoV (9%, n=93/400). Among the HRV infected controls, there was a lower prevalence of Influenza virus (1% vs. 3%, *P*<0.001) than controls without HRV infections, whilst the detection of RSV, AdV, HMPV and PIV did not differ in HRV-infected controls compared to controls without HRV infections; Table 3.1.

Amongst the HRV controls, those with other co-infecting viruses were clinically indistinguishable from children with HRV mono-infection and no differences were observed

in HRV viral load between controls with mono- and mixed infections; Table 3.3. Controls with HRV mono-infections were, however, associated with premature birth (14%) compared to controls with HRV mixed infections (10%, P=0.002) but only trends for the association of HRV mono-infections with premature birth were seen in comparison to the individual co-infecting viruses. For individual co-infecting viruses, those with RSV were more likely to have RTI (46% vs. 27%, P=0.031), tachypnea (17% vs. 10%, P=0.044) and whole blood *LytA* positivity (18% vs. 6%, P=0.007) compared to controls with HRV mono-infections. No other differences in clinical characteristics were observed between HRV mono- and co-infections due to any of the other respiratory viruses, including AdV, HMPV (A and B), PIV (Type 1-4), HCoV (Types 43, 64, 229 and HKU), Influenza virus (A, B and C) and HBoV; Table 3.3.

Table 3.3: The demographics, risk factors, clinical and laboratory characteristics of community controls with HRV mono-infections compared to HRV mixed infections

	Mono-infections (n=656)	Mixed infections (n=400)	*P*-value	HRV-RSV (n=24)	*P*-value	HRV-AdV (n=134)	*P*-value	HRV-HMPV (n=59)	*P*-value	HRV-PIV (n=68)	*P*-value	HRV-HBoV (n=139)	*P*-value	HRV-HCoV (n=93)	*P*-value
Age in months, mean (SD)	12.4 (12.8)	14.5 (13.0)	0.045	10.0 (8.4)	0.499	18.4 (13.6)	*P*<0.001	12.6 (11.1)	0.450	15.3 (14.5)	0.191	14.9 (12.9)	0.164	12.0 (11.0)	0.740
Female, n(%)	321 (49%)	194 (49%)	0.900	14 (58%)	0.423	66 (49%)	0.977	30 (51%)	0.243	31 (46%)	0.902	69 (50%)	0.696	45 (48%)	0.847
Never breast fed, n(%)	52 (8%)	25 (6%)	0.952	3 (13%)	0.409	9 (7%)	0.544	3 (5%)	0.330	2 (3%)	0.691	9 (6%)	0.969	7 (8%)	0.627
Under weight, n (%)[a]	75 (11%)	52 (13%)	0.926	4 (17%)	0.364	20 (15%)	0.526	6 (10%)	0.328	10 (15%)	0.787	16 (12%)	0.750	11 (12%)	0.785
HEU, n(%)[b]	58 (9%)	25 (6%)	0.680	1 (4%)	0.330	9 (7%)	0.543	3 (5%)	0.201	0	0.304	8 (6%)	0.702	10 (11%)	0.356
Day care attendance, n(%)	86 (13%)	816 (19%)	0.089	5 (21%)	0.059	31 (23%)	0.623	1 (2%)	0.554	13 (19%)	0.180	25 (18%)	0.575	21 (23%)	0.132
Smoker in household, n(%)	243 (37%)	1667 (39%)	0.768	7 (29%)	0.426	53 (40%)	0.602	28 (49%)	0.174	25 (37%)	0.949	5 (38%)	0.908	29 (31%)	0.263
Premature birth, n(%)[c]	92 (14%)	414 (10%)	0.004	3 (13%)	0.720	13 (10%)	0.388	2 (4%)	0.121	2 (3%)	0.092	14 (10%)	0.464	12 (13%)	0.526
Birth weight, mean (SD)	3.0 (0.6)	3.0 (0.5)	0.319	2.8 (0.6)	0.081	3.0 (0.6)	0.228	3.0 (0.7)	0.760	3.1 (0.5)	0.286	3.1 (0.5)	0.059	3.0 (0.5)	0.809
RTI control, n(%)[d]	176 (27%)	124 (31%)	0.720	11 (46%)	0.031	42 (31%)	0.698	18 (31%)	0.710	22 (32%)	0.861	47 (34%)	0.798	30 (32%)	0.250
Clinical Features:															
Tachypnea, n(%)[e]	62 (10%)	41 (10%)	0.598	4 (17%)	0.044	14 (10%)	0.394	5 (8%)	0.315	9 (13%)	0.655	16 (12%)	0.552	6 (7%)	0.358
Cough, n(%)	68 (10%)	52 (13%)	0.583	5 (21%)	0.135	17 (12%)	0.649	9 (15%)	0.585	14 (21%)	0.088	20 (14%)	0.622	10 (11%)	0.763
Fever, n(%)[f]	32 (5%)	24 (6%)	0.872	1 (4%)	0.968	7 (5%)	0.800	8 (14%)	0.823	4 (6%)	0.707	8 (6%)	0.466	4 (4%)	0.903
Diarrhoea, n(%)	10 (2%)	3 (1%)	0.128	0	0.742	1 (1%)	0.441	1 (2%)	0.363	0	0.397	2 (1%)	0.555	0	
Rhinorrhoea, n(%)	132 (20%)	91 (23%)	0.981	8 (33%)	0.110	34 (25%)	0.657	8 (14%)	0.729	16 (24%)	0.806	34 (25%)	0.712	24 (16%)	0.155
Laboratory markers:															
CRP ≥40mg/l, n(%)[g]	2 (0%)	0	0.685	0	0.317	0	0.806	0	0.656	0	0.991	0	0.606	0	0.870
***LytA* positive, n(%)[h]**	37 (6%)	23 (6%)	0.938	4 (18%)	0.007	6 (5%)	0.760	3 (6%)	0.624	5 (8%)	0.500	5 (4%)	0.278	6 (7%)	0.667
HDP, n(%)[i]															
-Blood	18 (3%)	18 (3%)	0.206	1 (5%)	0.504	5 (4%)	0.533	3 (6%)	0.237	5 (8%)	0.099	5 (4%)	0.533	5 (3%)	0.210
-NP	69 (11%)	44 (11%)	0.806	4 (17%)	0.339	17 (13%)	0.463	2 (3%)	0.123	7 (10%)	0.954	13(9%)	0.681	14 (15%)	0.192
HRV viral load, mean (SD)[j]	3.5 (0.9)	3.4 (0.9)	0.424	3.4 (0.9)	0.383	3.4 (0.9)	0.203	3.5 (0.8)	0.945	3.4 (0.9)	0.472	3.4 (0.9)	0.572	3.5 (1.0)	0.838

Abbreviations - SD: Standard deviation; NP: Nasopharyngeal; HRV: Human rhinovirus; HIV: Human immunodeficiency virus; HEU: HIV exposed but uninfected; RTI: Respiratory tract infection; CRP: C-reactive protein; HDP: High density pneumococcus.

P-values from Chi-squared and Wilcoxon tests, logistic regression models adjusted for confounding variates (<0.2 in univariate analysis) where applicable, *P*-values could not be calculated for variables where both values are 0, thus cells left blank.

[a] - Underweight defined as weight for age <-2SD of the median age-sex specific WHO reference; [b] - HEU defined as HIV-uninfected but HIV-exposed. Undetectable viral load, HIV seronegative in the child with a positive maternal history. Positive maternal status based on self-report was accepted, except for seronegative children < 7 months of age where documented positive maternal status was required; [c] - Premature birth defined as gestational age <37 weeks; [d] - RTI were controls showing any signs or symptoms of respiratory tract infections including any of the symptoms listed under clinical features; [e] - Tachypnea defined as respiratory rate >60 breaths/minute if aged <2 months, respiratory rate >50 breaths/minute if aged 2-12 months, respiration rate >40 breaths/minute if aged >12 months; [f] - Fever defined as temperature ≥38^{o}C; [g] - CRP defined as levels ≥40mg/mL which are considered to potentially indicate bacterial infection; [h] - Blood sample positive for *S. pneumoniae* colonisation by *LytA* PCR; [i] - HDP defined as *S. pneumoniae* density in nasopharynx >6.9 and/or density in whole blood sample >2.2 $\log_{10}$ copies/mL; [i] - HRV viral load in the nasopharynx expressed as $\log_{10}$ copies/mL.

3.1.3 Case and control comparison of HRV infection in children

In multivariate analysis adjusting for multiple confounding variables including co-infecting bacteria and viruses, HRV was 1.45-fold (aOR 95% CI: 1.29-1.62, *P*<0.001) more likely to be present among pneumonia cases (n=912, 24% overall; range 17% in Mali to 37% in Bangladesh) than controls (n=1 056, 21% overall; range by site 14% in Thailand to 28% in The Gambia); Table 3.4. Figure 3.1 details the seasonal distribution of HRV positive cases and controls by month of enrolment. HRV were found in both cases and controls throughout the study period, with the highest prevalence of HRV among cases in August 2013 (40%) and the lowest in October 2011 (13%), and among controls the highest prevalence was in November 2013 and January 2014 (both 37%) and the lowest in October 2011 (3%). There were no obvious patterns of seasonality in cases or controls as HRV prevalence fluctuated month-on-month.

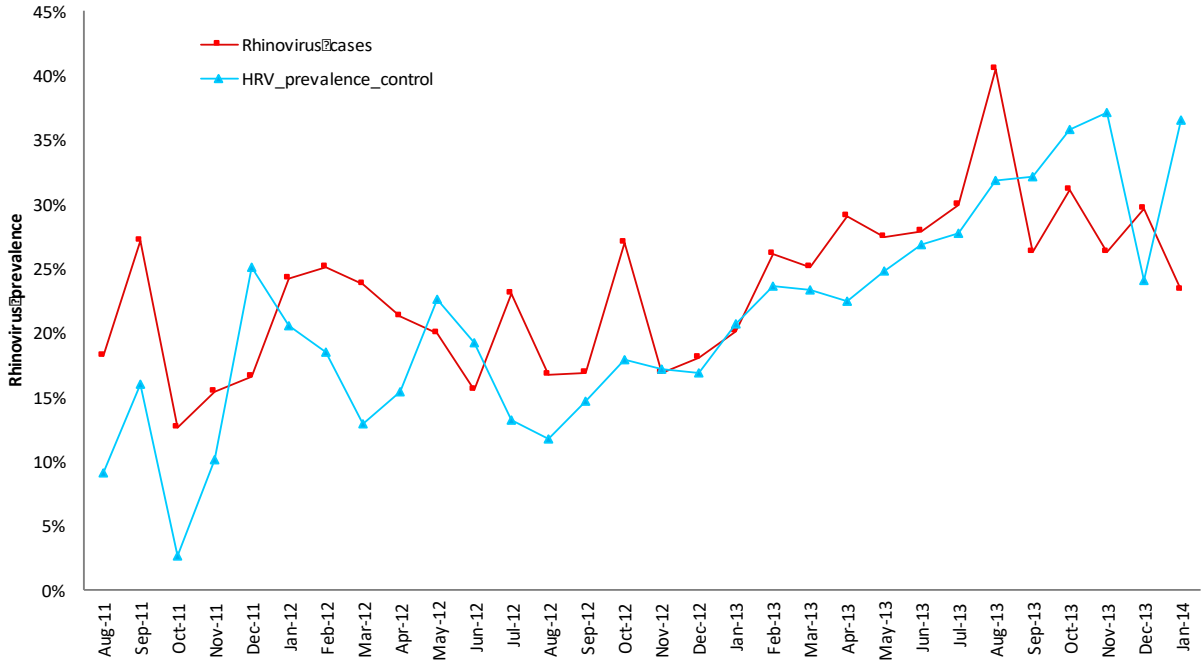

Figure 3.1: Distribution of HRV positive cases and controls by month of enrolment

The prevalence of HRV among cases varied by age group, with the lowest prevalence in the 1-5 month (21%) and 6-12 month age groups (22%) and highest (28%) in the 12-59 month age-group. Furthermore, the prevalence of HRV among cases 1-5 month of age was lower compared to controls of the same age-group (25%, aOR 0.86, 95% CI: 0.74-0.90, *P*=0.049); which was evident across all sites except Bangladesh and including being significant in The

Gambia (20% vs. 32%, aOR 0.51, 95% CI: 0.33-0.79, *P*=0.002). In contrast, the prevalence of HRV positivity was similar between cases (22%) and controls (22%) among the 6 to 12-month age group (OR 1.0, 95 CI: 0.81-1.24, *P*=0.96); Table 3.4. Conversely, in children between 1-5 years of age, HRV identification was more common among cases (28%) than controls (18%, aOR 2.08, 95% CI: 1.75-2.47, *P*<0.001), which was evident across all sites, including being significant in Kenya (26% vs. 21%, aOR 2.09, 95% CI: 1.41-3.11, *P*<0.001), Zambia (25% vs. 12%, aOR 4.22, 95% CI: 1.91-9.38, *P*<0.001) and Bangladesh (43% vs. 23%, aOR 3.33, 95% CI: 2.29-4.85, *P*<0.001); Table 3.4.

Table 3.4: Number of study subjects enrolled and tested for HRV, percent positive by age and site

Age groups			Kenya	Gambia	Mali	Zambia	South Africa	Thailand	Bangladesh	Total
All	Enrolled	Cases	628	609	650	449	794	221	525	3876
		Controls	855	624	724	533	823	650	768	4977
	HRV positive, n (%)	Cases	152 (24%)	139 (23%)	112 (17%)	95 (21%)	182 (23%)	40 (18%)	192 (37%)	912 (24%)
		Controls	162 (19%)	177 (28%)	143 (20%)	93 (17%)	194 (24%)	92 (14%)	195 (25%)	1056 (21%)
		P-value	0.018	0.134	0.093	0.156	0.838	0.140	*P*<0.001	*P*<0.001
1-5 months	Enrolled	Cases	208	249	299	248	399	37	136	1576
		Controls	231	192	247	253	318	90	221	1552
	HRV positive, n (%)	Cases	43 (21%)	49 (20%)	60 (20%)	44 (18%)	80 (20%)	3 (8%)	45 (33%)	324 (21%)
		Controls	57 (25%)	62 (32%)	65 (26%)	53 (21%)	76 (24%)	10 (11%)	68 (31%)	391 (25%)
		P-value	0.391	0.002	0.084	0.363	0.183	0.612	0.647	0.049
6-11 months	Enrolled	Cases	130	134	147	108	192	50	121	882
		Controls	190	127	188	135	227	150	167	11843
	HRV positive, n (%)	Cases	35 (27%)	22 (16%)	20 (14%)	28 (26%)	48 (25%)	10 (20%)	32 (26%)	195 (22%)
		Controls	33 (17%)	36 (28%)	36 (19%)	23 (17%)	64 (28%)	25 (17%)	39 (23%)	256 (22%)
		P-value	0.041	0.114	0.177	0.091	0.462	0.591	0.548	0.960
12-59 months	Enrolled	Cases	290	226	204	93	203	134	268	1418
		Controls	434	305	289	145	278	410	380	2241
	HRV positive, n (%)	Cases	74 (26%)	68 (30%)	32 (16%)	23 (25%)	54 (27%)	27 (20%)	115 (43%)	393 (28%)
		Controls	71 (17%)	79 (26%)	42 (15%)	17 (12%)	54 (19%)	57 (14%)	88 (23%)	409 (18%)
		P-value	*P*<0.001	0.286	0.724	*P*<0.001	0.062	0.082	*P*<0.001	*P*<0.001

Abbreviations: HRV - Human rhinovirus.

P-values from chi-square tests with logistic regression models adjusted for confounding variates (<0.2 in univariate analysis).

To identify potential risk factors for HRV-associated hospitalisation, we compared the HRV-associated cases to controls (regardless of HRV status). HRV-associated cases compared to controls were younger (mean 13.1 vs. 15.4 months, P=0.001) and less likely to be female (41% vs. 50%, aOR 0.70, 95% CI: 0.60-0.81, P<0.001), but 1.33-fold (aOR 95% CI: 1.01-1.74) more likely to be HIV-exposed (10% vs. 8, P=0.046), 3.21-fold (aOR 95% CI: 2.70-3.81) more likely to be underweight (30% vs. 12%, P<0.001) and have high nasopharyngeal colonisation densities (>6.9 $\log_{10}$ copies/mL) of *S. pneumoniae* in the nasopharynx (12% vs. 8%, aOR 1.56, 95% CI: 1.24-1.96, P<0.001); Table 3.5.

Table 3.5: Associations of demographic, clinical and respiratory pathogen co-infections of HRV-associated cases and controls regardless of HRV-associations

	HRV+ cases (n=912)	All controls (n=4977)	Unadjusted *P*-values	OR(95%CI)	Adjusted *P*-value	aOR (95%CI)
Age in months, mean (SD)	13.1 (12.1)	15.4 (14.3)	*P*<0.001		0.001	
Female, n(%)	373 (41%)	2 472 (50%)	*P*<0.001	0.70 (0.61-0.81)	*P*<0.001	0.70 (0.60-0.81)
Never breast fed, n(%)	80 (9%)	389 (8%)	0.327	1.13 (0.88-1.46)	0.302	1.17 (0.87-1.56)
Under weight, n (%)[a]	277 (30%)	598 (12%)	*P*<0.001	3.19 (2.71-3.77)	*P*<0.001	3.21 (2.70-3.81)
HEU, n(%)[b]	94 (10%)	378 (8%)	0.006	1.40 (1.10-1.77)	0.046	1.33 (1.01-1.74)
Day Care attendance, n(%)	126 (14%)	902 (18%)	0.001	0.72 (0.59-0.88)	0.280	0.86 (0.65-1.13)
Smoker in household, n(%)	340 (37%)	1910 (38%)	0.523	0.95 (0.82-1.10)	0.568	0.96 (0.82-1.11)
Premature birth, n(%)[c]	87 (10%)	506 (10%)	0.591	0.94 (0.74-1.19)	0.334	0.88 (0.69-1.14)
Birth weight, mean (SD)	2.8 (0.7)	3.0 (0.5)	*P*<0.001		*P*<0.001	
Clinical features:						
Tachypnea, n(%)[d]	785 (86%	560 (11%)	*P*<0.001	49.6 (40.2-61.1)	*P*<0.001	53.73 (43.06-67.03)
Cough, n(%)	647 (71%)	421 (8%)	*P*<0.001	26.6 (22.3-31.6)	*P*<0.001	5.65 (4.80-6.66)
Fever, n(%)[e]	707 (78%)	268 (5%)	*P*<0.001	60.6 (49.7-73.9)	*P*<0.001	117.2 (90.31-152.1)
Diarrhoea, n(%)	118 (13%)	95 (2%)	*P*<0.001	7.6 (5.8-10.1)	*P*<0.001	1.31 (1.15-1.50)
Rhinorrhoea, n(%)	402 (44%)	852 (17%)	*P*<0.001	3.82 (3.28-4.44)	*P*<0.001	2.19 (1.85-2.61)
Markers for Bacterial co-infection:						
***LytA* positive, n (%)[f]**	52 (6%)	253 (5%)	0.507	1.11 (0.82-1.51)	0.818	1.04 (0.76-1.42)
***S. pneu* load, mean (SD)[g]**	4.5 (2.4)	4.6 (2.2)	0.210		0.210	
HDP, n(%)[h]						
-Blood	32 (4%)	140 (3%)	0.289	1.24 (0.84-1.83)	0.491	1.17 (0.80-1.75)
-NP	111 (12%)	379 (8%)	*P*<0.001	1.68 (1.34-2.10)	*P*<0.001	1.56 (1.24-1.96)
Viral infections in the nasopharynx:						
-RSV, n(%)	121 (13%)	140 (3%)	*P*<0.001	5.29 (4.10-6.82)	*P*<0.001	5.68 (4.34-7.42)
-AdV, n(%)	108 (12%)	593 (12%)	0.950	0.99 (0.80-1.24)	0.578	1.06 (0.85-1.34)
-HMPV, n(%)	49 (5%)	206 (4%)	0.093	1.32 (0.96-1.81)	0.341	1.18 (0.84-1.66)
-HBoV, n(%)	138 (15%)	660 (13%)	0.129	1.17 (0.96-1.42)	0.023	1.27 (1.03-1.56)
-InFV A-C, n(%)	5 (1%)	113 (3%)	0.002	0.24 (0.10-0.58)	*P*<0.001	0.19 (0.07-0.47)
-PIV, n(%)	76 (8%)	314 (6%)	0.024	1.35 (1.04-1.75)	0.137	1.23 (0.94-1.61)
-HCoV, n(%)	56 (6%)	501 (10%)	*P*<0.001	0.58 (0.44-0.78)	0.049	0.55 (0.41-0.75)

Abbreviations - OR: Odds ratio; aOR: Adjusted odds ratio; CI: Confidence interval; SD: Standard deviation; NP: Nasopharyngeal; HRV: Human rhinovirus; HIV: Human immunodeficiency virus; HEU: HIV exposed but uninfected; HDP: High density pneumococcus; RSV: Respiratory Syncytial Virus, HMPV: human metapneumovirus; AdV: adenovirus; PIV: parainfluenza type 1-4; HBoV: Human Bocavirus; HCoV: Human Coronavirus (OC43, NL63, 229E and HKU1) and InFV: Influenza Virus (A, B and C); *S. pneu: Streptococcus pneumoniae*.

P-values from Chi-squared and Wilcoxon tests, logistic regression models adjusted for confounding variates (<0.2 in univariate analysis) where applicable, Odds ratio could not be calculated for continuous variables or variables with 0 values, thus cells left blank.

[a] - Underweight defined as weight for age <-2SD of the median age-sex specific WHO reference; [b] - HEU defined as HIV-uninfected but HIV-exposed. Undetectable viral load, HIV seronegative in the child with a

positive maternal history. Positive maternal status based on self-report was accepted, except for seronegative children < 7 months of age where documented positive maternal status was required; [c] - Premature birth defined as gestational age <37 weeks; [d] - Tachypnea defined as respiratory rate >60 breaths/minute if aged <2 months, respiratory rate >50 breaths/minute if aged 2-12 months, respiration rate >40 breaths/minute if aged >12 months; [e] - Fever defined as temperature ≥38^{o}C; [f] - Blood sample positive for *S. pneumoniae* colonisation by *LytA* PCR; [g] - Bacterial load of *S. pneu* in the nasopharynx, expressed as $\log_{10}$ copies/mL; [h] - HDP defined as *S. pneumoniae* density in nasopharynx >6.9 and/or density in whole blood sample >2.2 $\log_{10}$ copies/mL.

Additionally, multi logistic regression analyses to ascertain whether HRV viral load and co-infections were associated with case-control status among the HRV-associated participants were conducted; Table 3.6. Among HRV-associated cases and controls, HRV was 1.71-fold (aOR 95% CI: 1.40-2.09) more likely to be detected as a co-infection among cases (47% vs. 38%, $P<0.001$) and to have higher HRV viral loads in the nasopharynx (3.7 vs. 3.5 $\log_{10}$ copies/mL, $P<0.001$) compared to controls with HRV co-infections. This association of higher viral loads was irrespective of whether HRV was detected as a mono or mixed infection; Table 3.6. No discernible optimal threshold densities for distinguishing HRV-associated cases from controls based on nasopharyngeal viral loads could be calculated from reverse cumulative plots or Youden indices; Figure 3.2.

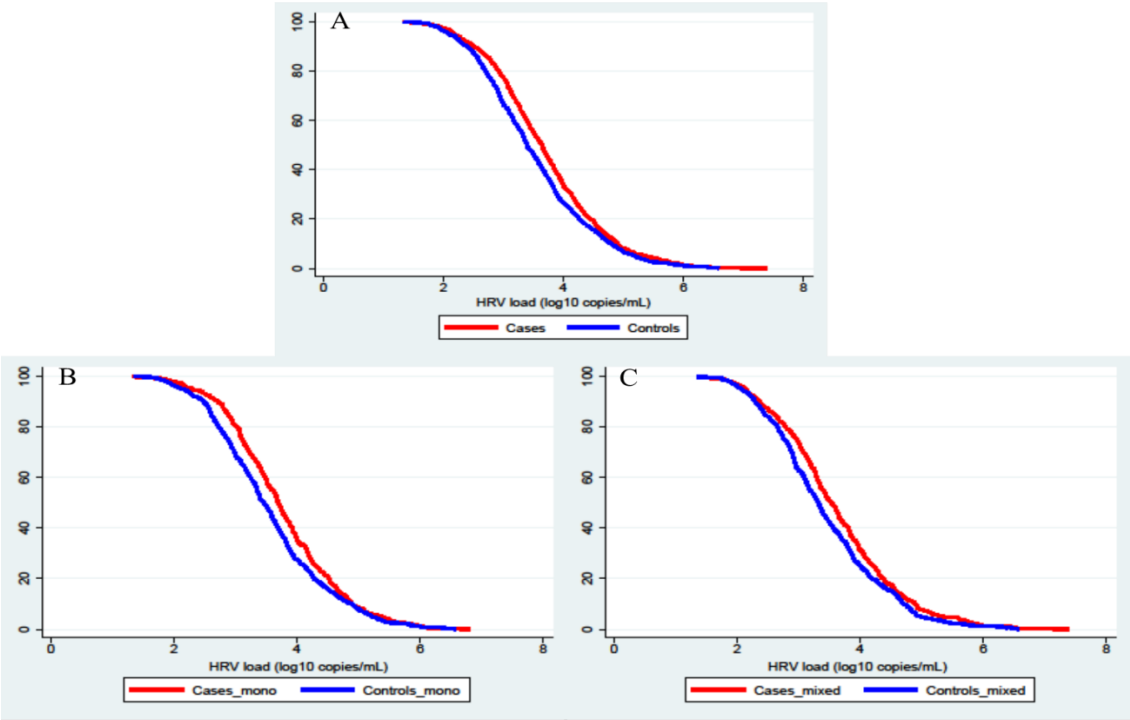

Figure 3.2: Reverse cumulative plot of HRV viral load in the nasopharynx among cases and controls. The viral loads of cases are shown in red and the controls in blue. Panel A.) the HRV viral load in all cases and controls; Panel B.) the HRV viral load in cases and controls with mono-infections; Panel C.) the HRV viral load in cases and controls with HRV co-infections.

Overall, HRV-RSV co-infections were 9.07-fold (aOR 95% CI: 5.54-14.9) more common among cases (13%) than in controls (2%, $P<0.001$). A similar trend was also observed for HRV-PIV co-infections (8% vs. 6%, $P=0.052$); Table 3.6. For HRV co-detection with bacteria in the nasopharynx, HRV co-infections with *S. pneumoniae* (81% vs. 72%, $P<0.001$) and *M. catarrhalis* (79% vs. 68%, $P<0.001$) were more prevalent among controls than cases. No HRV co-infections with bacteria were more prevalent among case than controls; Table 3.6.

Table 3.6: Multivariate analysis of HRV viral loads and co-infections among HRV-associated cases and controls

	Cases (n=912)	Controls (n=1056)	Unadjusted *P*-value	OR (95%CI)	Adjusted *P*-value	aOR (95%CI)
HRV Viral load, Mean (SD)[a]	3.7 (0.94)	3.5 (0.94)	*P*<0.001		*P*<0.001	
HRV Mono-infection, n(%)	481 (53%)	656 (62%)	*P*<0.001	0.69 (0.57-0.81)	*P*<0.001	0.58 (0.47-0.71)
-Viral load of mono-infections	3.8 (0.90)	3.6 (0.93)	*P*<0.001		0.001	
HRV Co-infections, n(%)[b]	431 (47%)	400 (38%)	*P*<0.001	1.47 (1.23-1.78)	*P*<0.001	1.71 (1.40-2.09)
-Viral load of mixed infections	3.6 (0.99)	3.4 (0.94)	0.009		0.020	
HRV Viral Co-infections in nasopharynx with:						
-HBoV, n(%)	138 (15%)	139 (13%)	0.024	1.35 (1.04-1.76)	0.107	1.24 (0.95-1.61)
-RSV, n(%)	121 (13%)	24 (2%)	*P*<0.001	6.88 (4.37-10.2)	*P*<0.001	9.07 (5.54-14.9)
-AdV, n(%)	108 (12%)	134 (13%)	0.507	1.1 (0.83-1.45)	0.663	0.94 (0.70-1.25)
-HMPV, n(%)	49 (5%)	59 (6%)	0.538	1.13 (0.76-1.68)	0.827	1.05 (0.69-1.59)
-PIV, n(%)	76 (8%)	68 (6%)	0.017	1.52 (1.06-2.15)	0.052	1.68 (0.99-2.64)
-InFV A-C , n(%)	5 (1%)	0	0.579		0.088	
-HCoV, n(%)	56 (6%)	93 (9%)	0.272	0.82 (0.58-1.17)	0.625	0.90 (0.60-1.35)
Bacterial Co-infections in nasopharynx with:						
-*S. pneu*, n(%)	661 (72%)	860 (81%)	*P*<0.001	0.60 (0.49-0.74)	*P*<0.001	0.56 (0.45-0.70)
-*H. inf*, n(%)	510 (56%)	603 (57%)	0.598	0.95 (0.80-1.14)	0.415	0.93 (0.77-1.11)
-HiB, n(%)	23 (3%)	23 (2%)	0.615	1.16 (0.65-2.09)	0.606	1.17 (0.65-2.12)
-*M. cat*, n(%)	616 (68%)	836 (79%)	*P*<0.001	0.55 (0.46-0.67)	*P*<0.001	0.49 (0.40-0.61)
-*S. aureus*, n(%)	129 (14%)	135 (13%)	0.377	1.12 (0.87-1.46)	0.346	1.14 (0.87-1.49)
***P. jiroveci* Co-infections, n(%)**	80 (9%)	102 (10%)	0.498	0.90 (0.66-1.22)	0.615	0.92 (0.66-1.28)

Abbreviations - OR: Odds ratio; aOR: Adjusted odds ratio; CI: Confidence interval; SD: Standard deviation; NP: Nasopharyngeal; HRV: Human rhinovirus; RSV: Respiratory Syncytial Virus, HMPV: human metapneumovirus; AdV: adenovirus; PIV: parainfluenza type 1-4; HBoV: Human Bocavirus; HCoV: Human Coronavirus (OC43, NL63, 229E and HKU1) and InFV: Influenza Virus (A, B and C); *S. pneu: Streptococcus pneumoniae*; H.inf: *Haemophilus influenza*, HiB: *Haemophilus influenza* Type B, M.cat: *Moraxella catarrahalis; P. jiroveci: Pneumocytosis jiroveci*

P-values from Chi-squared and Wilcoxon tests, logistic regression models adjusted for confounding variates (<0.2 in univariate analysis) where applicable, Odds ratio could not be calculated for continuous variables or variables with 0 values, thus cells left blank.

[a] - Rhinovirus viral load in the nasopharynx, expressed as $\log_{10}$ copies/mL; [b] - Any viral respiratory coinfection with RSV (A and B), HMPV, AdV, InFV (A, B and C), PIV type 1-4, HCoV (OC43, NL63, 229E and HKU1).

3.1.4 Characteristics of cases by HRV status

Of the 3 876 case participants enrolled, 68% (n=2 630) had severe pneumonia and 32% (n=1 246) had very severe pneumonia. HRV was detected in 24% (n=912) of the cases and after logistic modelling there were no differences in the prevalence of HRV between the severe (24%, n=621/2 630) and very severe pneumonia cases (23%, n=291/1 246, P=0.128). The HRV-associated very severe pneumonia cases; however, had higher HRV viral loads (3.8 $\log_{10}$ copies/mL) compared to the HRV-associated severe pneumonia cases (3.6 $\log_{10}$ copies/mL, P=0.009). No clinically beneficial threshold densities capable of discriminating more severe HRV-associated disease could be determined from reverse cumulative plots or Youden indices, Figure 3.3.

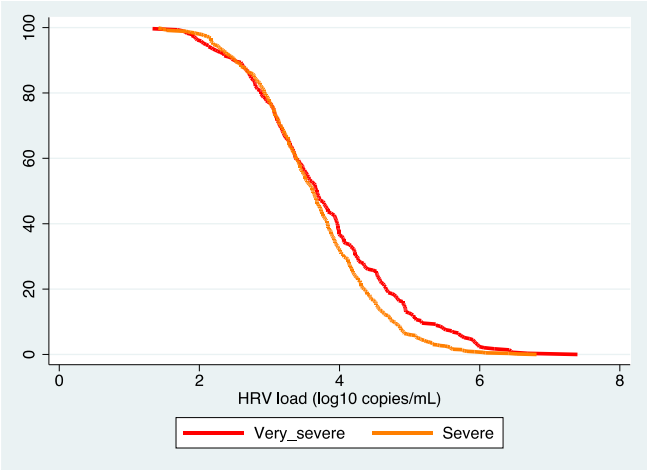

Figure 3.3: Reverse cumulative plot of HRV viral load in the nasopharynx among children with severe and very severe pneumonia

In the multivariate logistic regression model, HRV-associated cases were significantly older (mean 13.1 months) than those without HRV infection (mean 11.2 months, $P<0.001$). No other associations were seen in risk factors for pneumonia including day care attendance (14% vs. 17%, $P=0.095$), having a smoker in the household (37% vs. 33%, $P=0.357$) or premature birth (10% vs. 11%, $P=0.695$) between HRV-associated cases compared to HRV uninfected cases; Table 3.7.

HRV-associated cases were also 1.73-fold (aOR 95% CI: 1.43-2.0) more likely to have wheezing (46% vs. 31%, $P<0.001$) and 1.36-fold (aOR 95% CI: 1.09-1.70) more likely to have tachypnea (86% vs. 81%, $P=0.005$) than in the absence of HRV infection. Conversely, HRV-associated cases were 0.78-fold (aOR 95% CI: 0.67-0.91) less likely to have radiographically confirmed pneumonia (chest X-ray with any infiltrate) (40% vs. 46%, $P=0.001$) and 0.70-fold (aOR 95% CI: 0.49-0.99) less likely to present with convulsions (5% vs. 7%, $P=0.047$). There was no other association observed for presence of HRV infection and hypoxia (33% vs. 37%, $P=0.871$), supplementary oxygen therapy (29% vs. 31%, $P=0.746$) or mechanical ventilation (2% vs. 3%, $P=0.0752$) and very severe pneumonia (32% vs. 32%, $P=0.128$) in HRV-associated cases compared to those without infection. HRV-associated cases were, however, 0.76-fold (aOR 95% CI: 0.65-0.89) less likely to be hospitalised for >3 days duration (52% vs 61, $P=0.001$) but had similar case fatality ratio (8% each, $P=0.300$) Table 3.7.

Table 3.7: The demographical and clinical characteristics of cases by HRV infection status for all sites data combined

	HRV+ (n=912)	HRV- (n= 2964)	Unadjusted *P*-value	Unadjusted OR (95% CI)	Adjusted *P*-value	Adjusted aOR (95% CI)
Age in months, mean (SD)	13.1 (12.1)	11.2 (11.3)	*P*<0.001		*P*<0.001	
Female, n(%)	373 (41%)	1261 (43%)	0.379	0.93 (0.80-1.09)	0.472	0.95 (0.81-1.10)
Never breast fed, n(%)	80 (9%)	300 (10%)	0.231	0.85 (0.66-1.11)	0.541	1.09 (0.82-1.45)
Under weight, n (%)[a]	277 (30%)	916 (30%)	0.761	0.98 (0.83-1.15)	0.396	0.93 (0.79-1.10)
HEU, n(%)[b]	94 (10%)	330 (11%)	0.484	0.92 (0.72-1.17)	0.895	1.02 (0.77-1.35)
Day care attendance, n(%)	126 (14%)	517 (17%)	0.003	0.77 (0.65-0.91)	0.095	0.87 (0.75-1.02)
Smoker in household, n(%)	340 (37%)	969 (33%)	0.113	1.10 (0.98-1.24)	0.357	1.06 (0.93-1.21)
Premature at birth, n(%)[c]	87 (10%)	328 (11%)	0.678	0.99 (0.93-1.05)	0.695	0.99 (0.93-1.05)
Birth weight, mean (SD)	2.9 (0.6)	2.9 (0.7)	0.998		0.882	
Clinical Features:						
Very severe pneumonia, n(%)	291 (32%)	955 (32%)	0.869	0.98 (0.84-1.16)	0.128	1.14 (0.96-1.35)
Chest X-ray abnormal, n(%)[d]	365 (40%)	1355 (46%)	0.003	0.79 (0.68-0.92)	0.001	0.78 (0.67-0.91)
Supplementary 02 therapy, n(%)	266 (29%)	928 (31%)	0.278	0.92 (0.80-1.06)	0.746	0.96 (0.76-1.22)
Mechanical Ventilation, n(%)	15 (2%)	91 (3%)	0.023	0.53 (0.30-0.92)	0.072	0.60 (0.34-1.05)
Hypoxic, n(%)[e]	297 (33%)	1086 (37%)	0.028	0.84 (0.72-0.98)	0.571	0.98 (0.82-1.19)
Tachycardia, n(%)[f]	439 (48%)	1514 (51%)	0.108	0.88 (0.76-1.03)	0.834	0.98 (0.84-1.15)
Tachypnea, n(%)[g]	785 (86%)	2384 (81%)	*P*<0.001	1.47 (1.19-1.82)	0.005	1.36 (1.09-1.70)
Wheezing, n(%)	421 (46%)	898 (31%)	*P*<0.001	1.96 (1.69-2.29)	*P*<0.001	1.73 (1.43-2.0)
Cough, n(%)	647 (71%)	2054 (69%)	0.430	1.05 (0.94-1.17)	0.521	0.94 (0.79-1.13)
Lethargic, n(%)	89 (10%)	311 (11%)	0.520	0.92 (0.72-1.18)	0.777	0.96 (0.74-1.25)
Convulsions, n(%)	43 (5%)	201 (7%)	0.026	0.68 (0.48-0.96)	0.047	0.70 (0.49-0.99)
Diarrhoea, n(%)	118 (13%)	451 (15%)	0.060	0.82 (0.66-1.0)	0.580	1.01 (0.81-1.27)
Head nodding, n(%)	162 (18%)	477 (16%)	0.225	1.13 (0.93-1.37)	0.051	1.31 (0.99-1.62)
Central cyanosis, n(%)	15 (2%)	65 (2%)	0.310	0.75 (0.42-1.31)	0.958	0.98 (0.55-1.75)
Unable to Feed, n(%)	58 (6%)	241 (8%)	0.082	0.76 (0.57-1.03)	0.529	0.91 (0.67-1.23)
Vomiting everything, n(%)	22 (2%)	94 (3%)	0.240	0.75 (0.47-1.21)	0.207	0.73 (0.45-1.19)
Lower chest wall indrawing, n(%)	846 (93%)	2712 (92%)	0.224	1.19 (0.90-1.58)	0.197	1.21 (0.90-1.63)
Stridor, n(%)	18 (2%)	77 (3%)	0.277	0.87 (0.68-1.12)	0.459	0.82 (0.48-1.39)
Grunting, n(%)	225 (13%)	566 (19%)	0.039	0.86 (0.74-0.99)	0.154	0.76 (0.59-0.99)
Nasal flaring, n (%)	499 (55%)	1757 (59%)	0.004	0.81 (0.71-0.94)	0.229	1.11 (0.93-1.33)
Hospital stay >3 days, n(%)	477 (52%)	1795 (61%)	*P*<0.001	0.71 (0.62-0.83)	0.001	0.76 (0.65-0.89)
Case fatality ratio, n(%)	63 (8%)	215 (8%)	0.671	0.94 (0.70-1.26)	0.300	1.18 (0.86-1.60)

Abbreviations - OR: Odds ratio; aOR: Adjusted odds ratio; CI: Confidence interval; SD: Standard deviation; NP: Nasopharyngeal; HRV: Human rhinovirus; HIV: Human immunodeficiency virus; HEU: HIV exposed but uninfected.

P-values from Chi-squared and Wilcoxon tests, logistic regression models adjusted for confounding variates (<0.2 in univariate analysis) where applicable, Odds ratio could not be calculated for continuous variables or variables with 0 values, thus cells left blank.

[a] - Underweight defined as weight for age <-2SD of the median age-sex specific WHO reference; [b] - HEU defined as HIV-uninfected but HIV-exposed. Undetectable viral load, HIV seronegative in the child with a positive maternal history. Positive maternal status based on self-report was accepted, except for seronegative children < 7 months of age where documented positive maternal status was required; [c] - Premature birth defined as gestational age <37 weeks; [d] - Abnormal Chest X-ray defined as radiographically confirmed end point pneumonia consolidation or any infiltrates; [e] - A child was considered to be hypoxic if 1) a room air pulse-oximetry reading indicated oxygen saturation <90% at the two sites at elevation (Zambia and South Africa) or <92% at all other sites, or 2) a room air oxygen saturation; [f] - Tachycardia defined as heart rate >160 beats per minute (bpm) if aged <11 months, heart rate >150 bpm if aged 12-35 months, heart rate >140 bpm if aged 36-59 months; [g] - Tachypnea defined as respiratory rate >60 breaths/minute if aged <2 months, respiratory rate >50 breaths/minute if aged 2-12 months, respiration rate >40 breaths/minute if aged >12 month.

Among markers of possible bacterial co-infection; Table 3.8, HRV-associated cases were less likely associated with fever (78% vs. 83%, P=0.007), alveolar consolidation on CXR (18% vs 24%, P=0.013) and CRP levels ≥40mg/L (19% vs 24%, P=0.008) compared to cases without HRV infection. Children with HRV-associated pneumonia, however, had significantly higher

neutrophil percentages (51% vs. 46%, $P<0.001$) and were 1.39-fold (aOR 95% CI: 1.19-1.63) more likely to have leucocytosis (51% vs. 41%, $P<0.001$); but conversely had significantly lower lymphocyte percentages (37.55% vs. 42.8%, $P<0.001$). There were no other associations between the presence of HRV detection and presence of MCPP (1% for both, $P=0.626$), prevalence of concurrent *S. pneumoniae* (*LytA*) bacteraemia (6% vs 7%, $P=0.837$) or high colonisation densities of *S. pneumoniae* in the blood (>2.2 $\log_{10}$ copies/ml; 4% vs. 5%, $P=0.482$) or nasopharynx (>6.9 $\log_{10}$ copies/mL; 12% vs 13%, $P=0.858$).

Among other commonly detected respiratory viruses; Table 3.8, HRV-associated cases were less likely to be infected with RSV (13% vs. 28%, $P<0.001$), Influenza virus (1% vs. 6%, $P<0.001$), HMPV (5% vs. 10%, $P<0.001$) and PIV (8% vs. 15%, $P<0.001$) compared to cases without HRV infections; and there was a trend for the HRV-associated cases to be more commonly associated with AdV co-infections than cases without HRV infection (12% vs. 10%, $P=0.098$). Individual site case-case evaluations are available in Table 3.9A and B, with similar trends observed as for the overall site comparisons above.

