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Title: Respiratory Syncytial Virus, its Co-infection and Paediatric Lower Respiratory Infections

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ABSTRACT: Comprehensive population-based data on the role of respiratory viruses in the development of lower respiratory infections (LRIs) remain unclear. We investigated the incidences and effect of respiratory viruses single and multiple infections on risk of LTIs in Vietnam.

Population-based prospective surveillance and case-control study of hospitalized paediatric ARI were conducted from April 2007 through March 2010. Healthy controls were randomly recruited from the same community. Nasopharyngeal samples were collected and tested for 13 respiratory viruses using multiplex-polymerase chain reactions.

1,992 hospitalized ARI episodes including 397(19.9%) with LRIs were enrolled. Incidence of hospitalized LRIs among children under 24 months was 2,171.9 per 100,000(95% confidence interval: 1,947.9 – 2,419.7). The majority of ARI cases(60.9%) were positive for at least one virus. Human rhinovirus (HRV)(24.2%), respiratory syncytial virus (RSV)(20.1%), and influenza A virus (FLUA)(12.0%) were the most common and 9.5% had multiple-viral infections. RSV and human metapneumovirus (HMPV) infections independently increased the risk of LRIs. RSV further increased the risk, when co-infected with HRV, HMPV and Parainfluenzavirus-3 but not with FLUA. The case-control analysis revealed that RSV and FLUA increased the risk of ARI hospitalization but not HRV.

RSV is the leading pathogens associated with risk of ARI hospitalization and LTIs in Vietnam.

(Word count: 200)

INTRODUCTION

Acute respiratory infection (ARI) is the leading cause of mortality and morbidity among children worldwide [1,2]. Studies have established that *Streptococcus pneumoniae* and *Haemophilus influenzae* type b are the major bacterial causes of childhood pneumonia in developed and developing countries [3,4]. Viruses also play critical roles in the development of ARI/pneumonia in young children. However, elucidating the roles of viral pathogens may be complex as a wide range of viruses, such as respiratory syncytial virus (RSV), human rhinovirus (HRV), influenza virus A and B (FLUA, B), parainfluenza virus (PIV), and human metapneumovirus (HMPV) associate with severe forms of childhood ARI including bronchiolitis and pneumonia [5-9]**.** A systematic review has estimated that 22% of severe ARI episodes among children are related to RSV infections [10]. Still, hard evidence or population based data on burden of respiratory viruses on paediatric ARI in developing countries, especially in Southeast Asia has been lacking.

We have previously reported that HRV, RSV and FLUA were the most commonly detected viral agents among hospitalized ARI patients based on our 14 months study result in central Vietnam [11]. However, yearly difference in viral outbreaks may influence the annual incidence of hospitalized ARI/pneumonia cases. Data among healthy children is also important since respiratory virus like HRV has been detected in 5-18% of asymptomatic children and its' role on paediatric ARI is still controversial [6,12]**.**

Recent progress in molecular microbiology technique such as multiplex polymerase chain reaction (PCR) has made it possible to detect multiple viruses simultaneously with high sensitivity and specificity [13] and has revealed that multiple virus infections in children with ARI are not uncommon [7,14]. However contribution of respiratory viruses to severe ARI, either as single or multiple infections

needs further clarification. In particular, the combination of respiratory viruses that increase the risk of childhood LRIs has not been thoroughly investigated yet.

Thus, we conducted a population-based prospective surveillance and a case control study covering multiple years to precisely elucidate the incidence and effect of single and multiple respiratory virus infections on paediatric ARI hospitalization and risk of LRIs in central Vietnam.

METHODS

Study site and study population

The surveillance was conducted at Khanh Hoa General Hospital (KHGH). KHGH, located in the center of Nha Trang, is the only hospital in the catchment area providing primary, secondary and tertiary care for the population. After the implementation of "The Law on Child Care, Protection and Education" in 2005, all Vietnamese children less than 6 years of age have been eligible to receive free medical care including treatment and regular check-ups in the public sector. Thus, we can reasonably expect that almost all children aged less than 5 years with severe ARI who require hospitalization have been admitted to the KHGH.

A population-based cross-sectional survey was conducted from 2006, June 8 to July 25, covering all residents of the catchment area. Overall, 198,729 individuals living in 42,770 households at 16 communes in Nha Trang were enrolled. Among them, 13,935 were children less than five years of age. Detailed methods and characteristics of study populations have been described previously [15]. This census population formed the source population for our case-control study. In addition, this population was treated as a cohort for the calculation of incidence rates. The coverage of extended program on

immunization reaches 95% in this area however pneumococcal conjugate vaccine and *H. influenzae* type b conjugate vaccine had not been introduced at the time of this study.

