ASSOCIATION BETWEEN RHINOVIRUS AND *STREPTOCOCCUS PNEUMONIAE* AMONG CASES AND CONTROLS IN THE PERCH STUDY: A PRELIMINARY ANALYSIS

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INTRODUCTION

- *Streptococcus pneumoniae* is one of the leading causes of bacterial invasive disease in children worldwide[1]. Asymptomatic colonisation of the nasopharynx by *S.pneumoniae* is common during childhood and generally self-resolves; however, it can also develop into invasive pneumococcal disease (IPD).
- Respiratory viruses have been shown to predispose individuals to secondary bacterial infections through the up-regulation of specific respiratory epithelial cells receptors which promotes bacterial adhesion[2].
- A similar up-regulation of *S.pneumoniae* adherence to epithelial cells post rhinovirus infection was observed in cultured human airway epithelial cells suggesting that rhinovirus infection might also predispose individuals to IPD[3].
- In a Finnish study, IPD rates correlated with rhinovirus activity, but not with RSV and influenza activity[4].
- The aim of the study was to characterise the relationship between rhinovirus infection and invasive pneumococcal disease in children living in low to middle income countries.

METHODS

- The Pneumonia Etiology Research for Child Health (PERCH) project is a 7-country case-control study of children 1-59 months hospitalized with WHO-defined severe or very severe pneumonia and age-frequency matched community controls.
- Flocked nasopharyngeal (NP) and rayon oropharyngeal (OP) swab specimens were collected from all cases and controls on enrolment into the study.
- Total nucleic acids were extracted from the NP/OP swabs and tested using the Fast-track Diagnostics real-time quantitative PCR assays which tests for 33 respiratory pathogens, including rhinovirus and *S.pneumoniae*.
- Rhinovirus positive samples from the South Africa, Mali and Zambia sites were serotyped[5].
- Whole blood (WB) samples from both cases and controls were tested for the presence of *S.pneumoniae* bacteraemia using a quantifiable RT-PCR assay for the *LytA* gene[6].
- Microbiologically confirmed pneumococcal pneumonia (MCPP) was defined as having *Streptococcus pneumoniae* cultured from a normally sterile fluid.
- In PERCH MCPP cases were positively associated with NP/OP pneumococcal PCR densities >6.9 log copies/mL or WB pneumococcal PCR densities >2.2 log copies/mL. Thus pneumococcus densities above these levels were defined as high density pneumococcus (HDP) and were used as markers, together with MCPP, for IPD.
- Using age- and site- adjusted logistic regression; we compared the odds of MCPP and HDP between rhinovirus-positive and negative cases as well as between the different rhinovirus species (A, B and C) and a two-sided p-value <0.05 was considered as statistically significant.

RESULTS

- A total of 4,113 pneumonia cases were enrolled into the PERCH project:
  - -2,863 were severe pneumonia cases of which 23% had rhinovirus infections (n=652)
  - -1,370 were very severe pneumonia cases of which 23% had rhinovirus infections (n=311, p=0.931).
  - -21% of the MCPP confirmed cases (n=12/56) and 22% of the HDP cases (n=150/671) were also co-infected with rhinovirus
- A total of 5,189 community controls were enrolled into the PERCH project:
  - -4,101 were asymptomatic controls of which 22% had rhinovirus infections (n=916)
  - -1,088 were controls with signs or symptoms of a respiratory tract infection (RTI) of which 28% had a rhinovirus infection (n=310, p<0.001).
  - -27% of the rhinovirus-associated asymptomatic controls (n=102/380) had HDP levels versus 31% of the rhinovirus-associated RTI controls (n=48/155, p=0.018)
- Rhinovirus infection was not associated with MCPP (aOR=1.51, 95%CI 0.68, 3.39, p=0.98) or HDP (aOR=0.98, 95% CI 0.79, 1.21, p=0.91) in cases. However, among the controls, rhinovirus infection was associated with HDP (aOR=1.47, 95%CI 1.19, 1.80, p<0.001); Table 1.
- No obvious correlation was seen between the rate of HDP or MCPP cases and the prevalence of rhinovirus detection in the pneumonia cases or community controls; Figure 1.
- Among the cases and controls testing positive for rhinovirus at the Mali, Zambia and South Africa site - the distribution of rhinovirus subtypes were similar between cases (A:B:C=48%:8%:44%) and controls (A:B:C=45%:10%:45%; p=0.17).
- The percent with HDP was similar by subtype among rhinovirus-positive cases (A=51%, B=42%, C=43%; p=0.3) but among controls HDP was less common for rhinovirus-B (10% vs. A=25% and C=29%; p=0.05); Table 2.