Table 3.8: Markers for possible bacterial co-infections and viral co-infections of cases by HRV infection status

	HRV+ (n=912)	HRV- (n= 2964)	Unadjusted *P*-value	Unadjusted OR (95% CI)	Adjusted *P*-value	Adjusted aOR (95% CI)
Bacterial co-infection markers:						
Fever, n(%)[a]	707 (78%)	2447 (83%)	0.001	0.73 (0.60-0.87)	0.007	0.76 (0.63-0.93)
Alveolar consolidation, n(%)[b]	156 (18%)	657 (24%)	0.001	0.72 (0.59-0.87)	0.013	0.78 (0.64-0.95)
Leucocytosis, n(%)[c]	443 (51%)	1135 (41%)	*P*<0.001	1.54 (1.32-1.80)	*P*<0.001	1.39 (1.19-1.63)
Neutrophils (%), median (IQR)	51 (35.4-67.9)	46 (31-60.3)	*P*<0.001		*P*<0.001	
Lymphocytes (%), median (IQR)	37.6 (24-52)	42.8 (29.2-56.9)	*P*<0.001		*P*<0.001	
Eosinophils (%), median (IQR)	1.3 (0.4-3.5)	0.9 (0.1-2.8)	0.031		0.126	
CRP ≥40mg/l, n(%)[d]	176 (19%)	718 (24%)	0.002	0.75 (0.62-0.90)	0.008	0.77 (0.64-0.94)
Blood culture positive, n(%)	29 (3%)	108 (4%)	0.507	0.87 (0.57-1.32)	0.931	0.98 (0.64-1.50)
***LytA* positive, n(%)[e]**	52 (6%)	192 (7%)	0.355	0.86 (0.63-1.18)	0.837	0.97 (0.70-1.33)
MCPP, n(%)[f]	8 (1%)	35 (1%)	0.445	0.74 (0.34-2.60)	0.626	0.82 (0.38-1.80)
HDP, n(%)[g]						
-Blood	32 (4%)	135 (5%)	0.155	0.75 (0.51-1.11)	0.482	0.86 (0.58-1.29)
-NP	111 (12%)	380 (13%)	0.604	0.94 (0.75-1.18)	0.858	0.98 (0.77-1.23)
Viral co-infections in the nasopharynx with:						
-RSV, n (%)	121 (13%)	833 (28%)	*P*<0.001	0.39 (0.32-0.48)	*P*<0.001	0.39 (0.31-0.49)
-AdV, n (%)	108 (12%)	283 (10%)	0.045	1.27 (1.0-1.61)	0.098	1.23 (0.96-1.57)
-InFV A-C, n (%)	5 (1%)	174 (6%)	*P*<0.001	0.09 (0.04-0.22)	*P*<0.001	0.09 (0.04-0.22)
-HBoV, n (%)	138 (15%)	365 (12%)	0.027	1.27 (1.02-1.57)	0.116	1.19 (0.96-1.48)
-HMPV, n (%)	49 (5%)	294 (10%)	*P*<0.001	0.52 (0.38-0.70)	*P*<0.001	0.50 (0.37-0.69)
-PIV, n (%)	76 (8%)	437 (15%)	*P*<0.001	0.53 (0.41-0.68)	*P*<0.001	0.51 (0.39-0.67)
-HCoV, n (%)	56 (6%)	232 (8%)	0.090	0.77 (0.57-1.04)	0.145	0.80 (0.59-1.08)

Abbreviations - OR: Odds ratio; aOR: Adjusted odds ratio; CI: Confidence interval; IQR: Inter quartile range; NP: Nasopharyngeal; HRV: Human rhinovirus; HDP: High density pneumococcus; CRP: C-reactive protein; RSV: Respiratory Syncytial Virus, HMPV: human metapneumovirus; AdV: adenovirus; PIV: parainfluenza type 1-4; HBoV: Human Bocavirus; HCoV: Human Coronavirus (OC43, NL63, 229E and HKU1) and InFV: Influenza Virus (A, B and C); *S. pneu: Streptococcus pneumoniae*.

P-values from Chi-squared and Wilcoxon tests, logistic regression models adjusted for confounding variates (<0.2 in univariate analysis) where applicable, Odds ratio could not be calculated for continuous variables or variables with 0 values, thus cells left blank.

[a] - Fever defined as temperature ≥38^{o}C; [b] - defined as primary end point pneumonia on the chest X-rays; [c] - Leucocytosis defined as white blood cell count >15 000 cells/uL if age <12 months, white blood cell count >13 000 cells/uL if age >12 months; [d] - CRP defined as levels ≥40mg/mL are considered to potentially indicate bacterial infection; [e] - Blood sample positive for *S. pneumoniae* colonisation by *LytA* PCR; [f] - MCPP defined as *S. pneumoniae* was cultured from a normally sterile body fluid - blood, pleural fluid or lung aspirate - or pleural fluid or lung aspirate was PCR *LytA* positive; [g] - HDP defined as *S. pneumoniae* density in nasopharynx >6.9 and/or density in whole blood sample >2.2 $\log_{10}$ copies/mL.

Table 3.9A: The demographical and clinical characteristics of cases by HRV infection status for each African site individually

	Kenya (n=628)			Gambia (n=609)			Mali (n=650)			Zambia (n=449)			South Africa (n=794)		
	HRV+ (n=152)	HRV- (n=476)	*P*-value	HRV+ (n=139)	HRV- (n=470)	*P*-value	HRV+ (n=112)	HRV- (n=538)	*P*-value	HRV+ (n=95)	HRV- (n=354)	*P*-value	HRV+ (n=182)	HRV- (n=794)	*P*-value
Age in months, mean (SD)	14.5(13.2)	14.2 (13.1)	0.800	14.4 (12.5)	11.3 (11.2)	0.006	9.3 (10.1)	10.0 (10.2)	0.525	9.9 (10.9)	7.6 (8.9)	0.036	9.9 (9.7)	8.5 (9.5)	0.082
Female, n(%)	60 (39%)	201 (42%)	0.516	55 (40%)	176 (37%)	0.973	50 (45%)	237 (44%)	0.896	47 (49%)	158 (45%)	0.441	80 (44%)	291 (48%)	0.414
Never breast fed, n(%)	3 (2%)	6 (1%)	0.477	3 (2%)	4 (1%)	0.285	7 (6%)	25 (5%)	0.380	4 (4%)	22 (6%)	0.510	52 (29%)	211 (34%)	0.150
Under weight, n (%)[a]	60 (39%)	190 (43%)	0.811	41 (30%)	119 (25%)	0.898	37 (33%)	195 (36%)	0.701	34 (36%)	87 (25%)	0.047	37 (20%)	174 (28%)	0.034
HEU, n(%)[b]	1 (1%)	3 (1%)	0.970	1 (1%)	1 (0%)	0.359	1 (1%)	5 (1%)	0.971	24 (25%)	94 (27%)	0.800	226 (37%)	67 (37%)	0.977
Day care attendance, n(%)	8 (5%)	29 (6%)	0,570	2 (1%)	11 (2%)	0.507	83 (74%)	350 (65%)	0.170	1 (1%)	5 (1%)	0.842	24 (13%)	92 (15%)	0.380
Smoker in household, n(%)	1 (1%)	10 (2%)	0.242	79 (57%)	251 (54%)	0.569	33 (30%)	153 (28%)	0.836	27 (28%)	61 (17%)	0.046	73 (40%)	217 (35%)	0.498
Premature birth, n(%)[c]	28 (18%)	87 (18%)	0.618	5 (4%)	19 (4%)	0.936	3 (3%)	25 (5%)	0.563	2 (2%)	21 (6%)	0.280	33 (18%)	119 (19%)	0.790
Birth weight, mean (SD)	2. 8 (0.7)	2.8 (0.7)	0.589	3.0 (0.6)	2.9 (0.5)	0.486	3.1 (0.6)	3.0 (0.7)	0.896	2.9 (0.6)	3.0 (0.7)	0.827	2.9 (0.7)	2.9 (0.8)	0.538
Clinical features:															
Very severe pneumonia, n(%)	80 (53%)	242 (51%)	0.758	25 (18%)	63 (13%)	0.317	65 (58%)	273 (51%)	0.145	32 (34%)	108 (31%)	0.580	65 (36%)	189 (31%)	0.210
Chest X-ray abnormal, n(%)[d]	65 (43%)	217 (46%)	0.508	58 (42%)	215 (46%)	0.400	34 (30%)	205 (38%)	0.154	32 (34%)	148 (42%)	0.154	85 (47%)	344 (56%)	0.023
Supplementary 02 therapy, n(%)	32 (21%)	99 (21%)	0.169	3 (2%)	18 (4%)	0.313	3 (3%)	25 (5%)	0.428	46 (48%)	173 (49%)	0.617	167 (92%)	540 (88%)	0.843
Mechanical ventilation, n(%)	0	0		0	0		1 (1%)	3(1%)	0.680	2 (2%)	6 (2%)	0.788	8 (4%)	57 (9%)	0.050
Hypoxic, n(%)[e]	48 (32%)	136 (29%)	0.321	7 (5%)	36 (8%)	0.277	57 (51%)	249 (46%)	0.407	29 (31%)	133 (38%)	0.260	135 (75%)	457 (75%)	0.900
Tachycardia, n(%)[f]	77 (51%)	270 (57%)	0.198	65 (47%)	264 (56%)	0.041	69 (62%)	300 (56%)	0.312	64 (67%)	220 (63%)	0.385	87 (48%)	299 (49%)	0.835
Tachypnea, n(%)[g]	115 (76%)	322 (68%)	0.060	121 (87%)	409 (87%)	0.808	101 (90%)	450 (84%)	0.059	84 (88%)	302 (86%)	0.736	150 (83%)	459 (77%)	0.100
Wheezing, n(%)	31 (21%)	52 (11%)	0.002	48 (35%)	142 (30%)	0.135	27 (24%)	87 (16%)	0.066	21 (22%)	35 (10%)	0.005	79 (44%)	188 (32%)	0.008
Cough, n(%)	76 (50%)	243 (51%)	0.222	77 (55%)	269 (57%)	0.204	65 (58%)	300 (56%)	0.714	60 (63%)	248 (70%)	0.162	161 (88%)	527 (86%)	0.453
Lethargic, n(%)	37 (24%)	107 (23%)	0.722	14 (10%)	37 (8%)	0.575	16 (14%)	74 (14%)	0.671	13 (14%)	44 (12%)	0.870	7 (4%)	31 (5%)	0.467
Fever, n(%)[h]	119 (78%)	416 (87%)	0.004	135 (97%)	466 (99%)	0.051	95 (85%)	472 (88%)	0.528	73 (77%)	300 (85%)	0.042	116 (64%)	382 (62%)	0.942
Convulsions, n(%)	14 (9%)	61 (13%)	0.148	8 (6%)	18 (4%)	0.552	11 (10%)	73 (14%)	0.370	4 (4%)	17 (5%)	0.624	5 (3%)	5 (1%)	0.052
Diarrhoea, n(%)	25 (16%)	72 (15%)	0.774	13 (9%)	41 (9%)	0.642	25 (22%)	140 (26%)	0.468	22 (23%)	69 (19%)	0.874	30 (16%)	89 (15%)	0.396
Head nodding, n(%)	25 (17%)	68 (14%)	0.346	8 (6%)	18 (4%)	0.311	40 (36%)	151 (28%)	0.124	12 (13%)	46 (13%)	0.976	58 (32%)	162 (26%)	0.131
Central cyanosis, n(%)	0	5 (1%)	0.390	2 (1%)	1 (0%)	0.117	7 (6%)	33 (6%)	0.958	5 (5%)	9 (3%)	0.141	0	10 (2%)	0.202
Unable to Feed, n(%)	19 (13%)	58 (12%)	0.671	2 (1%)	13 (3%)	0.393	26 (23%)	92 (17%)	0.131	7 (7%)	30 (8%)	0.940	2 (1%)	27 (4%)	0.051
Vomiting everything, n(%)	13 (9%)	54 (11%)	0.225	2 (1%)	6 (1%)	0.980	1 (1%)	11 (2%)	0.484	2 (2%)	1 (0%)	0.114	2 (1%)	13 (2%)	0.321
Lower chest wall indrawing, n(%)	126 (83%)	364 (76%)	0.040	128 (92%)	442 (94%)	0.825	10 (90%)	496 (92%)	0.340	88 (93%)	342 (97%)	0.147	173 (95%)	588 (96%)	0.574
Stridor, n(%)	0	8 (2%)	0.241	2 (1%)	5 (1%)	0.392	5 (4%)	13 (2%)	0.299	3 (3%)	4 (1%)	0.228	6 (3%)	37 (6%)	0.733
Grunting, n(%)	5 (3%)	4 (1%)	0.330	10 (7%)	37 (8%)	0.299	68 (61%)	342 (64%)	0.550	18 (19%)	96 (27%)	0.116	10 (5%)	42 (7%)	0.936
Nasal flaring, n (%)	80 (53%)	236 (50%)	0.358	74 (53%)	246 (52%)	0.427	89 (79%)	430 (80%)	0.691	55 (58%)	225 (64%)	0.320	161 (88%)	506 (83%)	0.275
Laboratory markers:															
Leucocytosis, n(%)[i]	77 (52%)	203 (43%)	0.110	50 (48%)	141 (41%)	0.431	28 (25%)	156 (29%)	0.566	43 (46%)	141 (41%)	0.557	93 (51%)	260 (43%)	0.067
Neutrophils (%), median (IQR)	49.9 (33.3-64)	46.9 (32.8-60)	0.171	53 (37.4-72.2)	48.8 (37-60.5)	0.336	46 (27-64)	42 (28-59)	0.222	48.1 (36.5-64)	45 (30-61.5)	0.109	50.3 (36.6-64.3)	44.1 (28.6-57.7)	0.003
Lymphocytes (%), median (IQR)	37.9 (24.4-53.8)	39.3 (27.4-53)	0.789	38.4 (20.6-52)	42.2 (31-54.1)	0.338	49 (31-67)	48 (34-62)	0.839	37.9 (24-50.5)	42.5 (26.3-6)	0.179	37.5 (26-49.3)	43.1 (29.7-56.4)	0.009
CRP >40mg/l, n(%)[j]	29 (19%)	125 (26%)	0.052	25 (18%)	104 (22%)	0.178	30 (27%)	159 (30%)	0.759	31 (33%)	107 (30%)	0.794	38 (21%)	161 (26%)	0.068
Blood culture positive, n(%)[k]	3 (2%)	9 (2%)	0.798	4 (3%)	23 (5%)	0.214	11 (10%)	28 (5%)	0.059	6 (6%)	22 (6%)	1.0	3 (2%)	16 (3%)	0.519
***LytA* positive, n(%)[l]**	3 (2%)	28 (7%)	0.059	15 (11%)	43 (10%)	0.550	14 (13%)	54 (10%)	0.349	5 (6%)	22 (7%)	0.610	10 (5%)	42 (7%)	0.443
MCPP, n(%)[m]	1 (1%)	4 (1%)	0.806	3 (2%)	12 (3%)	0.667	4 (4%)	14 (3%)	0.514	0	2 (1%)	0.839	0	3 (0%)	0.624
HDP, n(%)[n]	12 (8%)	34 (7%)	0.800	29 (21%)	103 (22%)	0.773	39 (35%)	161 (30%)	0.234	8 (8%)	37 (10%)	0.580	19 (10%)	96 (16%)	0.091
Hospital stay >3 days, n(%)	25 (16%)	106 (22%)	0.159	10 (7%)	45 (20%)	0.451	47 (42%)	266 (49%)	0.133	38 (40%)	131 (37%)	0.472	77 (42%)	358 (59%)	*P*<0.001
Case fatality ratio, n(%)	9 (6%)	27 (6%)	0.788	7 (5%)	14 (3%)	0.333	25 (23%)	87 (17%)	0.134	14 (26%)	55 (28%)	0.779	7 (5%)	19 (4%)	0.506

Abbreviations - HRV: Human rhinovirus; SD: Standard deviation; IQR: Inter quartile range; CXR: Chest X-ray; HIV: Human immunodeficiency virus; HEU: HIV-uninfected but HIV exposed; CRP: C-reactive protein; MCPP: Microbiologically confirmed pneumococcal pneumonia; HDP: High Density pneumococcus; NP: Nasopharyngeal.
P-values from Chi-squared and Wilcoxon tests, logistic regression models adjusted for confounding variates (<0.2 in univariate analysis) where applicable. *P*-values could not be calculated for variables where both groups were zero, thus left blank.
[a] - Underweight defined as weight for age <-2SD of the median age-sex specific WHO reference; [b] - HEU defined as HIV-uninfected but HIV-exposed. Undetectable viral load, HIV seronegative in the child with a positive maternal history. Positive maternal status based on self-report was accepted, except for seronegative children < 7 months of age where documented positive maternal status was required; [c] - Premature birth defined as gestational age <37 weeks; [d] - Abnormal Chest X-ray defined as radiographically confirmed end point pneumonia consolidation or any infiltrates; [e] - A child was considered to be hypoxic if 1) a room air pulse-oximetry reading indicated oxygen saturation <90% at the two sites at elevation (Zambia and South Africa) or <92% at all other sites, or 2) a room air oxygen saturation; [f] - Tachycardia defined as heart rate >160 beats per minute (bpm) if aged <11 months, heart rate >150 bpm if aged 12-35 months, heart rate >140 bpm if aged 36-59 months; [g] - Tachypnea defined as respiratory rate >60 breaths/minute if aged <2 months, respiratory rate >50 breaths/minute if aged 2-12 months, respiration rate >40 breaths/minute if aged >12 month; [h] - Fever defined as temperature >38^{o}C; [i] - Leucocytosis defined as white blood cell count >15 000 cells/uL if age <12 months, white blood cell count >13 000 cells/uL if age >12 months; [j] - CRP defined as levels ≥40mg/mL which are considered to potentially indicate bacterial infection; [k] - Blood culture positive for any significant non contaminate bacteria; [l] - Blood sample positive for *S. pneumoniae* colonisation by *LytA* PCR; [m] - MCPP defined when *S. pneumoniae* was cultured from a normally sterile body fluid - blood, pleural fluid or lung aspirate - or pleural fluid or lung aspirate was PCR *LytA* positive; [n] - HDP defined as *S. pneumoniae* density in nasopharynx >6.9 and/or density in whole blood sample >2.2 $\log_{10}$ copies/mL.

Table 3.9B: The demographical and clinical characteristics of cases by HRV infection status for each Southeast Asia site individually

	Thailand (n=221)			Bangladesh (n=525)		
	HRV+ (n=40)	**HRV- (n=181)**	***P*-value**	**HRV+ (n=192)**	**HRV- (n=333)**	***P*-value**
Age in months, mean (SD)	21.9 (15.1)	17.2 (12.9)	0.046	16.0 (11.7)	13.9 (11.8)	0.041
Female, n(%)	15 (38%)	73 (40%)	0.713	66 (34%)	125 (38%)	0.417
Never breast fed, n(%)	9 (23%)	29 (16%)	0.334	2 (1%)	3 (1%)	0.714
Under weight, n (%)[a]	6 (15%)	47 (26%)	0.181	62 (32%)	114 (34%)	0.315
HEU, n(%)[b]	0	1 (1%)	0.638	0	0	
Day care attendance, n(%)	7 (18%)	20 (17%)	0.888	1 (1%)	0	0.315
Smoker in household, n(%)	22 (55%)	105 (58%)	0.830	105 (55%)	172 (52%)	0.502
Premature birth, n(%)[c]	7 (18%)	37 (20%)	0.500	9 (5%)	21 (6%)	0.474
Birth weight, mean (SD)	2.9 (0.7)	2.8 (0.7)	0.718	2.8 (0.6)	2.8 (0.6)	0.851
Clinical Features:						
Very severe pneumonia, n(%)	6 (15%)	45 (25%)	0.235	18 (9%)	35 (11%)	0.774
Chest X-ray abnormal, n(%)[d]	13 (33%)	85 (47%)	0.109	78 (41%)	141 (42%)	0.599
Supplementary 02 therapy, n(%)	6 (15%)	58 (32%)	0.051	9 (5%)	15 (5%)	0.915
Mechanical ventilation, n(%)	6 (15%)	47 (26%)	0.198	15 (8%)	28 (8%)	0.678
Hypoxic, n(%)[e]	17 (43%)	81 (45%)	0.813	60 (31%)	80 (24%)	0.150
Tachycardia, n(%)[f]	32 (82%)	135 (78%)	0.553	182 (95%)	307 (92%)	0.415
Tachypnea, n(%)[g]	30 (75%)	70 (39%)	*P*<0.001	185 (96%)	324 (97%)	0.482
Wheezing, n(%)	33 (83%)	155 (86%)	0.356	175 (91%)	312 (94%)	0.197
Cough, n(%)	1 (3%)	8 (4%)	0.675	1 (1%)	10 (3%)	0.099
Lethargic, n(%)	36 (90%)	169 (93%)	0.235	133 (69%)	242 (73%)	0.494
Fever, n(%)[h]	0	23 (13%)	0.084	1 (1%)	4 (1%)	0.451
Convulsions, n(%)	3 (8%)	39 (22%)	0.061	0	1 (0%)	0.736
Diarrhoea, n(%)	3 (8%)	5 (3%)	0.133	16 (8%)	27 (8%)	0.796
Head nodding, n(%)	1 (3%)	6 (3%)	0.929	0	1 (0%)	0.736
Central cyanosis, n(%)	0	8 (4%)	0.347	2 (1%)	13 (4%)	0.083
Unable to Feed, n(%)	1 (3%)	7 (4%)	0.723	1 (1%)	2 (1%)	0.882
Vomiting everything, n(%)	39 (98%)	152 (84%)	0.044	191 (99%)	328 (99%)	0.307
Lower chest wall indrawing, n(%)	1 (3%)	9 (5%)	0.470	1 (1%)	1 (0%)	0.465
Stridor, n(%)	1 (3%)	5 (3%)	0.970	3 (2%)	10 (3%)	0.326
Grunting, n(%)	23 (58%)	91 (50%)	0.407	17 (9%)	23 (7%)	0.629
Nasal flaring, n (%)						
Laboratory Markers:						
Leucocytosis, n(%)[i]	28 (70%)	89 (49%)	0.037	124 (69%)	145 (48%)	*P*<0.001
Neutrophils (%), median (IQR)	63.7 (46-76.3)	58 (44.5-70)	0.513	52.95 (36.8-68)	42.1 (30.5-58.6)	*P*<0.001
Lymphocytes (%), median (IQR)	34.3 (22-47.8)	27.1 (17.5-44)	0.436	33.85 (23.6-47)	45.55 (30.3-58)	*P*<0.001
Eosinophils, median (IQR)	1 (0.2-2.3)	0 (0-1.65)	0.006	3.7 (1.8-6.9)	2.4 (0.9-5.05)	0.002
CRP >40mg/l, n(%)[j]	6 (15%)	30 (17%)	0.804	17 (9%)	32 (10%)	0.784
Blood culture positive, n(%)[k]	0	7 (4%)	0.396	2 (1%)	3 (1%)	0.962
***LytA* positive, n(%)[l]**	1 (3%)	2 (1%)	0.426	4 (2%)	1 (0%)	0.088
MCPP, n(%)[m]	0	0		0	0	
HDP, n(%)[n]	0	3 (2%)	0.761	26 (14%)	43 (13%)	0.959
Hospital stay >3 days, n(%)	4 (10%)	76 (42%)	0.001	38 (20%)	70 (21%)	0.927
Case fatality ratio, n(%)	0	9 (5%)	0.297	1 (1%)	4 (1%)	0.640

Abbreviations - HRV: Human rhinovirus; SD: Standard deviation; IQR: Inter quartile range; CXR: Chest X-ray; HIV: Human immunodeficiency virus; HEU: HIV-uninfected but HIV exposed; CRP: C-reactive protein; MCPP: Microbiologically confirmed pneumococcal pneumonia; HDP: High Density pneumococcus; NP: Nasopharyngeal.

P-values from Chi-squared and Wilcoxon tests, logistic regression models adjusted for confounding variates (<0.2 in univariate analysis) where applicable. *P*-values could not be calculated for variables where both groups were zero, thus left blank.

[a] - Underweight defined as weight for age <-2SD of the median age-sex specific WHO reference; [b] - HEU defined as undetectable viral load, HIV seronegative in the child with a positive maternal history. Positive maternal status based on self-report was accepted, except for seronegative children < 7 months of age where documented positive maternal status was required; [c] - Premature birth defined as gestational age <37 weeks; [d] - Abnormal Chest X-ray defined as radiographically confirmed end point pneumonia consolidation or any infiltrates; [e] - A child was considered to be hypoxic if 1) a room air pulse-oximetry reading indicated oxygen saturation <90% at the two sites at elevation (Zambia and South Africa) or <92% at all other sites, or 2) a room

air oxygen saturation; [f] - Tachycardia defined as heart rate >160 beats per minute (bpm) if aged <11 months, heart rate >150 bpm if aged 12-35 months, heart rate >140 bpm if aged 36-59 months; [g] - Tachypnea defined as respiratory rate >60 breaths/minute if aged <2 months, respiratory rate >50 breaths/minute if aged 2-12 months, respiration rate >40 breaths/minute if aged >12 month; [h] - Fever defined as temperature >38^{o}C; [i] - Leucocytosis defined as white blood cell count >15 000 cells/uL if age <12 months, white blood cell count >13 000 cells/uL if age >12 months; [j] - CRP defined as levels ≥40mg/mL which are considered to potentially indicate bacterial infection; [k] - Blood culture positive for any significant non contaminate bacteria; [l] - Blood sample positive for *S. pneumoniae* colonisation by *LytA* PCR; [m] - MCPP defined when *S. pneumoniae* was cultured from a normally sterile body fluid - blood, pleural fluid or lung aspirate - or pleural fluid or lung aspirate was PCR *LytA* positive; [n] - HDP defined as *S. pneumoniae* density in nasopharynx >6.9 and/or density in whole blood sample >2.2 $\log_{10}$ copies/mL.

HRV were detected as a mono-infection in 53% of cases (n=481/912). We further evaluated the demographic and clinical characteristics amongst HRV mono-infection cases and those with other respiratory virus co-infections; Table 3.10. HRV-associated cases with mono-infections were older (mean 14.1 months) than cases with co-infections (10.3 months, $P<0.001$). Additionally, the HRV mono-infected cases were more likely to have been born prematurely (12%) compared to the cases with HRV mixed infections (7%, $P=0.026$). There were no differences in other characteristics between cases with HRV mono-infections than those with co-infections, including prevalence of very severe pneumonia (34% vs. 30%, $P=0.781$), hypoxia (33% vs. 33%, $P=0.267$) or requiring supplementary oxygen therapy (32% vs. 26%, $P=0.596$). Furthermore, cases with HRV mono-infections showed no associations with HIV exposure compare to cases with any HRV co-infections (11% vs. 10%, $P=0.787$) or individual co-infecting respiratory virus; Table 3.10. However, after multivariate analysis - adjusting for potentially confounding risk factors for death such as age categories, HIV exposure, malnutrition, hypoxia, very severe pneumonia diagnosis and markers for bacterial co-infections - the HRV mono-infection cases had 2.83-fold (aOR 95% CI: 1.44-5.53) higher case fatality ratio than cases with HRV mixed infections (10% vs. 5%, $P=0.002$).

We further evaluated for differences between HRV mono-infections compared to other individual co-infecting respiratory viruses; Table 3.10. Comparing HRV mono-infection cases to those with RSV co-infections (n=121, 28%), cases with HRV mono-infections were older (14.1 months) than the HRV-RSV co-infected cases (14.1 months, $P<0.001$) and less likely to present with cough (68% vs. 81%, $P=0.004$) than the RSV co-infected cases. Furthermore, the HRV mono-infected cases were 2.14-fold (aOR 95% CI: 1.62-2.84) more likely to be associated with leucocytosis (55% vs. 28%, $P=0.011$) and had 4.56-fold (aOR 95% CI: 2.34-18.70) higher case fatality ratio (10% vs. 6%, $P=0.007$) compared to the RSV co-infected cases.

Similarly, the HRV-associated cases with PIV co-infection (n=76/431, 18%) were younger (11.0 months) than those with HRV mono-infections (14.1 months, *P*=0.001). Cases with HRV mono-infections were 2.25-fold (aOR 95% CI: 1.45-3.37) more likely to have very severe pneumonia (34% vs. 17%, *P*=0.028) but less likely to present with stridor (2% vs. 8%, *P*=0.006) than those with HRV-PIV co-infections. Nonetheless, there were no other differences in clinical characteristics or markers for bacterial co-infections including CRP ≥40mg/mL (22% vs. 13%, *P*=0.158), microbiologically confirmed pneumococcal pneumonia (1% vs. 1%, *P*=0.709), or leucocytosis (55% vs. 42%, *P*=0.109) among cases with HRV mono-infections and HRV-PIV co-infections. There was however a trend for case with HRV mono-infections to have more prolonged hospital stay (>3 days; 51% vs. 38%, *P*=0.062) but no differences in case fatality ratios (10% vs. 9%, *P*=0.768) compared to the cases with HRV-PIV co-infections.

The HRV mono-infected cases were also older (14.1 months) than the HMPV co-infected cases (10.3 months, *P*<0.001), but there were no other differences in clinical characteristics and symptoms including very severe pneumonia (34% vs. 27%, *P*=0.691) and hypoxic (33% vs. 16%, *P*=0.355) compared to among cases with HRV-HMPV co-infections. The cases with HRV mono-infections were, however, associated with less tachycardia (50% vs. 59%, *P*=0.049) but more leucocytosis (55% vs. 40%, *P*=0.041) compared to cases with HRV mono-infections. There were no associations for case fatality ratio (10% vs. 2%, *P*=0.346) and prolonged hospital stays >3 days (51% vs. 47%, *P*=0.762) between the mono-infections and HRV-HMPV co-infections.

Additionally, cases with HRV mono-infections were older (14.1 months) than case with HRV-HCoV co-infections (9.3 months, *P*=0.012) but were clinically indistinguishable for all other clinical characteristics and symptoms, markers for bacterial co-infections as well as hospital stay >3 days (51% vs. 50%, *P*=0.641) and case fatality ratio (10% for both, *P*=0.881). Similarly, cases with HRV-AdV co-infections and HRV-HBoV were indistinguishable from cases with HRV mono-infections including case fatality ratio (5% vs. 10%, *P*=0.124 and 6% vs. 10%, *P*=0.694 respectively); Table 3.10.

Table 3.10: The demographics and clinical characteristics of cases with HRV mono-infections compared to cases with HRV mixed infections

	Mono-infection (n=481)	Any coinfection (n=431)	*P*-value[a]	HRV-RSV (n=121)	*P*-value[b]	HRV-AdV (n=108)	*P*-value[b]	HRV-HMPV (n=49)	*P*-value[b]	HRV-PIV (n=76)	*P*-value[b]	HRV-HBoV (n=138)	*P*-value[b]	HRV-HcoV (n=56)	*P*-value[b]
Age in months, mean (SD)	14.1 (12.6)	10.3 (11.3)	*P*<0.001	7.0 (8.7)	*P*<0.001	13.9 (9.5)	0.708	10.3 (10.0)	*P*<0.001	11.0 (10.7)	0.001	14.7 (12.5)	0.590	9.3 (7.1)	0.012
Female, n(%)	196 (41%)	177 (41%)	0.542	54 (45%)	0.271	59 (55%)	0.754	20 (21%)	0.518	28 (37%)	0.784	61 (42%)	0.212	17 (30%)	0.279
Never breast fed, n(%)	47 (10%)	33 (8%)	0.274	7 (6%)	0.060	15 (14%)	0.310	3 (6%)	0.843	2 (3%)	0.120	10 (7%)	0.405	9 (16%)	0.216
Under weight, n (%)[c]	156 (32%)	121 (28%)	0.451	24 (20%)	0.050	39 (36%)	0.461	14 (19%)	0.870	25 (33%)	0.544	30 (22%)	0.042	15 (27%)	0.632
HEU, n(%)[d]	52 (11%)	42 (10%)	0.787	14 (12%)	0.992	12 (11%)	0.437	3 (6%)	0.912	5 (7%)	0.888	3 (9%)	0.787	9 (16%)	0.153
Day care attendance, n(%)	60 (12%)	66 (15%)	0.300	35 (29%)	0.095	15 (14%)	0.828	1 (2%)	0.087	9 (12%)	0.912	13 (9%)	0.531	7 (13%)	0.943
Smoker in household, n(%)	163 (34%)	177 (41%)	0.061	46 (38%)	0.636	42 (39%)	0.573	20 (41%)	0.610	34 (45%)	0.183	61 (44%)	0.081	25 (45%)	0.278
Premature birth, n(%)[e]	57 (12%)	30 (7%)	0.026	8 (7%)	0.169	6 (6%)	0.140	4 (8%)	0.740	3 (4%)	0.11	11 (8%)	0.057	2 (4%)	0.129
Birth weight, mean (SD)	2.9 (0.6)	2.9 (0.7)	0.447	2.9 (0.6)	0.539	2.8 (0.7)	0.508	3.0 (0.8)	0.224	2.9 (0.6)	0.513	2.9 (0.7)	0.506	3.0 (0.7)	0.403
Clinical Features:															
Very severe pneumonia, n(%)	163 (34%)	128 (30%)	0.781	42 (35%)	0.419	40 (37%)	0.087	13 (27%)	0.691	13 (17%)	0.028	37 (27%)	0.759	19 (34%)	0.365
Chest X-ray abnormal, n(%)[f]	188 (39%)	177 (41%)	0.430	51 (42%)	0.349	40 (37%)	0.995	22 (45%)	0.556	35 (46%)	0.297	58 (42%)	0.475	25 (45%)	0.388
Supplementary 02 therapy, n(%)	154 (32%)	112 (26%)	0.596	39 (33%)	0.616	23 (21%)	0.307	9 (18%)	0.820	15 (20%)	0.655	32 (23%)	0.448	20 (36%)	0.399
Hypoxic , n(%)[g]	156 (33%)	141 (33%)	0.267	56 (47%)	0.625	32 (30%)	0.539	8 (16%)	0.355	17 (22%)	0.806	36 (26%)	0.734	23 (41%)	0.100
Tachycardia, n(%)[h]	240 (50%)	199 (46%)	0.662	56 (46%)	0.333	44 (41%)	0.192	29 (59%)	0.049	44 (58%)	0.131	56 (41%)	0.247	21(38%)	0.202
Tachypnea, n(%)[i]	405 (84%)	380 (89%)	0.171	106 (88%)	0.108	93 (86%)	0.590	43 (88%)	0.640	68 (89%)	0.371	122 (89%)	0.669	51 (91%)	0.192
Wheezing, n(%)	214 (45%)	207 (48%)	0.543	54 (45%)	0.053	60 (56%)	0.803	22 (45%)	0.051	32 (43%)	0.053	76 (55%)	0.496	22 (39%)	0.051
Cough, n(%)	326 (68%)	321 (74%)	0.041	98 (81%)	0.004	79 (73%)	0.306	32 (65%)	0.406	46 (61%)	0.193	109 (79%)	0.288	40 (71%)	0.819
Lethargic, n(%)	57 (12%)	32 (7%)	0.336	6 (5%)	0.107	14 (13%)	0.498	4 (8%)	0.854	5 (7%)	0.498	11 (8%)	0.926	7 (13%)	0.348
Fever, n(%)[j]	369 (77%)	338 (78%)	0.886	93 (77%)	0.96	82 (76%)	0.675	44 (90%)	0.085	65 (86%)	0.230	106 (77%)	0.813	38 (68%)	0.119
Convulsions, n(%)	30 (6%)	12 (3%)	0.078	2 (2%)	0.065	5 (5%)	0.512	1 (2%)	0.522	1 (1%)	0.185	7 (5%)	0.802	2 (4%)	0.642
Diarrhoea, n(%)	67 (14%)	51 (12%)	0.411	15 (12%)	0.109	10 (9%)	0.171	6 (12%)	0.840	13 (17%)	0.207	11 (8%)	0.191	9 (16%)	0.551
Head nodding, n(%)	81 (17%)	81 (19%)	0.437	32 (27%)	0.309	23 (21%)	0.118	8 (16%)	0.497	7 (9%)	0.221	22 (16%)	0.611	9 (16%)	0.951
Central cyanosis, n(%)	12 (3%)	4 (1%)	0.065	1 (1%)	0.117	0	0.206	1 (2%)	0.854	0	0.312	1 (1%)	0.355	1 (2%)	0.965
Unable to Feed, n(%)	35 (7%)	23 (5%)	0.245	11 (9%)	0.414	7 (7%)	0.944	1 (2%)	0.309	2 (3%)	0.107	3 (2%)	0.162	3 (5%)	0.802
Vomiting everything, n(%)	14 (3%)	8 (2%)	0.844	3 (2%)	0.474	4 (4%)	0.531	1 (2%)	0.968	0	0.416	2 (1%)	0.575	3 (5%)	0.138
Lower chest wall indrawing, n(%)	436 (91%)	410 (95%)	0.105	120 (99%)	0.068	96 (89%)	0.572	48 (98%)	0.261	74 (97%)	0.180	130 (94%)	0.541	51 (91%)	0.587
Stridor, n(%)	8 (2%)	10 (2%)	0.363	0	0.187	3 (3%)	0.271	0	0.787	6 (8%)	0.006	1 (1%)	0.315	1 (2%)	0.877
Grunting, n(%)	51 (11%)	64 (15%)	0.109	29 (24%)	0.136	16 (15%)	0.305	4 (8%)	0.965	6 (8%)	0.160	17 (12%)	0.707	7 (13%)	0.055
Nasal Flaring, n(%)	268 (56%)	231 (54%)	0.831	75 (62%)	0.597	54 (50%)	0.412	24 (49%)	0.444	39 (51%)	0.374	71 (51%)	0.757	30 (54%)	0.909
Laboratory markers:															
Leucocytosis, n(%)[k]	256 (55%)	187 (27%)	0.099	31 (28%)	0.011	55 (54%)	0.357	17 (40%)	0.041	26 (42%)	0.109	74 (57%)	0.489	30 (59%)	0.562
Neutrophils (%), median (IQR)	53.2 (36-68.2)	48.7 (34-66.1)	0.507	40 (26-52.5)	0.185	59.8 (41.1-74)	0.535	43.9 (36.3-58)	0.423	42.3 (32-61.3)	0.164	54.2 (38.7-70)	0.812	52.8 (40.1-70)	0.174
Lymphocyte (%), median (IQR)	35.9 (24-49)	41 (24.5-55.6)	0.382	50.7 (39-61.6)	0.037	32.1 (19.6-49)	0.733	45.4 (31-55)	0.320	44.4 (30.7-58)	0.426	22 (13.2-35.1)	0.966	36.85 (20-50.1)	0.230
Eosinophils (%), median (IQR)	2.3 (0.5-3.5)	1.2 (0.3-3.6)	0.631	0.9 (0.2-2.4)	0.741	1.7 (0.5-3.9)	0.812	0.7 (0.1-2)	0.160	1.1 (0.1-2.9)	0.317	2.2 (0.7-4.5)	0.771	1 (0.4-2.4)	0.795
CRP ≥40mg/l, n(%)[l]	108 (22%)	68 (16%)	0.031	22 (18%)	0.242	15 (14%)	0.107	7 (14%)	0.454	10 (13%)	0.158	18 (13%)	0.068	13 (23%)	0.669
Blood culture positive, n(%)[m]	18 (4%)	11 (3%)	0.158	2 (2%)	0.052	4 (4%)	0.864	0	0.392	1 (1%)	0.147	4 (3%)	0.764	2 (4%)	0.843
***LytA* positive, n(%)[n]**	23 (5%)	19 (7%)	0.584	6 (5%)	0.360	9 (9%)	0.230	4 (9%)	0.260	9 (12%)	0.090	9 (7%)	0.515	3 (6%)	0.930
MCPP, n(%)[o]	6 (1%)	2 (0.5%)	0.116	0	0.141	1 (1%)	0.818	0	0.946	1 (1%)	0.709	1 (1%)	0.643	0	0.702
HDP, n(%)[p]	63 (13%)	70 (16%)	0.858	14 (12%)	0.073	27 (25%)	0.012	7 (14%)	0.869	18 (24%)	0.171	23 (17%)	0.379	8 (14%)	0.877
HRV viral load, mean (SD)[q]	3.8 (0.9)	3.6 (1.0)	0.121	3.3 (0.9)	*P*<0.001	3.7 (1.0)	0.461	3.6 (1.1)	0.303	3.5 (1.1)	0.173	3.7 (0.9)	0.468	3.9 (1.1)	0.160
Hospital stay >3 days, n(%)	247 (51%)	230 (53%)	0.654	79 (65%)	0.544	53 (49%)	0.677	23 (47%)	0.762	29 (38%)	0.062	70 (51%)	0.625	28 (50%)	0.641
Case fatality, n(%)	44 (10%)	19 (5%)	0.002	6 (6%)	0.007	5 (5%)	0.124	1 (2%)	0.346	6 (9%)	0.768	8 (6%)	0.694	5 (10%)	0.881

Abbreviations - HRV: Human rhinovirus; SD: Standard deviation; IQR: Inter quartile range; CXR: Chest X-ray; HIV: Human immunodeficiency virus; HEU: HIV-uninfected but HIV exposed; CRP: C-reactive protein; MCPP: Microbiologically confirmed pneumococcal pneumonia; HDP: High Density pneumococcus; NP: Nasopharyngeal.

P-values from Chi-squared and Wilcoxon tests, logistic regression models adjusted for confounding variates (<0.2 in univariate analysis) where applicable. *P*-values could not be calculated for variables where both values are 0, thus cells left blank

[a] - *P*-values from logistic regression models for HRV mono-infections compared to all HRV co-infections; [b] - *P*-values from logistic regression models for HRV mono-infections compared to each HRV co-infection individually; [c] - Underweight defined as weight for age <-2SD of the median age-sex specific WHO reference; [d] - HEU defined as undetectable viral load, HIV seronegative in the child with a positive maternal history. Positive maternal status based on self-report was accepted, except for seronegative children < 7 months of age where documented positive maternal status was required; [e] - premature birth defined as <37 weeks gestational age; [f] - defined as radiographically confirmed endpoint pneumonia or any infiltrates; [g] - A child was considered to be hypoxic if 1) a room air pulse-oximetry reading indicated oxygen saturation <90% at the two sites at elevation (Zambia and South Africa) or <92% at all other sites, or 2) a room air oxygen saturation reading was not available and the child was on oxygen; [h] - Tachycardia defined as heart rate >160 beats per minute (bpm) if aged <11 months, heart rate >150 bpm if aged 12-35 months, heart rate >140 bpm if aged 36-59 months; [i] - Tachypnea defined as respiratory rate >60 breaths/minute if aged <2 months, respiratory rate >50 breaths/minute if aged 2-12 months, respiration rate >40 breaths/minute if aged >12 months; [j] - Fever defined as temperature >38^{o}C; [k] - Leucocytosis defined as white blood cell count >15 000 cells/uL if age <12 months, white blood cell count >13 000 cells/uL if age >12 months; [l] - CRP defined as levels ≥40mg/mL which are considered to potentially indicate bacterial infection; [m] - blood culture positive for any significant bacteria (non-contaminant); [n] - Blood sample positive for *S. pneumoniae* colonisation by *LytA* PCR; [o] - MCPP defined as *S. pneumoniae* was cultured from a normally sterile body fluid - blood, pleural fluid or lung aspirate - or pleural fluid or lung aspirate was PCR *LytA* positive; [p] - HDP defined as *S. pneumoniae* density in nasopharynx>6.9 and/or density in whole blood sample>2.2 $\log_{10}$ copies/mL; [q] - HRV viral loads in the nasopharynx and expressed in $\log_{10}$ copies/mL.