Hospital case enrollments and data collection

Children admitted to the KHGH from April 1st 2007 through March 31st 2010, (i) with cough and/or difficulty breathing, (ii) aged between 1 month to 60 months on admission, and (iii) who were residing in our catchment area (16 communes in Nha Trang) were enrolled to the study. On admission, after obtaining informed consent from the parents or guardians, clinical-epidemiological information was collected by paediatricians in KHGH. Nasopharyngeal and blood samples were taken within 24 hours of admission by trained research nurses. To achieve good quality of data, all processes from the enrollment to data and sample collection were monitored by a research doctor and a research nurse. The information on disease onset had been collected since January 2009.

Case definitions of LRIs

Case categories were defined using modified WHO Integrated Management of Childhood Illnesses (IMCI) algorithms [16]. Children with general danger signs, chest indrawing or stridor were categorized as severe LRIs. The presence of severe LRIs or tachypnea (a respiratory rate >60 per min for children aged 1 month, >50 per min for those aged 2-11 months, and >40 per min for those aged 12-59 months) were categorized as LRIs.

Enrollment of healthy controls

Three hundred and fifty healthy children less than 5 years of age were randomly selected from two communes in the study area based on the census data. Eligible children were free of fever, signs and symptoms of ARI and had no history of antibiotic usage within one month of evaluation. In July 2008, children were invited to commune health centers where a paediatrician performed clinical examination to confirm their healthy status. After obtaining informed consent, nasopharyngeal swab samples were collected in the same way as children hospitalized for ARI.

Laboratory analysis

Nasopharyngeal samples collected at the time of admission were stored at –80^{o}C. Nucleic acid extraction was done using QIAamp viral RNA extraction kit. Four multiplex-PCR assays (I: FLUA, FLUB, RSV, HMPV; II: PIV-1, -2, -3, and -4; III: HRV, human coronavirus 229E (HCoV-229E), human coronavirus OC43 (hCoV-OC43); IV: adenovirus (AdV) and human bocavirus (HBoV) were performed to detect 13 respiratory viruses in each sample, as described previously [11].

Data management and analysis

All data were double-entered and validated by trained data management staffs at the Khanh Hoa Provincial Health Service using FoxPro 9.0 (Microsoft, USA) database. The incidences of hospitalized ARIs, hospitalized viral respiratory infections, and hospitalized LRIs among the study population were calculated. The 95% confidence intervals for incidences were calculated using Wilson's score method. Clinical characteristics were compared between LRIs categories using chi-square test for trend and analysis of variance (ANOVA).

To evaluate the effect of respiratory viral infection on the risk of ARI admissions, case-control analysis was performed. The dataset comprised ARI patients hospitalized from June to August in 2008 and 350 healthy control subjects enrolled in July 2008. Odds ratios were calculated using logistic regression models.

The effects of the respective respiratory viral infection on LRIs among hospitalized children were investigated by analyzing the frequency of viral co-infections among each respiratory viral infection. The effect of each viral infection on the risk of ARI being diagnosed as LRIs was assessed using Poisson regression [17]. Confidence intervals for risk ratios were adjusted for individual-level correlation using robust standard errors. Multivariate analysis was performed to adjust for the effects of other viruses. Finally, to evaluate the risk ratios of single- and multiple-infection, Mantel's score tests for trend were performed adjusting for other viruses. STATA 10 (Stata corp., USA) was used for all statistical analyses.

Ethics

The study was approved by institutional review boards in National Institute of Hygiene and Epidemiology, Vietnam and Institute of Tropical Medicine, Nagasaki University, Japan.

RESULTS

Characteristics of hospitalized ARI cases

During the study period, a total of 2035 cases from the catchment area were admitted and 1,786 children with 1,992 hospitalized ARI episodes (97.9%) who gave informed consent were enrolled to the study. Among them, 1,622 had one episode while 264 and 106 were from children with two and three or more ARI episodes, respectively. Characteristics of hospitalized ARI cases are shown in Table 1. Among 1,992 ARI cases, 397 (19.9%) were categorized as LRIs including 113 cases of severe LRIs (5.7% of all episodes). The mean duration from disease onset to admission was 2.9 +/- 3.5 days (N=753). Younger age, lower body temperature, higher pulse rate, presence of wheeze and malnutrition were associated with higher clinical severity/LRIs. Children with severe LRIs were hospitalized longer than those without severe LRIs and received steroid therapy more frequently. Antibiotics usage before admission tended to be less among severe LRIs.