TABLE 1 – The clinical epidemiology of rhinovirus

	Pneumonia cases (n=4113)			Community controls (n=5189)		
	Rhinovirus+ (n=963)	Rhinovirus- (n=3150)	P-value*	Rhinovirus+ (n=1088)	Rhinovirus- (n=4101)	P-value*
0-5 months (n, %)	347 (36%)	1338 (42%)		398 (37%)	1219 (30%)	
6-11 months (n, %)	211 (22%)	731 (23%)	0.0029	28 (25%)	974 (24%)	p<0.001
12-23 months (n, %)	244 (25%)	668 (21%)		262 (24%)	1007 (25%)	
24-59 months (n, %)	161 (17%)	413 (13%)		160 (15%)	901 (22%)	
Sex, male n(%)	561 (58%)	1794 (57%)	0.622	558 (51%)	2046 (50%)	0.372
HIV+, n(%)	51 (5%)	186 (6%)		32 (3%)	180 (4%)	
HIV-, n(%)	832 (86%)	2669 (85%)	0.66	906 (83%)	3482 (85%)	p<0.001
HIV unknown, n(%)	80 (8%)	295 (9%)		150 (14%)	439 (11%)	
Clinical Outcomes						
ARI control, n(%)				310 (28%)	916 (22%)	p<0.001
Very severe pneumonia, n(%)	311 (32%)	1022 (32%)	0.112			
Tachypnea, n(%)	829 (86%)	2537 (81%)	0.003	104 (10%)	459 (12%)	0.053
Lab results						
Leukocytosis, n(%)	465 (51%)	1231 (41%)	p<0.001	28 (33%)	154 (30%)	0.701
Neutrophils, mean (SD)	51.29 (30.42)	45.93 (19.49)	p<0.001	32.87 (13.27)	30.15 (13.82)	0.024
CRP >40mg/l (n, %)	196 (20%)	788 (25%)	0.012	5 (0.5%)	19 (0.5%)	0.926
LytA positive, n (%)	59 (6%)	221 (7%)	0.77	61 (6%)	210 (5%)	0.752
MCPP (n, %)	12 (1%)	44 (1%)	0.976			
HDP, n (%)	150 (16%)	521 (17%)	0.912	150 (14%)	385 (9%)	p<0.001

* Adjusted for site and age. (Site variable adjusted for age only. Age Variable adjusted for site only)

Figure 1: MCPP and HDP in children compared to rhinovirus prevalence and rhinovirus-associated HDP over a 2 year period

