Association of Picornavirus Infections With Acute Otitis Media in a Prospective Birth Cohort Study

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Picornavirus Infections Are Associated with Acute Otitis Media in a Prospective Birth Cohort Study

Summary of main point

Human rhinoviruses (HRV) and human enteroviruses (HEV) are associated with acute otitis media (AOM) in young children and may contribute to the development of AOM in a relatively large proportion of cases.

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Footnotes

Abstract

Background. Human rhinoviruses (HRV), enteroviruses (HEV) and parechoviruses (HPeV) have been linked to acute otitis media (AOM). We evaluated this association in a prospective birth cohort setting.

Methods. 324 healthy infants were followed up from birth to the age of 3 years. Nasal swab samples were collected at the age of 3, 6, 12, 18, 24, and 36 months, and screened for HRV and HEV using real-time RT-qPCR. Stool samples were collected monthly and analyzed for HRV, HEV and HPeV. AOM episodes diagnosed by physician were reported by parents in a diary. The association of viruses with AOM was analyzed using Generalized Estimation Equation and their relative contributions using population attributable risk per cent (PAR%).

Results. A clear association was found between AOM episodes and simultaneous detection of HEV (adjusted Odds Ratio (aOR) for the detection of virus in stools 2.04, 95% CI 1.06-3.91) and HRV (aOR 1.54, 95% CI 1.04-2.30). HPeV showed a similar, yet non-significant trend (aOR 1.44, 95% CI 0.81-2.56). HRV and HEV showed higher PAR% (25% and 20%) than HPeV (11%).

Conclusions. HEVs and HRVs may contribute to the development of AOM in a relatively large proportion of cases.

Key words

Acute otitis media, picornaviruses, human rhinovirus, human enterovirus, human parechovirus, child

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Globally, upper respiratory infections (URI) continue to be the leading cause of acute morbidity in all age groups.[1] URIs, most of which have a viral etiology, are often complicated by acute otitis media (AOM), especially in young children.[2, 3] In studies carried out among children, approximately one third of all URIs have been complicated by AOM, which usually occurs within the first 7 days of URI onset.[2-5] Thus, due to the high frequency of viral URIs they constitute a significant risk factor of AOM, making it one of the most common diseases in childhood. Furthermore, children encountering AOM early in life have been shown to be at increased risk of concurrent AOM later on.[6] These episodes result in widespread use of antibiotics and in chronic cases may lead to otologic surgery, causing significant financial losses to families and society.[7]

AOM has generally been considered a bacterial complication of URI. However, viruses alone or together with bacterial co-pathogens have also been shown to cause AOM.[8-10] Furthermore, according to current consensus, the mucosal damage caused by virus infection is a prerequisite for bacteria to be able to enter the middle ear.[11] Thus, elimination of these viruses could offer efficient means for prevention of AOM. Of all viruses causing URIs, picornaviruses have been found to be among the most common causes, with human rhinoviruses (HRV; family *Picornaviridae*, genus *Enterovirus*) accounting for up to 73% of all respiratory infections recorded during the first year of life.[12, 13] HRVs as well as human enteroviruses (HEV; family *Picornaviridae*, genus *Enterovirus*) and human parechoviruses (HPeV; family *Picornaviridae*, genus *Parechovirus*) have been linked to AOM in a number of studies. In Finnish and Australian studies, HRVs and HEVs were the predominant viruses detected in children with AOM and recurrent AOM.[9, 14, 15] HPeVs have been found to play a smaller role in the development of AOM.[16-18]

The aforementioned studies have mostly relied on methods based on reverse transcriptase polymerase chain reaction (RT-PCR) assays, the high sensitivity of which has led to the questioning of the clinical relevance of the results. These viruses have also been found in children without concurrent respiratory symptoms.[19] Hence, the presence of HRVs, HEVs and HPeVs in respiratory or stool samples alone does not establish causality of the concurrent illness. In this study we

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investigated the possible causal relationship by comparing the frequency of HRV, HEV and HPeV infections during AOM episodes and AOM-free periods in a cohort of children who were followed from birth to the age of 3 years. We also calculated the population attributable risk (PAR) of these picornaviruses to the development of AOM.