Incidences of hospitalized paediatric ARI, LRIs and viral infections

The annual incidences of hospitalized episodes of ARI and LRIs among children under 5 years old were 4,765 and 949.6 per 100,000 child-years, respectively. Higher incidences were observed among children less than 24 months of age than 24-60 months. There was no significant difference in the incidences between children less than 12 months and those less than 24 months (Table 2).

Among 1,992 episodes, 1,214 (60.9%) were positive for any of the 13 viruses tested. HRV (481, 24%), RSV (401, 20%), and FLUA (239, 12%) were the most common viruses detected, followed by AdV (111, 6%), PIV3 (49, 2.4%), HMPV (44, 2%), and hBoV (40, 2%). Co-infections with two or more respiratory viruses were common. Of all ARI episodes, 189 (9.5%) were multiple viral infections

which included 174 (8.7%) dual infections and 15 (0.8%) triple infections (details in supplementary figure 1, online only). Among 15 triple infections, 10 included RSV; five were combinations of RSV+HRV+FLUA, two were RSV+HRV+AdV, another two were RSV+FLUA+AdV and one was RSV+FLUA+HMPV. High incidences of paediatric hospitalized ARI cases associated with HRV, RSV and FLUA infection, were observed among all age groups of children in Nha Trang (Table 2). The incidence rates of HMPV and FLUB considerably differed year by year but not the other viruses (Supplementary figure 2, online only).

In terms of seasonality, our data covering three consecutive years, including the peak of swine H1N1 flu in October 2009, showed that in every year there is a clear seasonal pattern for RSV infection during summer seasons (from June to November), whereas other viruses including HRV and FLUA did not show any discrete seasonality (supplementary figure 2).

Virus detection in apparently healthy children and the risk of ARI hospitalization

Subclinical HRV infection was reported to be common since it has been detected in apparently healthy children [6,12]. In order to investigate pathological role of respiratory viruses, including HRV among children, we compared the virus detection rates between the ARI inpatients and the 350 healthy children. Among the healthy controls enrolled in July 2008, 147 (42%) were positive for any of the tested viruses; HRV (35%) was by far most commonly detected, followed by AdV (4.6%), RSV (2.9%) and FLUA (1%). Comparison with ARI children hospitalized during the same period, (2008, June to August, n=148) showed that RSV and FLUA but not HRV were associated with the increased risk of hospitalization (Table 3).

Viral ARI and risk of LRIs

The effect of respiratory viral infection on risk of ARI being clinically diagnosed as LRIs is shown in Table 4. In univariate analysis, RSV and HMPV were associated with the diagnosis of LRIs. In multivariate analysis adjusting for other viral co-infections, HRV, RSV and HMPV infections were independent risk factors of LRIs among children. These three viruses also demonstrated increasing trend of risks between non-severe and severe LRIs (supplementary table 1). Child sex, age and month of admission were not included in these models as confounders because they did not change the effect size of viral infections. FLUA infection was not associated with increased risk of LRIs among hospitalized ARI even after adjusting for other viruses (adjusted risk ratio: 0.77, 95% confident interval: 0.56 to 1.06, data not shown in table).

Viral co-infection and risk of LRIs

To investigate the effects of respiratory virus co-infections on the development of paediatric LRIs, we further analyzed the risk of LRIs by comparison of three groups: virus-negative, single virus-positive and dual-positive. Overall, viral co-infection did not increase the risk of LRIs (test for interaction, $p>0.1$). However, there was good evidence (score test for trend) that dual infection with RSV+HRV, RSV+PIV3, or RSV+HMPV led to a higher risk of pneumonia than single infection with any of these viruses (Figure 1). Interestingly, FLUA+RSV or FLUA+ any other virus co-infection did not increase the risk for LRIs. These associations were observed regardless of child's age and months of admission.

DISCUSSIONS

This large scale comprehensive multiyear population-based study elucidated the burden of respiratory viruses on paediatric ARI hospitalization and LRIs in central Vietnam that enabled us to elucidate the impact of each viral infection or co-infection on the development of LRIs.