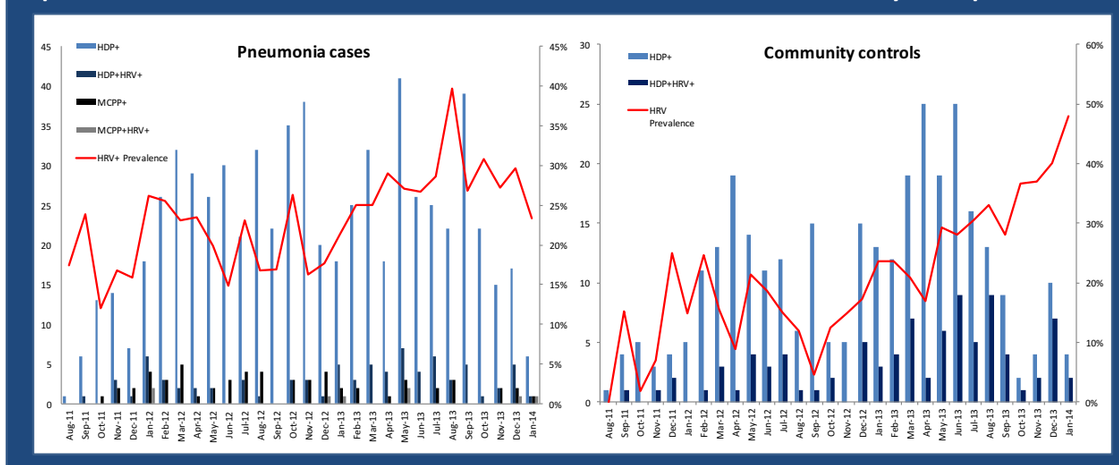

Table 2: The molecular epidemiology of rhinovirus

	Pneumonia Cases				HPD+ pneumonia cases			
	Rhinovirus-A (n=199)	Rhinovirus-B (n=31)	Rhinovirus-C (n=185)	P-value	Rhinovirus-A (n=57)	Rhinovirus-B (n=7)	Rhinovirus-C (n=41)	P-value
0-5 months (n, %)	110 (55%)	21 (68%)	67 (36%)		26 (46%)	5 (71%)	18 (44%)	
6-11 months (n, %)	46 (23%)	8 (26%)	51 (28%)	0.001	17 (30%)	2 (29%)	11 (27%)	0.6674
12-23 months (n, %)	22 (11%)	1 (3%)	42 (23%)		8 (14%)	0	11 (27%)	
24-59 months (n, %)	21 (11%)	1 (3%)	25 (14%)		6 (11%)	0	1 (2%)	
Sex, male n(%)	117 (59%)	14 (45%)	93 (50%)	0.135	32 (56%)	5 (71%)	19 (46%)	0.4153
HIV+, n(%)	30 (15%)	2 (6%)	16 (9%)		18 (32%)	0	2 (5%)	
HIV-, n(%)	150 (75%)	22 (71%)	153 (83%)	0.063	30 (53%)	5 (71%)	30 (72%)	0.0395
HIV unknown, n(%)	19 (10%)	7 (23%)	16 (9%)		9 (16%)	2 (29%)	9 (22%)	
Clinical Outcomes								
Very severe pneumonia, n(%)	88 (44%)	12 (39%)	72 (39%)	0.4348	29 (51%)	2 (29%)	24 (59%)	0.2798
Tachypnea, n(%)	169 (85%)	24 (77%)	161 (87%)	0.6581	50 (88%)	5 (71%)	38 (93%)	0.5641
Lab results								
LytA positive, n (%)	17 (9%)	4 (14%)	13 (7%)	0.4883	13 (24%)	3 (43%)	10 (24%)	0.5654
MCPP (n, %)	6 (3%)	0	2 (1%)	0.3569	6 (11%)	0	2 (5%)	0.6699
HDP, n (%)	57 (29%)	7 (23%)	41 (22%)	0.2413				

	Community controls				HPD+ community controls			
	Rhinovirus-A (n=190)	Rhinovirus-B (n=40)	Rhinovirus-C (n=191)	P-value	Rhinovirus-A (n=56)	Rhinovirus-B (n=7)	Rhinovirus-C (n=54)	P-value
0-5 months (n, %)	88 (46%)	24 (60%)	74 (39%)		27 (48%)	4 (57%)	26 (48%)	
6-11 months (n, %)	54 (28%)	8 (20%)	63 (33%)	0.3087	13 (23%)	1 (14%)	12 (22%)	0.8009
12-23 months (n, %)	33 (17%)	5 (13%)	35 (18%)		13 (23%)	1 (14%)	9 (17%)	
24-59 months (n, %)	15 (8%)	3 (7%)	19 (10%)		3 (5%)	1 (14%)	7 (13%)	
Sex, male n(%)	97 (51%)	17 (43%)	102 (53%)	0.4653	31 (55%)	1 (14%)	31 (57%)	0.1445
HIV+, n(%)	14 (7%)	1 (3%)	13 (7%)		4 (7%)	0	5 (9%)	
HIV-, n(%)	138 (73%)	23 (58%)	126 (66%)	0.6709	37 (66%)	2 (29%)	31 (57%)	0.9960
HIV unknown, n(%)	38 (20%)	16 (40%)	52 (27%)		15 (27%)	5 (71%)	18 (33%)	
Clinical Outcomes								
ARI controls, n(%)	33 (38%)	5 (6%)	48 (56%)	0.4395	13 (38%)	2 (6%)	19 (56%)	0.8058
Tachypnea, n(%)	16 (9%)	5 (12%)	13 (7%)	0.5674	8 (16%)	0	4 (8%)	0.4512
Lab results								
LytA positive, n (%)	19 (10%)	4 (10%)	7 (4%)	0.0308	13 (23%)	3 (43%)	5 (9%)	0.0442
HDP, n (%)	56 (29%)	7 (18%)	54 (29%)	0.17				

P-values adjusted for age and site. Age variables adjusted by site and site variables by age

CONCLUSIONS

- There was no clear relationship between rhinovirus infection and MCPP in children hospitalized with severe or very severe pneumonia.
- In the community controls children, high levels of pneumococcal colonization were associated with rhinovirus infections and this was especially true for community controls with RTIs.
- Longitudinal studies are needed to establish whether children with mild rhinovirus-associated disease and high levels of pneumococcal colonization progress to more severe pneumococcal disease.
- Interactions between viruses and pneumococcus are likely complex; thus longitudinal studies controlling for the presence of other viruses and bacteria might better define these interactions.

References

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