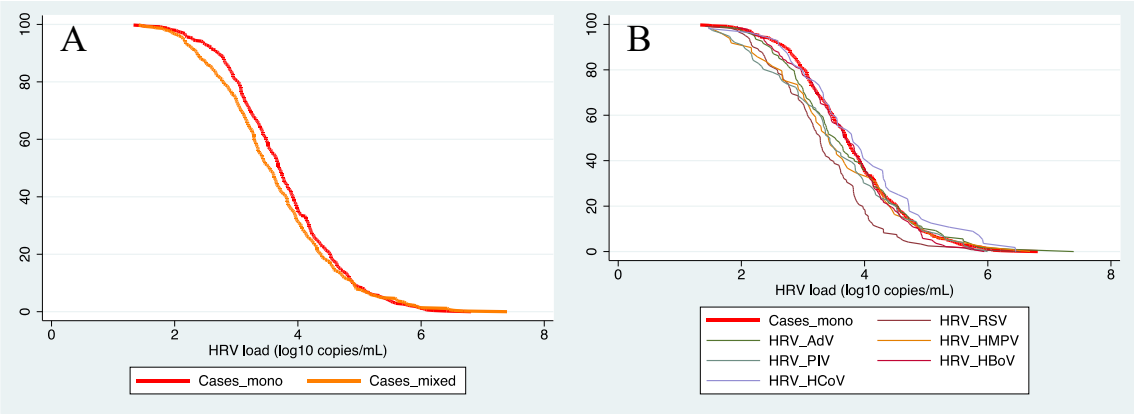

Figure 3.4: Reverse cumulative plot of HRV viral load in the nasopharynx among cases with HRV mono-infections and HRV co-infections with other common respiratory viruses. The viral loads of HRV mono-infected cases are shown in red. Panel A.) the HRV viral load in HRV mono-infected cases and all HRV co-infections combined; Panel B.) the HRV viral load in HRV mono-infected cases and HRV co-infections with common respiratory viruses separately.

3.1.3.1 HRV in radiographically confirmed cases

Based on HRV-associated cases being more likely to have a normal chest X-ray (CXR) (60% vs. 54%, aOR 1.28, 95% CI: 1.10-1.50, *P*=0.001); Table 3.7, further analyses were undertaken which was limited to pneumonia cases in whom the CXR showed evidence of any infiltrate; Table 3.11 and 3.12. Similar trends were observed as for the all pneumonia case comparison; however, among children with radiographically confirmed pneumonia, HRV-associated cases were of similar age (12.3 months) as those without HRV infection (11.2 months, *P*=0.260) and did not differ in any other characteristics including presence of tachypnea (88% vs. 84%, *P*=0.362) or convulsions (2% vs. 3%, *P*=0.544). Additionally, the radiographically confirmed HRV-associated cases were still associated with more wheeze (46% vs. 28%, *P*<0.001) and head nodding (21% vs. 16%, *P*=0.003) than cases without HRV infection. Moreover, among the radiologically confirmed cases, HRV-associated cases were 1.51-fold (aOR 95% CI: 1.16-1.97) more likely to have very severe pneumonia (34%) compared to the HRV negative case (28%, *P*=0.002). The radiologically confirmed cases with HRV infections, however, showed no associations with hospital stay of >3 days (58% vs. 68%, *P*=0.069), requirement of supplementary oxygen therapy (33% vs. 38%, *P*=0.317), mechanical ventilation (3% vs. 4%, *P*=0.810) or case fatality ratio (8% vs. 7%, *P*=0.087) compared to cases without HRV infections; Table 3.11.

Among the markers for bacterial co-infection, the radiographically confirmed HRV-associated cases were associated with leucocytosis (58% vs. 45%, P=0.001) and higher neutrophil percentages (50.9% vs. 47.1%, P<0.001), but lower lymphocyte percentages (37.5% vs. 41.3%, P=0.002) compared to the HRV negative radiographically confirmed cases. There were no other differences in markers for bacterial co-infections, including CRP≥40mg/mL (27% vs. 30%, P=0.698), blood culture positivity (4% vs. 9%, P=0.801) and microbiologically confirmed pneumonia (1% vs. 2%, P=0.378) between the radiographically confirmed cases associated with HRV and those without HRV.

Additionally, similarly to the overall study findings, among other commonly detected respiratory viruses the HRV-associated cases were less likely to be co-detected together with RSV (14% vs. 30%, P<0.001), Influenza virus (0 vs. 7%, P=0.006), HMPV (6% vs. 12%, P=0.001) and PIV (10% vs. 16%, P=0.002) compared to the cases without HRV infections; Table 3.11.

Table 3.11: The demographical and clinical characteristics of HRV-associated and non-HRV cases among children with infiltrates observed on chest X-rays

	HRV+ (n=365)	HRV- (n=1355)	Unadjusted *P*-value	Unadjusted OR (95%CI)	Adjusted *P*-value	Adjusted aOR (95% CI)
Age in months, mean (SD)	12.3 (11.0)	11.2 (12.3)	0.072		0.260	
Female, n(%)	157 (43%)	593 (44%)	0.798	0.97 (0.77-1.22)	0.795	0.96 (0.76-1.23)
Never breast fed, n(%)	28 (8%)	161 (12%)	0.024	0.62 (0.41-0.94)	0.121	0.70 (0.44-1.10)
Under weight, n (%)[a]	127 (35%)	481 (36%)	0.803	0.97 (0.76-1.24)	0.487	0.92 (0.71-1.17)
HEU, n(%)[b]	43 (12%)	175 (13%)	0.563	0.90 (0.63-1.29)	0.737	1.07 (0.71-1.63)
Day Care attendance, n(%)	37 (10%)	226 (17%)	0.002	0.56 (0.39-0.81)	0.144	0.70 (0.45-1.13)
Smoker in household, n(%)	140 (38%)	470 (34%)	0.194	1.17 (0.92-1.49)	0.562	1.08 (0.82-1.41)
Premature birth, n(%)[c]	41 (11%)	175 (13%)	0.402	0.86 (0.60-1.23)	0.555	0.89 (0.61-1.30)
Birth weight, mean (SD)	2.8 (0.7)	2.9 (0.7)	0.819		0.825	
Clinical Features						
Very severe pneumonia, n(%)	123 (34%)	381 (28%)	0.038	1.30 (1.01-1.66)	0.002	1.51 (1.16-1.97)
Supplementary 02 therapy, n(%)	120 (33%)	516 (38%)	0.070	0.80 (0.62-1.02)	0.317	0.83 (0.58-1.19)
Mechanical ventilation, n(%)	12 (3%)	59 (4%)	0.363	0.75 (0.40-1.40)	0.810	0.92 (0.48-1.79)
Hypoxic, n(%)[d]	140 (38%)	589 (44%)	0.084	0.82 (0.65-1.03)	0.992	1.00 (0.75-1.32)
Tachycardia, n(%)[e]	178 (49%)	703 (52%)	0.285	0.88 (0.70-1.11)	0.985	1.00 (0.78-1.28)
Tachypnea, n(%)[f]	320 (88%)	1130 (84%)	0.101	1.34 (0.95-1.90)	0.362	1.18 (0.82-1.69)
Wheezing, n(%)	168 (46%)	377 (28%)	*P*<0.001	1.20 (1.73-2.80)	*P*<0.001	1.92 (1.43-2.57)
Cough, n(%)	268 (73%)	999 (74%)	0.948	0.99 (0.76-1.29)	0.486	0.90 (0.68-1.20)
Lethargic, n(%)	33 (9%)	119 (9%)	0.842	1.05 (0.70-1.56)	0.778	1.06 (0.70-1.62)
Fever, n(%)[g]	285 (78%)	1151 (85%)	0.002	0.63 (0.47-0.84)	0.013	0.67 (0.49-0.92)
Convulsions, n(%)	9 (2%)	45 (3%)	0.416	0.74 (0.36-1.53)	0.544	0.80 (0.38-1.67)
Diarrhoea, n(%)	46 (13%)	194 (14%)	0.401	0.86 (0.61-1.22)	0.472	1.14 (0.79-1.64)
Head nodding, n(%)	76 (21%)	217 (16%)	0.031	1.38 (1.03-1.85)	0.003	1.60 (1.17-2.18)
Central cyanosis, n(%)	11 (3%)	29 (2%)	0.303	1.42 (0.70-2.87)	0.051	2.07 (0.99-4.28)
Unable to Feed, n(%)	19 (5%)	100 (7%)	0.147	0.69 (0.42-1.14)	0.266	0.75 (0.44-1.25)
Vomiting everything, n(%)	4 (1%)	30 (2%)	0.182	0.49 (0.17-1.40)	0.219	0.51 (0.18-1.49)
Lower chest wall indrawing, n(%)	351 (96%)	1291 (95%)	0.470	1.24 (0.69-1.24)	0.512	1.22 (0.67-2.23)
Stridor, n(%)	4 (1%)	29 (2%)	0.205	0.50 (0.18-1.45)	0.251	0.53 (0.18-1.55)
Grunting, n(%)	42 (12%)	240 (18%)	0.005	0.61 (0.43-0.86)	0.266	0.79 (0.53-1.19)
Nasal flaring, n (%)	211 (58%)	832 (61%)	0.190	0.85 (0.68-1.09)	0.123	1.25 (0.95-1.67)
Markers for bacterial co-infection:						
Leucocytosis, n(%)[h]	198 (58%)	573 (45%)	*P*<0.001	1.66 (1.30-2.10)	0.001	1.51 (1.18-1.94)
Neutrophils (%), median (IQR)	50.9 (36-68.4)	47.1 (33-60.1)	0.001		*P*<0.001	
Lymphocytes (%), median (IQR)	37.5 (24-51.3)	41.3 (28.5-55)	0.002		0.002	
Eosinophils (%), median (IQR)	1.1 (0.4-3.4)	0.6 (0.1-2)	0.050		0.113	
CRP ≥40mg/l, n(%)[i]	99 (27%)	409 (30%)	0.255	0.86 (0.66-1.11)	0.698	0.94 (0.73-1.24)
Blood culture positive, n(%)[j]	13 (4%)	52 (9%)	0.801	0.92 (0.50-1.72)	0.920	1.03 (0.55-1.93)
***LytA* positive, n(%)[k]**	20 (6%)	103 (8%)	0.149	0.69 (0.42-1.14)	0.329	0.78 (0.47-1.29)
MCPP, n(%)[l]	4 (1%)	25 (2%)	0.327	0.59 (0.20-1.70)	0.378	0.62 (0.21-1.81)
HDP, n(%)[m]						
-Blood	14 (4%)	71 (6%)	0.255	0.71 (0.40-1.28)	0.477	0.80 (0.45-1.46)
-NP	46 (13%)	189 (14%)	0.504	0.88 (0.68-1.21)	0.525	0.89 (0.62-1.27)
Viral co-infections in the nasopharynx with:						
-RSV, n(%)	51 (14%)	410 (30%)	*P*<0.001	0.37 (0.27-0.51)	*P*<0.001	0.36 (0.26-0.50)
-AdV, n(%)	40 (11%)	124 (9%)	0.297	1.22 (0.84-1.78)	0.234	1.27 (0.86-1.87)
-InFV A-C, n(%)	0	89 (7%)	0.005		0.006	
-HBoV, n(%)	58 (16%)	173 (13%)	0.122	1.29 (0.93-1.78)	0.187	1.25 (0.89-1.75)
-HMPV, n (%)	22 (6%)	163 (12%)	0.001	0.47 (0.30-0.75)	0.001	0.46 (0.29-0.74)
-PIV, n(%)	35 (10%)	215 (16%)	0.003	0.56 (0.39-0.82)	0.002	0.54 (0.36-0.79)
-HCoV, n(%)	25 (7%)	99 (7%)	0.765	0.93 (0.59-1.47)	0.792	0.94 (0.59-1.49)
Hospital stay >3 days, n(%)	212 (58%)	915 (68%)	0.001	0.66 (0.53-0.84)	0.069	0.77 (0.59-1.05)
Case fatality ratio, n(%)	27 (8%)	86 (7%)	0.490	1.17 (0.75-1.83)	0.087	1.50 (0.94-2.40)

Abbreviations - HRV: Human rhinovirus; OR: Odds ratios; aOR: Adjusted odds ratio; CI: Confidence interval; SD: Standard deviation; IQR: Inter quartile range; CXR: Chest X-ray; HIV: Human immunodeficiency virus; HEU: HIV-uninfected but HIV exposed; CRP: C-reactive protein; MCPP: Microbiologically confirmed pneumococcal pneumonia; HDP: High Density pneumococcus; NP: Nasopharyngeal; RSV: Respiratory Syncytial Virus, HMPV: human metapneumovirus; AdV: adenovirus; PIV: parainfluenza type 1-4; HBoV:

Human Bocavirus; HCoV: Human Coronavirus (OC43, NL63, 229E and HKU1) and InFV: Influenza Virus (A, B and C) ; *S. pneumoniae: Streptococcus pneumoniae*.
P-values from Chi-squared and Wilcoxon tests, logistic regression models adjusted for confounding variates (<0.2 in univariate analysis) where applicable, Odds ratio could not be calculated for continuous variables or variables with 0 values, thus cells left blank.
[a] - Underweight defined as weight for age <-2SD of the median age-sex specific WHO reference; [b] - HEU defined as HIV-uninfected but HIV-exposed. Undetectable viral load, HIV seronegative in the child with a positive maternal history. Positive maternal status based on self-report was accepted, except for seronegative children < 7 months of age where documented positive maternal status was required; [c] - Premature birth defined as gestational age <37 weeks; [d] - A child was considered to be hypoxic if 1) a room air pulse-oximetry reading indicated oxygen saturation <90% at the two sites at elevation (Zambia and South Africa) or <92% at all other sites, or 2) a room air oxygen saturation; [e] - Tachycardia defined as heart rate >160 beats per minute (bpm) if aged <11 months, heart rate >150 bpm if aged 12-35 months, heart rate >140 bpm if aged 36-59 months; [f] - Tachypnea defined as respiratory rate >60 breaths/minute if aged <2 months, respiratory rate >50 breaths/minute if aged 2-12 months, respiration rate >40 breaths/minute if aged >12 month; [g] - Fever defined as temperature >38^{o}C; [h] - Leucocytosis defined as white blood cell count >15 000 cells/uL if age <12 months, white blood cell count >13 000 cells/uL if age >12 months; [i] - CRP defined as levels ≥40mg/mL which are considered to potentially indicate bacterial infection; [j] - Blood culture positive for any significant non contaminate bacteria; [k] - Blood sample positive for *S. pneumoniae* colonisation by *LytA* PCR; [l] - MCPP defined when *S. pneumoniae* was cultured from a normally sterile body fluid - blood, pleural fluid or lung aspirate - or pleural fluid or lung aspirate was PCR *LytA* positive; [m] - HDP defined as *S. pneumoniae* density in nasopharynx >6.9 and/or density in whole blood sample >2.2 $\log_{10}$ copies/mL.

The same analysis as described in detail in Table 3.10 was undertaken but limited to cases with radiographically confirmed cases (any evidence of infiltrates on the CXR); Table 3.12, which yielded similar trends to the comparison of overall HRV case analysis of HRV mono-infected cases and those with other respiratory virus co-infections.

Overall among the radiological confirmed cases, episodes associated with HRV mono-infections were also older (mean 13.8) than cases with other co-infecting viruses (10.8, P=0.013); including compared to coinfections specifically to RSV (5.4 months, P<0.001), HMPV (7.5 months, P=0.003) and PIV (7.5 months, P<0.001). Additionally, the HRV-associated mono-infected cases were associated with lower birth weight (2.8kg) compared to HRV co-infected cases (3.0kgs, P=0.038). Among the radiologically confirmed HRV-associated cases, HRV mono-infections were no longer associated with higher case fatality ratio (9%) than cases with co-infecting viruses (7%, P=0.342); although there remained a trend for a higher case fatality ratio among cases with mono-infections than those with RSV co-infection (9% vs. 6%, P=0.075); Table 3.9.

Table 3.12: The demographics and clinical characteristics of radiologically confirmed cases with HRV mono-infections compared to those with coinfections by other respiratory viruses

	HRV mono-infections (n=188)	HRV co-infections (n=177)	*P*-value[a]	HRV-RSV (n=51)	*P*-value[b]	HRV-AdV (n=40)	*P*-value[b]	HRV-HMPV (n=22)	*P*-value[b]	HRV-PIV (n=35)	*P*-value[b]	HRV-HBoV (n=58)	*P*-value[b]	HRV-HCoVs (n=25)	*P*-value[b]
Age in months, mean (SD)	13.8 (11.5)	10.8 (10.5)	0.013	5.4 (6.5)	*P*<0.001	16.4 (10.4)	0.193	7.5 (6.6)	0.003	7.5 (7.8)	*P*<0.001	14.3 (11.3)	0.940	14.44(15.1)	0.878
Female, n(%)	88 (47%)	69 (39%)	0.316	22 (43%)	0.765	16 (40%)	0.374	7 (32%)	0.771	10 (29%)	0.438	22 (38%)	0.508	9 (36%)	0.452
Never breast fed, n(%)	10 (5%)	18 (10%)	0.060	6 (12%)	0.459	8 (20%)	0.017	1 (5%)	0.446	1 (3%)	0.670	4 (7%)	0.609	6 (24%)	0.011
Under weight, n (%)[c]	73 (39%)	54 (31%)	0.295	11 (22%)	0.085	15 (38%)	0.982	7 (32%)	0.649	12 (34%)	0.656	13 (22%)	0.075	7 (28%)	0.612
HEU, n(%)[d]	19 (10%)	24 (14%)	0.498	9 (18%)	0.733	8 (20%)	0.243	2 (9%)	0.567	3 (9%)	0.954	5 (9%)	0.627	4 (16%)	0.760
Day Care attendance, n(%)	18 (10%)	19 (11%)	0.714	10 (20%)	0.099	4 (10%)	0.935	0	0.129	3 (9%)	0.852	3 (5%)	0.294	3 (12%)	0.702
Smoker in household, n(%)	65 (35%)	75 (42%)	0.136	20 (40%)	0.556	13 (33%)	0.785	9 (41%)	0.568	19 (55%)	0.348	28 (48%)	0264	12 (48%)	0.196
Premature birth, n(%)[e]	24 (13%)	17 (10%)	0.633	5 (10%)	0.408	1 (3%)	0.135	1 (5%)	0.483	1 (3%)	0.163	8 (14%)	0.907	2 (8%)	0.748
Birth weight, mean (SD)	2.8 (0.7)	3.0 (0.7)	0.038	2.8 (0.7)	0.519	3.0 (0.5)	0.117	3.2 (0.5)	0.159	2.9 (0.4)	0.301	3.1 (0.7)	0.087	3.0 (0.8)	0.207
Clinical Features:															
Very severe pneumonia, n(%)	64 (34%)	59 (33%)	0.648	19 (37%)	0.903	14 (35%)	0.456	7 (32%)	0.365	6 (34%)	0.080	19 (33%)	0.229	11 (44%)	0.150
Supplementary 02 therapy, n(%)	61 (32%)	59 (33%)	0.415	22 (43%)	0.602	13 (33%)	0.892	4 (18%)	0.950	7 (20%)	0.273	16 (28%)	0.788	11 (44%)	0.117
Mechanical ventilation, n(%)	6 (3%)	6 (3%)	0.915	2 (4%)	0.797	1 (3%)	0.818	0	0.395	1 (3%)	0.917	1 (2%)	0.557	3 (12%)	0.040
Hypoxic, n(%)[f]	68 (36%)	72 (41%)	0.096	30 (59%)	0.399	16 (40%)	0.173	4 (18%)	0.978	8 (23%)	0.653	18 (31%)	0.589	12 (48%)	0.144
Tachycardia, n(%)[g]	91 (48%)	87 (49%)	0.445	22 (43%)	0.706	20 (50%)	0.721	15 (68%)	0.104	20 (57%)	0.273	27 (47%)	0.840	9 (36%)	0.280
Tachypnea, n(%)[h]	258 (84%)	162 (92%)	0.023	45 (88%)	0.112	36 (90%)	0.369	22 (100%)	0.146	33 (94%)	0.123	53 (93%)	0.188	23 (92%)	0.166
Wheezing, n(%)	87 (47%)	81 (46%)	0.958	22 (43%)	0.212	24 (60%)	0.347	8 (36%)	0.828	14 (41%)	0.695	29 (51%)	0.599	9 (36%)	0.262
Cough, n(%)	133 (71%)	135 (76%)	0.280	41 (80%)	0.181	31 (78%)	0.951	14 (64%)	0.531	20 (57%)	0.683	46 (79%)	0.586	20 (80%)	0.477
Lethargic, n(%)	18 (10%)	15 (8%)	0.497	5 (10%)	0.435	3 (8%)	0.943	2 (9%)	0.506	2 (6%)	0.425	6 (10%)	0.195	2 (8%)	0.806
Fever, n(%)[i]	145 (77%)	140 (79%)	0.772	39 (76%)	0.861	33 (83%)	0.218	18 (82%)	0.556	29 (83%)	0.501	48 (83%)	0.345	17 (68%)	0.269
Convulsions, n(%)	6 (3%)	3 (2%)	0.637	2 (4%)	0.771	0	0.755	0	0.394	0	0.283	3 (5%)	0.176	0	0.373
Diarrhoea, n(%)	22 (12%)	24 (14%)	0.845	9 (18%)	0.904	3 (8%)	0.538	3 (14%)	0.805	6 (17%)	0.374	5 (9%)	0.699	4 (16%)	0.717
Head nodding, n(%)	36 (19%)	40 (23%)	0.551	14 (27%)	0.797	10 (25%)	0.279	4 (18%)	0.415	4 (11%)	0.254	12 (21%)	0.360	7 (28%)	0.401
Central cyanosis, n(%)	8 (4%)	3 (2%)	0.166	0	0.169	0	0.345	1 (5%)	0.803	0	0.214	1 (2%)	0.545	1 (4%)	0.974
Unable to Feed, n(%)	11 (6%)	8 (5%)	0.794	5 (10%)	0.657	2 (5%)	0.421	0	0.244	0	0.142	2 (3%)	0.578	2 (8%)	0.209
Vomiting everything, n(%)	4 (2%)	0	0.205	0	0.813	0	0.503	0	0.490	0	0.384	0	0.263	0	0.462
Lower chest wall indrawing, n(%)	179 (95%)	172 (97%)	0.437	51 (100%)	0.749	39 (98%)	0.388	22 (100%)	0.294	35 (100%)	0.186	54 (93%)	0.631	24 (96%)	0.733
Stridor, n(%)	2 (1%)	2 (1%)	0.791	0	0.450	1 (3%)	0.677	0	0.627	1 (3%)	0.625	1 (2%)	0.853	0	0.604
Grunting, n(%)	17 (9%)	25 (14%)	0.188	9 (18%)	0.547	4 (10%)	0.789	1 (5%)	0.514	1 (3%)	0.058	7 (12%)	0.399	6 (24%)	0.056
Nasal Flaring, n(%)	106 (56%)	105 (59%)	0.963	34 (67%)	0.722	23 (58%)	0.515	14 (64%)	0.632	20 (57%)	0.689	29 (50%)	0.677	19 (76%)	0.120
Laboratory markers:															
Leucocytosis, n(%)[j]	113 (62%)	85 (53%)	0.533	16 (32%)	0.081	24 (65%)	0.796	8 (47%)	0.965	11 (41%)	0.388	34 (64%)	0.954	15 (65%)	0.565
Neutrophils (%), median (IQR)	53.4 (38-68.3)	47.3 (32.8-66.0)	0.332	41.2 (26.3-53.8)	0.071	60.5 (43.1-73)	0.867	41.5 (38.7-53)	0.497	38.8 (31-55)	0.077	52.4 (41-71)	0.892	58.1 (41-71)	0.783
Lymphocytes (%), median (IQR)	34.2 (22.5-48)	41.8 (26.1-55.7)	0.205	49.2 (33.5-59.4)	0.126	31.7 (20.5-46)	0.846	47.8 (35.6-53)	0.407	48.4 (34-58)	0.196	35.7 (21-45.5)	0.775	33.1 (20-51.4)	0.981
Eosinophils (%), median (IQR)	1.1 (0.6-3.9)	1.1 (0.3-3)	0.542	0.75 (0.2-2.4)	0.805	1.85 (0.9-3.7)	0.902	0.2 (0.1-0.9)	0.101	1.2 (0.4-2.6)	0.430	1.3 (0.4-5)	0.435	1 (0.1-2.4)	0.335
CRP >40mg/l, n(%)[k]	51 (27%)	48 (27%)	0.914	17 (33%)	0.328	8 (20%)	0.439	7 (32%)	0.925	8 (23%)	0.363	14 (24%)	0.388	9 (36%)	0.512
Blood culture positive, n(%)[l]	8 (4%)	5 (3%)	0.391	1 (2%)	0.322	2 (5%)	0.876	0	0.324	0	0.214	2 (4%)	0.388	1 (4%)	0.794
***LytA* positive, n(%)[m]**	11 (6%)	9 (5%)	0.345	0	0.130	2 (5%)	0.885	1 (6%)	0.184	4 (11%)	0.845	5 (9%)	0.723	1 (5%)	0.434
MCPP, n(%)[n]	3 (2%)	1 (1%)	0.247	0	0.387	0	0.817	0	0.551	0	0.452	1 (2%)	0.933	0	0.533
HDP, n(%)[o]															
-Blood	6 (3%)	8 (5%)	0.471	0	0.208	1 (3%)	0.856	1 (6%)	0.615	4 (11%)	0.783	5 (9%)	0.409	1 (5%)	0.757
-NP	24 (13%)	22 (12%)	0.923	4 (8%)	0.332	9 (23%)	0.112	1 (5%)	0.486	9 (16%)	0.591	9 (16%)	0.591	3 (12%)	0.914
HRV viral load, Mean (SD)[p]	3.77 (0.92)	3.68 (1.04)	0.977	3.45 (0.96)	0.068	3.91 (1.05)	0.535	3.35 (1.14)	0.941	3.77 (0.92)	0.935	3.77 (0.84)	0.767	4.03 (1.22)	0.071
Hospital stay >3 days, n(%)	111 (59%)	101 (57%)	0.996	39 (76%)	0.681	19 (48%)	0.247	8 (36%)	0.794	15 (43%)	0.524	30 (52%)	0.743	13 (52%)	0.538

Case fatality ratio, n(%)	16 (9%)	11 (7%)	0.342	3 (6%)	0.075	3 (8%)	0.870	1 (6%)	0.817	3 (9%)	0.786	6 (12%)	0.257	3 (13%)	0.771

Abbreviations - HRV: Human rhinovirus; SD: Standard deviation; IQR: Inter quartile range; CXR: Chest X-ray; HIV: Human immunodeficiency virus; HEU: HIV-uninfected but HIV exposed; CRP: C-reactive protein; MCPP: Microbiologically confirmed pneumococcal pneumonia; HDP: High Density pneumococcus; NP: Nasopharyngeal.

P-values from Chi-squared and Wilcoxon tests, logistic regression models adjusted for confounding variates (<0.2 in univariate analysis) where applicable. *P*-values could not be calculated for variables where both values are 0, thus cells left blank.

[a] - *P*-values from logistic regression models for HRV mono-infections compared to all HRV co-infections; [b] - *P*-values from logistic regression models for HRV mono-infections compared to each HRV co-infection individually; [c] - Underweight defined as weight for age <-2SD of the median age-sex specific WHO reference; [d] - HEU defined as undetectable viral load, HIV seronegative in the child with a positive maternal history. Positive maternal status based on self-report was accepted, except for seronegative children < 7 months of age where documented positive maternal status was required; [e] - premature birth defined as <37 weeks gestational age; [f] - A child was considered to be hypoxic if 1) a room air pulse-oximetry reading indicated oxygen saturation <90% at the two sites at elevation (Zambia and South Africa) or <92% at all other sites, or 2) a room air oxygen saturation reading was not available and the child was on oxygen; [g] - Tachycardia defined as heart rate >160 beats per minute (bpm) if aged <11 months, heart rate >150 bpm if aged 12-35 months, heart rate >140 bpm if aged 36-59 months; [h] - Tachypnea defined as respiratory rate >60 breaths/minute if aged <2 months, respiratory rate >50 breaths/minute if aged 2-12 months, respiration rate >40 breaths/minute if aged >12 months; [i] - Fever defined as temperature >38^{o}C; [j] - Leucocytosis defined as white blood cell count >15 000 cells/uL if age <12 months, white blood cell count >13 000 cells/uL if age >12 months; [k] - CRP defined as levels ≥40mg/mL which are considered to potentially indicate bacterial infection; [l] - blood culture positive for any significant bacteria (non-contaminant); [m] - Blood sample positive for *S. pneumoniae* colonisation by *LytA* PCR; [n] - MCPP defined as *S. pneumoniae* was cultured from a normally sterile body fluid - blood, pleural fluid or lung aspirate - or pleural fluid or lung aspirate was PCR *LytA* positive; [o] - HDP defined as *S. pneumoniae* density in nasopharynx>6.9 and/or density in whole blood sample>2.2 $\log_{10}$ copies/mL; [p] - HRV viral loads in the nasopharynx and expressed in $\log_{10}$ copies/mL.

3.1.4.2 HRV-associated mortality in the cases

The case fatality ratio and requirement for mechanical ventilation of HRV-associated cases (8%, n=63/826 and 1%, n=3/912 respectively) was not different compared to HRV-negative cases (8%, n=215/2659, *P*=0.300 and 1%, n=23/2 964, *P*=0.172 respectively). Similarly, among the radiologically confirmed cases, the case fatality ratio among HRV-associated cases (8%, n=27/330) was similar to those in whom HRV was not identified (7%, n=86/1 217, *P*=0.096), as was the requirement for mechanical ventilation (1%, n=3/365 vs. 1%, n=16/1 355, *P*=0.810).

Of the 63 HRV-associated fatalities, 75% (n=47) occurred during hospitalisation. In addition, the remainder occurred within 30 days of discharge, including 19% (n=12) who were well when discharged, 5% (n=3) who were discharged moribund or against medical advice and 2% (n=1) who had been transferred to another care facility. Case fatalities with HRV infections were seen at all sites except Thailand and in all age groups, ranging from 5% in South Africa to 26% in Zambia. The most deaths occurred in the infants less than 5 months of age (54%, n=34/63) and the least deaths in the children older than 1 year of age (22%, n=14/63).

A case-case comparison at all sites to ascertain factors that might help distinguish between HRV positive cases that resulted in death compared to HRV positive cases that survived as well as distinguish between mortalities in HRV-associated cases compared to mortalities in cases without HRV infections was performed; Table 3.13. The HRV-associated deaths were younger than the HRV-associated cases that survived (mean 7.9 vs. 13.9 months, *P*=0.002) and they were 5.75-fold (aOR 95% CI: 3.17-10.42) more likely to be underweight (63% vs. 27%, *P*<0.001). There were no other differences between the HRV-associated deaths and survivors such as attending day-care facilities (30% vs. 13%, *P*=0.101), having a smoker leaving in the household (32% vs. 38%, *P*=0.487) or premature birth (13% vs. 9%, *P*=0.556). There was, however, an association for the HRV-associated cases that died to have been born at lower birth weight than cases that survived (2.7Kgs vs. 2.9Kgs, *P*=0.028).

The HRV-associated deaths were also 4.75-fold (aOR 95% CI: 2.75-8.20) more likely to present with very severe pneumonia (67% vs. 30%, *P*<0.001) and 4.06-fold (aOR 95% CI: 2.38-6.91) more likely to be hypoxic (62% vs. 29%, *P*<0.001) compared to the cases that

survived. There were, however, no differences between the requirement of oxygen therapy (33% vs. 25%, P=0.146) or mechanical ventilation (2% vs. 1%, P=0.989). Additionally, the HRV-associated deaths were not associated with more abnormal chest X-rays which was defined as primary end point consolidation or any infiltrates (43% vs. 40%, P=0.216). The HRV positive case deaths were however more likely to present with lethargy (35% vs. 8%, P<0.001), concurrent diarrhoea (38% vs. 10%, P<0.001), central cyanosis (11% vs. 1%, P=0.002) and were unable to feed (30% vs. 5%, P<0.001) compared to the HRV positive cases that recovered. Conversely, the HRV positive case survivors were more likely to be associated with wheezing (51% vs. 8%, P=0.001), coughing (72% vs. 51%, P=0.044) and lower chest wall indrawing (93% vs. 79%, P=0.001) compared to the HRV-associated deaths.

For markers of potential bacterial co-infections, the HRV-associated deaths were 6.59-fold (aOR 95% CI: 2.69-16.10) more likely to have positive blood cultures (19% vs. 2%, P<0.001) and 8.67-fold (aOR 95% CI: 1.80-41.73) more likely to have microbiologically confirmed pneumococcal pneumonia (6% vs. 0.5%, P=0.007) compared to the HRV-associated case survivors. Additionally, the HRV-associated deaths were more likely to present with fever (90% vs. 78%, P=0.033), CRP levels ≥40mg/mL (35% vs. 17%, P=0.022) and high colonisation densities of *S. pneumoniae* in the nasopharynx (>6.9 $\log_{10}$ copies/mL) which was shown to be a predictor of pneumococcal pneumonia in the PERCH study [117] (27% vs. 23%, P=0.021) compared to the HRV-associated survivors. The HRV-associated deaths were also 2.75-fold (aOR 95% CI: 1.42-4.92) more likely to have HRV as the only respiratory virus detected in the nasopharynx compared to survivors (70% vs. 51%, P=0.002). Upon comparison of the other common respiratory viruses there were no specific associations observed between HRV co-infections and mortality; Table 3.13.

The HRV-associated deaths fatalities were indistinguishable from the HRV negative case fatalities for all demographics, risk factors for infection, clinical signs and symptoms as well as markers for bacterial and viral co-infections. The only trend that was evident was that the HRV-associated deaths were less likely to be HIV-exposed but uninfected (8% vs. 16%, P=0.050), but more likely to have high colonisation densities of *S. pneumoniae* in the nasopharynx (>6.9 $\log_{10}$ copies/mL) (27% vs. 15%, P=0.035) compared to the HRV negative deaths; Table 3.13.

Table 3.13: The demographical and clinical characteristics of the pneumonia cases by mortality and HRV infection status

	HRV Case fatalities (n=63)	HRV Case recovery (n=763)	Unadjusted *P*-value[a]	OR (95%CI)	Adjusted *P*-value[a]	aOR (95% CI)	All other fatalities (n=215)	Unadjusted *P*-value[b]	OR (95%CI)	Adjusted *P*-value[b]	aOR(95%CI)
Age in months, mean (SD)	7.9 (7.80)	13.9 (12.36)	0.0002		0.001		9.25 (9.81)	0.332		0.228	
Female, n(%)	35 (56%)	301 (39%)	0.014	1.91 (1.14-3.22)	0.034	1.83 (1.05-3.19)	114 (53%)	0.723	1.10 (0.63-1.95)	0.798	1.08 (0.61-1.91)
Never breast fed, n(%)	8 (13%)	63 (8%)	0.231	1.62 (0.74-3.54)	0.065	2.41 (0.95-6.15)	20 (9%)	0.433	1.42 (0.59-3.39)	0.361	1.54 (0.61-3.93)
Under weight, n (%)[c]	40 (63%)	207 (27%)	*P*<0.001	4.67 (2.73-7.99)	*P*<0.001	5.75 (3.17-10.42)	135 (63%)	0.919	1.04 (0.58-1.85)	0.955	1.01 (0.56-1.85)
HEU, n (%)[d]	5 (8%)	62 (8%)	0.958	0.97 (0.38-2.52)	0.561	0.73 (0.25-2.13)	35 (16%)	0.105	0.44 (0.17-1.18)	0.050	0.34 (0.11-0.99)
Day Care attendance, n(%)	19 (30%)	102 (13%)	0.036	1.59 (1.03-2.45)	0.101	0.85 (0.36-2.00)	55 (26%)	0.790	0.95 (0.65-1.40)	0.885	0.97 (0.64-1.47)
Smoker in household, n(%)	20 (32%)	286 (38%)	0.377	0.78 (0.45-1.34)	0.487	1.19 (0.72-1.97)	48 (23%)	0.120	1.64 (0.88-3.04)	0.093	1.79 (0.90-3.53)
Premature birth, n(%)[e]	8 (13%)	71 (9%)	0.326	083 (0.56-1.21)	0.556	0.90 (0.64-1.27)	23 (11%)	0.245	0.77 (0.49-1.20)	0.204	0.74 (0.46-1.18)
Birth weight, mean (SD)	2.7 (0.7)	2.9 (0.6)	0.097		0.028		2. 8 (0.6)	0.219		0.195	
Clinical Features:											
Very severe pneumonia, n(%)	42 (67%)	226 (30%)	*P*<0.001	4.75 (2.75-8.20)	*P*<0.001	3.21 (1.76-5.84)	139 (65%)	0.768	1.10 (0.60-1.98)	0.766	1.09 (0.59-2.03)
Chest X-ray abnormal, n(%)[f]	27 (43%)	303 (40%)	0.624	1.14 (0.68-1.92)	0.216	1.43 (0.81-2.49)	86 (40%)	0.685	1.13 (0.64-1.99)	0.872	1.05 (0.57-1.92)
Alveolar consolidation, n(%)	21 (33%)	116 (15%)	*P*<0.001	2.78 (1.59-4.88)	0.001	2.77 (1.49-5.16)	59 (27%)	0.365	1.32 (0.72-2.42)	0.421	1.29 (0.69-2.42)
Supplementary 02 therapy, n(%)	21 (33%)	193 (25%)	0.340	1.20 (0.83-1.74)	0.146	2.81 (1.22-6.50)	94 (44%)	0.143	0.64 (0.36-1.16)	0.101	0.51 (0.22-1.14)
Mechanical ventilation, n(%)	1 (2%)	11 (1%)	0.926	1.10 (0.14-8.68)	0.989	1.01 (0.10-9.38)	16 (7%)	0.123	0.20 (0.03-1.55)	0.103	0.15 (0.02-1.46)
Hypoxic, n(%)[g]	39 (62%)	217 (29%)	*P*<0.001	4.06 (2.38-6.91)	*P*<0.001	3.92 (2.06-7.44)	121 (56%)	0.427	1.26 (0.71-2.24)	0.338	1.36 (0.72-2.56)
Tachycardia, n(%)[h]	33 (52%)	353 (46%)	0.351	1.28 (0.76-2.14)	0.563	0.85 (0.49-1.48)	109 (51%)	0.840	1.06 (0.60-1.86)	0.808	1.07 (0.6-1.90)
Tachypnea, n(%)[i]	53 (84%)	655 (86%)	0.651	0.85 (0.42-1.72)	0.923	0.96 (0.45-2.06)	171 (81%)	0.625	1.21 (0.57-2.58)	0.339	1.47 (0.67-3.26)
Wheezing, n(%)	5 (8%)	390 (51%)	*P*<0.001	0.08 (0.03-0.21)	0.001	0.17 (0.06-0.46)	21 (10%)	0.664	0.80 (0.29-2.21)	0.612	0.73 (0.22-2.42)
Cough, n(%)	32 (51%)	552 (72%)	*P*<0.001	0.39 (0.23-0.65)	0.049	0.56 (0.32-0.98)	94 (44%)	0.778	1.06 (0.73-1.53)	0.335	1.33 (0.74-2.41)
Lethargic, n(%)	22 (35%)	62 (8%)	*P*<0.001	6.07 (3.40-10.83)	*P*<0.001	5.91 (2.99-11.69)	75 (35%)	0.996	1.00 (0.56-1.81)	0.895	1.04 (0.55-1.95)
Fever, n(%)[j]	57 (90%)	592 (78%)	0.021	2.75 (1.16-6.50)	0.033	2.65 (1.08-6.50)	184 (86%)	0.318	1.60 (0.64-4.03)	0.200	1.91 (0.71-5.13)
Convulsions, n(%)	9 (14%)	33 (4%)	0.001	3.67 (1.67-8.08)	0.014	2.98 (1.24-7.10)	29 (13%)	0.871	1.07 (0.48-2.40)	0.716	1.17 (0.49-2.80)
Diarrhoea, n(%)	24 (38%)	78 (10%)	*P*<0.001	5.40 (3.08-9.45)	*P*<0.001	3.35 (1.80-6.23)	64 (30%)	0.213	1.45 (0.81-2.61)	0.105	1.66 (0.89-3.08)
Head nodding, n(%)	12 (19%)	136 (18%)	0.815	1.08 (0.56-2.08)	0.296	0.68 (0.34-1.39)	52 (24%)	0.395	0.74 (0.37-1.49)	0.234	0.64 (0.31-1.33)
Central cyanosis, n(%)	7 (11%)	6 (1%)	*P*<0.001	15.77 (5.13-48.5)	0.002	7.48 (2.09-26.80)	26 (12%)	0.823	0.90 (0.37-2.19)	0.848	0.91 (0.36-2.29)
Unable to Feed, n(%)	19 (30%)	36 (5%)	*P*<0.001	8.70 (4.62-16.38)	*P*<0.001	4.27 (2.10-8.66)	60 (28%)	0.743	1.11 (0.60-2.05)	0.851	1.06 (0.57-1.99)
Vomiting everything, n(%)	4 (6%)	18 (2%)	0.070	2.80 (0.92-8.56)	0.061	3.32 (0.95-11.70)	9 (4%)	0.478	1.55 (0.46-5.21)	0.349	1.85 (0.51-6.72)
Lower chest wall indrawing, n(%)	50 (79%)	713 (93%)	*P*<0.001	0.27 (0.14-0.53)	0.001	9.27 (0.12-0.58)	187 (87%)	0.137	0.58 (0.28-1.19)	0.099	0.51 (0.23-1.13)
Stridor, n(%)	1 (2%)	12 (2%)	0.801	0.84 (0.21-3.34)	0.610	0.57 (0.06-4.93)	2 (1%)	0.687	0.81 (0.31-2.17)	0.640	1.80 (0.15-21.44)
Grunting, n(%)	21 (33%)	85 (11%)	0.102	1.21 (0.96-1.51)	0.581	1.23 (0.59-2.54)	88 (41%)	0.230	0.71 (0.40-1.24)	0.227	0.67 (0.35-1.28)
Nasal flaring, n (%)	42 (67%)	401 (53%)	0.033	1.80 (1.05-3.11)	0.839	0.94 (0.51-1.74)	146 (68%)	0.435	0.83 (0.53-1.32)	0.863	0.94 (0.49-1.82)
Laboratory markers:											
Leucocytosis, n(%)[k]	27 (46%)	371 (52%)	0.384	0.79 (0.46-1.34)	0.097	1.66 (0.91-3.03)	84 (40%)	0.460	1.25 (0.70-2.23)	0.217	1.47 (0.79-2.72)
Neutrophils (%), median (IQR)	48 (30.4-65)	51.3 (36.1-69)	0.138		0.950		47.9 (32.4-64)	0.826		0.945	
Lymphocytes (%), median (IQR)	40 (28-58.3)	37 (23.4-51.4)	0.096		0.618		40 (26.3-55)	0.449		0.539	
Eosinophils (%), median (IQR)	0.9 (0-2)	1.5 (0.5-3.9)	0.021		0.329		0.8 (0.1-3.15)	0.066		0.059	
CRP >40mg/l, n(%)[l]	22 (35%)	131 (17%)	0.001	2.58 (1.49-4.49)	0.022	1.99 (1.10-3.59)	65 (30%)	0.481	1.24 (0.68-2.24)	0.379	1.33 (0.71-2.48)

Blood culture positive, n(%)[m]	12 (19%)	15 (2%)	*P*<0.001	11.58 (5.15-26.0)	*P*<0.001	6.59 (2.69-16.10)	32 (15%)	0.453	1.33 (0.64-2.75)	0.481	1.31 (0.61-2.81)
***LytA* positive, n(%)[n]**	8 (14%)	39 (5%)	0.013	2.80 (1.24-6.30)	0.183	1.83 (0.75-4.45)	20 (10%)	0.471	1.38 (0.57-3.32)	0.520	1.35 (0.55-3.33)
MCPP, n(%)[o]	4 (6%)	4 (0.5%)	*P*<0.001	12.69 (3.09-52.1)	0.007	8.67 (1.80-41.73)	7 (3%)	0.287	1.99 (0.56-7.01)	0.342	1.77 (0.48-6.50)
HDP, n(%)[p]											
-Blood	7 (12%)	22 (3%)	0.001	4.36 (1.78-10.7)	0.056	2.65 (0.98-7.22)	15 (8%)	0.316	1.62 (0.63-4.19)	0.356	1.59 (0.59-4.28)
-NP	17 (27%)	88 (23%)	0.001	2.83 (1.56-5.16)	0.021	2.21 (1.12-4.32)	33 (15%)	0.037	2.04 (1.05-3.97)	0.035	2.17 (0.16-4.47)
HRV viral load, mean (SD)[q]	3.52 (0.93)	3.71 (0.94)	0.122		0.889						
HRV mono-infection, n(%)	44 (70%)	391 (51%)	0.004	2.75 (1.37-5.52)	0.002	2.64 (1.42-4.92)					
Viral co-infections in the nasopharynx with:											
-RSV, n(%)	6 (10%)	102 (14%)	0.387	0.68 (0.29-1.62)	0.017	0.31 (0.12-0.81)	16 (7%)	0.591	1.31 (0.49-3.50)	0.772	1.16 (0.42-3.20)
-AdV, n(%)	5 (8%)	97 (13%)	0.273	0.59 (0.23-1.51)	0.401	0.65 (0.23-1.79)	26 (12%)	0.360	0.63 (0.23-1.71)	0.433	0.66 (0.23-1.86)
-InFV A-C, n(%)	0	4 (1%)	0.849		0.668		13 (6%)	0.140		0.185	
-HBoV, n(%)	8 (13%)	117 (15%)	0.575	0.80 (0.37-1.71)	0.730	1.16 (0.49-2.72)	23 (11%)	0.658	1.21 (0.51-2.87)	0.680	1.20 (0.50-2.90)
-HMPV, n(%)	1 (2%)	41 (5%)	0.218	0.28 (0.04-2.10)	0.309	0.34 (0.04-2.70)	11 (5%)	0.252	0.30 (0.04-2.36)	0.187	0.24 (0.03-1.99)
-PIV, n(%)	6 (10%)	61 (8%)	0.670	1.21 (0.50-2.92)	0.682	1.22 (0.46-3.21)	19 (9%)	0.867	1.09 (0.41-2.85)	0.836	1.11 (0.40-3.02)
-HCoV, n(%)	5 (8%)	47 (6%)	0.578	1.31 (0.50-3.42)	0.216	1.94 (0.70-5.54)	22 (10%)	0.589	0.75 (0.27-2.08)	0.554	0.73 (0.26-2.06)

Abbreviations - HRV: Human rhinovirus; SD: Standard deviation; IQR: Inter quartile range; CXR: Chest X-ray; HIV: Human immunodeficiency virus; HEU: HIV-uninfected but HIV exposed; CRP: C-reactive protein; MCPP: Microbiologically confirmed pneumococcal pneumonia; HDP: High Density pneumococcus; NP: Nasopharyngeal.
P-values from Chi-squared and Wilcoxon tests, logistic regression models adjusted for confounding variates (<0.2 in univariate analysis) where applicable. Odds ratio could not be calculated for continuous variables or variables with 0 values, thus cells left blank.
[a] - *P*-values from logistic regression models for HRV-associated mortalities compared to all HRV associated cases that survived; [b] - *P*-values from logistic regression models for HRV-associated case mortalities compared to all other case fatalities; [c] - Underweight defined as weight for age <-2SD of the median age-sex specific WHO reference; [d] - HEU defined as undetectable viral load, HIV seronegative in the child with a positive maternal history. Positive maternal status based on self-report was accepted, except for seronegative children < 7 months of age where documented positive maternal status was required; [e] - premature birth defined as <37 weeks gestational age; [f] - Defined as primary end point pneumonia or any infiltrates observed on the chest X-rays; [g] - A child was considered to be hypoxic if 1) a room air pulse-oximetry reading indicated oxygen saturation <90% at the two sites at elevation (Zambia and South Africa) or <92% at all other sites, or 2) a room air oxygen saturation reading was not available and the child was on oxygen; [h] - Tachycardia defined as heart rate >160 beats per minute (bpm) if aged <11 months, heart rate >150 bpm if aged 12-35 months, heart rate >140 bpm if aged 36-59 months; [i] - Tachypnea defined as respiratory rate >60 breaths/minute if aged <2 months, respiratory rate >50 breaths/minute if aged 2-12 months, respiration rate >40 breaths/minute if aged >12 months; [j] - Fever defined as temperature >38^{o}C; [k] - Leucocytosis defined as white blood cell count >15 000 cells/uL if age <12 months, white blood cell count >13 000 cells/uL if age >12 months; [l] - CRP defined as levels ≥40mg/mL which are considered to potentially indicate bacterial infection; [m] - blood culture positive for any significant bacteria (non-contaminant); [n] - Blood sample positive for *S. pneumoniae* colonisation by *LytA* PCR; [o] - MCPP defined as *S. pneumoniae* was cultured from a normally sterile body fluid - blood, pleural fluid or lung aspirate - or pleural fluid or lung aspirate was PCR *LytA* positive; [p] - HDP defined as *S. pneumoniae* density in nasopharynx>6.9 and/or density in whole blood sample>2.2 $\log_{10}$ copies/mL; [q] - HRV viral loads in the nasopharynx and expressed in $\log_{10}$ copies/mL.