RSV as leading viral pathogen causing ARI hospitalization in Nha Trang, Central Vietnam

We quantitatively clarified the burden of each viral pathogen on paediatric ARI and demonstrated that RSV is by far the most important viral pathogen to cause paediatric ARI requiring hospitalization in Vietnam, which is compatible with a number of other studies [5,18,19]. Interestingly, our three-consecutive-year observation showed the peak of RSV diseases always occurred in the dry hot season. This endemic pattern is distinct from that in countries in the temperate zone where RSV outbreaks normally occur during winter season [9,18,20]. We found that Influenza virus was the second most important virus causing paediatric ARI hospitalization circulating throughout the year in this area without a clear peak. Seasonality information of respiratory viruses is very important for future preventive strategies against viral ARI in Vietnam.

Respiratory virus co-infection and their interaction in the development of LRIs

Interestingly, our study found that co-infections of RSV with certain respiratory viruses increased risk for LRIs. Such effects appeared to depend on the combination with different viruses; RSV with HRV, PIV3 or HMPV significantly increased the risk while RSV with FLUA did not increase the risk. Recent studies have also shown that multiple viral co-infections were not rare in paediatric ARI cases [7,14,21] but these studies have not evaluated the effects of viral co-infection on the risk of LRIs. Our observation may reflect the pathological effects of such synergistic interactions on childhood LRIs.

Cytokines are thought to play an important role in the pathogenesis of RSV infection. It has been shown that RSV and FLUA stimulate host immune system differently, especially in the context of Th1-Th2 balance. RSV infection has been shown to have enhanced Th2 effect with increase production of IL4, IL5 and decrease in INF-γ level where as a predominant Th1effect with increase INF-γ production is observed in FLUA infection [22-24]. Therefore, co-infection of these two viruses might have neutralized their immunopathological effects in the respiratory tract, leading to this phenomenon. Future studies of viral co-infection on cytokines are necessary to clarify the interaction and underlying mechanism.

Detection of HRV, RSV, HMPV or FLUA and risk of LRIs

Among children hospitalized for ARI, HRV, RSV and HMPV were associated with LRIs, categorized by WHO IMCI guideline, even after adjusting for the presence of other viruses. Although HRV infection was most commonly detected, the direct etiological role of HRV in hospitalized ARI cases is questionable. Previous reports showed frequent detection of HRV in apparently healthy children [12]. Similarly, our case-control study demonstrated that one third of healthy children were also positive for HRV and detection of HRV did not increase any risk for hospitalization.

There could be several underlying reasons for why HRV was associated with LRIs independent upon the co-infection with other viruses, while high HRV detection rate among healthy children. Viral loads [25] and serotypes of HRV[26] may play a role. ARI patients may have higher HRV loads than healthy control, and/or may be infected with more pathogenic HRV while healthy control subjects harbor less pathogenic HRV. Other possibilities may be co-infection with other undetected

respiratory viruses or bacteria. Further prospective studies to investigate the aforementioned possibilities are warranted to clarify the pathological role of HRV in ARI and LRIs.

Interestingly, FLUA, one of the major causes of ARI hospitalization, was not associated with the risk of LRIs. This finding was consistent even after analyzing seasonal influenza A and swine H1N1 influenza A separately (data not shown). Lack of association of swine H1N1 influenza A with LRIs was further surprising, since it has been associated with LRIs/pneumonia more frequently than seasonal influenza worldwide [27,28].

Limitations of our study

In this study we used multiplex PCR assays to detect 13 common respiratory viruses with high sensitivity. However, there is a possibility that some other viruses which were not in our detection system might be circulating in Nha Trang [29]. Another limitation was lack of bacteria blood culture results of these cases. According to our previous study, there was a high rate of antibiotic usage before hospitalization and thus blood culture-positive rate was very low (<1%) [30]. Therefore, we did not conduct blood culture testing in this study.

This study used the modified WHO-IMCI algorisms to define LRIs instead of radiologically confirmed pneumonia (RCP) as outcome. RCP has been used as an evaluation endpoint, especially for vaccine trials as the definitions of the standardized radiological interpretation are very strict [31]. However, in this study we relied on case definition of WHO-IMCI algorisms which is globally standardized and has been widely used, especially in the settings of developing countries.

In this study, severe LRIs cases were less likely to receive antibiotics before admission, associated with wheezing and the pediatricians in KHGH used steroid more frequently among them.

Early treatment with antibiotics or medical attention or hospitalization may reduce the risk of LRIs in this study population. Our severe LRIs cases defined by WHO IMCI algorisms may have included many bronchiolitis cases. This may be the limitation of WHO IMCI algorisms since it does not differentiate clearly between pneumonia and bronchiolitis cases.