METHODS

Subjects

This study is a secondary analysis of data collected during the prospective international DIABIMMUNE study carried out from September 2008 to October 2013. The birth cohort included in this study comprises 324 healthy infants (169 boys and 155 girls) born between September 2008 and May 2010 who were enrolled in the DIABIMMUNE study at Jorvi Hospital, Espoo, Finland. All newborns, whose families gave written informed consent to the study, were first screened for HLA DR/DQ alleles conferring susceptibility to type 1 diabetes using cord blood. Those with an eligible HLA genotype were recruited to prospective follow-up, constituting approximately 5 % of all newborn infants.[20] The local Ethics Committee approved the DIABIMMUNE study protocol.

Recording of Otitis Media Episodes and Background Characteristics

During the study period, subjects visited the study clinics at the age of 3, 6, 12, 18, 24 and 36 months, making altogether 1836 visits to the study clinics. On the first visit, the parents were given a diary in which they were advised to record information related to the child's diet, allergies, environmental exposures (such as type of daycare, pets, living environment and parental smoking), infections, use of medications, and vaccinations in between the visits. The number of siblings was also documented and updated during the follow-up period. All data from the diaries were transferred to a central database during each visit. The number of otitis media (OM) episodes, which the child had encountered since their last visit to the clinic, was recorded in the database on each visit. The date, ICD-10 (International Statistical Classification of Diseases and Related Health

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Problems 10) codes and type of OM diagnosis (parental report or diagnosed by a physician) were also recorded.

After data extraction, OM diagnoses were classified as acute or chronic based on the ICD-10 codes recorded. As customary in Finland for those cases diagnosed by otoscopy and without myringotomy, more than 99% of the episodes were recorded as H66.9 (unspecified otitis media). These were assumed to be acute. All OM episodes reported by the parents were counted as separate episodes regardless of the time period between two consecutive episodes.

Detection of Viruses

To assess HRV, HEV and HPeV infections, a study nurse collected nasal swab (NS) samples on each visit to the study clinic using a pediatric flocked swab (Copan Diagnostics, Inc. USA) After sampling, the swab was put into a tube containing UTM-RT medium (Copan Diagnostics, Inc.). The samples were frozen immediately and stored at -80°C until analyzed. Parents were also asked to collect stool samples from the subjects at home every month starting at the age of 1 month. Parents stored the samples at home at -20°C and brought them to the study clinic on scheduled visits. The frozen samples were then stored at -80°C until analyzed.

Altogether, 1387 NS samples were collected at the age of 3, 6, 12, 18, 24 and 36 months (mean 4.3 samples per child), and 2861 stool samples were collected monthly until the age of one year (mean 8.8 samples per child). At least one NS and stool sample was obtained and analyzed from 279 and 316 children, respectively.

NS and stool samples were screened for the presence of viral RNA with real-time RT-qPCR. First, a 10% stool suspension was prepared from each original stool sample using Hanks solution. Next, RNA was extracted from 140 µl of suspension using the modified Qiagen RNeasy96 kit (QIAGEN, Germany) to obtain a final volume of 60 µl extracted RNA. Similarly, 140 µl of each NS sample was subjected to RNA extraction to obtain a final volume of 60 µl of extracted RNA. The RT-qPCR methods used for detection of HRVs[21], HEVs[22] and HPeVs[23] utilized QuantiTect Probe kit

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(QIAGEN, Germany) as described in the references given. All samples were analyzed in triplicate paralleling RT-qPCR runs, and a sample was considered positive if one or more of the triplicate runs yielded a positive result. The cut off values for positivity were 44 for HRV and 42 for HEV and HPeV. Among the virus positive NS samples, 77.9 % and 73.3 % of were positive for HRV and HEV in all three runs, respectively. Of the virus positive stool samples, 63.3 %, 68.8 % and 69.7 % yielded a positive result in all three runs for HRV, HEV and HPeV, respectively.

Only one fourth of the NS samples were screened for HPeV. These analyses were discontinued due to the very low number of positive results (0.5%). Therefore, the HPeV findings of NS samples were excluded from statistical analyses.

HRV and HEV PCRs were repeatedly validated in regular external quality control rounds (Quality Control for Molecular Diagnostics, QCMD) where they got the best possible scores.