3.2 DISCUSSION

In children 1-59 months of age hospitalised with severe (n=2 630) and very severe (n=1 246) pneumonia, significantly higher rates of HRV detection (24%) were noted when compared to rates (21%, $P<0.001$) among community control children (n=4 977). However, in terms of clinical severity - such as hypoxia, requiring mechanical ventilation and very severe pneumonia diagnosis - there were no significant differences among HRV-associated cases and cases without HRV infections suggesting that there were no obvious differences in clinical presentation of respiratory viruses causing severe LRTI. There was however evidence that HRV-bacterial co-infections resulted in higher case fatality ratios. To date, this is the largest multi-country, case-control surveillance study providing a detailed clinical analysis of HRV epidemiology in the developing world.

Among the community control population over the full two-year period, HRV detection (21%) was ubiquitous which is consistent with other African and Southeast Asian studies which have reported HRV prevalence of between 12-50% in children without severe respiratory illness [54, 83, 92, 93, 95, 99, 106]. These studies reporting on HRV detection among a control population often only enrolled very limited numbers of controls with all of the studies enrolling less than half of the number of controls compared to the number of cases, the highest number of controls (n=369 compared to 810 cases) were enrolled during a study conducted in Kenya [95]. Furthermore, in the majority of these studies the controls were a convenient sample of children presenting for routine immunisation at local clinics [54, 83, 92, 93, 106] thus they might not provide a true representation of the actual community.

Additionally, our study also analysed the prevalence of HRV between asymptomatic controls at the time of sampling and controls with RTI that did not warrant hospitalisation; and found that controls with RTI symptoms were 1.55-fold more likely to have HRV detected in the nasopharynx. The high prevalence of HRV detection in asymptomatic controls (20%) was in concordance with other studies (10-24%); [31, 47, 50, 51, 53, 122, 123] and could potentially be linked to the highly sensitive detection capabilities of PCR and the prolonged shedding periods of HRV. In a longitudinal study of infants, following HRV RTI, shedding of virus was evident for more than 30 days in 4.5% of the cases; although the majority of the episodes were negative within 7 days of infection [124]. In our study only a single sample was taken on enrolment, and controls were not interviewed about disease episodes more than three days

prior to enrolment, thus we were unable to determine the temporal association of detection of HRV in relation to the onset of current or previous RTI symptoms. Additionally, controls were not followed up to determine whether they became ill post sampling, thus we could not rule out the possibility they were in the incubation period of disease at the time of sampling. This was seen in a Finnish study which enrolled children under the age of 15 years with acute wheezing (n=161) as well as surgical controls (n=79). They found that HRV prevalence was 16% and 8% in the cases and controls respectively. Although the controls were asymptomatic at the time of sampling; 5 of the 13 HRV-positive controls went on to develop respiratory symptoms in the following week [51].

Thus, although the detection of HRV in respiratory samples does not necessarily infer causality with any concurrent illness/symptom, the higher detection prevalence of HRV in controls with RTI than those who are asymptomatic infers a causal association between HRV and respiratory illness. Furthermore, in the case-control comparison of HRV in this study, HRV detection was more prevalent among cases (24%) compared to controls (21%); and after adjustment for multiple potentially confounding variables, including presence of other respiratory viruses, the cases were 1.45-fold (95% CI: 1.29-1.62, $P<0.001$) more likely to have HRV detected compared to community controls. Moreover, in the 12-59 month age group, the odds were even higher with the hospitalised cases having a 2.08-fold (95% 1.75-2.47, $P<0.001$) increased odds of HRV detection compared to controls. Conversely, among infants the HRV prevalence was 1.29-fold (95% 1.09-1.53, $P=0.004$) greater among controls compared to cases. Viral infections, particularly RSV, are extremely common during infancy [112] with approximately 63% of all PERCH cases falling in the 1-12 month age group, thus the lower HRV prevalence among infants could be proportion to the higher prevalence's of the other common respiratory viruses. Population based attack rates are required to study the differences in HRV prevalence among cases and controls between the different age groups further; however, our study was not set up to do these kind of analyses.

When comparing controls to the HRV-associated cases, several differences were observed that might help to discriminate for more severe clinical outcomes. Firstly, more cases were males compared to controls and cases were more likely to be malnourished and HIV-exposed but uninfected than controls. In addition, cases were associated with significantly higher HRV nasopharyngeal viral load than controls, suggesting higher viral load could lead to more

severe disease. This association between increased viral load and more severe disease was also seen in the hospitalised children, with very severe pneumonia cases having higher viral loads than the severe pneumonia case. However, we were unable to calculate an optimal HRV nasopharyngeal density threshold capable of discriminating between cases and controls or between more severe disease among the cases. The association between higher HRV viral loads and more severe disease has also been described in other studies [59, 64, 125, 126], where viral load correlated with illness severity scores.

Another important factor for the development of severe disease could be co-infection with other respiratory pathogens, with several studies reporting on HRV co-infections with other respiratory viruses [85, 107, 127] and bacteria [72-75, 110] to be associated with more severe disease. On comparison of the HRV-associated cases and controls, we however, did not find any compelling evidence of a significant relationship between HRV and co-infection with either bacteria or common respiratory viruses resulting in increased disease severity. In fact, on comparison of the HRV-associated cases and controls, *Moraxella catarrahalis* and *Streptococcus pneumoniae* were more commonly detection in the nasopharynx of controls rather than cases and there were no associations between HRV and *S. pneumoniae* (*LytA)* detection in whole blood of cases and controls. These analyses of HRV-bacterial co-infections were, however, limited as the majority of the data on bacterial co-infection was based on identification from nasopharyngeal swabs and it is well known that young children have a high prevalence of colonisation of different bacteria during the first years of life.

HRV detection was also very ubiquitous among children hospitalised with severe (24%) and very severe pneumonia (23%), and was the second most common respiratory virus detected after RSV (25%). HRV and RSV being detected in similar proportions among children with LRTI have been reported in other studies, and the HRV prevalence in this study (24%) was in concordance with other studies conducted in similar socioeconomic settings where HRV detection ranged between 17% to 53% [80, 81, 94, 96, 101, 102].

Overall 53% of HRV-associated cases had mono-infections, which concurs with previous studies conducted in Africa and Southeast Asia which also investigated for similar respiratory viruses as done in our study and reported mono-infection prevalence between 50-80% [52, 54, 83, 85-87, 127, 128]. We found no differences in clinical severity - including markers such as

hypoxia, requiring mechanical ventilation and hospital stays longer than 3 days - between cases with HRV mono and mixed viral co-infections. However, cases with HRV mono-viral infections had fatality ratio 2.83-fold higher than cases with HRV viral co-infections and on closer inspection of the HRV-associated fatalities, the case fatalities were 2.2-fold more likely to have HRV mono-infections compared to the HRV-associated cases that survived. Conversely, the HRV-associated deaths had greater evidence of bacterial co-infection including alveolar consolidation, MCPP, positive blood culture, greater prevalence of fever, CRP levels ≥40mg/mL and high colonisation densities of *S. pneumoniae* in the nasopharynx and blood, compared to HRV-associated cases who survived. It has been shown *in vivo*, that epithelial cells infected with HRV enhance the adherence of *Staphylococcus* and *Streptococcus* bacteria to the cells, potentially increasing the potential for bacterial co-infection [109]. Additionally, HRV have been shown to impair the immune response of macrophages to bacterial products [110]. Furthermore, a Finnish ecology study has found a link between HRV infections in the community and an increased incidence of invasive pneumococcal disease [73, 74]. Therefore, the HRV infection could have predisposed these cases to bacterial super-infections which subsequently lead to the fatal outcomes.

Several previous studies have looked at the effects of HRV co-infections including a South African study [54] which enrolled children less than 5 years of age and which similarly to our study also found that HRV detection was more prevalent among cases requiring hospitalisation (35.8%) compared to community controls (18.8%, P=0.047). Furthermore, in that study 80% of the HRV-associated hospitalised cases had mono-infections, and significantly more of the cases with HRV mono-infections required hospitalisation (88%) than cases with HRV and other respiratory virus co-infections (58%), suggesting that HRV mono-infection caused more severe disease [54]. Conversely, a Vietnamese study [85] in children <15 years of age hospitalised with respiratory infection, reported that HRV co-infections were associated with more severe disease (more chest refraction, fever and trend of longer hospital stays) than HRV mono-infections, and likewise a study in the USA also reported that HRV co-infections resulted in longer hospital stay (their indicator of more severe disease) than cases with mono-infections in children <2 years hospitalised with bronchiolitis [127]. Other studies such is from Thailand [52], United States [53, 129, 130] and Greece [128] did not identify significant differences in disease severity between HRV mono-infections and co-infections in patients hospitalised with respiratory tract infections. None of these studies, however, investigated for bacterial co-infections as undertaken in our study and

they were not powered to study case fatalities; furthermore, the majority of them focused on bronchiolitis which we attempted to exclude from our study.

There were however several limitations in our co-infection analysis - the bacterial co-infections were for the most part based on hypothesised markers for bacterial co-infections including fever, alveolar consolidation, whole blood cell counts, raised CRP levels (≥40mg/mL), high *S. pneumoniae* colonisation densities in the blood and nasopharynx as well as physical evidence of bacterial co-infections such as positive blood cultures and detection of bacteria at the site of infection. This is because blood culture are very insensitive (15-30%) and collecting samples from the site of infection remains challenging with direct lung aspiration rarely being performed [95, 111] thus greatly limiting the detection of invasive bacterial disease. Additionally, we only analysed for the most common respiratory viruses which are well documented to cause the majority of respiratory infections there are however other respiratory viruses which we could have overlooked, although the clinical relevance of these other respiratory viruses (e.g Polyomaviruses) remains to be fully elucidated during severe disease episodes [89].

In the PERCH study we found that children with HRV-associated pneumonia were more likely to present with wheezing, even though children were given a bronchodilator challenge in an attempt to exclude cases with bronchiolitis, prior to enrolment into this study. If the lower chest wall indrawing resolved itself post challenge, irrespective of its effects on wheeze, then the child was considered to have bronchiolitis and was excluded from the study. Thus the estimates of wheezing in children with HRV-associated severe and very severe pneumonia in this study are likely underestimated. Nevertheless, even after adjustment for potentially confounding variables including age, gender, site of enrolment, HIV exposure, malnutrition, other co-infecting respiratory viruses and markers for bacterial co-infections, HRV were almost 2-fold more likely to be detected in children presenting with wheeze. Additionally, the HRV-associated cases were more likely to have leucocytosis and higher neutrophil levels than cases without HRV infections. High white blood cell counts and neutrophil levels are also a predictor of reactive airway disease and chronic obstructive pulmonary disease [87] whereby the accumulation of excessive neutrophils at the site of inflammation can actually damage the host tissues [131] and this destructive process is linked to both asthma development and increased wheezing which was well documented to be

associated with HRV in this study. The association between HRV infection and increased wheezing has been recorded in several other studies in similar socio-economic settings in children with bronchiolitis and LRTI [35, 85-87, 132] and has long term implication as wheezing in some children has been shown to be the first clinical manifestation of asthma development [132, 133].

In conclusion, this is the first study to our knowledge which reports on HRV-associated deaths and the interactions of HRV with other respiratory pathogens during fatal infections. This study is also strengthened by drawing on a large samples size and data from a diversity of settings over a two-year period. The large study size allowed us to analyses many different variables, relevance of HRV co-infections, and included control to account for potential confounders for HRV-associated disease severity and death. The study findings suggest that HRV clinical outcomes are influenced by multiple host-specific factors, including age, malnutrition, HIV-exposure as well as HRV viral loads and the presence of viral and bacterial co-infections. It also highlights the need to test for both viral and bacterial pathogens. These risk factors for infection that we have identified point the way to interventions but they are long-term development challenges and are not easy to correct. New treatment and prevention strategies are necessary to reduce what appears to be a substantial morbidity associated with HRV disease.

4.0 A case-control study of the impact of HIV-1 on the clinical and molecular subtyping of Human rhinovirus (HRV) in South African and Zambian children

HIV-1 infection in children is an established risk factor for respiratory viral associated LRTI morbidity and mortality probably due to impaired humoral and cell-mediated immunity [5]. This has been described for RSV, HMPV and Influenza viruses [134-136]; however, not much is known on the role of HRV in the pathogenesis of severe LRTI disease in HIV-1-infected children.

In this chapter, we analyse the clinical epidemiology of HRV in children hospitalised with pneumonia and age-frequency matched community controls in settings with a high burden of HIV-1 infection.

4.1 RESULTS

4.1.1 Study populations

A total of 1 449 children hospitalised with WHO-defined pneumonia in South Africa (n=911, 63%) and Zambia (n=538, 37%) between August 2011 and August 2013 were enrolled into the PERCH project, of whom 14% (n=204) were HIV-1-infected children. HRV prevalence was 22% (n=323/1 449) overall and 23% (n=46/204) compared to 22% (n=277/1 245) in HIV-1-infected and -uninfected cases, respectively, P=0.934.

Furthermore, 1 566 community controls were enrolled into the PERCH project in South Africa (n=959, 61%) and Zambia (n=607, 39%) of whom 13% (n=210/1 566) were HIV-1-infected. Additionally, of the 1 566 controls enrolled into the study, 1 424 (91%) were asymptomatic at the time of sampling and 142 (9%) had signs of respiratory tract infections (RTI); and of the 210 HIV-1-infected controls, 166 (79%) were asymptomatic and 44 (21%) had signs of RTI (P<0.001). HRV was detected in 20% (n=319/1 556) of controls overall, but less commonly so among HIV-1-infected (15%, n=32/210) than HIV-uninfected controls (21%, n=287/1 356, P=0.045).

4.1.2 HRV in the community controls

A total of 319 HRV-associated controls were enrolled into the study, of these 10% (n=32) were HIV-1-infected controls and 287 (90%) were HIV-uninfected. After multivariate regression analysis controlling for potential confounding variables, as well presence of other respiratory viruses and markers for bacterial co-infection, HRV was found to be 1.56-fold (aOR 95% CI: 1.05-2.40) more prevalent among HIV-uninfected controls (21%) compared to HIV-1-infected controls (15%, P=0.045); Table 4.1. Conversely, among the HRV-associated controls, HRV was 3.45-fold (aOR 95% CI: 1.22-9.80) more likely to be present in HIV-1-infected children with RTI (31%) than HIV-uninfected children with RTI (7%, P=0.02). Subsequently, HRV was less likely to be detected in HIV-1-infected asymptomatic controls (69%) than HIV-uninfected asymptomatic controls (93%, aOR 0.29, 95% CI: 0.11-0.83, P=0.020).

Among the HIV-uninfected controls, 29% were HIV-exposed uninfected (HEU) (22% were HRV positive, n=83/287), 51% were HIV-unexposed (HUU) (24% were HRV positive, n=147/287) and 20% were HIV-uninfected with an unknown HIV exposure status (HU-UE) (16% were HRV positive, n=57/287). There were no differences in the demographics and clinical epidemiology observed between these three HIV-uninfected strata, except that the HEU group were more likely to have never been breastfed, as such, further analysis focussed on comparing HRV-associated controls between the HIV-1-infected compared to the overall group of HIV-uninfected controls.

The demographics of HIV-1-infected and HIV-uninfected community controls with HRV infections are shown in Table 4.1. HIV-1-infected controls infected with HRV were older (mean 14.4 vs. 9.9 months, P=0.010) and 6.52-fold (aOR 95% CI: 2.64-16.1) more likely to be underweight (34% vs. 7%, P<0.001) than HIV-uninfected controls. Additionally, the HIV-1-infected controls were 4.23-fold (aOR 95% CI: 1.39-12.09) more likely to have a cough (19% vs. 4%, P=0.011) compared to HIV-uninfected controls. There were, however, no other associations between the HIV-1-infected compared to HIV-uninfected children with HRV infection among the controls including for markers of bacterial infections such as blood samples positive for *LytA*, or high densities of *S. pneumoniae* in colonisation the blood or nasopharyngeal samples. Additionally, there were no overall differences in the prevalence of

HRV mono- and co-viral infections between the HIV-1-infected and -uninfected controls, including for any individual respiratory virus co-infections.

Table 4.1: Demographical and clinical characteristics of HRV associated HIV-1-infected and HIV-uninfected control children living in the community

	HIV+ (n=32)	HIV- (n=287)	Unadjusted *P*-value	OR (95% CI)	Adjusted *P*-value	aOR (95% CI)
Age in months, mean (SD)	14.4 (12.9)	9.9 (10.1)	0.019		0.010	
Female, n(%)	17 (53%)	142 (49%)	0.780	0.90 (0.43-1.87)	0.895	1.05 (0.50-2.24)
Never breast fed, n(%)	11 (34%)	68 (24%)	0.188	1.68 (0.77-3.67)	0.082	2.27 (0.90-5.73)
Under weight, n(%)[a]	11 (34%)	19 (7%)	*P*<0.001	7.3 (3.11-17.55)	*P*<0.001	6.52 (2.64-16.1)
Day care attendance, n(%)	3 (9%)	28 (10%)	0.849	0.88 (0.25-3.09)	0.921	0.94 (0.2203.87)
Smoker in household, n(%)	11 (34%)	86 (30%)	0.607	1.22 (0.56-2.64)	0.641	1.21 (0.55-2.64)
Premature birth, n(%)[b]	4 (13%)	69 (24%)	0.660	0.82 (0.34-1.98)	0.962	0.98 (0.37-2.54)
Birth weight, mean (SD)	2.9 (0.7)	2.9 (0.5)	0.928		0.831	
Symptoms features:						
Cough, n(%)	6 (19%)	12 (4%)	0.003	4.87 (1.71-13.87)	0.011	4.23 (1.39-12.09)
Fever, n(%)[c]	2 (6%)	0	0.014		0.055	
Diarrhoea, n(%)	0	1 (0%)	0.718		0.959	
Rhinorrhoea, n(%)	2 (6%)	5 (2%)	0.175	3.12 (0.60-16.16)	0.282	2.61 (0.45-15.14)
Laboratory markers:						
***LytA* positive, n(%)[d]**	1 (3%)	21 (7%)	0.400	0.42 (0.05-3.20)	0.418	0.42 (0.05-3.37)
HDP, n(%)[e]						
-Blood	1 (8%)	30 (10%)	0.856	0.82 (0.10-6.60)	0.837	0.80 (0.10-6.66)
-NP	5 (12%)	27 (10%)	0.622	1.29 (0.47-3.57)	0.464	1.48 (0.52-4.18)
HRV load, mean (SD)[f]	3.83 (1.81)	3.63 (0.89)	0.288		0.289	
Single HRV infection, n(%)[g]	21 (66%)	205 (71%)	0.494	0.76 (0.35-1.65)	0.555	0.78 (0.36-1.74)
Mixed HRV infection, n(%)[h]	11 (34%)	82 (29%)	0.494	1.30 (0.60-2.84)	0.555	1.27 (0.57-2.81)
HRV co-infections in the nasopharynx with:						
-RSV, n(%)	0	8 (3%)	0.642		0.600	
-HBoV, n(%)	6 (19%)	24 (8%)	0.064	2.53 (0.95-6.75)	0.089	2.54 (0.55-6.58)
-HMPV, n(%)	0	6 (2%)	0.784		0.696	
-AdV, n(%)	2 (6%)	26 (9%)	0.597	0.67 (0.15-2.96)	0.453	0.56 (0.13-2.53)
-PIV, n(%)	2 (6%)	6 (2%)	0.175	3.12 (0.60-16.16)	0.158	3.42 (0.62-18.79)
-HCoV, n(%)	3 (9%)	27 (9%)	0.995	0.99 (0.28-3.49)	0.921	1.07 (0.30-3.81)
-InFV A-C, n(%)	1 (3%)	3 (1%)	0.340	3.05 (0.31-30.26)	0.254	4.15 (0.36-48.0)
Fungal co-infections:						
-*P. jiroveci*, n(%)	3 (9%)	35 (12%)	0.642	0.75 (0.22-2.58)	0.651	1.36 (0.36-5.25)

Abbreviations - OR: Odds ratio; aOR: Adjusted odds ratio; CI: Confidence interval; SD: Standard deviation; n: Number; NP: Nasopharyngeal; HRV: Human rhinovirus; HIV: Human immunodeficiency virus; HDP: High density pneumococcus; RSV: Respiratory Syncytial Virus, HMPV: human metapneumovirus; AdV: adenovirus; PIV: parainfluenza type 1-4; HBoV: Human Bocavirus; HCoV: Human Coronavirus (OC43, NL63, 229E and HKU1) and InFV: Influenza Virus (A, B and C); *P. jiroveci: Pneumocystis jiroveci.*

P-values from Chi-squared and Wilcoxon tests, logistic regression models adjusted for confounding variates (<0.2 in univariate analysis) where applicable, Odds ratio could not be calculated for continuous variables or variables with 0 values, thus cells left blank.

[a] - Underweight defined as weight for age <-2SD of the median age-sex specific WHO reference; [b] - Premature birth defined as gestational age <37 weeks; [c] - Fever defined as temperature ≥38^{o}C; [d] - Blood sample positive for *S. pneumoniae* colonisation by *LytA* PCR; [e] - HDP defined as *S. pneumoniae* density in nasopharynx >6.9 and/or density in whole blood sample >2.2 $\log_{10}$ copies/mL; [f] - HRV viral load in the nasopharynx, expressed as log copies/mL; [g] - HRV was the only respiratory virus detected in the nasopharynx; [h] - HRV was detected in the nasopharynx together with other respiratory viruses.

4.1.3 Case-control comparison of HRV associated HIV-1-infected children

Overall, there were no differences in the HRV prevalence between cases (22%) and controls (20%, *P*=0.198); however, in the children 12-59 months, HRV detection was significantly more prevalent among cases (25%) compared to controls (16%, *P*=0.003). In the HIV-1-infected participants, there was a trend for higher prevalence of HRV among cases (23%) compared to controls (15%, *P*=0.056), which was mainly evident in South Africa (24% vs. 13% among cases vs. controls, *P*=0.025); Table 4.2. However, after multivariate regression analysis - adjusting for multiple potentially confounding risk factors for hospitalisation including malnutrition, age categories and markers for bacterial, fungal and virus co-infections - HIV-1 infection was not an independent risk factor for HRV-associated hospitalisation (aOR 1.49, 95% CI: 0.92-2.42, *P*=0.104).

Table 4.2: Number of study subjects enrolled and tested for HRV - percent positive by age and HIV-1 infection status

Age groups			**Zambia HIV+**	**South Africa HIV+**	**Total HIV+**	**Overall**
All	Enrolled	Cases	89	115	204	1449
		Controls	74	136	210	1566
	HRV positive, n (%)	Cases	18 (20%)	28 (24%)	46 (23%)	323 (22%)
		Controls	14 (19%)	18 (13%)	32 (15%)	319 (20%)
			P=0.808	*P*=0.025	*P*=0.056	*P*=0.198
1-5 months	Enrolled	Cases	45	49	94	743
		Controls	20	45	65	636
	HRV positive, n (%)	Cases	8 (18%)	13 (27%)	21 (22%)	145 (20%)
		Controls	4 (20%)	3 (7%)	7 (11%)	136 (21%)
			P=0.831	*P*=0.017	*P*=0.061	*P*=0.374
6-11 months	Enrolled	Cases	23	34	57	357
		Controls	18	40	58	420
	HRV positive, n (%)	Cases	6 (26%)	10 (29%)	16 (28%)	92 (26%)
		Controls	3 (17%)	9 (23%)	12 (21%)	99 (24%)
			P=0.473	*P*=0.499	*P*=0.333	*P*=0.478
12-59 months	Enrolled	Cases	21	32	53	349
		Controls	36	51	87	510
	HRV positive, n (%)	Cases	4 (19%)	5 (16%)	9 (19%)	86 (25%)
		Controls	7 (19%)	6 (12%)	13 (15%)	84 (16%)
			P=0.971	*P*=0.615	*P*=0.734	*P*=0.003

Multivariate analysis of HRV prevalence between cases and controls by adjusting for confounding variates (<0.2 in univariate analysis) where applicable.

We examined for risk factors and associations for hospitalisation by comparing HRV positive case to all HIV-1-infected community controls irrespective of HRV status; Table 4.3. Among the HIV-1-infected population, HRV associated cases were younger (median 8.9 months) than HIV-1-infected control population (16 months, *P*=0.005). Furthermore, the HRV-associated HIV-1-infected cases were 0.39-fold (aOR 95% CI: 0.2-0.9) less likely to have been breastfed (24% vs. 39%, *P*=0.026) and 2-fold (aOR 95% CI: 1.1-3.9) more likely to be malnourished

(50% vs. 31%, P=0.035) compared to HIV-1-infected controls. The HRV positive cases were also more likely to have higher densities of *S. pneumoniae* colonisation in the nasopharynx (i.e. >6.9 $\log_{10}$ copies/mL; 28% vs 11%, P=0.001) compared to the HIV-1-infected control population, which in PERCH was a relative measure for microbiologically confirmed pneumococcal pneumonia [117]. There were no significant differences in the CD4+ counts (%) between the HRV associated HIV-1-infected cases compared to HIV-1-infected controls (mean 18.2 vs. 24.5%, P=0.294); although, CD4+ counts were only available for 12 of the HRV associated participants, 4 cases and 8 controls. There were no associations between hospitalisation and co-infection with other common respiratory viruses; however, the HIV-1-infected HRV associated cases were 5.72-fold (aOR 95% CI: 2.27-14.39) more likely to be co-infected with *Pneumocystis jiroveci* compared to the HIV-1-infected controls (30% vs 6%, P<0.001).

Table 4.3: Associations of demographic, clinical and laboratory findings of HIV-1-infected cases and controls by HRV status

	HIV+ Cases (n=204)	HIV+ HRV+ Cases (n=46)	HIV+ controls (n=210)	Unadjusted *P*-value*	OR (95%CI)	Adjusted *P*-value*	aOR (95% CI)
Age in months, mean (SD)	10.8 (12.3)	8.9 (9.7)	16.0 (15.3)	0.003		0.005	
Female, n(%)	104 (51%)	26 (57%)	111 (53%)	0.654	1.15 (0.61-2.21)	0.910	1.03 (0.5-2.0)
Never breast fed, n(%)	50 (25%)	11 (24%)	82 (39%)	0.057	0.49 (0.24-1.02)	0.026	0.39 (0.2-0.9)
Under weight, n(%)[a]	115 (56%)	23 (50%)	66 (31%)	0.018	2.18 (1.14-4.17)	0.035	2.04 (1.1-3.9)
Day care attendance, n(%)	11 (5%)	4 (9%)	18 (8%)	0.629	1.29 (0.46-3.67)	0.110	2.70 (.80-9.14)
Smoker in household, n(%)	55 (27%)	11 (24%)	76 (36%)	0.098	0.54 (0.26-1.12)	0.226	0.63 (0.30-1.33)
Premature birth, n(%)[b]	21 (10%)	6 (13%)	32 (15%)	0.105	0.49 (0.21-1.16)	0.72	0.44 (0.19-1.08)
Birth weight, mean (SD)	2.9 (0.6)	3.0 (0.8)	2.8 (0.6)	0.224		0.114	
Clinical features:							
Tachypnea, n(%)[c]	169 (84%)	39 (87%)	3 (27%)	*P*<0.001	17.3 (3.57-84.2)	0.003	13.2 (2.97-84.01)
Cough, n(%)	149 (73%)	32 (70%)	18 (9%)	*P*<0.001	22.9 (10.5-50.4)	*P*<0.001	36.96 (14.12-96.3)
Fever, n(%)[d]	166 (81%)	43 (93%)	9 (4%)	*P*<0.001	320 (83.2-1231)	*P*<0.001	651.0 (120-3516)
Diarrhoea, n(%)	44 (22%)	9 (20%)	4 (2%)	*P*<0.001	12.5 (3.65-42.6)	*P*<0.001	14.25 (3.92-51.89)
Rhinorrhoea, n(%)	59 (29%)	15 (33%)	16 (8%)	*P*<0.001	5.49 (2.5-12.12)	*P*<0.001	5.91 (2.50-13.95)
Markers for Bacterial co-infection:							
***LytA* positive, n (%)[e]**	26 (13%)	5 (12%)	18 (9%)	0.521	1.41 (0.49-4.05)	0.283	1.8 (0.6-5.5)
***S. pneu* load, mean (SD)[f]**	4.6 (2.5)	4.7 (2.7)	4.0 (2.5)	0.089		0.033	
HDP, n(%)[g]							
-Blood	18 (9%)	4 (10%)	9 (5%)	0.183	2.31 (0.67-7.88)	0.105	2.89 (0.80-10.42)
-NP	46 (23%)	13 (28%)	24 (11%)	0.005	3.04 (1.41-6.56)	0.001	3.96 (1.73-9.10)
CD4 counts(%), mean (SD)	12.3 (9.0)	18.2 (5.34)	24.5 (1.20)	0.292		0.294	0.95 (0.86-1.07)
Viral infections in the nasopharynx:							
-RSV, n(%)	19 (9%)	3 (7%)	5 (2%)	0.161	2.86 (0.66-12.4)	0.196	2.70 (0.60-12.18)
-AdV, n(%)	30 (15%)	4 (9%)	15 (7%)	0.716	1.24 (0.39-3.92)	0.603	1.39 (0.41-4.72)
-HMPV, n(%)	7 (3%)	2 (4%)	2 (3%)	0.601	1.55 (0.30-7.91)	0.703	1.38 (0.26-7.30)
-HBoV, n(%)	29 (14%)	9 (20%)	24 (11%)	0.141	1.89 (0.81-4.38)	0.217	1.74 (0.72-4.20)
-InFV A-C, n(%)	6 (3%)	1 (2%)	4 (2%)	0.905	1.14 (0.12-10.5)	0.972	0.96 (0.09-9.72)
-PIV, n(%)	25 (12%)	6 (13%)	13 (6%)	0.116	2.27 (0.82-6.33)	0.139	2.24 (0.77-6.49)
-HCoV, n(%)	16 (8%)	3 (7%)	21 (10%)	0.467	0.62 (0.18-2.20)	0.659	0.75 (0.21-2.69)
Fungal infections:							
-*P. jiroveci*, n(%)	66 (32%)	14 (30%)	13 (6%)	*P*<0.001	6.6 (2.84-15.31)	*P*<0.001	5.72 (2.27-14.39)

Abbreviations - OR: Odds ratio; aOR: Adjusted odds ratio; CI: Confidence interval; SD: Standard deviation; NP: Nasopharyngeal; HRV: Human rhinovirus; HIV: Human immunodeficiency virus; HDP: High density pneumococcus; RSV: Respiratory Syncytial Virus, HMPV: human metapneumovirus; AdV: adenovirus; PIV: parainfluenza type 1-4; HBoV: Human Bocavirus; HCoV: Human Coronavirus (OC43, NL63, 229E and HKU1) and InFV: Influenza Virus (A, B and C); *P. jiroveci: Pneumocystis jiroveci.*

**P*-values from Chi-squared and Wilcoxon tests by comparing HIV-1-infected HRV associated cases to all controls (regardless of HRV status), logistic regression models adjusted for confounding variates (<0.2 in univariate analysis) where applicable, Odds ratio could not be calculated for continuous variables or variables with 0 values, thus cells left blank.

[a] - Underweight defined as weight for age <-2SD of the median age-sex specific WHO reference; [b] - Premature birth defined as gestational age <37 weeks; [c] - Tachypnea defined as respiratory rate >60 breaths/minute if aged <2 months, respiratory rate >50 breaths/minute if aged 2-12 months, respiration rate >40 breaths/minute if aged >12 months; [d] - Fever defined as temperature ≥38^{o}C; [e] - Blood sample positive for *S. pneumoniae* colonisation by *LytA* PCR; [f] - *S. pneumoniae* bacterial load in the Nasopharynx and expressed as $\log_{10}$ copies/mL; [g] - HDP defined as *S. pneumoniae* density in nasopharynx >6.9 and/or density in whole blood sample >2.2 $\log_{10}$ copies/mL.

On comparison of HIV-1-infected HRV associated cases compared to HIV-1-infected HRV associated controls, there were no obvious associations between case status and HRV viral load or the presence of other respiratory or bacterial co-infections; except that the HIV-1-infected HRV associated cases (30%) were more than 9.11-fold (aOR 95% CI: 2.15-38.7) likely to have *P. jiroveci* co-infections than controls (9%, *P*=0.003); Table 4.4.

Table 4.4: Multivariate analysis for associations of HRV viral loads and co-infections between HIV-1-infected HRV associated cases and controls

	HIV+ Cases (n=46)	**HIV+ Controls (n=32)**	**Unadjusted *P*-value**	**OR (95%CI)**	**Adjusted *P*-value**	**aOR (95%CI)**
HRV Viral load, Mean (SD)[a]	3.5 (0.8)	3.8 (1.8)	0.330		0.472	
Single viral infection, n(%)[b]	27 (59%)	21 (66%)	0.537	0.74 (0.29-1.90)	0.459	0.68 (0.25-1.87)
-HRV Viral load, mean (SD)[c]	3.5 (0.6)	36 (0.9)	0.824		0.245	
HRV Co-infections, n(%)[d]	19 (41%)	11 (34%)	0.539	1.52 (0.10-5.75)	0.459	1.46 (0.53-3.99)
-HRV Viral load, mean (SD)[e]	3.5 (1.0)	4.3 (2.8)	0.297		0.405	
Viral infections in the nasopharynx:						
-HBoV, n(%)	9 (20%)	6 (19%)	0.928	1.05 (0.33-3.23)	0.804	0.86 (0.24-2.98)
-RSV, n(%)	3 (7%)	0	0.279		0.141	
-AdV, n(%)	4 (9%)	2 (6%)	0.691	1.43 (0.25-8.31)	0.970	0.96 (0.13-6.96)
-HMPV, n(%)	2 (4%)	0	0408		0.846	
-PIV, n(%)	6 (13%)	2 (6%)	0.341	2.25 (0.42-11.9)	0.179	3.3 (0.58-18.37)
-InFV A-C, n(%)	1 (2%)	1 (3%)	0.795	0.69 (0.04-11.4)	0.816	1.4 (0.08-24.43)
-HCoV, n(%)	3 (7%)	3 (9%)	0.644	0.67 (0.13-3.57)	0.305	0.4 (0.05-2.59)
HRV Fungal Co-infections with:						
-*P. jiroveci*, n(%)	14 (30%)	3 (9%)	0.036	4.23 (1.10-16.2)	0.003	9.11 (2.15-38.7)

Abbreviations - OR: Odds ratio; aOR: Adjusted odds ratio; CI: Confidence interval; SD: Standard deviation; NP: Nasopharyngeal; HRV: Human rhinovirus; HIV: Human immunodeficiency virus; RSV: Respiratory Syncytial Virus, HMPV: human metapneumovirus; AdV: adenovirus; PIV: parainfluenza type 1-4; HBoV: Human Bocavirus; HCoV: Human Coronavirus (OC43, NL63, 229E and HKU1) and InFV: Influenza Virus (A, B and C); *P. jiroveci: Pneumocystis jiroveci.*

P-values from Chi-squared and Wilcoxon tests, logistic regression models adjusted for confounding variates (<0.2 in univariate analysis) where applicable, Odds ratio could not be calculated for continuous variables or variables with 0 values, thus cells left blank.

[a] - HRV viral load in the nasopharynx, expressed as log10copies/mL; [b] - HRV was the only Respiratory virus detected in the nasopharynx; [c] - The HRV viral load in participants were HRV was the only respiratory virus detected in the nasopharynx, expressed as $\log_{10}$copies/mL; [d] - HRV was detected together with other common respiratory viruses in the nasopharynx; [e] - The HRV viral load in participants were HRV was detected together with other common respiratory viruses in the nasopharynx, expressed as $\log_{10}$ copies/mL.

4.1.4 HRV infection in the cases by HIV-1 infection status

A total of 323 HRV associated cases were enrolled into the study, of whom 14% (n=46) were HIV-1-infected and 86% (n=277) HIV-uninfected. Additionally, among the 1 245 HIV-uninfected cases, 413 (33%) were HEU and 509 (41%) were HIV- unexposed, with the remaining 26% (n=323) being HIV-uninfected albeit the maternal HIV status being unknown (HU-UE). Twenty-two percent (n=91/413) of HEU, 21% (n=108/509) of HIV-unexposed and 24% (n=78/323) of the HU-UE were positive for HRV infection (*P*=0.711). There were no

differences in the demographics and clinical characteristics of HRV associated cases between these three HIV-uninfected groups (data no shown), as such, further analysis focussed on comparing HRV-associated cases between the HIV-1-infected compared to the overall group of HIV-uninfected cases. The demographics of children with HRV associated pneumonia is shown in Table 4.5.

There were no differences in age, gender and being breastfed between the HIV-1-infected and HIV-uninfected HRV associated case; however, the HIV-1-infected cases were 2.87-fold (aOR 95% CI: 1.49-5.54) more likely to be clinically underweight (50% vs. 26%, P=0.002). In addition, the HRV associated HIV-1-infected cases were 2.85-fold (aOR 95% CI: 1.02-7.75) more likely to require supplementary oxygen therapy (87% vs. 77%, P=0.045) than the HRV associated HIV-uninfected cases. Furthermore, they were 2.42-fold (aOR 95% CI: 1.26-4.66) more likely to have abnormal chest X-rays, defined as primary endpoint pneumonia or any infiltrates, than the HIV-uninfected cases (63% vs. 42%, P=0.008). A sub-analysis which excluded cases with a normal CXR (HIV-1-infected and HIV-uninfected HRV associated cases), showed similar trend as the overall case analysis (Data not shown). The HIV-1-infected HRV associated case were also 2.99-fold (aOR 95% CI: 1.24-7.21) more likely to be associated with lethargy (20% vs. 7%, P=0.010); whereas, the HIV-1-infected HRV associated cases presented 0.08-fold (aOR 95% CI: 0.02-0.33) less commonly with wheeze (4% vs. 36%, P=0.001) than the HIV-uninfected HRV-associated cases. Moreover, after multivariate analysis with adjustments for potentially confounding variables including other co-infecting respiratory viruses, *P. jiroveci* and markers for bacterial co-infections, the HRV associated HIV-1-infected cases were 2.38-fold (aOR 95% CI: 1.10-5.17) more likely to present with hypoxia (74% vs. 60%, P=0.028) and were associated with a 4.89-fold (aOR 95% CI: 1.84-15.54) greater case fatality ratios (42%) compared to the HIV-uninfected cases (10%, P=0.001). There were no other associations between HIV-1 infection status among the HRV-association cases and the other markers for severe disease including prolonged hospital stays (>3 days), requiring mechanical ventilation and a diagnosis of very severe pneumonia.