Conclusion

RSV is the leading pathogens associated with the risk of ARI hospitalization and LRIs in central Vietnam. RSV played a major etiologic role either by itself or combination with other respiratory viruses. Thus, vaccine or antiviral agents against RSV are urgently required to reduce severe paediatric ARI hospitalization.

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Competing interest

The authors have no competing interest with regard to the content of this manuscript.

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Table 1. Demographic and clinical features of hospitalized acute respiratory infection cases grouped by clinical severity, Khanh Hoa Province, Vietnam, 2007–2010.

Characteristic	Total	Non-LRIs	LRIs		p-value *
			Non-severe	Severe	
	N=1,992	N=1,595	N=284	N=113	
Gender					
Male	1,211 (60.8)	965 (60.5)	170 (59.9)	76 (67.3)	1
Female	781 (39.2)	630 (39.5)	114 (40.1)	37 (32.7)	
Age (month)	18.0 ± 12.4	18.2 ± 12.7	17.8 ± 11.1	14.9 ± 12.0	0.0231†
Clinical findings					
Body temperature (℃)	38.2 ± 0.9	38.2 ± 0.8	38.2 ± 0.9	38.0 ± 0.8	0.0241†
Pulse rate (/min)	113.9 ± 11.4	112.8 ± 10.5	116.8 ± 12.1	122.0 ± 16.3	<0.0001†
Wheeze					
Yes	1,070 (53.7)	777 (48.7)	200 (70.4)	93 (82.3)	<0.0001
No	922 (46.3)	818 (51.3)	84 (29.6)	20 (17.7)	
WBC count	12.8 ± 7.1	12.8 ± 6.5	13.2 ± 9.8	13.0 ± 6.3	0.6†
RBC count	4.5 ± 1.1	4.5 ± 1.2	4.4 ± 0.6	4.5 ± 1.1	0.8†
Breast feeding					
Yes	793 (40.0)	637 (40.1)	105 (37.4)	51 (45.1)	0.7
No	1,189 (60.0)	951 (59.9)	176 (62.6)	62 (54.9)	
Malnutrition					
Yes	4 (0.2)	1 (0.1)	2 (0.7)	1 (0.9)	0.0069
No	1,988 (99.8)	1,594 (99.9)	282 (99.3)	112 (99.1)	
Number of household member	5.0 ± 2.1	5.1 ± 2.0	5.0 ± 2.1	4.8 ± 2.4	0.6 †
Kindergarden attended					
Yes	864 (43.4)	700 (43.9)	130 (45.8)	34 (30.1)	0.06
No	1,128 (56.6)	895 (56.1)	154 (54.2)	79 (69.9)	

Antibiotics given before admission						
	Yes	595 (42.7)	481 (43.3)	92 (46.0)	22 (26.8)	0.06
	No	799 (57.3)	631 (56.7)	108 (54.0)	60 (73.2)	
Management						
Antibiotics used						
	Yes	1,974 (99.1)	1581 (99.1)	283 (99.7)	110 (97.4)	0.3
	No	18 (0.9)	14 (0.9)	1 (0.3)	3 (2.6)	
Steroid used						
	Yes	945 (47.5)	706 (44.3)	161 (56.7)	78 (69.0)	<0.0001
	No	1,046 (52.5)	888 (55.7)	123 (43.3)	35 (31.0)	
Hospitalized day		4.8 ± 2.7	4.6 ± 2.5	5.3 ± 3.0	6.1 ± 3.6	<0.0001[†]

[*]Chi-square test for trend otherwise indicated. [†]Analysis of variance (ANOVA), LRIs: lower respiratory infections

Table 2. Annual incidence of hospitalizations with acute respiratory infection, viral respiratory infections, and lower respiratory infection (LRIs) among children < 5 years, Khanh Hoa Province, Vietnam.

	Children aged <24 months		Children aged <60 months	
	Annual incidence (per 100,000)	95% CI	Annual incidence (per 100,000)	95% CI
Hospitalized ARI	10282.7	9847.1-10728.8	4765	4574.5-4962
LRIs	2171.9	1947.9-2419.7	949.6	861.8-1046.1
Severe LRIs	626.6	509.5-770.1	270.3	225-324.7
HRV	2663.3	2415.7-2933.8	1150.6	1053.7-1256
RSV	2342.8	2110.3-2598.8	959.2	870.9-1056.2
InfA	1039.7	886.1-1218.9	571.7	504.2-648.1
AdV	662.3	541.5-809.2	265.5	220.6-319.4
PIV3	284.8	209.4-387.2	117.2	88.7-154.8
HMPV	220.8	155.7-312.8	105.3	78.4-141.2
HBoV	242.1	173.4-337.7	95.7	70.3-130.2
InfB	99.7	59.4-167.1	52.6	34.8-79.6
PIV1	71.2	38.7-130.9	43.1	27.2-68
PIV2	28.5	11.1-73.2	26.3	14.7-47.1
Corona	0	0-0	4.8	1.3-17.4
PIV4	7.1	1.3-40.3	4.8	1.3-17.4

Table 3. Comparison between hospitalized ARI children and healthy subjects*, Khanh Hoa Province, 2008.

	Case N=148		Control N=350		Odds Ratio
	N	%	N	%	
Any Virus	100	67.6	147	42	2.88 (1.92 to 4.31)
HRV	43	29.1	121	34.6	0.78 (0.51 to 1.18)
RSV	58	39.2	10	2.9	21.91 (10.77 to 44.58)
FLUA	13	8.9	4	1.1	8.33 (2.67 to 26)
AdV	8	5.4	16	4.6	1.19 (0.5 to 2.85)
PIV3	0	0	0	0	-
HMPV	0	0	0	0	-
HBoV	1	0.7	4	1.1	0.59 (0.07 to 5.31)
FLUB	1	0.7	1	0.3	2.37 (0.15 to 38.21)
PIV1	0	0	0	0	-
PIV2	0	0	1	0.3	-
HCoV	0	0	2	0.6	-
PIV4	0	0	0	0	-

* Cases are ARI children aged < 5 years hospitalized from June to August 2008. Controls are healthy children < 5 years old randomly selected from two communes in the catchment area.

Table 4. Effects of respiratory viral infection on the risk of lower respiratory infections (LRIs) among hospitalized children < 5 years, Khanh Hoa Province, Vietnam, 2007-2010.

RT-PCR N=1,992	Non LRIs N=1,595 (%)	LRIs N=397 (%)	Unadjusted RR (95% CI)	Adjusted RR (95% CI)
Rhino				
Positive	371 (77.1)	110 (22.9)	1.2 (0.99 to 1.46)	1.26 (1.03 to 1.54)
Negative	1,224 (81.0)	287 (19.0)	1	1
RSV				
Positive	307 (76.6)	94 (23.4)	1.23 (1.00 to 1.51)	1.3 (1.05 to 1.59)
Negative	1,288 (81.0)	303 (19.0)	1	1
InfA				
Positive	203 (84.9)	36 (15.1)	0.73 (0.53 to 1.00)	
Negative	1,392 (79.4)	361 (20.6)	1	
Adeno				
Positive	95 (85.6)	16 (14.4)	0.71 (0.44 to 1.16)	
Negative	1,500 (79.7)	381 (20.3)	1	
PIV3				
Positive	38 (77.5)	11 (22.5)	1.13 (0.67 to 1.92)	
Negative	1,557 (80.1)	386 (19.9)	1	
HMPV				
Positive	30 (68.2)	14 (31.8)	1.62 (1.04 to 2.52)	1.72 (1.1 to 2.68)
Negative	1,565 (80.3)	383 (19.7)	1	1
HBoV				
Positive	33 (82.5)	7 (17.5)	0.88 (0.44 to 1.73)	
Negative	1,562 (80.0)	390 (20.0)	1	
InfB				
Positive	19 (86.4)	3 (13.6)	0.68 (0.24 to 1.95)	
Negative	1,576 (80.0)	394 (20.0)	1	
PIV1				
Positive	17 (94.4)	1 (5.56)	0.28 (0.04 to 1.87)	
Negative	1,578 (79.9)	396 (20.1)	1	
PIV2				
Positive	9 (81.8)	2 (18.2)	0.91 (0.26 to 3.2)	
Negative	1,586 (80.1)	395 (19.9)	1	

Supplementary table 1. Effects of respiratory viral infection on the risk of non-severe and severe lower respiratory infection (LRIs) among hospitalized children < 5 years, Khanh Hoa Province, Vietnam, 2007-2010.