Table 4.5: Demographical and clinical characteristics of HIV-1-infected and HIV-uninfected children hospitalised with HRV associated pneumonia

	HIV+ (n=46)	HIV- (n=277)	Unadjusted *P*-value	OR (95%CI)	Adjusted *P*-value	aOR (95% CI)
Age, months (SD)	8.9 (1.4)	9.9 (10.1)	0.564		0.561	
Gender, female, n(%)	26 (57%)	127 (46%)	0.182	1.54 (0.82-2.88)	0.204	1.50 (0.80-2.84)
Never breastfed, n(%)	11 (24%)	56 (20%)	0.567	1.24 (0.59-2.59)	0.384	1.43 (0.65-3.16)
Underweight, n(%)[a]	23 (50%)	71 (26%)	0.001	2.90 (1.53-5.49)	0.002	2.87 (1.49-5.54)
Day care attendance, n(%)	4 (9%)	25 (9%)	0.232	1.23 (0.88-1.72)	0.151	1.29 (0.91-1.83)
Smoker in household, n(%)	11 (24%)	100 (36%)	0.104	0.55 (0.27-1.13)	0.164	0.59 (0.29-1.24)
Premature birth, n(%)[b]	6 (13%)	35 (13%)	0.637	0.94 (0.73-1.21)	0.835	0.97 (0.75-1.26)
Birth weight, mean (SD)	3.0 (0.8)	2.9 (0.7)	0.751		0.624	
Clinical features:						
Very severe pneumonia, n(%)	16 (35%)	97 (35%)	0.975	0.99 (0.51-1.91)	0.961	0.97 (0.50-1.88)
Chest X-ray abnormal, n(%)[c]	29 (63%)	117 (42%)	0.010	2.33 (1.22-4.44)	0.008	2.42 (1.26-4.66)
Supplementary 02 therapy, n(%)	40 (87%)	213 (77%)	0.051	2.0 (0.94-4.94)	0.045	2.85 (1.02-7.75)
Mechanical ventilation, n(%)	3 (7%)	10 (4%)	0.359	1.86 (0.49-7.04)	0.434	1.72 (0.44-6.76)
Hypoxic, n(%)[d]	34 (74%)	164 (60%)	0.049	1.90 (1.0-3.87)	0.028	2.38 (1.10-5.17)
Tachycardia, n(%)[e]	28 (62%)	151 (55%)	0.336	1.37 (0.72-2.63)	0.401	1.33 (0.68-2.60)
Tachypnea, n(%)[f]	39 (87%)	234 (85%)	0.782	1.14 (0.45-2.86)	0.747	1.17 (0.46-2.97)
Wheezing, n(%)	2 (4%)	100 (36%)	0.001	0.10 (0.02-0.35)	0.001	0.08 (0.02-0.33)
Cough, n(%)	32 (70%)	221 (79%)	0.106	0.57 (0.29-1.13)	0.151	0.60 (0.29-1.22)
Lethargic, n(%)	9 (20%)	20 (7%)	0.009	3.13 (1.32-7.38)	0.010	2.99 (1.24-7.21)
Convulsions, n(%)	0	9 (3%)	0.213		0.391	
Diarrhoea, n(%)	9 (20%)	52 (19%)	0.899	1.05 (0.48-2.32)	0.944	0.98 (0.44-2.16)
Head nodding, n(%)	7 (15%)	70 (25%)	0.144	0.53 (0.27-1.24)	0.160	0.54 (0.22-1.28)
Central cyanosis, n(%)	2 (4%)	5 (2%)	0.288	2.47 (0.47-13.14)	0.315	2.42 (0.44-13.69)
Unable to Feed, n(%)	1 (2%)	9 (3%)	0.699	0.66 (0.08-5.34)	0.590	0.56 (0.07-4.66)
Vomiting everything, n(%)	1 (2%)	4 (1%)	0.712	1.52 (0.17-13.88)	0.793	1.34 (0.15-12.46)
Lower chest wall indrawing, n(%)	44 (96%)	261 (94%)	0.697	1.34 (0.30-6.07)	0.586	1.53 (0.33-7.03)
Stridor, n(%)	0	9 (3%)	0.312		0.443	
Grunting, n(%)	20 (22%)	28 (10%)	0.017	1.49 (1.08-2.06)	0.028	2.64 (1.11-6.31)
Nasal flaring, n(%)	34 (74%)	216 (78%)	0.542	0.80 (0.39-1.64)	0.694	0.86 (0.40-1.84)
Hospital stay>3 days, n(%)	35 (76%)	195 (70%)	0.431	1.34 (0.65-2.76)	0.398	1.38 (0.65-2.93)
Case fatality ratio, n(%)	14 (42%)	21 (10%)	*P*<0.001	6.45 (2.83-14.73)	0.007	4.89 (1.84-15.54)

Abbreviations - OR: Odds ratio; aOR: Adjusted odds ratio; CI: Confidence interval; SD: Standard deviation; NP: Nasopharyngeal; HRV: Human rhinovirus; HIV: Human immunodeficiency virus

P-values from Chi-squared and Wilcoxon tests, logistic regression models adjusted for confounding variates (<0.2 in univariate analysis) where applicable, Odds ratio could not be calculated for continuous variables or variables with 0 values, thus cells left blank.

[a] - Underweight defined as weight for age <-2SD of the median age-sex specific WHO reference; [b] - Premature birth defined as gestational age <37 weeks; [c] - Abnormal chest X-rays defined as defined as primary end point pneumonia or infiltrates; [d] - Hypoxic defined as 1) a room air pulse-oximetry reading indicated oxygen saturation <90% at the two sites at elevation (Zambia and South Africa) or <92% at all other sites, or 2) a room air oxygen saturation; [e] - Tachycardia defined as heart rate >160 beats per minute (bpm) if aged <11 months, heart rate >150 bpm if aged 12-35 months, heart rate >140 bpm if aged 36-59 months; [f] - Tachypnea defined as respiratory rate >60 breaths/minute if aged <2 months, respiratory rate >50 breaths/minute if aged 2-12 months, respiration rate >40 breaths/minute if aged >12 months.

Among markers for possible bacterial co-infection; Table 4.6, after multivariate analysis, the HRV associated HIV-1-infected cases had a 6.55-fold (aOR 95% CI: 1.97-21.76) higher prevalence of fever (93% vs. 68%, *P*=0.002), alveolar consolidation on CXR (52% vs. 26%, *P*=0.002), as well as high colonisation densities (>6.9 copies/ml) of *S. pneumoniae* in their nasopharynx (28% vs. 8%, *P*<0.001) compared to HRV associated HIV-uninfected cases. Additionally, the HRV associated HIV-1-infected cases were 2.19-fold (aOR 95% CI: 1.13-

4.20) more likely to have CRP ≥40mg/mL (41% vs. 25%, *P*=0.020), microbiologically confirmed pneumococcal pneumonia (4% versus 0%, *P*=0.027) and 3.42-fold (aOR 95% CI: 1.06-10.99) more likely to have positive blood culture for non-contaminate bacteria (11% vs. 3%, *P*=0.038) compared to the HIV-uninfected HRV-associated cases. After regression analysis, there were no association between HRV associated HIV-1-infected cases and whole blood counts - elevated white blood cells, neutrophils, eosinophils or lymphocytes.

Table 4.6: Markers for possible bacterial co-infections of HIV-1-infected and HIV-uninfected children hospitalised with HRV associated pneumonia

	HIV+ (n=46)	HIV- (n=277)	Unadjusted		Adjusted	
			P-value	OR(95%CI)	*P*-value	aOR (95% CI)
Fever, n(%)[a]	43 (93%)	189 (68%)	0.002	6.68 (2.01-22.10)	0.002	6.55 (1.97-21.76)
Alveolar consolidation, n(%)	22 (52%)	67 (26%)	0.001	3.05 (1.56-5.94)	0.001	3.02 (1.54-5.93)
Leucocytosis, n(%)[b]	20 (45%)	136 (49%)	0.638	0.86 (0.45-1.63)	0.767	0.91 (0.47-1.74)
Neutrophils (%), median (IQR)	43.4 (19.1)	50 (36.6-64.3)	0.042		0.062	
Lymphocyte (%), median (IQR)	44.1 (17.6)	37.5 (25.4-50)	0.048		0.074	
Eosinophil (%), median (IQR)	0.2 (0.1-0.9)	0.7 (0.1-1.8)	0.202		0.206	
CRP ≥40mg/l, n(%)[c]	19 (41%)	69 (25%)	0.023	2.12 (1.11-4.05)	0.020	2.19 (1.13-4.20)
Blood culture positive, n(%)	5 (11%)	9 (3%)	0.027	3.63 (1.16-11.37)	0.038	3.42 (1.06-10.99)
***LytA* positive, n(%)[d]**	5 (12%)	15 (6%)	0.123	2.32 (0.80-6.78)	0.159	2.17 (0.74-6.42)
MCPP, n(%)[e]	2 (4%)	0	0.027		0.036	
HDP, n(%)[f]						
-Blood	4 (10%)	8 (3%)	0.050	3.48 (1.0-12.15)	0.063	3.30 (0.94-11.62)
-NP	13 (28%)	22 (8%)	*P*<0.001	4.57 (2.10-9.92)	*P*<0.001	4.75 (2.13-10.52)

Abbreviations - HIV: Human Immunodeficiency virus; OR: Odds ratio; aOR: Adjusted odds ratio; CI: Confidence interval; IQR: Inter quartile range; CRP: C-reactive protein; MCPP: Microbiologically confirmed pneumococcus pneumonia; HDP: High density pneumococcus; NP: Nasopharyngeal; HRV: Human rhinovirus.
P-values from chi-square and Wilcoxon tests - logistic regression models adjusted for confounding variates (<0.2 in univariate analysis) where applicable; odds ratios could not be calculated for variables with zero variables.
[a] - Fever was defined as body temperate ≥38^{o}C; [b] - Leucocytosis defined as white blood cell count >15 000 cells/uL if age <12 months, white blood cell count >13 000 cells/uL if age >12 months; [c] - CRP defined as levels ≥40mg/mL are considered to be medically significant of indirect evidence of bacterial co-infection; [d] - Blood sample positive for *S. pneumoniae* colonisation by *LytA* PCR; [e] - MCPP defined when *S. pneumoniae* was cultured from a normally sterile body fluid - blood, pleural fluid or lung aspirate - or pleural fluid or lung aspirate was PCR *LytA* positive; [f] - HDP defined as *S. pneumoniae* density in nasopharynx>6.9 and density in whole blood sample>2.2 $\log_{10}$ copies/mL.

Additional analysis was conducted in order to determine whether HRV viral loads and co-infection were associated with HIV-1 infection status in the HRV-associated cases; Table 4.7. We found that the prevalence of HRV mono-infections were similar between HIV-1-infected (59%) compared to HIV-uninfected cases (57%, *P*=0.798) in whom HRV was identified, and similarly the prevalence of other respiratory virus co-infections did not differ by HIV-status, except for a trend for the HIV-1-infected cases to have more HRV-PIV co-infections (13% vs. 5%, aOR 2.87, 95% CI: 1.02-8.06, *P*=0.045) compared to the HIV-uninfected cases. Additionally, the HRV associated HIV-1-infected cases were 3.23-fold (aOR 95% CI: 1.47-7.11) more likely to have co-infections with *P. jiroveci:* (30% vs. 12%, *P*=0.003) and 4.09-

fold (aOR 95% CI: 1.75-9.59) more likely to have pulmonary Tuberculosis (14%) than HIV-uninfected HRV associated cases (4%, *P*=0.001).

Table 4.7: Multivariate analysis for associations between HRV viral loads and co-infections among HRV-associated HIV-1-infected and HIV-uninfected cases

	HIV+ (n=46)	HIV- (n=277)	Unadjusted *P*-value	OR (95%CI)	Adjusted *P*-value	aOR (95% CI)
HRV Viral load, Mean (SD)[a]	3.5 (0.8)	3.7 (3.6)	0.158		0.197	
Single viral infection, n(%)[b]	27 (59%)	159 (57%)	0.869	1.05 (0.56-1.99)	0.798	1.09 (0.57-2.08)
-HRV Viral load, Mean (SD)[c]	3.5 (0.6)	3.7 (0.9)	0.254		0.264	
HRV Co-infections, n(%)[d]	19 (41%)	118 (43%)	0.869	0.95 (0.50-1.79)	0.798	0.92 (0.48-1.75)
-HRV Viral load, Mean (SD)[e]	3.5 (0.9)	3.7 (0.9)	0.399		0.537	
Viral co-infections in the nasopharynx[g]:						
-HBoV, n(%)	9 (20%)	36 (13%)	0.237	1.62 (0.73-3.65)	0.258	1.61 (0.70-3.70)
-RSV, n(%)	3 (7%)	41 (15%)	0.142	0.40 (0.12-1.36)	0.118	0.37 (0.11-1.28)
-AdV, n(%)	4 (9%)	27 (10%)	0.823	0.823 (0.29-2.65)	0.824	0.87 (0.28-2.74)
-HMPV, n(%)	2 (4%)	9 (3%)	0.705	1.35 (0.28-6.47)	0.771	1.26 (0.26-6.07)
-PIV, n(%)	6 (13%)	13 (5%)	0.033	3.05 (1.10-8.47)	0.045	2.87 (1.02-8.06)
-InFV A-C, n(%)	1 (2%)	3 (1%)	0.544	2.03 (0.21-19.49)	0.593	1.88 (0.19-18.83)
-HCoV, n(%)	3 (7%)	17 (6%)	0.920	1.07 (0.30-3.80)	0.905	1.08 (0.30-8.87)
Fungal Co-infections with:						
-*P. jiroveci*, n(%)	14 (30%)	34 (12%)	0.002	3.12 (1.52-6.45)	0.003	3.23 (1.47-7.11)
Co-infection with TB, n(%)	11 (14%)	24 (4%)	0.003	3.13 (1.49-7.35)	0.001	4.09 (1.75-9.59)

Abbreviations - OR: Odds ratio; aOR: Adjusted odds ratio; CI: Confidence interval; SD: Standard deviation; NP: Nasopharyngeal; HRV: Human rhinovirus; HIV: Human immunodeficiency virus; RSV: Respiratory Syncytial Virus, HMPV: human metapneumovirus; AdV: adenovirus; PIV: parainfluenza type 1-4; HBoV: Human Bocavirus; HCoV: Human Coronavirus (OC43, NL63, 229E and HKU1) and InFV: Influenza Virus (A, B and C); *P. jiroveci: Pneumocystis jiroveci*: TB: Tuberculosis.

P-values from Chi-squared and Wilcoxon tests, logistic regression models adjusted for confounding variates (<0.2 in univariate analysis) where applicable, Odds ratio could not be calculated for continuous variables or variables with 0 values, thus cells left blank.

[a] - HRV viral load in the nasopharynx, expressed as $\log_{10}$copies/mL; [b] - HRV was the only Respiratory virus detected in the nasopharynx; [c] - The HRV viral load in participants were HRV was the only respiratory virus detected in the nasopharynx, expressed as $\log_{10}$copies/mL; [d] - HRV was detected together with other common respiratory viruses in the nasopharynx; [e] - The HRV viral load in participants were HRV was detected together with other common respiratory viruses in the nasopharynx, expressed as $\log_{10}$ copies/mL.

4.2 DISCUSSION

In this study, it was found that HIV-1 infection was not an independent risk factor for hospitalisation with HRV-associated pneumonia; however, HIV-1 infection was associated with a 4.89-fold higher case fatality ratio among children hospitalised with HRV-associated severe and very severe pneumonia. This study provides evidence that HRV is ubiquitous during severe childhood disease in both HIV-1-infected (23%) and HIV-uninfected children (22%). Although HRV was one of the most prevalent respiratory viruses detected in the hospitalised children with pneumonia (22%), irrespective of HIV-1 infection status, it was also detected at similar prevalence in the age-group matched control population (20% overall, 15% and 21% among HIV-1-infected and -uninfected controls). The detection rates reported in this study are similar to those reported in other studies conducted in Africa (24%-32%) which have looked at HIV-infection and HRV co-infections in children with LRTI [54, 80, 89, 132].

To the best of our knowledge, this is one of the first studies reporting on HRV prevalence in HIV-1-infected community control children living in Africa. There was however a study conducted in South African in all age groups which looked at the attributable role of rhinovirus and reported on the HIV prevalence (29% among cases and 43% among controls which was reflective of the control enrolment criteria); however, this study did not report on HIV-1 incidences in children <5 years of age and did not report on HRV prevalence among the HIV positive cases and controls. They did however determine that HRV was mildly associated with severe disease with an attributable fraction of 46.9% among the entire population and 45.7% among children under the age of 5 years [137]. Another study conducted in South Africa [54] was the only other study which reported on HRV prevalence and HIV-infection status among sick children; and although they enrolled 46 asymptomatic controls, their HIV status was unknown. The prevalence of HRV was reported to be 18.8% among the control population in that study which is similar to other studies reporting on HRV prevalence in control populations (18.8-50%) [99]. This is comparable to the overall control population prevalence of HRV reported in our study (20%). Furthermore, in our study we found that the prevalence of HRV was 1.56-fold higher in HIV-uninfected controls (21%) compared to HIV-1-infected controls (15%, P=0.045). The HRV associated HIV-1-infected controls were, however, 3.45-fold more likely to have respiratory tract infection symptoms compared to HRV associated HIV-uninfected controls thus among the controls HIV-1-

infection was an independent risk factor for HRV-associated RTI not requiring hospitalisation. Nonetheless, the detection of HRV does not necessarily imply causality of disease as HRV have been shown to shed for several days post infection [124] and it has been shown for other respiratory viruses (namely RSV) that virus shedding can be considerably longer in HIV-1-infected individuals [138]. Our study was not designed to distinguish between HRV detection in relation to shedding from previous infections, incubation period of current infections and controls which have asymptomatic infections thus additional longitudinal studies are needed to fully clarify the role of HIV-1-infections during HRV-associated disease episodes as well as the effects and length of HRV shedding in HIV-1-infected individuals.

Nevertheless, the association between HRV detection and RTI in the HIV-1-infected controls infers a pathogenic role of HRV causing RTI in the HIV-1-infected population not requiring hospitalisation. Moreover, there was a trend for HRV detection to be associated with case status in the HIV-1-infected participants (23% vs. 15% for the cases vs. controls, P=0.059) which was mainly driven by 1-5 month age group (22% vs. 11% for the cases vs. controls, P=0.061). Thus suggesting that immunosuppression and young age group might be risk factors for development of HRV associated severe pneumonia. Future studies designed to analyse population based attack rates are required to study the associations between immunosuppression and HRV-infection among different age groups further; however, the association between HIV and HRV was also seen in a study conducted in Mozambique, which determined that HIV was associated with an increased risk of HRV-associated LRTI [80].

Additional risk factors for HRV-associated disease were seen in the case-control comparison of HIV-1-infected participants, with the cases being younger and clinically malnourished compared to the controls. Conversely, on comparison of viral co-infections in HIV-1-infected participants there were no observed relationships between HRV-associated cases compared to the controls thus viral co-infections do not appear to be a risk factor for hospitalisation. The relationship between bacterial co-infection is more difficult to fully clarify as current methods for detecting bacterial infections are insensitive i.e. blood culture has a sensitivity of only 15-30% and direct sampling from site of infection remains challenging and highly invasive [95, 111]. Thus the majority of bacterial data are from nasopharynx sampling which is unreliable

as children are well known to be colonised with large numbers of different bacteria during the first years of life. Consequently, markers for potential bacterial co-infections were utilised to study bacterial co-infections, these include fever, alveolar consolidation, CRP levels ≥40mg/mL and elevated white blood cell, lymphocyte and neutrophil counts are generally indicative of bacterial infections. Additionally, in PERCH it was found that *S. pneumoniae* colonisation densities above a threshold (>6.9 log10copies/mL in the nasopharynx and >2.2 log10copies/mL in the blood) were strongly correlated with pneumococcal pneumonia [117]. Blood counts, CRP levels and chest X-rays were not performed on the controls so of the remaining potential bacterial markers available for both the cases and controls - fever and high *S. pnuemoniae* colonisation densities - the HRV positive HIV-1-infected cases were more associated with both compared to the HIV-1-infected controls. Thus we can conclude that HRV positive HIV-1-infected cases were most likely associated with more bacterial co-infected than the HIV-1-infected controls. This concurs with reports which have suggested that bacterial infections are in fact an essential part in the pathogenesis of viral infections progressing to severe respiratory disease [80, 139]. Additionally, the HIV-1-infected cases positive for HRV infections were 5.72-fold more likely to be co-infected with *Pneumocystis jiroveci* than the HIV-1-infected controls. None of the other common risk factors for respiratory virus associated RTI - including day care attendance, a regular smoker in the household, premature birth or low birth weight - appeared to be relevant in our study population.

The large study population of children hospitalised with pneumonia (n=1 449) of which 14% (n=204) were HIV-1-infected allowed us to describe the epidemiology of HRV-associated pneumonia (n=323) in more detail than any previous study on HRV in children living in a high HIV burden setting. Among the HRV-associated cases, there were no differences in socio-economic or demographic characteristics between HIV-1-infected and HIV-uninfected cases, except the HIV-1-infected cases were almost 3-fold more likely to be malnourished that the HIV-uninfected cases. Additionally, the HRV positive HIV-1-infected cases were more associated with fever, alveolar consolidation, increased CRP levels, MCPP, positive blood cultures and high colonisation densities of *S. pneumoniae* in the nasopharynx and blood compared to the HRV positive HIV-uninfected cases. Thus providing evidence that the HRV positive HIV-1-infected cases were more likely to have concurrent bacterial infections compared to the HIV-uninfected HRV positive cases. Furthermore, in comparison the HIV-uninfected cases, the HIV-1-infected cases associated with HRV-infections were also more

likely to be co-infected with tuberculosis and *P. jiroveci* which is a fungal parasite well known to cause opportunistic infections in immunocompromised patients often with fatal outcomes [6, 140].

The associations of the HRV-associated HIV-1-infected cases with more concurrent bacterial and fungal infections could account for the differences in the clinical presentation of HRV-associated disease between the HIV-1-infected and HIV-uninfected cases with the HIV-1-infected cases being 1.90-fold more likely to present with hypoxia and subsequently 2.0-fold more likely to require supplementary oxygen therapy than the HRV-associated HIV-uninfected cases. Additionally, the HRV-associated HIV-1-infected cases were 2.33-fold more likely to have abnormal chest X-ray findings and 3.13-fold more likely to present with lethargy than the HRV-associated HIV-uninfected cases. Furthermore, among the HRV-associated cases, the HIV-1-infected cases had a 6.45 increased odds of death. However, these associations of HRV-associated cases with concurrent HIV-1 infections with more severe disease remained even after multi-logistic regression analysis adjusting for potentially confounding by co-infecting viruses, bacteria and *P. jiroveci*. After multivariate modelling, the HRV-associated HIV-1-infected cases were 2.38-fold more likely to present with hypoxia and 2.85-fold more likely to require supplementary oxygen therapy; furthermore, they were 2.42-fold more likely to have abnormal chest X-ray findings and 2.99-fold more likely to present with lethargy than the HIV-uninfected cases. Moreover, even after adjusting for co-infecting viruses, bacteria, *P. jiroveci* and other potentially confounding variables the HRV-associated cases with concurrent HIV-1-infections had a 4.89-fold increased odds of death compared to the HRV-associated HIV-uninfected cases.

A major study limitation is that due to the very sensitive detection capabilities of multiplex PCR the detection of several potential pathogens among the cases was highly likely but the extent to which each of the detected pathogens was contributing to the disease episode could not be determined. This is especially true for HRV which is frequently detected in the airways of asymptomatic individuals. Thus the burden of HRV disease reported in this study could potentially be over-estimating the true incidence of HRV related LRT disease.

In conclusion, the present study contributes to the understanding of the prevalence of HRV associated disease in children with respiratory tract infections, including children hospitalised

with pneumonia, across different age-groups living in high HIV-1-infection setting. HIV-infection was found to be a risk factor for RTI among the HRV-associated community controls not requiring hospitalisation and a risk factor for concurrent bacterial and fungal superinfections and death among children hospitalised with HRV-associated pneumonia.

5.0 The molecular subtyping of Human rhinovirus (HRV) in children from three Sub-Saharan African countries

HRV is currently classified into three different species: HRV-A, HRV-B and HRV-C [19-22]. The three species are detected perennially **[45]** and have been associated with ARI episodes of varying severity in children **[35-39]**. In this chapter we characterise the molecular subtyping of HRV in children living in Zambia, Mali and South Africa. The children were 1-59 months of age, and included those hospitalised for severe or very severe pneumonia and age-frequency matched community controls.

5.1 RESULTS

5.1.1 The study population

A total of 2 120 children hospitalised for severe or very severe pneumonia were enrolled between the three Sub-Saharan African sites into the PERCH project. Of these children, 439 (21%) tested positive for HRV by PCR analysis of nasopharyngeal swabs, which were subtyped. Among the pneumonia cases the prevalence of HRV associated pneumonia was highest in South Africa (23%, n=210/911), followed by Zambia (21%, n=113/538) and lowest in Mali (17%, n=116/671, P=0.020).

In addition, 2 290 age-frequency matched community controls were enrolled, among whom the prevalence of HRV infection (20%, n=462) was similar to cases (23%, P=0.852). The HRV prevalence among controls was similar across the sites, including 22% in South Africa (n=212/964), 20% in Mali (n=143/725) and 18% in Zambia (n=107/686, P=0.093). There was no difference in the prevalence of HRV detection between cases and controls (P=0.693); furthermore, the prevalence of HRV identification did not differ between cases and controls at the individual sites (P=0.148). By age group there was however a difference in HRV prevalence among children 1-5 years of age with HRV detection being 1.82-fold (aOR 95% CI: 1.31-2.53) higher among cases (21%, n=120/563) compared to among controls (15%, n=126/797, P=0.001); conversely, among infants there were no differences in HRV prevalence among cases (20%, n=319/1 557) and controls (23%, n=336/ 1 487, P=0.503).

5.1.2 HRV clinical and molecular subtyping among community controls

Of the 462 HRV positive samples identified among community controls, 97% (n=446) were successfully amplified, 3% (n=14) failing to amplify and 0.5% (n=2) being unavailable for serotyping due to insufficient volume of the archived sample. Of the 446 successfully amplified samples, 5% (n=24) were typed as enterovirus, one as an Echovirus, and 92% (n=421) successfully speciated for HRV. The dominant HRV species among controls were HRV-C (45%, n=191) and HRV-A (45%, n=190), whilst 10% were HRV-B (n=40). There were no differences in HRV species distribution by age groups with HRV-A (46%) and HRV-C (44%) being most prevalent among infants followed by HRV-B (10%); and similarly among children 1-5 years of age HRV-C was most prevalent (49%) followed by HRV-A (44%) and HRV-B (7%, P=0.520).

HRV-B, the least prevalent species (10%), was identified sporadically throughout the year, Figure 5.1. There were, however no statistical differences in demographics and clinical symptoms among controls with HRV-B compared to those with HRV-A and HRV-C infection; Table 5.1, albeit the low numbers limited statistical comparisons. Thus, to further investigate for clinical difference between HRV species, we specifically analysed HRV-A to HRV-C associated controls; Table 5.1 and 5.2. Except for HRV-A associated controls being 3.25-fold (aOR 95% CI: 1.30-8.14) more likely to have *S. pneumoniae* detected in whole blood (*LytA* positive) than HRV-C associated controls (10% vs. 4%, P=0.012), there were no other differences in demographic, risk factors associated with developing respiratory infections (including day care attendance, regular smoker in the household, premature birth and low birth weight) and symptoms of RTI between controls associated with HRV-A compared to HRV-C infections. Additionally, there were no differences in the nasopharyngeal viral loads of the HRV species. Although there was no difference in the overall prevalence of other respiratory virus co-infections between HRV-A (32%) and HRV-C (36%, P=0.713) infected controls, co-infection with PIV was 3.8-fold (aOR 95% CI: 1.17-12.42) more common among HRV-C (6%) than HRV-A (2%, P=0.026) associated community controls. The individual site analysis is shown in Table 5.3.

Table 5.1: The demographical and clinical characteristics of the Community controls infected with the three HRV species

	HRV-A (n=190)	HRV-B (n=40)	HRV-C (n=191)	Unadjusted *P*-value	OR (95% CI)	Adjusted *P*-value	aOR (95% CI)
Age in months, mean (SD)	9.7 (9.9)	7.3 (7.6)	10.8 (10.7)	0.300		0.230	
Female, n(%)	93 (49%)	23 (58%)	89 (47%)	0.646	1.10 (0.73-1.64)	0.854	1.04 (0.69-1.57)
HIV+, n (%)	14 (7%)	1 (3%)	13 (7%)	0.967	0.98 (0.45-2.17)	0.999	0.99 (0.47-2.24)
HEU, n (%)[a]	32 (18%)	6 (15%)	39 (22%)	0.382	0.79 (0.47-1.34)	0.178	0.60 (0.34-1.26)
Never breast fed, n(%)	49 (26%)	12 (30%)	49 (26%)	0.340	1.29 (0.76-2.18)	0.420	0.82 (0.50-1.34)
Under weight, n (%)[b]	22 (12%)	7 (18%)	16 (8%)	0.299	1.43 (0.73-2.82)	0.151	1.67 (0.83-3.36)
Day care attendance, n(%)	44 (23%)	14 (35%)	65 (34%)	0.023	0.59 (0.38-0.93)	0.323	0.74 (0.40-1.35)
Smoker in household, n(%)	53 (28%)	6 (15%)	52 (27%)	0.884	1.04 (0.66-1.62)	0.905	0.97 (0.62-1.54)
Premature birth, n(%)[c]	32 (17%)	6 (15%)	34 (18%)	0.795	0.98 (0.82-1.17)	0.618	0.95 (0.79-1.15)
Birth weight, mean (SD)	2.9 (0.6)	3.0 (0.7)	3.1 (0.5)	0.196		0.328	
Clinical features:							
Tachypnea, n(%)[d]	16 (9%)	5 (13%)	13 (7%)	0.530	1.28 (0.60-2.74)	0.433	1.37 (0.62-3.0)
Cough, n(%)	13 (7%)	2 (5%)	13 (7%)	0.993	1.00 (0.73-1.38)	0.757	1.14 (0.50-2.57)
Fever, n(%)[e]	1 (1%)	0	3 (2%)	0.343	0.33 (0.03-3.23)	0.481	0.44 (0.04-4.39)
Diarrhoea, n(%)	1 (1%)	1 (3%)	0	0.364		0.462	
Rhinorrhoea, n(%)	18 (9%)	4 (10%)	33 (17%)	0.605	0.91 (0.62-1.32)	0.310	0.69 (0.34-1.41)
Laboratory markers:							
CRP≥40mg/l, n(%)[f]	2 (1%)	0	2 (1%)	0.996	1.0 (0.14-7.21)	0.847	1.22 (0.16-9.0)
***LytA* positive, n(%)[g]**	19 (10%)	4 (10%)	7 (4%)	0.019	2.91 (1.20-7.10)	0.012	3.25 (1.30-8.14)
HDP, n(%)[h]							
-Blood	12 (6%)	3 (8%)	5 (3%)	0.092	2.50 (0.87-7.23)	0.058	2.88 (0.97-8.16)
-NP	29 (15%)	3 (8%)	35 (18%)	0.425	0.80 (0.47-1.38)	0.458	0.81 (0.47-1.41)
HRV viral load, mean (SD)[i]	3.5 (0.9)	3.3 (0.7)	3.5 (0.9)	0.542		0.138	
HRV mono-infection, n(%)[j]	130 (68%)	27 (67%)	123 (64%)	0.406	1.20 (0.78-1.83)	0.713	1.10 (0.70-1.68)
Viral co-infections in the nasopharynx:							
-AdV, n(%)	17 (9%)	0	22 (12%)	0.409	0.75 (0.39-1.47)	0.635	0.85 (0.43-1.68)
-RSV, n(%)	5 (3%)	4 (10%)	5 (3%)	0.993	1.00 (0.29-3.53)	0.915	0.93 (0.26-3.31)
-HBoV, n(%)	21 (11%)	2 (5%)	25 (13%)	0.542	0.83 (0.44-1.53)	0.797	0.92 (0.49-1.76)
-HMPV, n(%)	4 (2%)	3 (8%)	0	0.044		0.168	
-InFV A-C, n(%)	1 (1%)	2 (5%)	0	0.179		0.175	
-PIVs, n(%)	4 (2%)	3 (8%)	12 (6%)	0.050	3.15 (1.0-9.95)	0.026	3.81 (1.17-12.42)
-HCoVs, n(%)	16 (8%)	0	29 (15%)	0.044	0.51 (0.27-0.98)	0.091	0.57 (0.29-1.10)

Abbreviations - HIV: Human Immunodeficiency virus; HEU: HIV exposed but uninfected; OR: Odds ratio; aOR: Adjusted odds ratio; CI: Confidence interval; SD: Standard deviation; HDP: High density pneumococcus; CRP: C-reactive protein; NP: Nasopharyngeal; HRV: Human rhinovirus; RSV: Respiratory Syncytial Virus (A and B), HMPV: Human Metapneumovirus; AdV: Adenovirus; PIV: Parainfluenza type 1-4; HBoV: Human Bocavirus; HCoV: Human Coronavirus (OC43, NL63, 229E and HKU1) and InFV A-C: Influenza Virus (A, B and C).

P-values calculated by comparing HRV-A to HRV-C using chi-square and Wilcoxon tests - logistic regression models adjusted for confounding variates (<0.2 in univariate analysis) where applicable; Odds ratios could not be calculated for variables with zero variables.

[a] - HEU defined as HIV-uninfected but HIV-exposed. Undetectable viral load, HIV seronegative in the child with a positive maternal history. Positive maternal status based on self-report was accepted, except for seronegative children < 7 months of age where documented positive maternal status was required; [b] - Underweight defined as weight for age <-2SD of the median age-sex specific WHO reference; [c] - Premature birth defined as gestational age <37 weeks; [d] - Tachypnea defined as respiratory rate >60 breaths/minute if aged <2 months, respiratory rate >50 breaths/minute if aged 2-12 months, respiration rate >40 breaths/minute if aged >12 months; [e] - Fever defined as temperature ≥38^{o}C; [f] - CRP defined as levels ≥40mg/mL which are considered to show potential bacterial infection. Only a subset of randomly chosen controls had CRP testing conducted at the South African site; [g] - Blood sample positive for *S. pneumoniae* colonisation by *LytA* PCR; [h] - HDP defined as *S. pneumoniae* density in nasopharynx >6.9 and/or density in whole blood sample >2.2 $\log_{10}$ copies/mL; [i] - HRV viral load in the nasopharynx, expressed as log copies/mL; j - HRV was the only respiratory virus detected in the nasopharynx.

Among controls with HRV infection, HRV was identified as the sole respiratory virus in the nasopharynx among 67% (n=280/421) of controls, including 68% (n=130/190) of HRV-A;

67% (n=27/40) of HRV-B and 64% (n=123/191) of HRV-C infections (P=0.70). There were no significant differences in demographics, clinical presentation or laboratory markers when comparing controls with only HRV infections to those with other respiratory virus coinfections, either overall or specifically for HRV-A and HRV-C; Table 5.2.

Table 5.2: The demographical and clinical characteristics of the Community controls by HRV species and mono vs. mixed infections

	HRV-A mono (n=130)	HRV-A mixed (n=60)	*P*-value[a]	HRV-B mono (n=27)	HRV-B mixed (n=13)	*P*-value[b]	HRV-C mono (n=123)	HRV-C mixed (n=47)	*P*-value[c]	*P*-value[d]
Age in months, mean (SD)	9.6 (10.1)	10.0 (9.3)	0.775	7.9 (8.8)	5.8 (4.1)	0.427	10.7 (11.3)	11.0 (9.5)	0.297	0.606
Female, n(%)	70 (54%)	23 (38%)	0.053	15 (56%)	8 (62%)	0.720	59 (48%)	30 (44%)	0.582	0.437
HIV+, n (%)	9 (7%)	5 (8%)	0.456	1 (4%)	0	0.210	9 (7%)	4 (6%)	0.444	0.931
HEU, n (%)[e]	22 (18%)	10 (18%)	0.996	4 (15%)	2 (15%)	0.998	27 (24%)	12 (19%)	0.445	0.299
Never breast fed, n (%)	27 (21%)	11 (18%)	0.612	3 (11%)	2 (15%)	0.702	18 (15%)	13 (19%)	0.161	0.388
Under weight, n (%)[f]	13 (10%)	9 (15%)	0.371	3 (11%)	4 (41%)	0.125	12 (10%)	4 (6%)	0.352	0.763
Day care attendance, n(%)	21 (16%)	23 (38%)	0.037	11 (41%)	3 (23%)	0.273	34 (28%)	31 (46%)	0.279	0.298
Smoker in household, n(%)	39 (30%)	14 (23%)	0.430	3 (11%)	3 (23%)	0.321	38 (31%)	14 (21%)	0.193	0.769
Premature birth, n(%)[g]	22 (17%)	12 (20%)	0.635	5 (19%)	1 (8%)	0.369	21 (17%)	11 (16%)	0.942	0.529
Birth weight, mean (SD)	2.9 (0.6)	3.1 (0.6)	0.065	2.9 (0.7)	2.8 (0.4)	0.528	3.0 (0.5)	3.1 (0.6)	0.658	0.763
Clinical features:										
Tachypnea, n(%)[h]	9 (8%)	7 (13%)	0.402	3 (12%)	2 (15%)	0.770	7 (6%)	6 (10%)	0263	0.607
Cough, n(%)	7 (5%)	6 (10%)	0.178	2 (8%)	0	0.314	8 (7%)	5 (7%)	0.397	0.761
Fever, n(%)[i]	1 (1%)	0	0.925	0	0		1 (1%)	2 (3%)	0.431	0.815
Diarrhoea, n(%)	1 (1%)	0	0.442	0	1 (8%)	0.144	0	0		0.509
Rhinorrhoea, n(%)	8 (6%)	10 (17%)	0.051	4 (15%)	0	0.144	19 (15%)	14 (21%)	0.488	0.101
Laboratory markers:										
CRP≥40mg/l, n(%)[j]	2 (1%)	0	0.573	0	0		2 (1%)	0	0.762	0.409
***LytA* positive, n(%)[k]**	10 (8%)	9 (15%)	0.293	3 (11%)	1 (8%)	0.736	4 (3%)	3 (4%)	0.780	0.078
HDP, n(%)[l]										
-Blood	6 (5%)	6 (10%)	0.342	2 (7%)	1 (8%)	0.974	3 (3%)	2 (3%)	0.987	0.258
-NP	19 (15%)	10 (17%)	0.847	3 (11%)	1 (8%)	0.736	23 (19%)	12 (18%)	0.843	0.471

Abbreviations - SD: Standard deviation; HRV: Human rhinovirus; HIV: Human immunodeficiency virus; HEU: HIV exposed but HIV-uninfected; CRP: C-reactive protein; HDP: High density pneumococcus; NP: Nasopharyngeal.

P-values from Chi-squared and Wilcoxon tests, logistic regression models adjusted for confounding variates (<0.2 in univariate analysis) where applicable. *P*-values could not be calculated for variables where both values are 0, thus cells left blank

[a] - *P*-values for the comparison of HRV-A mono vs. mixed infections; [b] - *P*-values for the comparison of HRV-B mono vs. mixed infections; [c] - *P*-values for the comparison of HRV-C mono vs. mixed infections; [d] - *P*-values for the comparison of HRV-A mono vs. HRV-C mono-infections, *P*-values could not be calculated for variables with zero variables for both groups; [e] - HEU defined as undetectable viral load, HIV seronegative in the child with a positive maternal history. Positive maternal status based on self-report was accepted, except for seronegative children < 7 months of age where documented positive maternal status was required; [f] - Underweight defined as weight for age <2SD of the median age-sex specific WHO reference; [g] - Premature birth defined as gestational age <37 weeks; [h] - Tachypnea defined as respiratory rate >60 breaths/minute if aged <2 months, respiratory rate >50 breaths/minute if aged 2-12 months, respiration rate >40 breaths/minute if aged >12 months; [i] - Fever defined as temperature ≥38^{o}C; [j] - CRP defined as levels ≥40mg/mL which are considered to show potential bacterial infection. Only a subset of randomly chosen controls had CRP testing conducted at the South African site; [k] - Blood sample positive for *S. pneumoniae* colonisation by *LytA* PCR; [l] - HDP defined as *S. pneumoniae* density in nasopharynx >6.9 and/or density in whole blood sample >2.2 $\log_{10}$ copies/mL.

Figure 5.3: The demographics and clinical characteristics of HRV-associated cases stratified by HRV species for each of the sites

	South-African HRV+ controls (n=189				Mali HRV+ controls (n=130)				Zambia HRV+ controls (n=102)			
	HRV-A (n=96)	**HRV-B (n=14)**	**HRV-C (n=79)**	***P*-value**	**HRV-A (n=46)**	**HRV-B (n=17)**	**HRV-C (n=67)**	***P*-value**	**HRV-A (n=48)**	**HRV-B (n=9)**	**HRV-C (n=45)**	***P*-value**
Age in months, mean (SD)	11.3 (10.7)	6.0 (6.4)	12.4 (12.7)	0.075	7.8 (7.6)	8.8 (9.1)	11.0 (10.3)	0.205	8.3 (9.7)	6.1 (6.0)	7.8 (5.7)	0.688
Female, n(%)	53 (55%)	9 (64%)	44 (56%)	0.854	19 (41%)	9 (53%)	31 (46%)	0.720	21 (44%)	5 (56%)	14 (31%)	0.296
HIV+, n (%)	9 (9%)	0	6 (8%)	0.395	0	0	0	-	9 (9%)	0	6 (8%)	0.658
Never breast fed, n(%)	38 (39%)	7 (50%)	34 (43%)	0.642	5 (11%)	3 (18%)	7 (10%)	0.655	6 (13%)	2 (22%)	8 (18%)	0.401
Under weight, n (%)[a]	7 (7%)	2 (14%)	3 (4%)	0.359	9 (20%)	4 (24%)	5 (7%)	0.034	6 (13%)	1 (11%)	8 (18%)	0.871
Day care attendance, n(%)	14 (14%)	0	14 (18%)	0.295	30 (65%)	14 (82%)	51 (76%)	0.286	0	0	0	-
Smoker in household, n(%)	29 (30%)	5 (36%)	25 (32%)	0.912	9 (20%)	0	14 (21%)	0.120	15 (31%)	1 (11%)	13 (29%)	0.468
Premature birth, n(%)[b]	30 (31%)	5 (36%)	29 (37%)	0.907	2 (4%)	1 (6%)	2 (3%)	0.864	2 (4%)	0	1 (2%)	0.759
Birth weight, mean (SD)	2.9 (0.6)	2.7 (0.7)	3.0 (0.6)	0.179	3.1 (0.6)	3.3 (0.5)	3.4 (0.6)	0.167	2.9 (0.4)	2.9 (0.4)	2.9 (0.6)	0.732
Clinical features:												
RTI control[c]	8 (8%)	0	3 (4%)	0.199	19 (41%)	4 (24%)	35 (52%)	0.112	6 (13%)	1 (11%)	10 (22%)	0.622
Tachypnea, n(%)[d]	5 (6%)	2 (15%)	3 (4%)	0.488	8 (17%)	3 (18%)	6 (9%)	0.467	3 (7%)	0	4 (10%)	0.493
Cough, n(%)	4 (4%)	0	2 (3%)	0.589	6 (13%)	1 (6%)	4 (6%)	0.472	3 (6%)	1 (11%)	7 (16%)	0.415
Fever, n(%)[e]	0	0	0		0	0	2 (3%)	0.346	1 (2%)	0	1 (2%)	0.819
Diarrhoea, n(%)	0	0	0		0	1 (6%)	0	0.076	1 (2%)	0	0	0.442
Rhinorrhoea, n(%)	1 (1%)	0	0	0.587	15 (33%)	4 (24%)	30 (45%)	0.254	2 (4%)	0	3 (7%)	0.748
Laboratory markers:												
CRP≥40mg/l, n(%)[f]	0	0	1 (1%)	0.405	1 (2%)	0	0	0.292	1 (2%)	0	1 (2%)	0.853
***LytA* positive, n(%)[g]**	13 (14%)	1 (7%)	3 (4%)	0.070	6 (13%)	3 (18%)	3 (4%)	0.089	0	0	1 (2%)	0.327
HDP, n(%)[h]												
-Blood	7 (7%)	0	10 (5%)	0.387	5 (11%)	3 (18%)	2 (3%)	0.076	0	0	0	-
-NP	13 (14%)	0	15 (19%)	0.161	10 (22%)	2 (12%)	15 (22%)	0.616	6 (13%)	1 (11%)	5 (11%)	0.977

Abbreviations: SD: Standard deviation; HRV: Human rhinovirus; HIV: Human immunodeficiency virus; RTI: Respiratory tract infections; CRP: C-reactive protein; HDP: High density pneumococcus; NP: Nasopharyngeal.