		No LRIs	Non-severe LRIs	Severe LRIs	Total	Test for trend
HRV	Positive	371 (23.3)	77 (27.1)	33 (29.2)	481	0.0227
	Negative	1,224 (76.7)	207 (72.9)	80 (70.8)	1,511	
		1,595	284	113	1,992	
		No LRIs	Non-severe LRIs	Severe LRIs	Total	Test for trend
RSV	Positive	307 (19.2)	67 (23.6)	27 (23.9)	401	0.0208
	Negative	1,288 (80.8)	217 (76.4)	86 (76.1)	1,591	
		1,595	284	113	1,992	
		No LRIs	Non-severe LRIs	Severe LRIs	Total	Test for trend
Inf A	Positive	203 (12.7)	31 (10.9)	5 (4.4)	239	0.0226
	Negative	1,392 (87.3)	253 (89.1)	108 (95.6)	1,753	
		1,595	284	113	1,992	
		No LRIs	Non-severe LRIs	Severe LRIs	Total	Test for trend
Adeno	Positive	95 (6.0)	13 (4.6)	3 (2.7)	111	0.1
	Negative	1,500 (94.0)	271 (95.4)	110 (97.3)	1,881	
		1,595	284	113	1,992	
		No LRIs	Non-severe LRIs	Severe LRIs	Total	Test for trend
HMPV	Positive	30 (1.9)	10 (3.5)	4 (3.5)	44	0.0373
	Negative	1,565 (98.1)	274 (96.5)	109 (96.5)	1,948	
		1,595	284	113	1,992	

Figure legend

Figure 1. Effects of mono-infection and co-infection of respiratory viruses on risk of lower respiratory infections (LRIs) among children age < 5years in Khanh Hoa Province, Vietnam

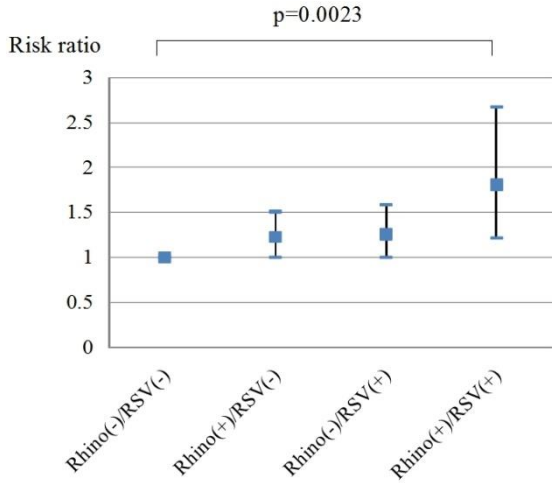
a. HRV and RSV[*]
b. HRV and Influenza A[†]
c. HRV and HMPV[‡]
d. RSV and Influenza A[¶]
e. RSV and PIV3[¶]
f. RSV and HMPV[π]

Risk ratios were calculated for each category adjusting for other viruses. To evaluate trends of effect of mono-infection and co-infection on pneumonia, chi-squared tests for trend were used combining either mono-infection as one dummy category (ie. HRV(+)/RSV(-) and HRV(-)/RSV(+) were regarded as one category) adjusting for other viruses.

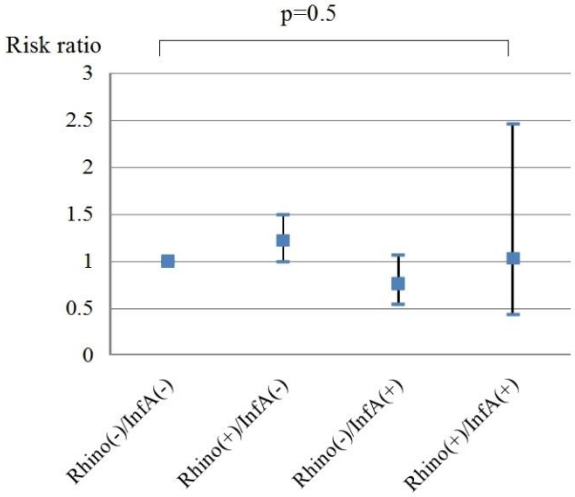
[*] Adjusted for HMPV. [†] Adjusted for RSV and HMPV. [‡] Adjusted for RSV. [¶] Adjusted for HRV and HMPV. [π] Adjusted for HRV.

Figure 1. Effect of respiratory viruses mono-infection and co-infection on risk of lower respiratory infections

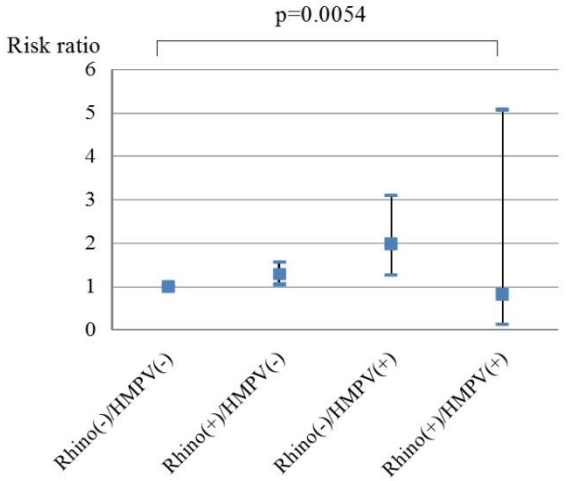
a. HRV and RSV*



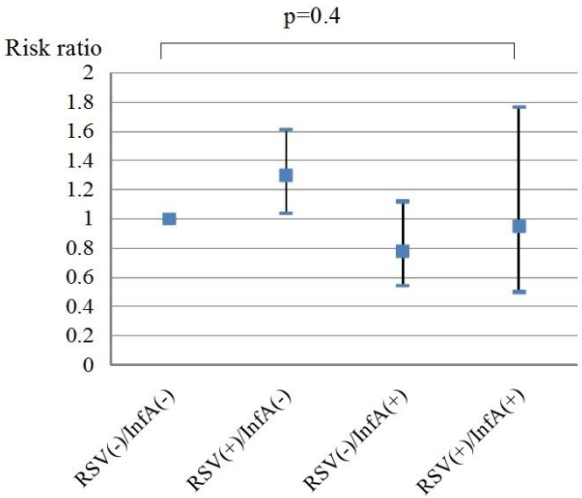
b. HRV and Influenza A‡



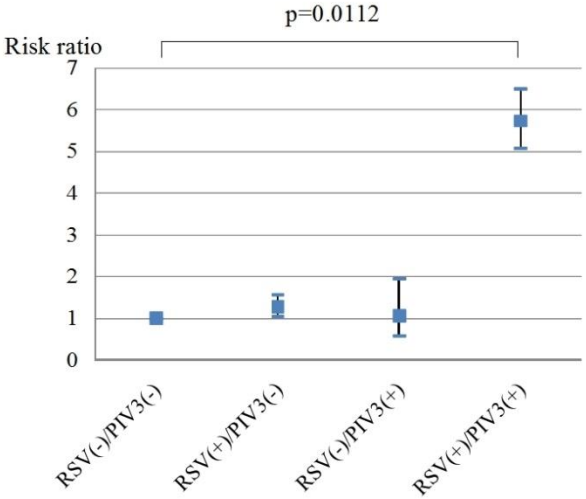
c. HRV and HMPV†



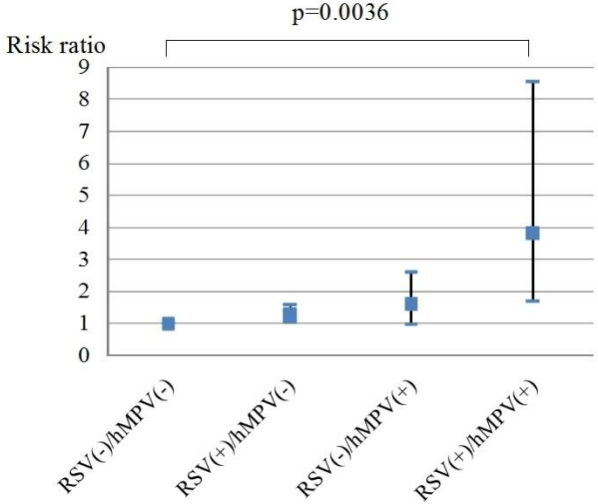
d. RSV and Influenza A ¶



e. RSV and PIV3 ¶



f. RSV and HMPV$^{\pi}$



Risk ratios were calculated for each category adjusting for other viruses. To evaluate trends of effect of mono-infection and co-infection on LRIs, chi-squared tests for trend were used combining either mono-infection as one dummy category (ie. HRV(+)/RSV(-) and HRV(-)/RSV(+) were regarded as one category) adjusting for other viruses.
*Adjusted for HMPV. ‡ Adjusted for RSV and HMPV. † Adjusted for RSV. ¶ Adjusted for HRV and HMPV. $^{\pi}$ Adjusted for HRV.