P-values from chi-square and Wilcoxon tests - logistic regression models adjusted for confounding variates (<0.2 in univariate analysis) where applicable; *P*-values could not be calculated for variables with zero values for all groups.

[a] - Underweight defined as weight for age <-2SD of the median age-sex specific WHO reference; [b] - Premature birth defined as gestational age <37 weeks; [c] - controls with any signs or symptoms of respiratory tract infections; [d] - Tachypnea defined as respiratory rate >60 breaths/minute if aged <2 months, respiratory rate >50 breaths/minute if aged 2-12 months, respiration rate >40 breaths/minute if aged >12 months; [e] - Fever defined as temperature ≥38^{o}C; [f] - CRP defined as levels ≥40mg/mL which are considered to show potential bacterial infection. Only a subset of randomly chosen controls had CRP testing conducted at the South African site; [g] - Blood sample positive for *S. pneumoniae* colonisation by *LytA* PCR; [h] - HDP defined as *S. pneumoniae* density in nasopharynx >6.9 and/or density in whole blood sample >2.2 $\log_{10}$ copies/mL.

5.1.3 Molecular subtyping of the HRV-associated cases

Of the 439 HRV-associated cases, 428 (97%) samples were successfully amplified and subtyped. The remaining 3% (n=11) of samples failed to amplify, all of which were of very low copy numbers (Ct >37) on the real-time PCR assay. Furthermore, of the 428 successfully amplified samples, 3% (n=13) were typed as enterovirus which is a closely related member of HRV among the *Picornaviridae* family. Thus, samples from 91% (n=415) of the HRV-associated cases were successfully speciated. The order of prevalence of the HRV species among cases were HRV-A (48%, n=199), HRV-C (45%, n=185) and least common HRV-B (7%, n=31). This distribution of HRV species differed significantly by age-group with HRV-A being most prevalent among infants (52%), followed by HRV-C (38%) then HRV-B (10%), compared to HRV-C (60%) being most prevalent among children 1-5 years of age, followed by HRV-A (38%) and HRV-B (2%, P=0.002).

Similar to among controls, HRV-B was the least prevalent species (7%) and appeared sporadically throughout the two years; Figure 5.1. This limited the ability to conduct any in-depth statistical analysis specific to HRV-B; nonetheless, there were no significant differences in demographics and clinical presentation or markers of more severe disease between HRV-B associated cases compared to HRV-A or HRV-C associated cases, except that HRV-B (4.8 months) cases were significantly younger than HRV-A (9.4 months, P=0.01) and HRV-C (12.1 months, P<0.001) associated cases; Table 5.4. Further species specific analyses were limited to comparing HRV-A to HRV-C associated cases; Table 5.4-5.6.

In the multivariate logistic regression model which was adjusted for confounding variates (P<0.2 in univariate analysis) as well as HIV-1-infection status, co-infecting viruses and bacteria; cases with HRV-A were younger (9.4 months) than HRV-C (12.1 months, P=0.033) cases. Furthermore, HRV-A associated cases were 1.57-fold (aOR 95% CI: 1.02-2.41) more likely to have radiographically confirmed pneumonia (abnormal chest X-ray defined as primary end point pneumonia or any infiltrates) than HRV-C (46% vs. 36%, P=0.040), and 2.23-fold (aOR 95% CI: 1.24-4.02) more likely to present with concurrent diarrhoea (26% vs. 14%, P=0.007). Conversely, cases with HRV-C were 1.60-fold (aOR 95% CI: 1.02-2.67) more likely to present with wheeze (35%) than HRV-A cases (25%, P=0.031).

There were no other differences in markers for more severe disease such as hypoxia at admission (55% vs. 74%, *P*=0.206), mechanical ventilation support (3% vs. 4%, *P* =0.362), hospital stay >3 days (71% vs. 16%, *P*=0.234) or clinical severity of pneumonia (44% vs. 39%, *P* =0.265) between HRV-A and HRV-C associated cases. Also, there were no differences in laboratory parameters and putative markers of bacterial co-infections such as CRP ≥40mg/mL (29% vs. 25%, *P*=0.267), leucocytosis (44% vs. 41%, *P*=0.209), microbiologically confirmed pneumococcal pneumonia (3% vs. 1%, *P*=0.143), blood culture positivity (5% vs. 6%, *P*=0.623) or high colonisation densities of *Streptococcus pneumoniae* (*LytA*) in the nasopharynx (19% vs. 13%, *P*=0.069) or blood (7% vs. 5%, *P*=0.471). Additionally, there were no differences in the HRV viral load between the species. For the viral co-infections in the univariate analysis, HRV-A was less likely to be co-detected with AdV (9% vs. 16%, *P*=0.024) and HBoV (9% vs. 17%, *P*=0.029) but more likely to be co-infected with PIV (9% vs. 3%, *P*=0.024) compared with HRV-C. After multivariate analysis the only association that remained was between HRV-A and PIV (*P*=0.036). Also, the case fatality ratio was similar between HRV-A (21%) compared to HRV-C (16%, *P*=0.522) associated cases; Table 5.4.

Table 5.4: The demographics and clinical characteristics of cases infected with HRV-A, HRV-B and HRV-C

	HRV-A (n=119)	HRV-B (n=31)	HRV-C (n=185)	Unadjusted		Adjusted	
				P-value	OR (95% CI)	*P*-value	aOR (95% CI)
Age in months, mean (SD)	9.4 (9.5)	4.8 (4.9)	12.1 (10.2)	0.023		0.033	
Female, n(%)	82 (41%)	17 (55%)	92 (50%)	0.094	0.71 (0.47-1.08)	0.126	0.72 (0.48-1.10)
HIV positive, n(%)	30 (15%)	2 (6%)	16 (9%)	0.044	1.56 (1.05-2.58)	0.059	1.96 (0.98-3.94)
HEU, n (%)[a]	41 (24%)	3 (10%)	45 (27%)	0.617	1.06 (0.83-1.36)	0.786	0.93 (0.53-1.62)
Never breast fed, n(%)	40 (20%)	4 (13%)	27 (14%)	0.157	1.47 (0.86-2.51)	0.099	1.78 (1.02-3.11)
Under weight, n (%)[b]	71 (36%)	7 (23%)	51 (28%)	0.089	1.46 (0.95-2.25)	0.061	1.54 (0.98-2.42)
Day care attendance, n(%)	55 (28%)	7 (23%)	43 (23%)	0.331	1.25 (0.80-1.98)	0.126	1.63 (0.87-3.05)
Smoker in household, n(%)	69 (35%)	9 (29%)	56 (30%)	0.421	1.19 (0.78-1.83)	0.290	1.28 (0.81-2.0)
Premature birth, n(%)[c]	26 (13%)	2 (6%)	15 (8%)	0.248	1.42 (0.79-2.53)	0.266	1.43 (0.76-2.71)
Birth weight, mean (SD)	2.9 (0.7)	3.0 (0.7)	3.0 (0.6)	0.237		0.235	
Clinical features:							
Very severe pneumonia, n(%)	88 (44%)	12 (39%)	72 (39%)	0.293	1.24 (0.83-1.87)	0.265	1.31 (0.85-2.01)
CXR abnormal, n(%)[d]	91 (46%)	15 (48%)	66 (36%)	0.046	1.52 (1.01-2.29)	0.040	1.57 (1.02-2.41)
Supplementary 02 therapy, n(%)	113 (57%)	20 (65%)	110 (59%)	0.596	0.89 (0.60-1.34)	0.791	0.92 (0.49-1.70)
Mechanical ventilation, n(%)	6 (3%)	0	7 (4%)	0.678	0.79 (0.26-2.40)	0.362	0.58 (0.18-1.86)
Hypoxic, n(%)[e]	108 (55%)	23 (74%)	120 (60%)	0.302	0.81 (0.54-1.21)	0.206	0.76 (0.49-1.19)
Tachycardia, n(%)[f]	113 (57%)	17 (55%)	108 (58%)	0.796	0.95 (0.63-1.42)	0.818	0.95 (0.62-1.46)
Tachypnea, n(%)[g]	169 (86%)	24 (77%)	161 (87%)	0.818	0.93 (0.52-1.68)	0.931	1.09 (0.59-2.01)
Wheezing, n(%)	49 (25%)	7 (23%)	65 (35%)	0.025	0.60 (.39-0.94)	0.031	0.61 (0.39-0.95)
Cough, n(%)	142 (71%)	23 (74%)	139 (75%)	0.339	0.80 (0.51-1.26)	0.494	0.82 (0.50-1.34)
Lethargic, n(%)	26 (13%)	3 (10%)	15 (8%)	0.119	1.70 (0.87-3.33)	0.056	1.97 (0.98-3.96)
Fever, n(%)[h]	152 (76%)	23 (74%)	139 (75%)	0.776	1.08 (0.67-1.71)	0.901	1.04 (0.64-1.69)
Convulsions, n(%)	14 (7%)	2 (6%)	4 (2%)	0.033	3.43 (1.11-10.60)	0.098	2.78 (0.82-9.45)
Diarrhoea, n(%)	51 (26%)	8 (26%)	26 (14%)	0.005	2.11 (1.25-3.55)	0.007	2.23 (1.24-4.02)
Head nodding, n(%)	54 (27%)	9 (29%)	51 (28%)	0.924	0.98 (0.62-1.53)	0.947	1.00 (0.63-1.61)
Central cyanosis, n(%)	9 (5%)	1 (3%)	3 (2%)	0.118	2.87 (0.77-10.78)	0.199	2.50 (0.64-9.62)
Unable to Feed, n(%)	17 (9%)	1 (3%)	17 (9%)	0.824	0.92 (0.46-1.87)	0.611	0.87 (0.41-1.86)
Vomiting everything, n(%)	4 (2%)	0	2 (1%)	0.470	1.87 (0.34-10.37)	0.510	2.17 (0.37-12.55)
Lower chest wall indrawing, n(%)	183 (92%)	28 (90%)	176 (95%)	0.212	0.58 (0.25-1.36)	0.078	0.46 (0.19-1.10)
Stridor, n(%)	7 (4%)	1 (3%)	6 (3%)	0.684	0.81 (0.29-2.27)	0.875	0.76 (0.26-2.22)
Grunting, n(%)	46 (23%)	10 (32%)	46 (25%)	0.597	0.88 (0.56-1.40)	0.333	0.75 (0.43-1.30)
Nasal Flaring, n(%)	152 (76%)	24 (77%)	148 (80%)	0.392	0.81 (0.50-1.32)	0.575	0.83 (0.50-1.40)
Laboratory markers:							
Leucocytosis, n(%)[i]	88 (44%)	13 (42%)	75 (41%)	0.524	1.14 (0.76-1.71)	0.209	1.27 (0.82-1.97)
Neutrophils %, median (IQR)	49.1 (32.9-60)	34.7 (24.1-58)	51 (36.5-67)	0.049		0.716	
Lymphocyte %, median (IQR)	41.1 (30.8-54)	52.7 (30.3-65)	37 (24.3-52)	0.052		0.779	
Eosinophils %, median (IQR)	1 (0.1-2.8)	1.05 (0.2-3.3)	0.6 (0.1-2)	0.044		0.058	

CRP≥40mg/l, n(%)[j]	58 (29%)	10 (32%)	47 (25%)	0.412	1.21 (0.77-1.90)	0.267	1.32 (0.83-2.10)
Blood culture positive, n(%)[k]	10 (5%)	5 (16%)	11 (6%)	0.692	0.84 (0.34-2.02)	0.623	0.83 (0.33-2.04)
***LytA* positive, n(%)[l]**	19 (9%)	4 (12%)	15 (7%)	0.514	1.28 (0.61-2.73)	0.469	1.30 (0.60-2.82)
MCPP, n(%)[m]	6 (3%)	0	2 (1%)	0.204	2.84 (0.57-14.27)	0.143	3.78 (0.70-20.50)
HDP, n(%)[n]							
-Blood	13 (7%)	3 (10%)	9 (5%)	0.433	1.42 (0.59-3.41)	0.471	1.40 (0.57-3.43)
-NP	38 (19%)	4 (13%)	24 (13%)	0.105	1.58 (0.91-2.76)	0.069	1.72 (0.96-3.09)
HRV NP viral load, Mean (SD)[o]	3.5 (3.0-4.1)	3.5 (2.8-3.8)	3.5 (3.1-4.3)	0.544		0.846	
HRV mono-infections, n(%)	110 (55%)	13 (42%)	100 (54%)	0.810	1.05 (0.70-1.57)	0.742	1.07 (0.70-1.63)
Viral co-infections in the nasopharynx:							
-AdV, n(%)	17 (9%)	3 (10%)	30 (16%)	0.024	0.58 (0.26-0.91)	0.116	0.58 (0.29-1.14)
-RSV, n(%)	36 (18%)	8 (26%)	24 (13%)	0.169	1.48 (0.85-2.60)	0.679	1.14 (0.62-2.07)
-HBoV, n(%)	18 (9%)	5 (16%)	31 (17%)	0.029	0.49 (0.27-0.92)	0.095	0.58 (0.30-1.10)
-HMPV, n(%)	5 (3%)	1 (3%)	6 (3%)	0.669	0.77 (0.23-2.56)	0.592	0.71 (0.20-2.45)
-InFV A-C, n(%)	2 (1%)	0	2 (1%)	0.942	0.93 (0.13-6.66)	0.954	1.06 (0.14-7.98)
-PIV, n(%)	18 (9%)	2 (6%)	6 (3%)	0.024	2.97 (1.15-7.65)	0.036	2.82 (1.07-7.40)
-HCoV, n(%)	11 (6%)	1 (3%)	14 (8%)	0.420	0.71 (0.32-1.62)	0.414	0.70 (0.30-1.63)
Hospital stay >3 days, n(%)	141 (71%)	23 (74%)	117 (63%)	0.113	1.41 (0.92-2.17)	0.234	1.34 (0.85-2.10)
Case fatality ratio, n(%)	32 (21%)	2 (9%)	25 (16%)	0.332	1.33 (0.75-2.37)	0.522	1.23 (0.66-2.29)

Abbreviations - HRV: Human rhinovirus; OR: Odds ratios; aOR: Adjusted odds ratio; CI: Confidence intervals, SD: Standard deviation; IQR: Inter quartile range; HIV: Human immunodeficiency virus; HEU: HIV-uninfected but HIV exposed; CXR: Chest X-ray; CRP: C-reactive protein; MCPP: Microbiologically confirmed pneumococcal pneumonia; HDP: High Density pneumococcus; NP: Nasopharyngeal; RSV: Respiratory Syncytial Virus, HMPV: human metapneumovirus; AdV: adenovirus; PIV: parainfluenza type 1-4; HBoV: Human Bocavirus; HCoV: Human Coronavirus (OC43, NL63, 229E and HKU1) and InFV: Influenza Virus (A, B and C).

Odds ratios and *P*-values were calculated by comparing HRV-A to HRV-C infected cases using Chi-squared and Wilcoxon tests, logistic regression models adjusted for confounding variates (<0.2 in univariate analysis) where applicable; Odds ratio could not be calculated for continuous variables or variables with 0 values, thus cells left blank. [a] - HEU defined as undetectable viral load, HIV seronegative in the child with a positive maternal history. Positive maternal status based on self-report was accepted, except for seronegative children < 7 months of age where documented positive maternal status was required; [b] - Underweight defined as weight for age <-2SD of the median age-sex specific WHO reference; [c] - Premature birth defined as gestational age <37 weeks; [d] - Abnormal Chest X-ray defined as radiographically confirmed end point pneumonia consolidation or any infiltrates; [e] - Hypoxic defined as 1) a room air pulse-oximetry reading indicated oxygen saturation <90% at the two sites at elevation (Zambia and South Africa) or <92% at all other sites, or 2) a room air oxygen saturation reading was not available and the child was on oxygen; [f] - Tachycardia defined as heart rate >160 beats per minute (bpm) if aged <11 months, heart rate >150 bpm if aged 12-35 months, heart rate >140 bpm if aged 36-59 months; [g] - Tachypnea defined as Respiratory rate >60 breaths/minute if aged <2 months, respiratory rate >50 breaths/minute if aged 2-12 months, respiration rate >40 breaths/minute if aged >12 months; [h] - Fever defined as temperature >38^{o}C; [i] - Leucocytosis defined as white blood cell count >15 000 cells/uL if age <12 months, white blood cell count >13 000 cells/uL if age >12 months; [j] - CRP defined as levels ≥40mg/mL which are considered to potentially indicate bacterial infection; [k] - Blood culture positive for any significant non contaminate bacteria; [l] - Blood sample positive for *S. pneumoniae* colonisation by *LytA* PCR; [m] - MCPP defined when *S. pneumoniae* was cultured from a normally sterile body fluid - blood, pleural fluid or lung aspirate - or pleural fluid or lung aspirate was PCR *LytA* positive; [n] - HDP defined as *S. pneumoniae* density in nasopharynx >6.9 and/or density in whole blood sample >2.2 $\log_{10}$ copies/mL; [o] - HRV viral load in the nasopharynx expressed as $\log_{10}$ copies/mL.

Among HRV-associated cases, HRV was identified as the sole respiratory virus in the nasopharynx 54% (n=223/415) of cases, including 55% (n=110/199) of HRV-A; 42% (n=13/31) of HRV-B and 54% (n=100/185) of HRV-C infections (P=0.380). There were no differences in the nasopharyngeal viral loads of HRV between the mono and mixed viral infections for any of the species. The clinical presentation, including pneumonia severity categorisation at admission, signs and symptoms of illness and laboratory parameters did not differ between cases with mono HRV-A compared to those with concurrent HRV-A infections with other respiratory viruses, including Influenza virus (A-B), RSV, HMPV, HBoV, PIV (1-4), AdV and HCoV. What was notable, was the higher case fatality ratio among HRV-A mono-infections (28%) than those with HRV-A together with other concurrent respiratory infections (10%, P=0.005). This association of higher case fatality ratio remained in the multivariate analysis which was adjusted for confounding variates (P<0.2 in univariate analysis) including HIV infection status, age categories, gender, site of enrolment, malnutrition and potential concurrent bacterial infection (aOR 5.81, 95% CI: 1.70-19.98, P=0.005); Table 5.5. Moreover, HRV-A mono-infected cases had 2.20-fold (aOR 95% CI: 1.20-4.04) higher case fatality ratio (28%) than all other cases (16%, P=0.011).

Among the HRV-C associated cases, HRV-C mono viral infections compared to HRV-C mixed-infection cases were 5.57-fold (aOR 95% CI: 1.77-17.47) more likely to have concurrent diarrhoea (18% vs. 9%, P=0.003) and 2.29-fold (aOR 95% CI: 1.20-4.38) more likely to present with tachycardia (68% vs. 47%, P=0.012). The HRV-C associated cases with mixed viral infections were however 2.19-fold (aOR 95% CI: 1.08-4.69) more likely to present with fever (82% vs. 69%, P=0.044), 2.07-fold (aOR 95% CI: 1.04-4.27) more likely to be categorised as very severe pneumonia (47% vs. 32%, P=0.49) and 2.84-fold (aOR 95% CI: 1.37-5.87) more likely to be hospitalised for >3 days (74% vs. 54%, P=0.005) compared to cases with HRV-C mono-infections; Table 5.5. There was no other association between HRV-C associated cases with mono compared to mixed viral infections, including no differences in presence of hypoxia, requiring mechanical ventilation and case fatality ratio (20% vs. 12% P=0.190); Table 5.5.

On comparison of cases with HRV-A and HRV-C mono-infections, the HRV-A mono-infected cases were 2.80-fold (aOR 95% CI: 1.05-7.51) more likely to present with lethargy (17% vs. 7%, P=0.041), 15.45-fold (aOR 95% CI: 1.39-172.40) more likely to present with

concurrent convulsions (8% vs. 1%, P=0.026) and 1.83-fold (aOR 95% CI: 1.07-3.49) more likely to be hospitalised for >3 days (72% vs. 54%, P=0.039). There were, however, no differences in case fatality ratio between HRV-A (28%) and HRV-C mono-infected cases (20%, P=0.297); Table 5.5.

Table 5.5: The demographics and clinical characteristics of cases with HRV-A, HRV-B and -C mono-infections compared to cases with HRV mixed infections

	HRV-A mono (n=110)	HRV-A mixed (n=89)	*P*-value[a]	HRV-B mono (n=13)	HRV-B mixed (n=18)	*P*-value[b]	HRV-C mono (n=100)	HRV-C mixed (n=85)	*P*-value[c]	*P*-value[d]
Age in months, mean (SD)	10.0 (11.7)	7.6 (8.6)	0.089	5.6 (6.5)	4.4 (3.6)	0.503	11.5 (11.0)	11.1 (9.2)	0.918	0.364
Female, n(%)	47 (43%)	35 (39%)	0.902	7 (54%)	10 (56%)	0.925	51 (51%)	41 (48%)	0.758	0.246
HIV positive, n(%)	17 (15%)	13 (15%)	0.613	1 (8%)	1 (6%)	0.124	10 (10%)	6 (7%)	0.456	0.311
HEU, n(%)[e]	24 (26%)	17 (22%)	0.604	0	3 (18%)	0.124	25 (28%)	20 (25%)	0.718	0.763
Never breast fed, n(%)	36 (33%)	24 (27%)	0.485	2 (15%)	2 (11%)	0.726	29 (29%)	25 (29%)	0.690	0.761
Under weight, n (%)[f]	42 (38%)	29 (33%)	0.497	4 (31%)	3 (17%)	0.352	25 (25%)	26 (31%)	0.437	0.059
Day care attendance, n(%)	25 (23%)	30 (34%)	0.547	1 (8%)	6 (33%)	0.092	37 (34%)	32 (36%)	0.513	0.756
Smoker in household, n(%)	37 (34%)	32 (36%)	0.400	2 (15%)	7 (39%)	0.155	26 (26%)	30 (35%)	0.150	0.194
Premature birth, n(%)[g]	15 (14%)	11 (12%)	0.833	1 (8%)	1 (6%)	0.675	8 (8%)	7 (8%)	0.592	0.482
Birth weight, mean (SD)	2.9 (0.7)	2.9 (0.7)	0.915	2.9 (0.8)	3.2 (0.6)	0.376	3.0 (0.6)	3.0 (0.7)	0.886	0.221
Clinical features:										
Very severe pneumonia, n(%)	48 (44%)	40 (45%)	0.777	7 (54%)	5 (28%)	0.141	32 (32%)	40 (47%)	0.045	0.064
CXR abnormal, n(%)[h]	46 (41%)	45 (51%)	0.103	7 (54%)	8 (44%)	0.605	32 (32%)	34 (40%)	0.230	0.156
CXR Alveolar consolidation, n(%)	18 (19%)	9 (11%)	0.210	4 (33%)	2 (12%)	0.158	10 (11%)	8 (10%)	0.877	0.168
Supplementary 02 therapy, n(%)	67 (61%)	46 (52%)	0.953	11 (85%)	9 (50%)	0.087	62 (62%)	48 (56%)	0.672	0.746
Mechanical ventilation, n(%)	4 (4%)	2 (2%)	0.569	0	0		3 (3%)	4 (5%)	0.482	0.901
Hypoxic, n(%)[i]	55 (50%)	53 (60%)	0.084	9 (70%)	14 (78$)	0.592	56 (57%)	54 (64%)	0.441	0.224
Tachycardia, n(%)[j]	66 (60%)	47 (53%)	0.458	5 (39%)	12 (67%)	0.119	68 (68%)	40 (47%)	0.010	0.273
Tachypnea, n(%)[k]	145 (85%)	79 (91%)	0.082	11 (85%)	13 (72%)	0.514	88 (88%)	73 (86%)	0.696	0.390
Wheezing, n(%)	27 (25%)	22 (25%)	0.956	2 (15%)	5 (28%)	0.415	35 (35%)	30 (35%)	0.687	0.149
Cough, n(%)	76 (69%)	66 (74%)	0.292	8 (62%)	15 (83%)	0.171	72 (72%)	67 (79%)	0.144	0.756
Lethargic, n(%)	19 (17%)	7 (8%)	0.066	3 (23%)	0	0.092	7 (7%)	8 (9%)	0.553	0.041
Fever, n(%)[l]	88 (80%)	64 (72%)	0.099	11 (85%)	12 (67%)	0.260	69 (69%)	70 (82%)	0.044	0.062
Convulsions, n(%)	9 (8%)	5 (6%)	0.296	2 (15%)	0	0.185	1 (1%)	3 (4%)	0.282	0.026
Diarrhoea, n(%)	28 (25%)	23 (26%)	0.843	4 (31%)	4 (2%)	0.592	18 (18%)	8 (9%)	0.003	0.280
Head nodding, n(%)	23 (21%)	31 (35%)	0.223	4 (31%)	5 (28%)	0.856	21 (21%)	30 (35%)	0.088	0.975
Central cyanosis, n(%)	7 (6%)	2 (2%)	0.189	1 (8%)	0	0.232	3 (3%)	11 (3%)	0.903	0.272
Unable to Feed, n(%)	11 (10%)	6 (7%)	0.158	0	1 (6%)	0.388	10 (10%)	7 (8%)	0.510	0.840
Vomiting everything, n(%)	4 (4%)	0	0.194	0	0		1 (1%)	1 (1%)	0.913	0.250
Lower chest wall indrawing, n(%)	99 (90%)	84 (95%)	0.154	11 (85%)	17 (94%)	0.361	96 (96%)	80 (94%)	0.631	0.072
Stridor, n(%)	2 (2%)	5 (6%)	0.286	0	1 (6%)	0.388	5 (5%)	1 (1%)	0.673	0.232
Grunting, n(%)	19 (17%)	27 (30%)	0.123	5 (38%)	5 (28%)	0.530	21 (21%)	25 (29%)	0.651	0.297
Nasal Flaring, n(%)	81 (74%)	71 (80%)	0.486	10 (77%)	14 (78%)	0.955	81 (81%)	67 (79%)	0.417	0.231
Laboratory markers:										
Leucocytosis, n(%)[m]	54 (50%)	34 (38%)	0.256	6 (46%)	7 (39%)	0.686	39 (40%)	36 (43%)	0.397	0.097
Neutrophils %, Median (IQR)	50.3 (36.9-66)	43.1 (28-55)	0.113	39.6 (31-52)	40.2 (30.9-48)	0.947	48.9 (36-64)	55.3 (37-69)	0.683	0.588
Lymphocyte %, Median (IQR)	37.5 (25.6-49)	46.2 (36-59.4)	0.052	42.8 (33.6-49)	37.9 (28.2-51)	0.840	37.2 (26-52)	34.1 (22-52.8)	0.823	0.495

Eosinophils %, Median (IQR)	0.5 (0-1.8)	0.5 (0-2)	0.664	0.6 (0-2.1)	0.8 (0-2.3)	0.868	1 (0.2-2.8)	1 (0.1-2.6)	0.887	0.148
CRP≥40mg/l, n(%)[n]	34 (31%)	24 (27%)	0.982	5 (38%)	5 (27%)	0.530	27 (27%)	20 (24%)	0.743	0.461
Blood culture positive, n(%)[o]	6 (5%)	4 (4%)	0.768	3 (23%)	2 (11%)	0.371	7 (7%)	4 (5%)	0.353	0.621
***LytA* positive, n(%)[p]**	7 (7%)	10 (12%)	0.416	1 (8%)	3 (18%)	0.474	10 (10%)	3 (4%)	0.064	0.337
MCPP, n(%)[q]	4 (4%)	2 (2%)	0.545	0	0		2 (2%)	0	0.299	0.446
HDP, n(%)[r]										
-Blood	4 (4%)	9 (10%)	0.098	0	3 (18%)	0.124	8 (8%)	1 (1%)	0.035	0.286
-NP	19 (21%)	19 (17%)	0.684	2 (15%)	3 (17%)	0.924	9 (9%)	15 (18%)	0.148	0.064
HRV NP viral load, Mean (SD)[s]	3.5 (3.0-4.1)	3.4 (3.0-4.1)	0.664	3.8 (0.5)	3.2 (0.9)	0.137	3.6 (3.1-4.2)	3.6 (3.0-4.3)	0.402	0.591
Hospital stay >3 days, n(%)	79 (72%)	62 (70%)	0.660	11 (85%)	12 (67%)	0.260	54 (54%)	63 (74%)	0.005	0.039
Case fatality ratio, n(%)	25 (28%)	7 (10%)	0.005	2 (25%)	0	0.150	16 (20%)	9 (12%)	0.190	0.297

Abbreviations - HRV: Human rhinovirus; OR: Odds ratios; aOR: Adjusted odds ratio; CI: Confidence intervals, CXR: Chest X-ray; HIV: Human immunodeficiency virus; HEU: HIV-uninfected but HIV exposed; CRP: C-reactive protein; MCPP: Microbiologically confirmed pneumococcal pneumonia; HDP: High Density pneumococcus; NP: Nasopharyngeal.

P-values from Chi-squared and Wilcoxon tests, logistic regression models adjusted for confounding variates (<0.2 in univariate analysis) where applicable. *P*-values could not be calculated for variables where both groups were zero, thus left blank.

[a] - *P*-values for the comparison of HRV-A mono vs. mixed infections; [b] - *P*-values for the comparison of HRV-B mono vs. mixed infections; [c] - *P*-values for the comparison of HRV-C mono vs. mixed infections; [d] - *P*-values for the comparison of HRV-A mono vs. HRV-C mono-infections; [e] - HEU defined as undetectable viral load, HIV seronegative in the child with a positive maternal history. Positive maternal status based on self-report was accepted, except for seronegative children < 7 months of age where documented positive maternal status was required; [f] - Underweight defined as weight for age <-2SD of the median age-sex specific WHO reference; [g] - Premature birth defined as gestational age <37 weeks; [h] - Abnormal Chest X-ray defined as radiographically confirmed end point pneumonia consolidation or any infiltrates; [i] - Hypoxic defined as 1) a room air pulse-oximetry reading indicated oxygen saturation <90% at the two sites at elevation (Zambia and South Africa) or <92% at all other sites, or 2) a room air oxygen saturation reading was not available and the child was on oxygen; [j] - Tachycardia defined as heart rate >160 beats per minute (bpm) if aged <11 months, heart rate >150 bpm if aged 12-35 months, heart rate >140 bpm if aged 36-59 months; [k] - Tachypnea defined as Respiratory rate >60 breaths/minute if aged <2 months, respiratory rate >50 breaths/minute if aged 2-12 months, respiration rate >40 breaths/minute if aged >12 months; [l] - Fever defined as temperature >38^{o}C; [m] - Leucocytosis defined as white blood cell count >15 000 cells/uL if age <12 months, white blood cell count >13 000 cells/uL if age >12 months; [n] - CRP defined as levels ≥40mg/mL which are considered to potentially indicate bacterial infection; [o] - Blood culture positive for any significant non contaminate bacteria; [p] - Blood sample positive for *S. pneumoniae* colonisation by *LytA* PCR; [q] - MCPP defined when *S. pneumoniae* was cultured from a normally sterile body fluid - blood, pleural fluid or lung aspirate - or pleural fluid or lung aspirate was PCR *LytA* positive; [r] - HDP defined as *S. pneumoniae* density in nasopharynx >6.9 and/or density in whole blood sample >2.2 $\log_{10}$ copies/mL; [s] - HRV viral load in the nasopharynx expressed as $\log_{10}$ copies/mL.

Similar trends as observed in the overall analysis of the three sites; Table 5.4, were evident when analysed for the individual three sites, with HRV-C being significantly associated with older children in South Africa (10 months vs. 5 and 3.5 months for HRV-A and -B respectively, $P<0.001$). Additionally, even after multivariate logistic regression adjusting for age group, gender, HIV infection status, malnutrition, bacterial and viral co-infection, other site specific significant differences included HRV-C being less associated with concurrent diarrhoea in South Africa (7% vs. 29% and 25% for HRV-A and HRV-B respectively, $P<0.001$), and more likely to have lower chest wall indrawing in Mali (98% vs. 83% and 89% for HRV-A and -B respectively, $P=0.009$). In Zambia, HRV-A associated cases were more likely to be HIV-infected (24%) compared to HRV-B (0%) and HRV-C cases (6%, $P=0.010$); Table 5.6. There were no other associations in clinical presentation, markers for bacterial co-infections, hospital stays >3 days or case fatality ratio between the three HRV species at any of the sites.

Table 5.6: The demographics and clinical characteristics of HRV-associated cases stratified by HRV species for each of the sites

	South-African HRV+ pneumonia cases (n=198)				Mali HRV+ pneumonia cases (n=108)				Zambia HRV+ pneumonia cases (n=109)			
	HRV-A (n=91)	**HRV-B (n=16)**	**HRV-C (n=91)**	***P*-value**	**HRV-A (n=53)**	**HRV-B (n=9)**	**HRV-C (n=46)**	***P*-value**	**HRV-A (n=55)**	**HRV-B (n=6)**	**HRV-C (n=48)**	***P*-value**
Age in months, median (IQR)	5 (2-9)	3.5 (2-7)	10 (5-17)	*P*<0.001	5 (3-11)	2 (1-6)	5.5 (3-12)	0.434	8.5 (3.5-14.5)	3 (3-7)	5 (3-11)	0.187
Female, n(%)	35 (38%)	7 (44%)	46 (51%)	0.1720	19 (36%)	6 (67%)	23 (50%)	0.132	28 (51%)	4 (67%)	23 (48%)	0.609
HIV positive, n(%)	15 (16%)	2 (13%)	11 (12%)	0.753	2 (4%)	0	2 (4%)	0.691	13 (24%)	0	3 (6%)	0.010
Never breast fed, n(%)	44 (48%)	9 (56%)	40 (44%)	0.930	7 (13%)	2 (22%)	8 (17%)	0.727	9 (16%)	0	6 (13%)	0.270
Under weight, n (%)[a]	26 (29%)	3 (19%)	17 (19%)	0.323	20 (38%)	3 (33%)	16 (35%)	0.903	25 (45%)	1 (17%)	18 (38%)	0.269
Clinical features:												
Very severe pneumonia, n(%)	37 (41%)	5 (31%)	30 (33%)	0.550	32 (60%)	5 (56%)	28 (61%)	0.956	19 (35%)	2 (33%)	14 (29%)	0.809
Chest X-ray abnormal, n(%)[b]	52 (57%)	9 (56%)	38 (42%)	0.152	14 (26%)	3 (33)%	16 (35%)	0.627	25 (45%)	3 (50%)	12 (25%)	0.112
Supplementary 02 therapy, n(%)	84 (92%)	15 (94%)	84 (92%)	0.976	0	0	3 (7%)	0.081	29 (53%)	5 (83%)	23 (48%)	0.295
Hypoxic, n(%)[c]	64 (71%)	14 (88%)	68 (76%)	0.256	22 (42%)	6 (67%)	28 (61%)	0.095	22 (40%)	3 (50%)	14 (29%)	0.525
Tachycardia, n(%)[d]	43 (48%)	7 (44%)	46 (51%)	0.919	30 (57%)	6 (67%)	28 (61%)	0.829	40 (73%)	4 (67%)	34 (71%)	0.950
Tachypnea, n(%)[e]	69 (78%)	12 (75%)	78 (86%)	0.546	49 (92%)	6 (67%)	42 (91%)	0.137	51 (93%)	6 (100%)	41 (85%)	0.102
Wheezing, n(%)	31 (34%)	4 (25%)	40 (44%)	0.665	12 (23%)	2 (22%)	11 (24%)	0.984	6 (11%)	1 (17%)	14 (29%)	0.104
Cough, n(%)	79 (87%)	12 (75%)	79 (87%)	0.547	29 (55%)	6 (67%)	27 (59%)	0.758	34 (62%)	5 (83%)	33 (69%)	0.478
Lethargic, n(%)	5 (5%)	0	6 (7%)	0.243	10 (19%)	1 (11%)	5 (11%)	0.335	11 (20%)	2 (33%)	4 (8%)	0.079
Fever, n(%)[f]	59 (65%)	11 (69%)	67 (74%)	0.399	46 (87%)	7 (78%)	38 (83%)	0.730	47 (85%)	5 (83%)	34 (71%)	0.405
Convulsions, n(%)	4 (4%)	0	1 (1%)	0.273	8 (15%)	1 (11%)	2 (4%)	0.159	2 (4%)	1 (17%)	1 (2%)	0.295
Diarrhoea, n(%)	26 (29%)	4 (25%)	6 (7%)	*P*<0.001	11 (21%)	3 (33%)	12 (26%)	0.656	14 (25%)	1 (17%)	8 (17%)	0.157
Head nodding, n(%)	31 (34%)	5 (31%)	25 (27%)	0.605	18 (34%)	4 (44%)	19 (41%)	0.694	5 (9%)	0	7 (15%)	0.339
Central cyanosis, n(%)	1 (1%)	0	0	0.559	3 (6%)	1 (11%)	3 (7%)	0.864	5 (9%)	0	0	0.047
Unable to Feed, n(%)	0	0	2 (2%)	0.201	12 (23%)	1 (11%)	12 (26%)	0.602	5 (9%)	0	3 (6%)	0.496
Vomiting everything, n(%)	2 (2%)	0	1 (1%)	0.664	0	0	1 (2%)	0.473	2 (4%)	0	0	0.244
Lower chest wall indrawing, n(%)	87 (96%)	15 (94%)	86 (95%)	0.930	44 (83%)	8 (89%)	45 (98%)	0.009	52 (95%)	5 (83%)	45 (94%)	0.699
Stridor, n(%)	2 (2%)	0	4 (4%)	0.214	4 (8%)	0	1 (2%)	0.307	1 (2%)	1 (17%)	1 (2%)	0.363
Grunting, n(%)	2 (2%)	3 (19%)	5 (5)	0.855	31 (58%)	7 (78%)	28 (61%)	0.554	13 (24%)	0	13 (27%)	0.207
Nasal Flaring, n(%)	82 (90%)	13 (81%)	78 (86%)	0.404	41 (77%)	7 (78%)	38 (83%)	0.790	29 (53%)	4 (67%)	32 (67%)	0.308
Laboratory markers:												
Leucocytosis, n(%)[g]	45 (49%)	10 (63%)	46 (51%)	0.517	15 (28%)	1 (11%)	10 (22%)	0.492	28 (52%)	2 (33%)	19 (41%)	0.464
Neutrophils %, median (IQR)	47 (33-56.9)	41 (24.3-60.4)	55.3 (40.7-70)	0.263	51 (30-61)	26 (23-54)	45 (27-64)	0.279	47 (36.5-64.8)	41.5 (29-58)	50 (34.7-63.9)	0.937
Lymphocytes %, median (IQR)	41.8 (34-51.4)	51.1 (28.7-65)	33.2 (22-43.9)	0.178	43 (31-61)	65 (44-75)	49 (29-69)	0.289	37 (23.3-52)	49.2 (25.6-61)	37.5 (27.7-50)	
Eosinophils %, median (IQR)	0.3 (0.1-1.2)	1.3 (0.3-3.3)	0.8 (0.1-2.1)	0.102	1 (0-3)	1 (0-5)	2 (0-4)	0.844	0.3 (0.1-0.9)	0.85 (0.2-4.4)	0.8 (0.2-1.8)	0.244
CRP≥40mg/l, n(%)[h]	27 (30%)	4 (25%)	19 (21%)	0.470	11 (21%)	4 (44%)	13 (28%)	0.302	20 (36%)	2 (33%)	15 (31%)	0.642
Blood culture positive, n(%)	1 (1%)	2 (13%)	2 (2%)	0.145	5 (9%)	2 (22%)	6 (13%)	0.563	4 (7%)	1 (17%)	3 (6%)	0.676
***LytA* positive, n(%)[i]**	5 (6%)	3 (19%)	4 (4%)	0.137	10 (19%)	1 (11%)	5 (11%)	0.483	2 (4%)	0	4 (9%)	0.582
MCPP, n(%)[j]	1 (1%)	0	0	0.429	4 (8%)	0	2 (4%)	0.444	1 (2%)	0	0	0.433
HDP, n(%)[k]	14 (15%)	4 (25%)	8 (9%)	0.222	21 (40%)	2 (22%)	17 (37%)	0.601	8 (15%)	0	6 (13%)	0.367
HRV NP viral load, Mean (SD)[l]	3.81 (3.2-4.6)	3.45 (3.2-3.8)	3.93 (3.2-4.7)	0.320	3.3 (2.9-3.9)	2.8 (2.4-4.5)	3.4 (2.8-4.2)	0.980	3.13 (2.9-3.7)	3.50 (2.9-3.8)	3.18 (2.9-3.9)	0.831
Hospital stay >3 days, n(%)	72 (79%)	13 (81%)	68 (75%)	0.995	33 (62%)	6 (67%)	26 (57%)	0.889	36 (66%)	4 (67%)	23 (50%)	0.247
Case fatality ratio, n(%)	7 (10%)	0	4 (5%)	0.212	13 (25%)	1 (11%)	13 (28%)	0.487	12 (39%)	1 (100%)	8 (28%)	0.194

Abbreviations - SD: Standard deviation; IQR: Inter quartile range; HRV: Human rhinovirus; HIV: Human immunodeficiency virus; CRP: C-reactive protein; MCPP: Microbiologically confirmed pneumococcal pneumonia; HDP: High Density pneumococcus; NP: Nasopharyngeal

P-values from Chi-square and Wilcoxon tests, logistic regression models adjusted for confounding variates (<0.2 in univariate analysis). *P*-values could not be calculated for variables where both values are 0, thus cells left blank.

[a] - Underweight defined as weight for age <-2SD of the median age-sex specific WHO reference; [b] - defined as primary end point pneumonia and/or infiltrates; [c] - Hypoxic defined as 1) a room air pulse-oximetry reading indicated oxygen saturation <90% at the two sites at elevation (Zambia and South Africa) or <92% at all other sites, or 2) a room air oxygen saturation reading was not available and the child was on oxygen; [d] - Tachycardia defined as heart rate >160 beats per minute (bpm) if aged <11 months, heart rate >150 bpm if aged 12-35 months, heart rate >140 bpm if aged 36-59 months; [e] - Tachypnea defined as respiratory rate >60 breaths/minute if aged <2 months, respiratory rate >50 breaths/minute if aged 2-12 months, respiration rate >40 breaths/minute if aged >12 month; [f] - Fever defined as body temperature ≥38^{o}C; [g] - Leucocytosis defined as white blood cell count >15 000 cells/uL if age <12 months, white blood cell count >13 000 cells/uL if age >12 months; [h] - CRP defined as levels ≥40mg/mL which are considered to potentially indicate bacterial infection; [i] - Blood sample positive for *S. pneumoniae* colonisation by *LytA* PCR; [j] - MCPP defined if *S. pneumoniae* was cultured from a normally sterile body fluid - blood, pleural fluid or lung aspirate - or pleural fluid or lung aspirate was PCR *LytA* positive; [k] - HDP defined as *S. pneumoniae* density in nasopharynx>6.9 and/or density in whole blood sample>2.2 $\log_{10}$ copies/mL; [l] - HRV viral load in the nasopharynx expressed as mean $\log_{10}$ copies/mL.

5.1.4 Case-control comparison of the molecular subtyping of HRV

Overall, the distribution of HRV species did not differ between cases and controls (P=0.496); Table 5.7.

Table 5.7: The molecular subtyping of the human HRV population in cases and community controls stratified by age groups

		Overall (n=836)	**HRV-A (n=389)**	**HRV-B (n=71)**	**HRV-C (n=376)**	***P*-value[a]**
All ages	Cases	415 (50%)	199 (49%)	31 (44%)	185 (49%)	0.496
	Community controls	421 (50%)	190 (51%)	40 (56%)	191 (51%)	
	P-value[b]	0.334	0.425	0.306	0.861	
1-5 months	Cases	198 (52%)	110 (56%)	21 (47%)	67 (48%)	0.270
	Community controls	186 (48%)	88 (44%)	24 (53%)	74 (52%)	
	P-value[b]	0.207	0.107	0.474	0.246	
6-12 months	Cases	105 (46%)	46 (46%)	8 (50%)	51 (45%)	0.921
	Community controls	125 (54%)	54 (54%)	8 (50%)	63 (55%)	
	P-value[b]	0.951	0.925	0.650	0.750	
1-5 years	Cases	112 (51%)	43 (47%)	2 (20%)	67 (55%)	0.072
	Community controls	110 (50%)	48 (53%)	8 (80%)	54 (44%)	
	P-value[b]	0.054	0.568	0.095	0.054	

Abbreviations: HRV - Human rhinovirus.

P-values chi-square tests with logistic regression models adjusted for confounding variates (<0.2 in univariate analysis).

[a] - *P*-value for difference between cases and controls across the three HRV species; [b] - *P*-value for comparison of individual HRV species between cases and controls for the different age groups.

Figure 5.1 details the seasonal circulation of the three HRV species over the full two-year period for the cases and controls. For both cases and controls, HRV-B appeared sporadically throughout the year with no obvious seasonality pattern. Furthermore, circulation of HRV-A and HRV–C was perennial; and there was no obvious pattern in the circulation of the species among both cases and controls. The overall positivity for HRV ranged from 33% in August 2013 to 4% in October 2011 among cases; and 44% in January 2014 to 2% in October 2011 among controls during the study period.

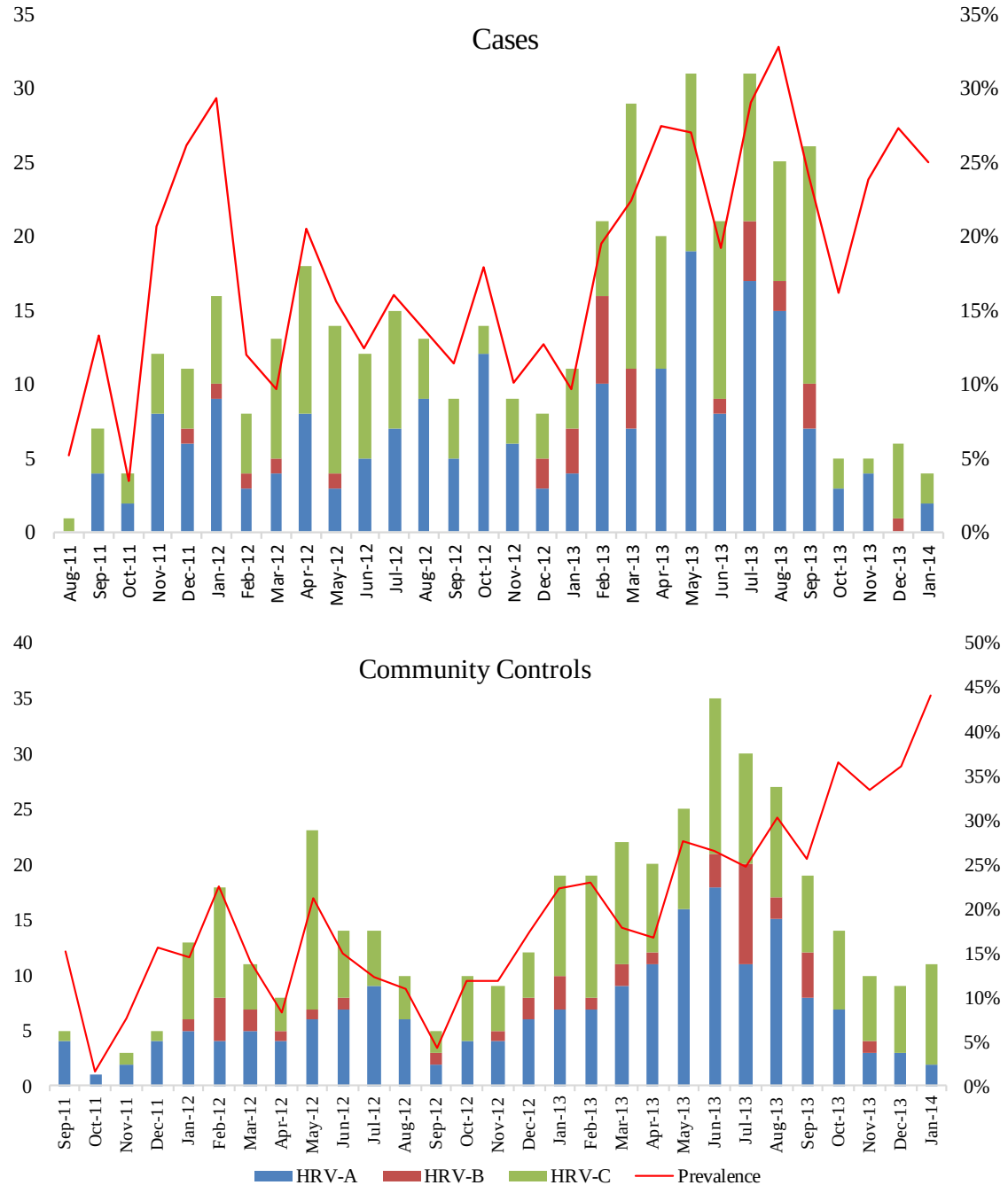

Figure 5.1: The seasonal distribution of HRV species over a period of two years in children hospitalised with pneumonia and age matched community controls. Prevalence is the % of tested samples for each month.

There were 60 different HRV-A strains, 17 different HRV-B strains and 28 different HRV-C strains circulating throughout the two year period; Figure 5.2. The most commonly detected HRV-A strains (n=389) were A78 (n=30, (8%); n=19 (10%) among cases and n=11 (6%) among controls), A101 (n=27 (7%); n=15 (8%) among cases and n=12 (6%) among controls), A12 (n=19 (5%); n=13 (7%) among cases and n=6 (3%) among controls), A80 (n=16 (4%); n=12 (6%) among cases and n=4 (2%) among controls) and A49 (n=12 (3%); n=10 (5%)

among cases and n=2 (1%) among controls). The most commonly detected HRV-B strains (n=71) were B17 (n=10 (14%); n=4 (13%) among cases and n=6 (15%) among controls), B72 (n=8 (11%), n=3 (10%) among cases and n=5 (13%) among controls) and B14 (n=8 (11%), all of which occurred among cases). Lastly; the most common HRV-C strains (n=376) were C11 (n=41 (11%), n=21 (11%) among cases and n=20 (10%) among controls), C35 (n=32 (8%), n=14 (8%) among cases and n=18 (9%) among controls), NAT045 (n=32 (8%), n=22 (12%) among cases and n=10 (5%) among controls) and C54 (n=26 (7%), n=12 (6%) among cases and n=14 (7%) among controls). No discernible differences were obvious in the distribution of strains between cases and controls; moreover, there were no apparent differences in the distribution of strains between the three sites (Figure 5.3-5.5). Additionally, no obvious patterns of temporal clustering of HRV species or strains were observed with strain distribution varying on a month to month basis. The study was not sufficiently powered for statistical analysis of case-control status or clinical epidemiology among cases for the different strains making up each of the HRV species.

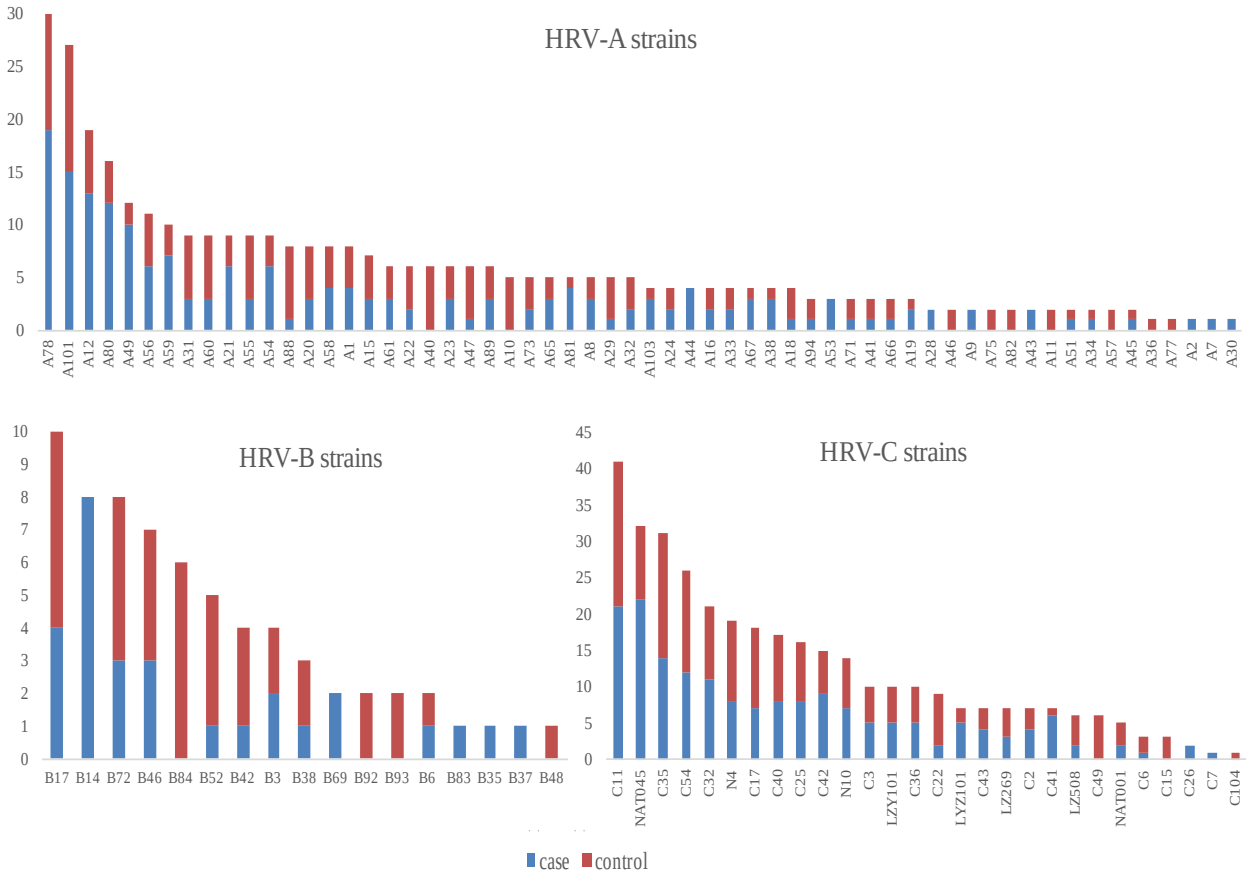

Figure 5.2: Frequency of HRV strains. For each species the prevalence of detected types is both cases and controls are indicated.

The HRV sequences for each of the species formed many unique clusters, with mean nucleotide diversity of 81.91% for HRV-A (nucleotide diversity range: 45.03% to 100%), 80.01% for HRV-B (nucleotide diversity range: 52.86% to 100%) and 73.54% for HRV-C (nucleotide diversity range 51.89% to 100%). The nucleotide diversity did not differ among cases and controls for the different HRV species. The HRV-A and HRV-B sequences clustered with statistically significant bootstrap support with the GenBank sequences of known HRV-A and -B strains; whereas, the HRV-C sequences tended to form numerous sub-clusters which did not always cluster closely with the GenBank sequences of known HRV-C strains. The numerous clusters and range of nucleotide diversities, especially in the HRV-C population, suggest considerable diversity in the strains present in the population (Figure 5.3-5.5).

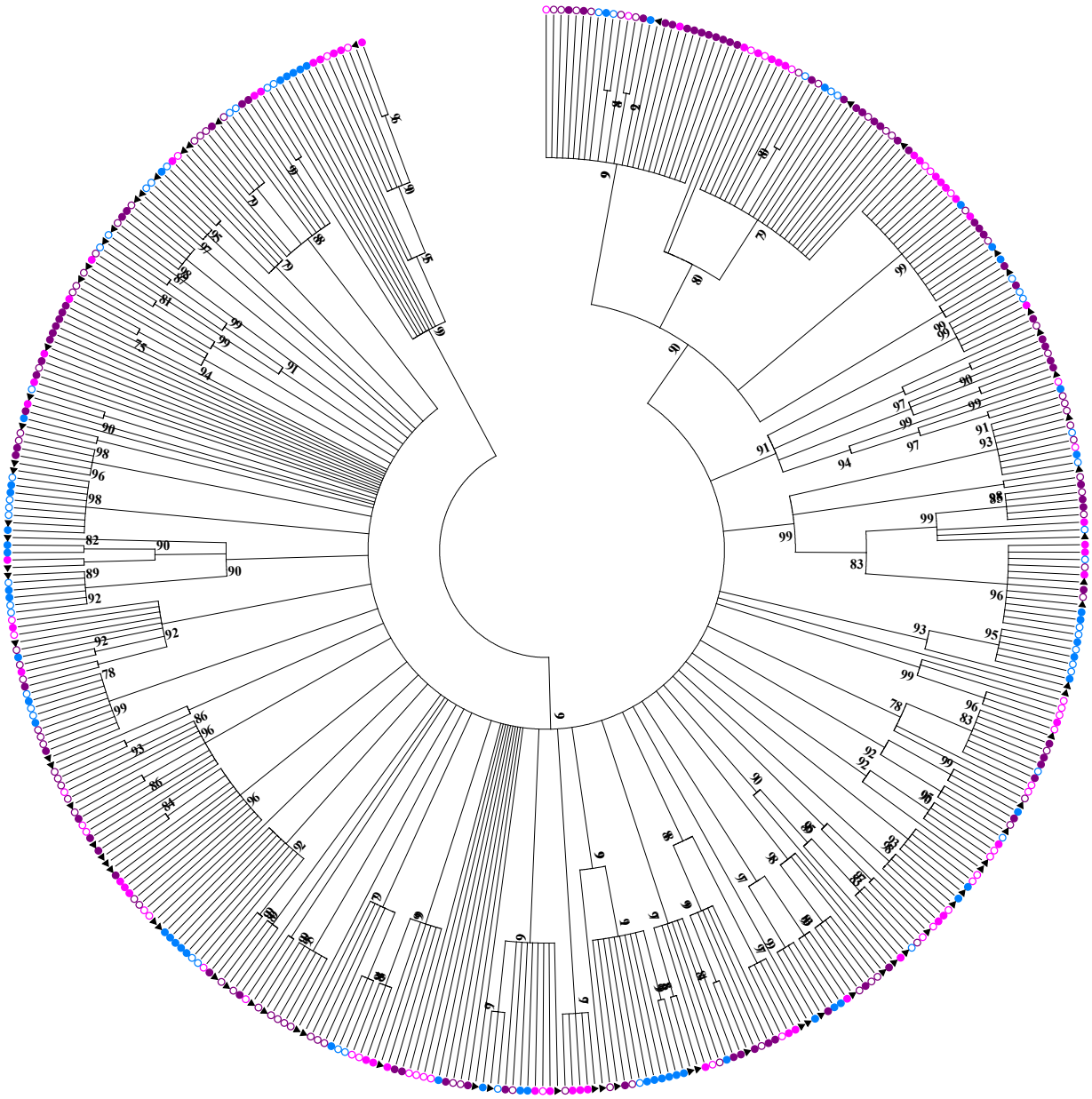

Figure 5.3: A phylogenetic analysis of HRV-A sequences from South Africa (●), Mali (●) and Zambia (●) alongside reference strains from GenBank (▲). Sequences with closed circles are from cases and those with open circles were detected in controls. Bootstrap values after 1000 replicates are shown next to the branches, values <70% have been omitted from the tree. The phylogenetic tree is drawn to scale and the branch length are in the same length of those used to infer the tree.

Among the HRV-A strains, the nucleotide similarities to the closest GenBank prototype reference strains varied from 82.3% to 99.4% and strain identity varied from 79.8% to 100% with other contemporaneous HRV-A strains.

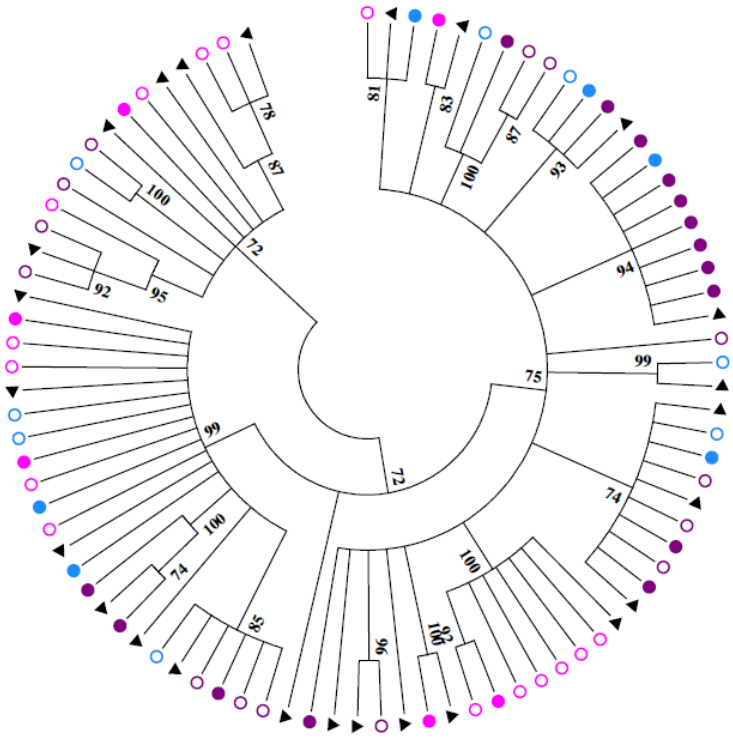

Figure 5.4: A phylogenetic analysis of HRV-B strains from South Africa (●), Mali (●) and Zambia (●) alongside reference strains from GenBank (▲). Sequences with closed circles are from cases and those with open circles were detected in controls. Bootstrap values after 1000 replicates are shown next to the branches, values <70% have been omitted from the tree. The phylogenetic tree is drawn to scale and the branch length are the same length of those used to infer the tree.

Among the HRV-B strains, the nucleotide similarities to the closest GenBank prototype reference strains varied from 87.9% to 99.8% and strain identity varied from 91.2% to 100% with other contemporaneous HRV-B strains.

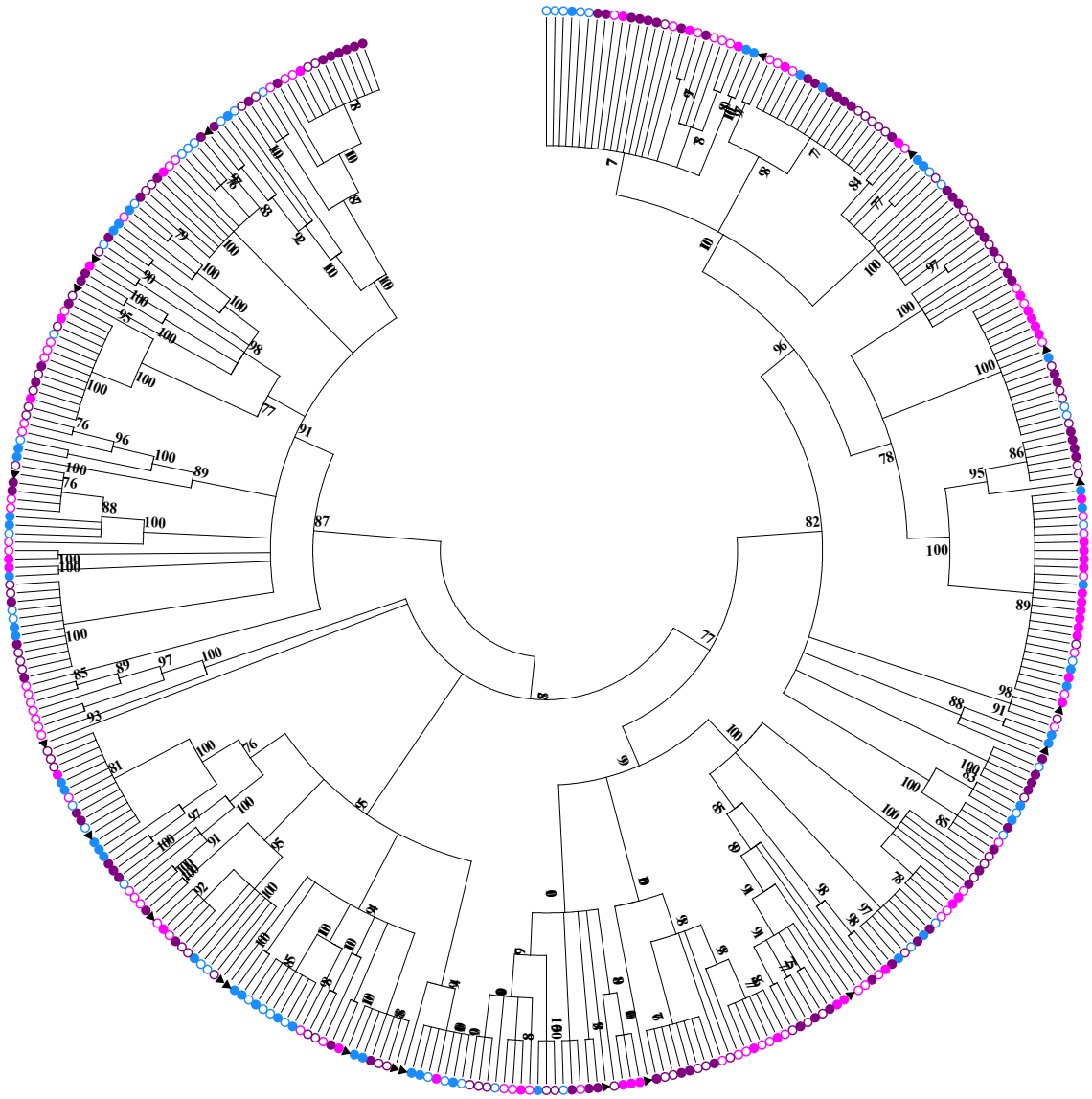

Figure 5.5: A phylogenetic analysis of HRV-C strains from South Africa (●), Mali (●) and Zambia (●) alongside reference strains from GenBank (▲). Sequences with closed circles are from cases and those with open circles were detected in controls. Bootstrap values after 1000 replicates are shown next to the branches, values <70% have been omitted from the tree. The phylogenetic tree is drawn to scale and the branch length are the same length of those used to infer the tree.

Among the HRV-C strains, the nucleotide similarities to the closest GenBank prototype reference strains showed much lower relatedness than the other two species and varied from 74.5% to 98.8%; however, there was a high degree of similarity with the other contemporaneous HRV-C strains with strain identity varying from 93.9% to 100%. According to the criteria proposed by McIntyre et al. (McIntyre et al. 2013) (pairwise distance >10.5 with prototype strains) no novel strains were identified in this study.

5.2 DISCUSSION

This study reports the strain typing of 836 HRV positive samples, including 415 samples from children hospitalised with severe and very severe pneumonia and 421 samples from community controls; making it the largest and most in-depth case-control study reporting on HRV molecular subtyping. HRV-A was the dominant species identified among cases (48%) and cases (45%), followed closely by HRV-C (45% each among cases and controls), whereas HRV-B was only seen intermittently and accounted for 7% and 10% of HRV strains among the HRV positive samples among cases and controls, respectively. Among the three Sub-Saharan countries, the overall prevalence of HRV detection did not differ between cases (21%) compared to controls (20%). The HRV prevalence was, however, associated with case status (21% vs. 15% among controls) among children 1-5 years of age, among whom there was a trend for higher percentage of the HRV belonging to HRV-C species among cases (60%) compared to controls (49%), albeit not significant. Furthermore, there was no evidence that HRV viral load differed significantly between the HRV species and there was no evidence that any of the HRV species were more likely to be associated with either more bacterial or viral co-infections. The HRV species distribution among cases and controls in our study are similar to that reported by others in both hospitalised and control populations [53, 92, 99, 123, 125].

Studies from Africa, Asia, Europe, America and Australia [26, 37, 42, 43] have all suggested that HRV-C may cause more severe illness and are more prevalent in lower respiratory tract infections than HRV-A and HRV-B. This was not evident in our study where both HRV-A and HRV-C were ubiquitous throughout the study period. Additionally, among controls there was no association between HRV species and signs and symptoms of respiratory tract infections. Similarly, there was no evidence that cases associated with HRV-C infection had more severe disease, based on presence of hypoxia, categorisation to being very severe pneumonia, prolonged hospital stay (>3 days duration), mechanical ventilation support or case fatality ratio. Instead, among HRV-associated cases those with HRV-A were more likely to have radiographically confirmed pneumonia and concurrent diarrhoea compared to those with HRV-C. Additionally, among cases where HRV was the only respiratory virus detected in the nasopharynx, those with HRV-A mono-infections where associated with more prolonged hospital stays, lethargy and convulsions compared to the cases with HRV-C mono-infections. Moreover, HRV-A mono-infected cases had 2.0-fold higher case fatality ratio than

all other cases. Thus in our study, HRV-A was associated with more severe disease than HRV-C.

HRV-C, rather, tended to be more frequent among older children compared to HRV-A. The greater proportion of HRV being HRV-C species among older children has also been reported by others [53, 86, 87], and they suggested this link between HRV-C and older children might be linked to the higher risk for asthma exacerbation and wheezing in older children. The link between HRV-C and wheeze is well established, with many studies reporting that HRV-C is more commonly associated with wheezing exacerbation than HRV-A or HRV-B [23, 35, 86, 87]. This was also seen in our study, whereby, cases presenting with wheeze were 1.64-fold more likely to have a HRV-C (35%) compared to HRV-A (25%, P=0.031). Our study was not, however, designed to fully study the association between HRV species and wheezing as a bronchodilator challenge was undertaken among prospective study participants, aimed at excluding children with hyper reactive airway disease, i.e. exclusion of children in whom the lower chest wall indrawing resolved post B2-agonist nebulisation challenger regardless of its effect on wheezing. This association of HRV-A with more severe LRTI and HRV-C with more wheezing disease has also been reported from a study in Burundi, where HRV-A was more prevalent among pneumonia and bronchitis cases and HRV-C among cases with acute wheezing [16]. This association between HRV-C and wheezing disease was also seen in a study which identified the cell receptors for HRV-C infection in humans, namely the CDHR-3 receptors, which allowed for HRV-C adhesion and replication and found that certain genetic coding mutations in the genes for these receptor proteins increased the binding and replication of HRV-C *in vivo.* This mutation (cysteine to tyrosine mutation at amino acid 529) has also associated with increased susceptibility to wheezing and asthma illnesses [29].

HRV-A and HRV-C strains were ubiquitous throughout the study period with a highly heterogeneous population of over 100 strains identified. HRV-A had the most diverse genetic population with 60 different strains, followed by HRV-C with 28 strains and then HRV-B with 17 different strains identified over the two-year period in both the cases and controls. Furthermore, there was no discernible relationships between strains identified among cases and controls and no real evidence of temporal clustering of strains over time. However, we failed to type 3% (n=25) of the HRV positive samples, similar percentages between cases and controls, which was largely due to PCR failure as a consequence of very low viral loads

which was inferred from Ct values greater than 37 cycle thresholds; although, it cannot be excluded that these PCR failures may be due to variability in the primer annealing sites thus some genetic variants might have been missed by our typing assays. Regardless, the considerable genetic diversity of HRV reported in this study highlights the heterogeneity of the HRV strains circulating within the general populations. However, the prevalence and the genetic population of HRV was similar among children hospitalised with pneumonia and community controls, which further highlights the difficulties in attributing causality of disease to HRV and also negate that some strains might be more virulent. This might be compounded by the fact HRV has been shown to shed for prolonged periods post infection [51, 124], thus we cannot determine whether the HRV species detected are from active infection or shedding from previous infection. Other studies have also reported on the diverse nature of the HRV genetic population among both cases and controls including studies from South Africa [123], Botswana [99], Kenya [92], and other countries [53, 122, 125] as well as the lack of obvious seasonal patterns among cases [16, 23, 52, 85, 128]. Additional longitudinal studies are required to further clarify this, as well as whether there are differences in species/strain specific duration of shedding.

A limitation of the study is that the non-coding region used to type the HRV strains is not involved in the immune evasion mechanisms of the virus, thus although it is ideal for differentiating between the >160 different HRV genotypes, the data it provides in terms of molecular epidemiology of disease is unclear. Future studies done in conjunction with surface protein epitopes such as VP1-4 sequencing are needed to study the molecular epidemiology in relation to clinical outcomes in greater depth.

In conclusion, this study emphasises the highly divergent nature of the HRV population circulating in both community controls as well as children hospitalised with pneumonia, as well as shows that similar strains are circulating over a large geographical location and strains are similar year to year. Additionally, it further highlights the difficulty in attributing causality of LRTI disease to HRV, as no differences were observed in prevalence of HRV detection between cases and controls (other than in the 1-5 year age group), nor were there differences in HRV genetic population between cases and controls, especially among infants. Nevertheless, among cases, HRV-A tended to be more important among younger children and was associated with more severe disease and radiographically confirmed pneumonia

compared to HRV-C infections, whereas HRV-C was more important among older children with wheezing disease. Furthermore, mono-viral infections with HRV-A was associated with higher case fatality ratio.

6.0 A case-control study on association of Human rhinovirus (HRV) nasopharyngeal viral load and viraemia in South African children with WHO-defined pneumonia

HRV is considered to be an important pathogen during childhood disease; however, it is ubiquitous in diseased and asymptomatic individuals thus obscuring the true etiological role in respiratory infections [47]. Previous studies conducted on children with respiratory illnesses have investigated the association of HRV nasopharyngeal viral load and presence of HRV viraemia [64, 65]; but have not simultaneously enrolled asymptomatic controls, hence, limiting any inferences on causality or them being markers of severity in children with pneumonia.

In this chapter, the epidemiology, including nasopharyngeal HRV viral load and prevalence of viraemia is reported from South African children hospitalised with WHO-defined pneumonia and age-frequency matched community controls.

6.1 RESULTS

6.1.1 HRV subtyping among cases and controls

A total of 911 children were enrolled at the South African PERCH site with WHO-defined severe (n=624, 68%) or very severe pneumonia (n=298, 32%), of whom 23% (n=210/911) had HRV identified on nasopharyngeal swab samples through diagnostic real-time PCR. Additionally, 959 children were enrolled as age-matched community controls, 911 of controls were asymptomatic for respiratory illness and 53 had signs and symptoms of ARI. The overall prevalence of identifying HRV on NP swab among controls was 22% (n=212/959). There was no difference in overall prevalence of HRV NP swab positivity among cases (23%) and controls (21%, P=0.66).

The cases and controls with HRV infection were similar with regard to mean age, gender and prevalence of HIV-infection; however, the HRV-associated cases compared to controls were 3.7-fold (aOR 95% CI: 1.33-10.40) more likely to be malnourished (8% vs. 2%, P=0.012), but less likely to have been born prematurely (18% vs. 33%, P=0.004); Table 6.1. Furthermore, cases hospitalised with HRV-associated pneumonia were 2-fold (aOR 95% CI:

1.22-3.38) more likely to have another respiratory virus co-infection (46% vs. 31%, P=0.001), which was specifically evident for RSV (16% vs. 2%, aOR 7.91, 95% CI 2.99-20.94, P<0.001); whilst the prevalence of coinfection by other respiratory viruses did not differ; Table 6.1.

The 5' NCR of the HRV genome was successfully amplified in 99% (n=207/210) of case samples and 96% (n=203/212, P=0.262) of the community control samples. The un-typed samples from case (1%, n=3/210) and community controls (4%, n=9/212) failed to amplify and on analysis of the real-time PCR results these samples had late cycle threshold values above 35 cycles. In addition, from the sequencing analysis it was determined that 4% (n=9/207) of HRV-associated case samples and 7% (n=14/203, P=0.262) of community controls respectively were in fact enteroviruses, a closely related virus member from the same family of *Picornaviridae* as HRV. The distribution of the three HRV species in the 198 HRV-associated cases and 189 HRV-associated community controls did not differ significantly between the cases compared to controls (P=0.618); Table 6.1 and Figure 6.1.

Table 6.1: Demographics of HRV-associated cases and community controls

	HRV+ cases (n=210)	HRV+ controls (n=212)	Unadjusted *P*-value	Unadjusted OR (95% CI)	Adjusted *P*-value	Adjusted aOR (95% CI)
Age in months, mean (SD)	9.7 (9.7)	11.4 (11.4)	0.090		0.082	
Female, n(%)	95 (45%)	114 (54%)	0.080	1.41 (0.96-2.07)	0.061	0.69 (0.47-1.02)
HIV+, n(%)	28 (13%)	18 (8%)	0.156	1.56 (0.84-2.90)	0.112	1.66 (0.90-3.10)
HEU, n(%)[a]	67 (32%)	52 (25%)	0.093	1.44 (0.94-2.21)	0.095	1.34 (0.90-2.23)
Never breast fed, n(%)	63 (30%)	75 (35%)	0.239	0.78 (0.52-1.18)	0.255	0.79 (0.52-1.19)
Under weight, n (%)[b]	17 (8%)	5 (2%)	0.013	3.64 (1.32-10.07)	0.012	3.73 (1.33-10.40)
Day Care attendance, n(%)	28 (13%)	31 (15%)	0.712	0.90 (0.53-1.55)	0.783	0.93 (0.52-1.63)
Smoker in household, n(%)	81 (39%)	66 (31%)	0.089	1.41 (0.95-2.12)	0.078	1.44 (0.96-2.16)
Premature birth, n(%)[c]	37 (18%)	69 (33%)	0.005	0.54 (0.35-0.83)	0.004	0.53 (0.35-0.82
Birth weight, mean (SD)	2.92 (0.7)	2.94 (0.6)	0.694		0.655	
Markers for Bacterial co-infection:						
***LytA* positive, n (%)[d]**	12 (6%)	21 (10%)	0.113	0.55 (0.26-1.15)	0.147	0.58 (0.27-1.22)
S. pneu load, mean (SD)[e]	5.73 (1.21)	5.88 (1.13)	0.274		0.176	
HDP, n(%)[f]						
-Blood	9 (4%)	12 (6%)	0.516	0.75 (0.31-1.81)	0.607	0.79 (0.32-1.93)
-NP	23 (11%)	29 (14%)	0.327	0.74 (0.41-1.34)	0.328	0.72 (0.39-1.38)
HRV subtyping:						
HRV Mono-infection, n(%)[g]	113 (54%)	146 (69%)	0.002	0.53 (0.34-0.78)	0.001	0.51 (0.34-0.76)
HRV-A, n(%)	91 (44%)	96 (47%)				
HRV-B, n(%)	16 (8%)	14 (7%)				
HRV-C, n(%)	91 (44%)	79 (38%)	0.688		0.618	
Un-typeable, n(%)	3 (3%)	9 (5%)				
Enterovirus, n(%)	9 (3%)	14 (6%)				
HRV viral load, mean (SD)[h]	4.0 (0.98)	3.7 (0.94)	0.062		0.060	
Viral co-infections in the nasopharynx:						
-RSV, n(%)	33 (16%)	5 (2%)	*P*<0.001	7.71 (2.95-20.19)	*P*<0.001	7.91 (2.99-20.94)
-AdV, n(%)	22 (10%)	24 (11%)	0.781	0.92-0.50-1.69)	0.985	0.99 (0.53-1.86)
-HMPV, n(%)	7 (3%)	5 (2%)	0.549	1.43 (0.45-4.57)	0.595	1.38 (0.42-4.46)
-HBoV, n(%)	31 (15%)	24 (11%)	0.295	1.36 (0.77-2.40)	0.177	1.50 (0.83-2.68)
-InFV A-C, n(%)	2 (1%)	2 (1%)	0.992	1.0 (0.14-7.23)	0.983	0.98 (0.13-7.13)
-PIV, n(%)	12 (6%)	7 (3%)	0.238	1.77 (0.68-4.60)	0.262	1.73 (0.66-4.52)
-HCoV, n(%)	15 (7%)	16 (8%)	0.874	0.94 (0.45-1.96)	0.920	0.96 (0.46-2.02)

Abbreviations - HIV: Human Immunodeficiency virus; HEU: HIV exposed but uninfected; OR: Odds ratio; aOR: Adjusted odds ratio; CI: Confidence interval; SD: Standard deviation; NP: Nasopharynx; HDP: High density pneumococcus; HRV: Human rhinovirus; RSV: Respiratory Syncytial Virus (A and B), HMPV: Human Metapneumovirus; AdV: Adenovirus; PIV: Parainfluenza type 1-4; HBoV: Human Bocavirus; HCoV: Human Coronavirus (OC43, NL63, 229E and HKU1) and InFV A-C: Influenza Virus (A, B and C); *S. pneu: Streptococcus pneumoniae*

P-values from chi-square and Wilcoxon tests - logistic regression models adjusted for confounding variates (<0.2 in univariate analysis) where applicable; odds ratios could not be calculated for variables with zero variables.

[a] - HEU defined as HIV-uninfected but HIV-exposed. Undetectable viral load, HIV seronegative in the child with a positive maternal history. Positive maternal status based on self-report was accepted, except for seronegative children < 7 months of age where documented positive maternal status was required; [b] - Underweight defined as weight for age <-2SD of the median age-sex specific WHO reference; [c] - Premature birth defined as gestational age <37 weeks; [d] - Blood sample positive for *S. pneumoniae* colonisation by *LytA* PCR; [e] - Bacterial load of *S. pneu* in the nasopharynx, expressed as $\log_{10}$ copies/mL; [f] - HDP defined as *S. pneumoniae* density in nasopharynx >6.9 and/or density in whole blood sample >2.2 $\log_{10}$ copies/mL; [g]- HRV was the only respiratory virus detected in the nasopharynx; [h] - HRV viral load in the nasopharynx, expressed as log copies/ml

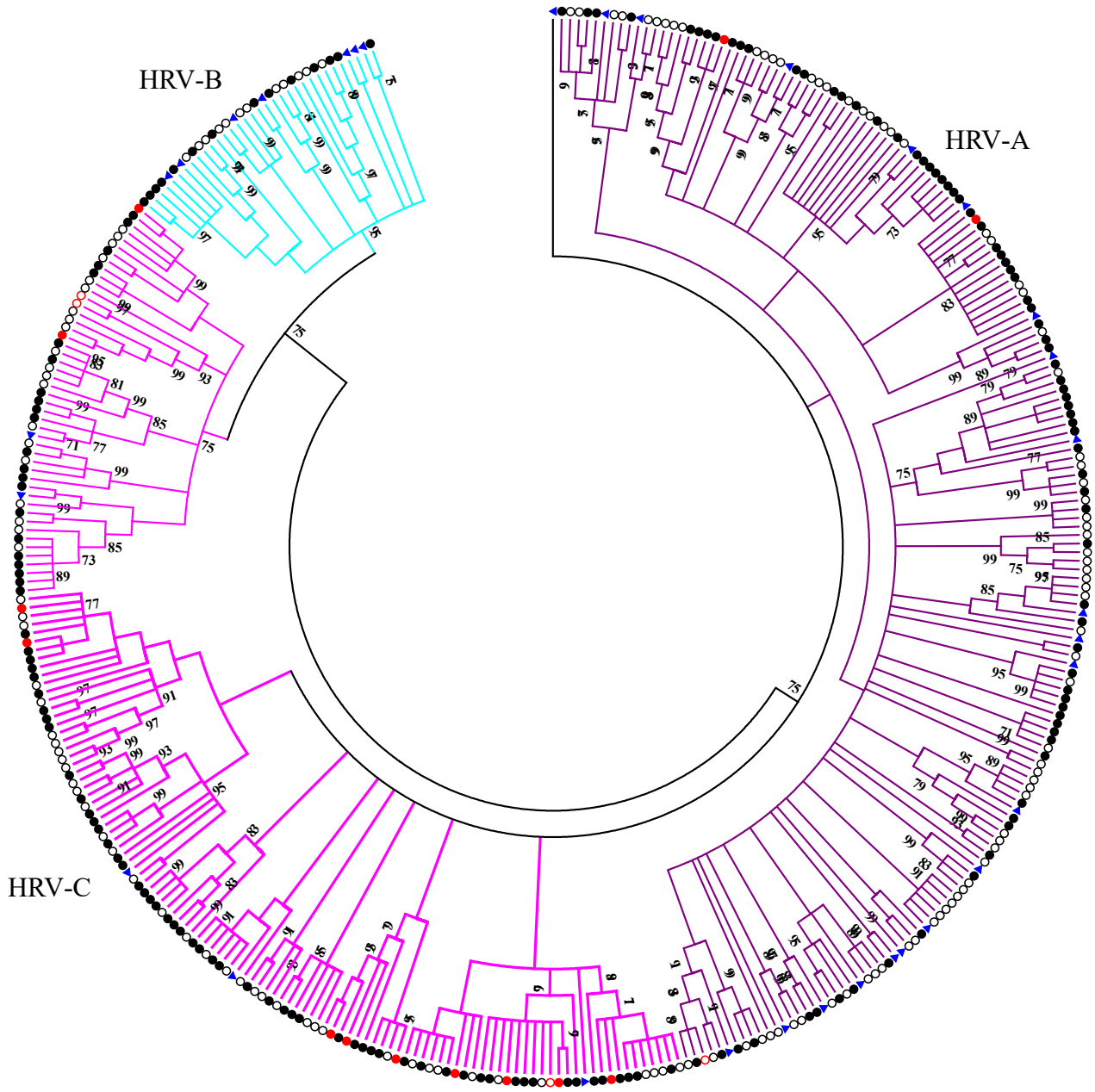

Figure 6.1: A phylogenetic analysis of HRV sequences. Sequences with closed circles denote types identified in nasopharyngeal case samples (●) and sequences with open circles denote types identified in nasopharyngeal control samples (O). Red, closed circles (●) denote types identified in viraemic cases and red, open circles (O) denoted types identified in control with viraemia. Closed triangles (▲) denote reference strains from GenBank. HRV-A types are indicated by purple branches, HRV-B types are indicated by light blue branches and HRV-C are indicated by pink branches. Bootstrap values after 1000 replicates are shown next to the branches, values <70% have been omitted from the tree. The phylogenetic tree is drawn to scale and the branch length are in the same length of those used to infer the tree.

6.1.2 Seasonal Distribution of HRV species

Case and controls with HRV infections were identified perennially during the 2-year study period, although the month-on-month prevalence of positivity ranged from 0 to 40%. The

month-on-month prevalence of HRV was similar between cases and controls, with the highest prevalence observed from December 2011-February 2012 (36%-39%), April 2012 (30%), February 2013 (32%) and July 2013-August 2013 (38%-40%); i.e. being independent of summer (December-February) and winter (June-August) in Johannesburg, South Africa. HRV-A and HRV-C co-circulated in most months, and their prevalence fluctuated continuously. HRV-B appeared sporadically through the study period in both cases and controls; Figure 6.2.

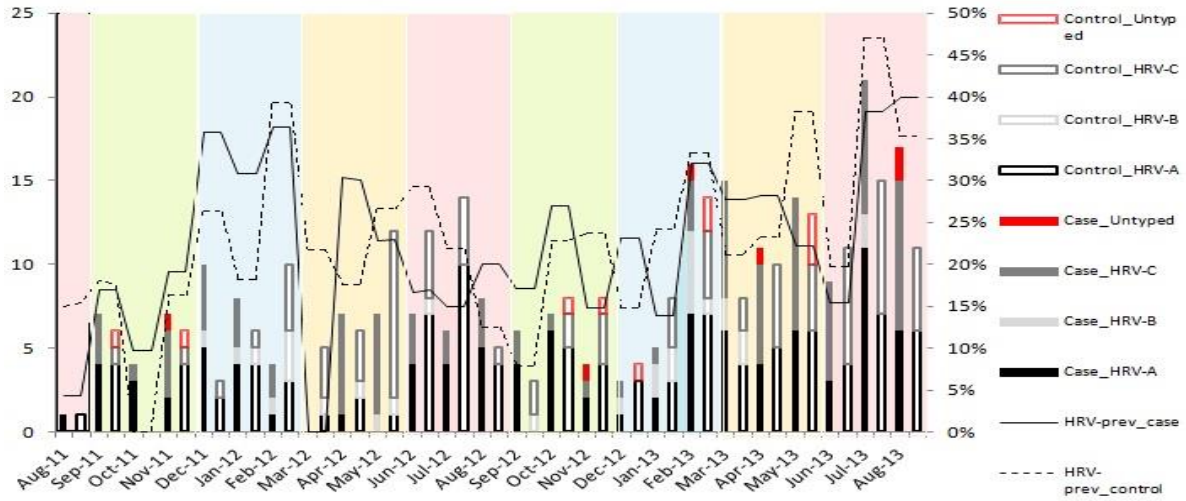

Figure 6.2: The monthly distribution of HRV species over a 2-year period in children hospitalised with pneumonia (cases) and community controls. On the y-axis are the proportion of samples from cases and controls positive for HRV infections. The seasons are indicated on the graph - red for winter months (June-end of August), green for spring (September-end of November), blue for summer (December- end of March) and orange for autumn months (April-end of May).

6.1.3 Nasopharyngeal HRV viral load

The nasopharyngeal HRV viral load (log_{10} copies/mL) in children hospitalised with HRV-associated pneumonia was not associated with any difference by age, gender, HIV status, disease diagnosis, clinical characteristics and length of hospital stay as well as independent of HRV species and whether HRV was the only virus detected in the nasopharynx. The presence of fever was however associated with a lower nasopharyngeal HRV viral load (3.82 log_{10} copies/mL) compared to HRV-associated cases without fever (4.25 log_{10} copies/mL, P=0.009); Table 6.2.

Table 6.2: Nasopharyngeal HRV viral load by demographics and clinical characteristics in HRV-associated cases

	n=198	Mean HRV viral load (log_{10} cp/ml)	*P*-value
Age			
<1 year	142	3.90 (0.95)	
>1year	56	4.04 (1.04)	0.368
Gender			
Male	110	3.8 (0.95)	
Female	88	4.02 (1.01)	0.312
HIV			
Negative	170	3.99 (0.99)	
Positive	28	3.65 (0.82)	0.071
HEU[a]			
Negative	133	3.90	
Positive	65	4.04 (0.93)	0.413
Diagnosis			
Severe pneumonia	126	3.86 (0.96)	
Very severe pneumonia	72	4.08 (0.99)	0.123
Chest X-ray[b]			
Normal	88	3.88 (0.93)	
Abnormal	99	4.00 (1.01)	0.384
Hypoxia[c]			
Yes	146	3.85 (0.95)	
No	50	3.98 0.99)	0.420
Supplementary Oxygen therapy			
Yes	183	3.95 (0.98)	
No	15	3.81 (0.97)	0.601
Mechanical ventilation			
Yes	10	3.90 (0.94)	
No	188	3.94 (0.98)	0.886
Wheeze			
Yes	75	3.95 (0.98)	
No	121	3.93 (0.97)	0.938
Fever[d]			
Yes	137	3.82 (0.85)	
No	61	4.25 (1.12)	0.009
Tachypnea[e]			
Yes	159	3.99 (0.98)	
No	36	3.67 (0.95)	0.073
Tachycardia[f]			
Yes	96	4.02 (1.02)	
No	101	3.86 (0.94)	0.250
Leucocytosis[g]			
Yes	101	4.01 0.89)	
No	97	3.87 (1.06)	0.292
Hospital stay			
>3 days	78	3.87 (0.91)	
<3 days	120	3.99 (1.02)	0.425
Case fatalities			
Yes	11	4.15 (0.97)	
No	153	3.94 (1.11)	0.494
HRV mono-infection	65	3.90 (0.97)	
HRV mixed infection	133	3.96 (0.98)	0.701
Type of HRV species			
-A	91	3.96 0.97)	
-B	16	3.57 (0.70)	
-C	91	3.99 (1.01)	0.271
HRV viraemia			
Yes	13	4.67 (0.73)	
No	185	3.90 (0.98)	0.028

HRV-A			
Viraemia present	2	3.75 (1.7)	
Viraemia absent	89	3.96 (0.9)	0.765
HRV-C			
Viraemia present	11	4.72 (0.45)	
Viraemia absent	80	3.87 (1.04)	0.016

Abbreviations - HIV: Human Immunodeficiency virus; HEU: HIV negative but HIV exposed; HRV: Human rhinovirus.

P-values from chi-square and Wilcoxon tests - logistic regression models adjusted for confounding variates (<0.2 in univariate analysis) where applicable.

[a] - HEU defined as undetectable viral load, HIV seronegative in the child with a positive maternal history. Positive maternal status based on self-report was accepted, except for seronegative children < 7 months of age where documented positive maternal status was required; [b] - Abnormal chest X-rays defined as defined as primary end point pneumonia or infiltrates; [c] - Hypoxic defined as 1) a room air pulse-oximetry reading indicated oxygen saturation <90% at the two sites at elevation (Zambia and South Africa) or <92% at all other sites, or 2) a room air oxygen saturation; [d] - Fever defined as body temperature >38^{o}C; [e] - Tachypnea defined as respiratory rate >60 breaths/minute if aged <2 months, respiratory rate >50 breaths/minute if aged 2-12 months, respiration rate >40 breaths/minute if aged >12 month; [f] - Tachycardia defined as heart rate >160 beats per minute (bpm) if aged <11 months, heart rate >150 bpm if aged 12-35 months, heart rate >140 bpm if aged 36-59 months; [g] - Leucocytosis defined as white blood cell count >15 000 cells/uL if age <12 months, white blood cell count >13 000 cells/uL if age >12 months.

Similarly to the HRV-associated cases, the nasopharyngeal HRV viral load among community controls was also not associated with any differences by age, gender, HIV-1-infected and HIV-exposure as well as independent of HRV species and whether HRV was detected as a mono viral infection in the nasopharynx. The nasopharyngeal HRV viral load was, however, higher among controls with symptoms of RTI (4.48 $\log_{10}$ copies/mL) compared to asymptomatic controls (3.77 $\log_{10}$ copies/mL, P=0.041); Table 6.3.

Table 6.3: Nasopharyngeal HRV viral load by demographics and clinical characteristics in HRV-associated community controls

	n=189	Mean NP RV load (log_{10} cp/ml)	*P*-value
Age			
<1 year	136	3.88 (0.86)	
>1year	52	3.65 (1.12)	0.141
Gender			
Male	83	3.89 (0.96)	
Female	106	3.75 (0.92)	0.336
HIV			
Yes	15	3.83 (0.88)	
No	173	3.82 (0.95)	0.848
HEU[a]			
Yes	46	3.67 (1.04)	
No	143	3.86 (0.90)	0.226
Diagnosis			
Asymptomatic control	178	3.77 (0.94)	
ARTI control	11	4.48 (0.63)	0.041
Tachypnea[b]			
Yes	10	8.40 (8.35)	
No	159	3.83 (0.97)	0.588
HRV mono-infection	60	3.82 (0.97)	
HRV mixed infection	129	3.81 (0.93)	0.964
Type of HRV			
-A	96	3.75 (0.90)	
-B	14	3.43 (0.82)	
-C	79	3.95 (0.99)	0.877
HRV viraemia			
Yes	4	4.83 (0.83)	
No	185	3.79 (0.93)	0.018
HRV-A			
Viraemia present	1	5.83 (-)	
Viraemia absent	95	3.73 (0.88)	NP
HRV-C			
Viraemia present	3	4.50 (0.60)	
Viraemia absent	76	3.93 (0.99)	0.181

Abbreviations - HIV: Human Immunodeficiency virus; HEU: HIV-uninfected but HIV-exposed; HRV: Human rhinovirus.

P-values from chi-square and Wilcoxon tests - logistic regression models adjusted for confounding variates (<0.2 in univariate analysis) where applicable.

[a] - HEU defined as undetectable viral load, HIV seronegative in the child with a positive maternal history. Positive maternal status based on self-report was accepted, except for seronegative children < 7 months of age where documented positive maternal status was required; [b] - Tachypnea = Respiratory rate >60 breaths/minute if aged <2 months, respiratory rate >50 breaths/minute if aged 2-12 months, respiration rate >40 breaths/minute if aged >12 month; NP - not powered to calculate *P*-value.

Although there was a trend for higher nasopharyngeal HRV viral load among cases (4.0 log_{10} copies/mL) compared to controls (3.7 log_{10} copies/mL, P=0.062; Table 6.1); there was no discernible nasopharyngeal density threshold for differentiating cases from controls as evident on the reverse cumulative plot; Figure 6.3.

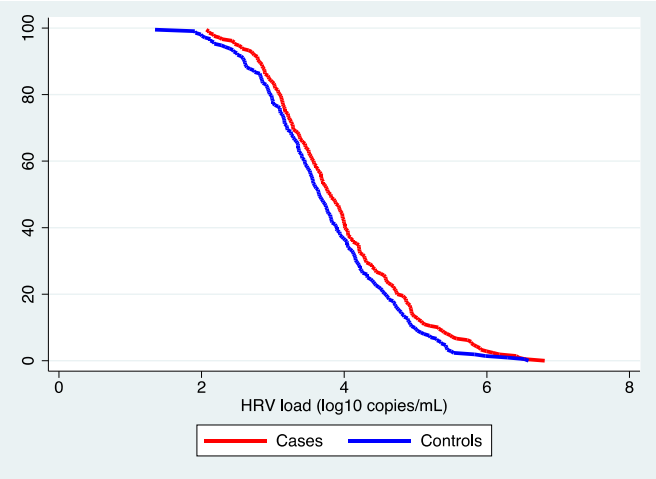

Figure 6.3: Reverse cumulative plot of HRV viral load in the nasopharynx among cases and controls. The viral loads of cases are shown in red and the controls in blue.

6.1.4 HRV viraemia

Overall, 7% (n=13/198) of HRV-associated cases and 2% (n=4/189, aOR 7.02, 95% CI 1.70-28.94, *P*=0.007) of controls were identified as having HRV viraemia using the HRV 5'NCR PCR analysis. For both cases and controls with viraemia, the HRV species detected on blood was identical to the respiratory species. The positivity rate of viraemia differed between HRV species among the cases, being highest for HRV-C (12%, n=11/91), lower for HRV-A (2%, n=2/91) and not identified for any of the HRV-B cases (0%, 0/16, *P*=0.014). Similar trends were seen in positivity rates of viraemia among controls, highest for HRV-C (4%, n=3/79), lower for HRV-A (1%, n=1/96) and no HRV-B viraemia cases (0%, n=0/14).

HRV-C viraemia was 4.43-fold (aOR 95% CI 1.22-16.04) more prevalent among cases (12%) than controls (4%, *P*=0.023). This was, however, not significantly different for HRV-A (2% vs. 1%, *P*=0.529) or for HRV-B (0%); Table 6.4. Furthermore, among HRV-associated cases, HRV-C was 2.59-fold (aOR 95% CI: 1.23-5.95) more likely to be associated with viraemia compared to HRV-A (12% vs. 2%, *P*=0.025).

Table 6.4: HRV molecular subtyping by sample type and viraemia status in HRV-associated pneumonia cases.

	Cases (n=198)			**Controls (n=189)**		
	Viraemia- (n=185)	**Viraemia+ (n=13)**	***P*-value**	**Viraemia- (n=185)**	**Viraemia+ (n=4)**	***P*-value**
HRV-A	89 (48%)	2 (15%)		95 (51%)	1 (25%)	
HRV-B	16 (9%)	0	0.001	14 (8%)	0	0.384
HRV-C	80 (43%)	11 (85%)		76 (41%)	3 (75%)	

P-values calculated using Fisher's exact test and t-test where necessary; HRV- Human rhinovirus. % refers to proportion of species within the column.

Among children with HRV-associated pneumonia, the presence of viraemia was associated with higher HRV nasopharyngeal load compared to non-viraemic cases (4.67 versus 3.90 $\log_{10}$ copies/mL, P=0.028). This difference was mainly driven by the HRV-C species, (viral load 4.72 compared to 3.87 $\log_{10}$ copies/ mL, P=0.016), whilst not evident for HRV-A (P=0.765); Table 6.2. Similarly, among HRV-associated controls, the nasopharyngeal viral load was higher in the presence of viraemia compared to the non-viraemic controls (4.83 vs. 3.79 $\log_{10}$ copies/mL, P=0.018), associations by individual HRV species were not significant due to the very small sample size of HRV viraemia positive controls; Table 6.3. There was no difference in the nasopharyngeal HRV viral load between viraemic cases (4.67 $\log_{10}$ copies/mL) compared to viraemic controls (4.83 $\log_{10}$ copies/mL, P=0.285).

Among HRV-associated cases; Table 6.5, those with viraemia were older (18.6 months) than children without viraemia (9.2 months, P=0.001). The presence of viraemia among cases was not associated with any of the indices of pneumonia severity, including requiring supplementary oxygen therapy, having abnormal chest X-rays or presence of leucocytosis. However, the HRV viraemic cases had a higher whole blood neutrophil percentage (68.1%), and lower lymphocyte percentage (23.3%) compared to cases without viraemia (48.5%, P=0.023 and 39.8%, P=0.024 respectively). Additionally, viraemic cases tended to be more likely associated with wheezing (62% vs. 37%, P=0.069) but less likely to be hospitalised for >3 days (8% vs. 42%, P=0.049) compared to non-viraemic cases. None of the HRV viraemic cases were fatal; with no significant difference in case fatality ratio between the HRV viraemic and non-viraemic cases (0% vs. 7%, P=0.382); Table 6.5.

Table 6.5: Characteristics and outcomes of children hospitalised with HRV-associated pneumonia by viraemia status

Characteristics	Viraemia+ (n=13)	Viraemia- (n=185)	Unadjusted *P*-value	Unadjusted OR (95% CI)	Adjusted *P*-value	Adjusted aOR (95% CI)
Age in months, mean (SD)	18.6 (14.1)	9.2 (9.2)	0.002		0.001	
Female, n(%)	7 (54%)	81 (44%)	0.480	1.50 (0.48-4.63)	0.370	1.70 (0.53-5.45)
HIV positive, n(%)	0	28 (15%)	0.130		0.269	
HEU, n(%)[a]	6 (46%)	59 (31%)	0.290	1.83 (0.59-5.69)	0.368	1.72 (0.53-5.57)
Never breast fed, n(%)	6 (46%)	54 (29%)	0.198	2.08 (0.67-6.47)	0.151	2.40 (0.73-7.84)
Under weight, n (%)[b]	0	17 (9%)	0.253		0.505	
Day care attendance, n(%)	4 (31%)	21 (11%)	0.667	1.11 (0.68-1.82)	0.791	1.09 (0.58-2.03)
Smoker in household, n(%)	5 (38%)	71 (38%)	0.965	0.91 (0.37-2.25)	0.652	0.81 (0.33-2.02)
Premature birth, n(%)[c]	2 (15%)	34 (18%)	0.619	0.65 (0.22-1.96)	0.529	0.75 (0.31-1.83)
Birth weight, mean (SD)	3.0 (0.4)	2.9 (0.7)	0.775		0.876	
Clinical Features:						
Very severe pneumonia, n(%)	7 (54%)	65 (35%)	0.175	2.15 (0.69-6.68)	0.168	2.31 (0.70-7.61)
Chest X-ray abnormal, n(%)[d]	6 (46%)	93 (53%)	0.611	0.75 (0.24-2.31)	0.522	0.67 (0.20-2.25)
Supplementary 0_2 therapy, n(%)	12 (92%)	171 (92%)	0.987	0.98 (0.12-8.11)	0.936	1.09 (0.12-9.63)
Mechanical ventilation, n(%)	0	10 (5%)	0.390		0.703	
Hypoxic, n(%)[e]	9 (69%)	137 (75%)	0.653	0.76 (0.22-2.57)	0.812	1.17 (0.33-4.15)
Tachycardia, n(%)[f]	9 (69%)	87 (47%)	0.126	2.51 (0.75-8.43)	0.153	2.49 (0.71-8.70)
Tachypnea, n(%)[g]	12 (92%)	147 (81%)	0.300	2.86 (0.36-22.71)	0.369	2.65 (0.32-22.2)
Fever, n(%)	7 (54%)	130 (70%)	0.223	0.49 (0.16-1.54)	0.192	0.46 (0.14-1.49)
Wheezing, n(%)	8 (62%)	67 (37%)	0.064	2.77 (0.91-8.81)	0.069	1.68 (0.89-5.57)
Cough, n(%)	13 (100%)	157 (85%)	0.552	1.21 (0.64-2.28)	0.864	1.07 (0.50-2.30)
Lethargic, n(%)	0	11 (6%)	0.366		0.687	
Convulsions, n(%)	0	5 (3%)	0.546		0.998	
Diarrhoea, n(%)	1 (8%)	35 (19%)	0.310	0.36 (0.05-2.83)	0.565	0.53 (0.06-4.53)
Head nodding, n(%)	7 (54%)	54 (29%)	0.063	2.83 (0.91-8.81)	0.079	2.90 (0.89-9.56)
Central cyanosis, n(%)	0	1 (1%)	0.790		0.181	
Unable to Feed, n(%)	0	2 (1%)	0.706		0.777	
Vomiting everything, n(%)	0	3 (2%)	0.644		0.720	
Lower chest wall indrawing, n(%)	12 (92%)	176 (95%)	0.653	0.61 (0.07-5.25)	0.739	0.68 (0.07-6.42)
Stridor, n(%)	1 (8%)	5 (3%)	0.561	0.93 (0.39-2.22)	0.704	0.84 (0.33-2.11)
Grunting, n(%)	0	10 (5%)	0.807		0.860	
Nasal Flaring, n(%)	13 (100%)	160 (86%)	0.156		0.391	
Laboratory markers:						
Leucocytosis, n(%)[h]	9 (69%)	92 (50%)	0.174	2.27 (0.68-7.65)	0.195	2.30 (0.65-8.09)
Neutrophils (%), median (IQR)	68.1 (15.2)	48.5 (19.1)	0.001		0.023	
Lymphocytes (%), median (IQR)	23.3 (13.3)	39.8 (16.5)	0.001		0.024	
Eosinophils (%), median (IQR)	1.3 (2.1)	1.4 (2.8)	0.788		0.089	
CRP≥40mg/l, n(%)[i]	3 (23%)	51 (28%)	0.803	0.76 (0.34-3.96)	0.629	0.56 (0.38-4.88)
Blood culture positive, n(%)	0	5 (3%)	0.548		0.196	
LytA positive, n(%)[j]	1 (7%)	10 (5%)	0.145	3.18 (0.62-16.33)	0.229	2.92 (0.51-16.7)
MCPP, n(%)[k]	0	1 (0.5%)	0.790		0.748	
HDP, n(%)[l]						
-Blood	1 (8%)	8 (4%)	0.573	1.84 (0.21-15.98)	0.539	2.07 (0.20-21.3)
-NP	1 (8%)	21 (11%)	0.685	0.65 (0.08-5.26)	0.896	0.87 (0.10-7.54)
HRV mono-infection, n(%)[m]	7 (54%)	99 (54%)	0.981	1.01 (0.33-3.13)	0.887	1.09 (0.33-3.61)
Hospital stay >3 days, n(%)	1 (8%)	77 (42%)	0.016	0.12 (0.14-0.62)	0.049	0.16 (0.02-0.98)
Case fatality ratio, n(%)	0	11 (7%)	0.382		0.756	

Abbreviations - HIV: Human Immunodeficiency virus; HEU: HIV exposed but uninfected; OR: Odds ratio; aOR: Adjusted odds ratio; CI: Confidence interval; SD: Standard deviation; IQR: Inter quartile range; NP: Nasopharynx; CRP: C-reactive protein; HDP: High density pneumococcus; MCPP: Microbiologically confirmed pneumococcal pneumonia; HRV: Human rhinovirus

P-values from Chi-squared and Wilcoxon tests, logistic regression models adjusted for confounding variates (<0.2 in univariate analysis) where applicable, Odds ratio could not be calculated for continuous variables or variables with 0 values, thus cells left blank.

[a] - HEU defined as undetectable viral load, HIV seronegative in the child with a positive maternal history. Positive maternal status based on self-report was accepted, except for seronegative children < 7 months of age where documented positive maternal status was required; [b] - Underweight defined as weight for age <-2SD of the

median age-sex specific WHO reference; [c] - Defined as primary end point pneumonia and/or infiltrates; [d] - Hypoxic defined 1) a room air pulse-oximetry reading indicated oxygen saturation <90% at the two sites at elevation (Zambia and South Africa) or <92% at all other sites, or 2) a room air oxygen saturation reading was not available and the child was on oxygen; [e] - Tachycardia defined as heart rate >160 beats per minute (bpm) if aged <11 months, heart rate >150 bpm if aged 12-35 months, heart rate >140 bpm if aged 36-59 months; [f] - Tachypnea defined as respiratory rate >60 breaths/minute if aged <2 months, respiratory rate >50 breaths/minute if aged 2-12 months, respiration rate >40 breaths/minute if aged >12 month; [g] - Fever defined as body temperature ≥38°C; [h] - Leucocytosis defined as white blood cell count >15 000 cells/uL if age <12 months, white blood cell count >13 000 cells/uL if age >12 months; [i] - CRP defined as levels ≥40mg/mL are considered to potentially indicate bacterial infection; [j] - Blood sample positive for *S. pneumoniae* colonisation by LytA PCR; [k] - MCPP defined as *S. pneumoniae* was cultured from a normally sterile body fluid - blood, pleural fluid or lung aspirate - or pleural fluid or lung aspirate was PCR LytA positive; [l] - HDP defined as *S. pneumoniae* density in nasopharynx >6.9 and/or density in whole blood sample >2.2 $\log_{10}$ copies/mL; [m] - HRV was the only virus detected in the nasopharynx.

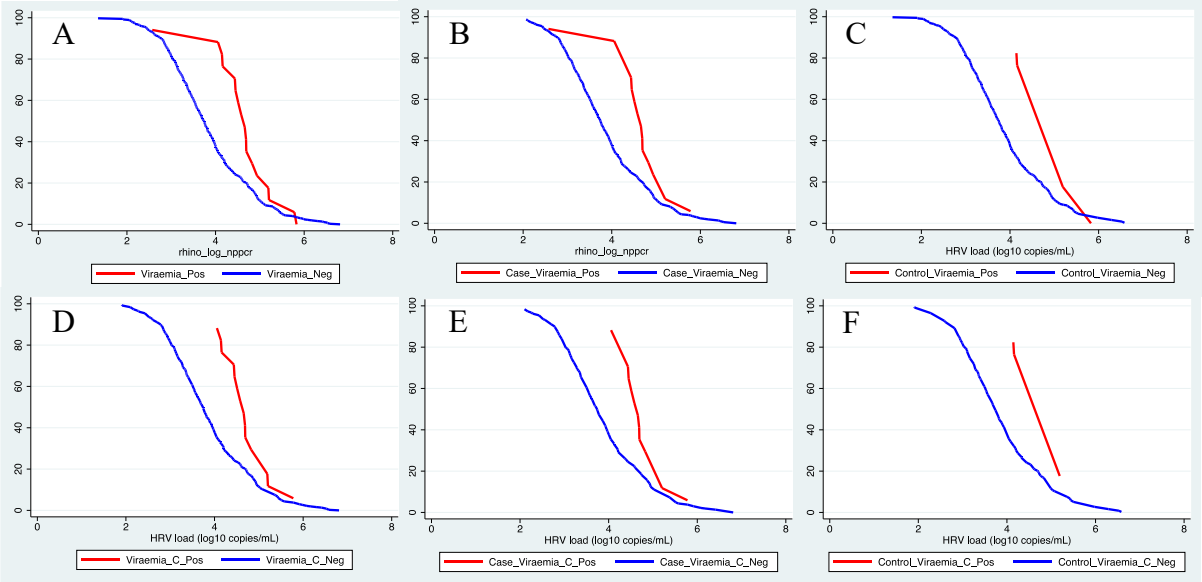

Figure 6.4: Reverse cumulative plots of nasopharyngeal HRV viral load in Panel A.) all HRV-infected viraemic and non-viraemic participants, Panel B.) cases positive for viraemia versus non-viraemic cases, Panel C.) community controls positive for viraemia versus non-viraemic controls, Panel D.) all HRV-C viraemic and non-viraemic participants, Panel E.) HRV-C viraemic cases compared to HRV-C non-viraemic cases, Panel F.) HRV-C viraemic community controls compared to HRV-C associated non-viraemic controls.

The higher nasopharyngeal HRV viral load in cases and controls with viraemia (red lines) compared to their counterpart without viraemia (blue lines) are shown in the reverse cumulative plot; Figure 6.4. Overall among the HRV-infected children, 95% (n=16/17) of viraemic participants had a nasopharyngeal viral load of ≥4 $\log_{10}$ copies/mL compared to 38% (n=140/370) of non-viraemic children (P<0.001); Figure 6.4 A. A greater proportion of viraemic compared to non-viraemic HRV infected children having nasopharyngeal viral load of ≥4 $\log_{10}$ copies/mL was also observed independently among the HRV-associated

pneumonia cases (93%, n=12/13 vs. 37%, n=70/185, $P<0.001$); Figure 6.4 B, and among the HRV-associated controls (100%, n=4/4 vs. 38%, n=70/185, $P=0.012$); Figure 6.4 C.

Furthermore, the same association of a higher percentage of viraemic compared to non-viraemic cases having nasopharyngeal viral loads of ≥4 $\log_{10}$ copies/mL was observed for infection by HRV-C overall (100%, n=14/14 vs. 39%, n=62/156, $P<0.001$); Figure 6.4 D; as well as being independently evident among HRV-C associated pneumonia cases (100%, n=11/11 vs. 37%, n=30/80, $P<0.001$); Figure 2E, and among the HRV-C infected controls (100%, n=3/3 vs. 42%, n=32/76, $P=0.048$); Figure 6.4 F.

The nasopharyngeal viral loads, as well as the case-control findings of HRV subtyping and HRV viral co-infections were similar to the all-site data presented in Chapters 3 and 5.

6.2 Discussion

The nasopharyngeal HRV viral loads were significantly higher among children with HRV viraemia than among children without HRV viraemia. The strength of this association increased as the viral load increased and a threshold density of ≥4 $\log_{10}$ copies/mL was found to significantly distinguish between HRV viraemia positive and negative participants. This was seen among both cases and controls with HRV viraemia and was mainly driven by HRV-C which was more likely to cause viraemic infections than both HRV-A and HRV-B. To our knowledge, this is the first study assessing HRV viraemia prevalence using highly sensitive PCR-based methods in both children hospitalised with severe respiratory disease as well as age-frequency matched community controls and found that HRV viraemia was 7-fold more prevalent among cases (7%) than controls (2%, P=0.007).

Of the four controls positive for HRV viraemia one had a respiratory tract infection and the remaining three were asymptomatic at the time of sampling. The nasopharyngeal load of HRV was substantially higher among the controls with HRV viraemia, thus the viraemia could have been a result of RNA shedding or "leakage" from the high nasopharyngeal colonisation density. The only way to detect infectious virus particles would be through culturing of the isolates; however, currently methods for culturing HRV-C have been unsuccessful. Alternatively, a study found that HRV viraemia was mainly detected during the early stages of disease symptoms [65] thus the 3 HRV viraemia positive controls could have been in the incubation period of illness at the time of sampling. The controls were not systematically followed up post sampling thus we were unable to conclusively determine whether the children developed pneumonia post enrolment into the study. This was seen in a case-control study conducted in Finland which enrolled asymptomatic controls in addition to cases and found that 38% of the HRV positive control developed respiratory symptoms within the week following sampling [51]. Nevertheless, the detection of HRV viraemia among community controls even at the very low prevalence seen in this study was unexpected, and further highlights the challenges of defining the etiological role of HRV during childhood disease.

This study confirms that HRV detection during disease episodes does not imply causality as HRV were frequently detected in the nasopharynx of both hospitalised children (23%) as well as age-frequency matched community controls (21%) living in South Africa. Moreover, the

molecular subtyping of the HRV population was similar between the cases and controls thus highlighting the need for additional techniques for determining the true etiological role of HRV in disease. In terms of clinical relevance among cases, no positive correlations were found between nasopharyngeal HRV viral loads and HRV species with markers for more severe disease - including hypoxia, requiring mechanical ventilation, hospital stays longer than 3 days, very severe pneumonia diagnosis and death; however, it was found that children with HRV viraemia had significantly higher nasopharyngeal viral loads than non-viraemic children. This concurs with a previous Italian study which also found an association between high viral loads and HRV viraemia [64]. They postulated that high viral loads were a prerequisite for viraemia and that viraemia was associated with more severe disease; however, they failed to enrol healthy controls into the study. This greater clinical severity was not evident in our study as there were very little clinical differences in the disease manifestation among cases with HRV viraemia compared to the HRV-associated without viraemia, including the markers for more severe disease. In fact, the HRV viraemia positive cases were associated with less prolonged hospital stays compared to the viraemia negative cases. However, the Italian study enrolled all paediatric patients younger than 14 years of age admitted to hospital with signs of respiratory tract infections, including both upper and lower respiratory tract infections and children presenting with wheezing [64] which could account for the difference in clinical involvement seen, as all our cases were children admitted to hospital with severe or very severe pneumonia. Additionally, the HRV viraemia detection rates reported in our study (7%) is substantially lower than reported in previous studies (11.4%-12.3%) [64-66]. These studies were all conducted in paediatric patients less than 14 years of age and enrolled all children hospitalised with severe respiratory infections, regardless of the site of infection. Furthermore, none of these studies enrolled control children into their studies. In the Greek study [65] they found 11.4% of hospitalised HRV positive children had HRV viraemia and the majority (70%) of the HRV viraemia cases were in children presenting with asthma exacerbations with no HRV viraemia cases detected among the children hospitalised with HRV-associated pneumonia. Similarly, in a Philippino study, [66] the majority (73%) of the HRV viraemia positive cases were in children presenting with wheezing disease. Thus the low viraemia detection rates reported in our study could be related to the cases receiving a bronchodilator challenge in order to exclude children with bronchiolitis; subsequently, a large proportion of cases with wheezing disease were excluded from our study. Regardless of this, a trend for wheezing to be more prevalent among HRV

viraemia positive cases was still evident in our study which is in accordance with the other studies reporting on an association between HRV viraemia and increased wheezing [64-66].

Although 73% of the HRV-associated cases (mean age of 9 months) were less than 1 year of age (including 44% under the age of 6 months), the majority of the HRV viraemia cases were more than 1 year of age (mean age of 18 months) with no viraemia cases detected in cases <6 months of age. This was also seen in the Phillipino study and they suggested that maternal antibodies in children less than 6 months of age might play a protective role against HRV infections developing into systemic viraemic infections [66]. This has been seen for other *enteroviruses*, in a Poliovirus vaccine study it was found that viraemia levels were lower in infants less than 6 months of age due to the presence of maternal antibodies [141] and in a mouse-model study it was found that pre-treating the mice with Coxsackievirus antibodies resulted in greatly reduced duration of viraemia due to the rapid clearance of the virus from infected tissues resulting in reduced fatality rates [142].

A limitation to this study was the small sample size of HRV viraemia positive cases and controls which could have limited the studies power to calculate statistical significance, especially among HRV-A positive participants (2% among cases and 1% among controls). Regardless, our findings provide strong evidence of a relationship between nasopharyngeal HRV viral loads and HRV viraemia. Furthermore, HRV viraemia was significantly more prevalent among children hospitalised with severe respiratory tract infections suggesting that high nasopharyngeal HRV viral loads ($\geq$4 $\log_{10}$ copies/mL) in conjunction with HRV viraemia could be used as a measure for attributing causality to HRV in children hospitalised for severe and very severe pneumonia. Additionally, the detection rates of HRV-C viraemia were higher than both HRV-A and HRV-B; in fact, no HRV-B viraemia cases were detected which is in line with other studies which have also never detected viraemia due to HRV-B [64-67]. This could be related to the much lower prevalence's of HRV-B in the population (9% among cases and 8% among controls) and provides additional evidence to the current hypothesis that HRV-B might have a lower pathogenicity than HRV-A and HRV-C [87, 143, 144]. Thus, our findings suggest that the pathogenicity of HRV-C is significantly different to that of HRV-A and HRV-C; however, additional studies are required to further understand how and why these differences exist.

7.0 Integrated Discussion and Conclusions

Through the work undertaken in this doctoral thesis, HRV was shown to be ubiquitous throughout childhood, and was found in similar prevalence to RSV among cases, which is well known to cause very severe disease and mortality in young children. Moreover, HRV was found to be 1.45-fold more prevalent among HIV-uninfected children hospitalised with WHO-defined pneumonia compared to children living in the community; with similar trends evident among HIV-1-infected children. Furthermore, in the 12-59 month age group, HRV prevalence was 2-fold greater among cases compared to controls; whereas, among infants the etiologic role of HRV was less clear.

This association of increased HRV detection among children 12-59 month old hospitalised with pneumonia was seen throughout the study, with the majority of the HRV viraemic infections also occurring among this age group of children. On analysis of the molecular genotyping, HRV-C was the dominant species among cases in the 12-59 month age-group, with the majority of the HRV viraemia cases being due to HRV-C. Furthermore, among the viraemia cases, there was minimal evidence of bacterial co-infection, suggesting that HRV-C was the most likely cause of pneumonia at least in this age group of children. HRV detection, and in particular HRV-C, in the nasopharynx and blood were more likely to be associated with wheezing disease as opposed to radiographically confirmed pneumonia. Also, HRV was the most prevalent virus detected among the children presenting with wheezing. Thus among children 12-59 months of age, there appears to be a causal association between HRV-C and respiratory wheezing illness.

Conversely, HRV-A among cases was associated with younger children and presence of radiographically confirmed pneumonia compared to HRV-C. Furthermore, among cases where HRV was the sole respiratory virus detected in the nasopharynx, HRV-A was associated with more severe disease than HRV-C. Moreover, cases with HRV-A mono-infections had a 5.81-fold greater case fatality ratio compared to HRV-A case with mixed viral infections. This association between cases with HRV mono-infections and increased case fatality ratio was also seen among the analysis of all HIV-uninfected children hospitalised with HRV-associated LRTI irrespective of HRV species. Where HRV was the sole respiratory virus detected in the nasopharynx, we found a 2.83-fold higher case fatality ratio compared to cases where HRV was detected together with other respiratory viruses.

Thus, in our study, HRV mono viral infections, and in particular HRV-A, were associated with higher case fatality ratio than HRV mixed viral infections. However, the HRV-associated fatalities were more likely to have bacterial co-infections compared to the HRV-associated cases that survived. Furthermore, bacterial co-infection was also more frequent among the HIV-1-infected HRV-associated cases compared to HIV-uninfected cases. Also HRV associated HIV-1-infected children had a 4.89-fold greater case fatality ratio than HIV-uninfected children. It has been hypothesised that infection with HRV might predispose individuals to bacterial super-infections [80] which could have contributed to the fatal outcomes observed in this study.

Other common trends were the nasopharyngeal HRV viral load which was higher among cases compared to controls. Moreover, controls with RTI were associated with higher HRV nasopharyngeal viral loads than asymptomatic controls and HRV viral loads were higher among cases categorised as having very severe pneumonia compared to cases categorised as severe pneumonia. Furthermore, the nasopharyngeal viral loads were significantly greater among viraemia positive cases, where the disease episode was most likely caused by HRV, compared to cases without viraemia with HRV nasopharyngeal viral loads ≥ 4 $\log_{10}$ copies/mL being significantly associated with HRV viraemia. Thus, HRV nasopharyngeal viral loads $\geq 4\log_{10}$ copies/mL in conjunction with HRV viraemia are potential markers for HRV-associated severe respiratory disease.

A major finding of this study, which limits attributing a role of HRV in the aetiology of pneumonia, was the detection of HRV in 1 out of every 5 community controls. Although one-third of the HRV detected among controls were in children with signs and symptoms of RTI, the rest were identified in asymptomatic children at the time of sampling. The sampling was, however, only done at a single time point using a highly sensitive diagnostic PCR assays which is able to detect very low copy numbers of HRV, thus detection could have been due to shedding or RNA leakage from a previous respiratory infection among the control participants, and more so if HIV-1-infected [124], [138]. Furthermore, controls were not systematically followed up post enrolment to determine whether they developed any signs of RTI. The only way to differentiate between infectious viral particles and RNA leakage would be viral cell culture, but this process is highly insensitive and laborious [30] and currently methods for culturing HRV-C strains have been unsuccessful [19].

What's more, the molecular subtyping of the HRV population was similar between the HRV-associated controls with RTI and asymptomatic controls; and also similar between cases and controls. This negates the possibility that some strains might be more virulent than others in causing pneumonia. Thus, host susceptibility factors, such as the availability of the necessary host receptors which allows for virus attachment and replication, might be more important factors for risk of developing severe disease rather than HRV strain virulence per se. The HRV-associated cases, among the HIV-1-infected and HIV-uninfected population, were significantly more likely to be malnourished compared to controls without HRV infections; and there was a stronger attributable role of HRV as a cause of the pneumonia in children >1 year of age. Additionally, among HIV-uninfected children with HRV infections, cases were more likely to be HIV-exposed and male than controls.

In conclusion, this is the largest case-control study to focus on both the clinical and molecular subtyping of HRV infection in children under the age of five living in Africa and Southeast Asia, where the burden of childhood morbidity and mortality due to pneumonia is greatest. The large sample size of the study, allowed us to analyse the epidemiology of HRV infection in both hospitalised and healthy children in greater detail than previously undertaken. Also, it allowed us to study HRV co-infections with other respiratory viruses and bacteria. Nevertheless, the etiologic role of HRV in causing severe childhood pneumonia remains tenuous especially among infants. Our study, however, identified several risk factors for HRV associated severe disease and highlighted some areas that require further research to get a better understanding of severe HRV disease. This includes addressing the issue of only sampling at a single time point so as to try identify HRV prevalence among truly asymptomatic individuals as well as the need to test for bacterial co-infections which appears to be of particular importance among HIV-1-infected individuals and potentially contributes to HRV-associated fatal outcomes. Thus longitudinal studies, with frequent nasopharyngeal sampling, are required in order to better understand the role HRV plays during different severities of LRTI episodes with intensive testing for both co-infection with respiratory virus and bacteria.

8.0 References

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Appendix 1: Certificate of approval granted by the University of Witwatersrand Human Research Ethics Committee (HREC number: M140906)

R14/49 Ms Vicky Lynne Baillie

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M140906

NAME: (Principal Investigator)	Ms Vicky Lynne Baillie
DEPARTMENT:	School of Public Health Respiratory and Meningeal Pathogens Research Chris Hani Baragwanath Academic Hospital
PROJECT TITLE:	Clinical and Molecular Epidemiology of Human Rhinovirus in Low-Middle Income Countries
DATE CONSIDERED:	03/10/2014
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof SA Madhi and Dr PV Adrian

APPROVED BY: Professor P Cleaton-Jones, Chairperson, HREC (Medical)

DATE OF APPROVAL: 17/10/2014

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.
I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**.

Principal Investigator Signature

Date 30/10/2014

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix 2: Certificate of approval granted by the University of Witwatersrand Human Research Ethics Committee (HREC number: M151042)

R14/49 Miss Vicky Lynne Baillie

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M151042

NAME: (Principal Investigator)	Miss Vicky Lynne Baillie
DEPARTMENT:	School of Pathology Chris Hani Baragwanath Academic Hospital Respiratory and Meningeal Pathogens Research Unit
PROJECT TITLE:	The Impacts of Human Rhinovirus Viremia and Respiratory Sample Viral Load on the Severity of Pneumonia in South African Children
DATE CONSIDERED:	30/10/2015
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof Shabir Madhi and Dr Peter V Adrian
APPROVED BY:	Professor P Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	02/09/2016

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 10004, 10th floor, Senate House/2nd Floor, Phillip Tobias Building, Parktown, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in October and will therefore be due in the month of October each year.

Principal Investigator Signature Date 10/09/2016

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix 3: Plagiarism/Turnitin report

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ORIGINALITY REPORT

%10	%8	%6	%2
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PRIMARY SOURCES

1	Submitted to Queensland University of Technology Student Paper	<%1
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4	TRAN, D. N., Q. D. TRINH, N. T. K. PHAM, T. M. H. PHAM, M. T. HA, T. Q. N. NGUYEN, S. OKITSU, H. SHIMIZU, S. HAYAKAWA, M. MIZUGUCHI, and H. USHIJIMA. "Human rhinovirus infections in hospitalized children: clinical, epidemiological and virological features", Epidemiology and Infection, 2015. Publication	<%1
5	www.ncbi.nlm.nih.gov Internet Source	<%1
6	www.ijponline.net Internet Source	<%1
7	"2016 ACR/ARHP Annual Meeting Abstract Supplement", Arthritis & Rheumatology, 2016	<%1