Statistical Methods

Background characteristics of children with and without AOM were compared with Mann-Whitney U test. The association between host and environmental factors and HRV, HEV and HPeV infections was analyzed with Fisher's exact test. The association between virus detection and AOM episodes was assessed first by univariate logistic regression. The covariates used in the final multivariable models were identified by Akaike Information Criterion (AIC) in a stepwise algorithm. Generalized Linear Models (GLM) with binomial family and logit link-function were used to fit statistical model(s). Generalized Estimation Equation (GEE) with binomial family and logit link-function was used to take account intra-individual correlation. Adjusted odds ratios (aOR) and their 95% confidence intervals (CIs) were presented as the measure of the associations analyzed. Only the AOM episodes diagnosed by a physician were included in the statistical analyses. Differences were considered statistically significant at $P < 0.05$. The analyses were run with R version 3.6.1 (2019-07-05, http://r-project.org). The packages used were "stats" and "gee". The contribution of HRV, HEV and HPeV to AOM was quantified with population attributable risk per cent (PAR%), which was calculated using the

following formula: PAR = P*(OR-1)/(P*(OR-1) +1) where P represents prevalence of infection in the population (here: the proportion of children who experienced at least one infection by the age of one year).

RESULTS

The total duration of follow-up was 930 child-years and the mean duration 34.4 (SD 6.1) months per child. Altogether 930 AOM episodes were recorded (median 2, range 0-18 per child) showing a peak at the age of 19-24 months (14 AOM episodes per 100 child months, Figure 1). Being first-born protected from AOM, whereas having a pet living mostly indoors was significantly associated with a higher number of AOM episodes. Table 1 shows the associations between different background factors and AOM.

Three hundred two (21.8%) NS samples and 594 (20.8%) stool samples were positive for HRV, and 25 (1.8%) and 83 (2.9%) for HEV, respectively. Furthermore, 145 (5.1%) stool samples tested positive for HPeV. The proportion of virus positive NS samples was highest at the age of 19-24 months for both HRV and HEV. In stool samples taken during the first year of life, the frequency of HEV and HPeV was highest at the age of 7-12 months, whereas HRV infections were most common at the age of 4-6 months (Figure 1). A clear seasonal pattern of HRV, HEV and HPeV infections was observed, with HRV infections peaking in late spring/early summer and in autumn, and HEV and HPeV infections peaking in late summer and autumn (Figure 2).

Having siblings significantly increased the proportion of HRV positive samples (Table 2). Conversely, having siblings and living in the countryside significantly reduced the proportion of HPeV positive samples. The median starting age of daycare was 17 months (inter-quartile range 13-23 months). Starting daycare under the age of 12 months significantly increased the number of HEV positive samples.

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A majority of the participants (82.4%) experienced at least one HRV infection, while 18.2% and 29.2% had at least one HEV or HPeV infection during the first year of life, respectively. At least one AOM episode was registered in 40.7% of the participants. Among those infants who were diagnosed with at least one AOM episode by the age of one year, the mean number of HRV and HEV positive stool samples was higher than among those who did not encounter any AOM episodes by that age (Table 3). Such an association was not seen in NS samples.

The time-association between virus infections and AOM was studied by comparing the virus detection rate in samples that were collected either within 2 weeks before or after the onset of AOM (101 NS and 184 stool samples) to that in all other samples of the same type (Table 4). HEV detection in NS and stool samples was strongly associated with experiencing AOM at the same time. Furthermore, detection of HRV in stool samples was significantly associated with simultaneous AOM. A similar trend was seen for HPeV, but the association was not statistically significant. The time-associations between HRV, HEV and HPeV detection and AOM episodes remained the same when a longer time-window (+/-3 weeks) was used in these analyses (data not shown).

PAR% was calculated for each virus using the aORs shown in Table 4 (combined stool and nasal swab results). Based on this estimate HRV and HEV could explain quite a significant proportion of AOM episodes, HRVs slightly more (25%) than HEVs (20%), while HPeVs was less significant (11%).

DISCUSSION

This study set out with the aim of assessing the frequency of HRV, HEV and HPeV infections and their association with AOM in a cohort of Finnish children who were followed from birth to the age of 3 years. The study setting allowed the evaluation of the role of these viruses in AOM in an unbiased manner in a child population that was not selected based on hospitalization, diagnosis of AOM or respiratory infection. This offered an opportunity to evaluate the contribution of HRV, HEV and HPeV infections to the development of AOM, taking into account the relatively high frequency of these

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infections in the background population, including children who do not develop AOM. Our findings suggest that HRV and HEV are associated with the development of AOM. According to the PAR% calculations, HRVs could theoretically contribute to the development of AOM in up to 25% of AOM cases, and HEVs in up to 20%. HPeVs seem to be less important as they showed a statistically non-significant trend that would equal to 10% PAR. Even though the PAR-prediction models are not accurate and should be interpreted with caution, the results point out that the role of these viruses can be significant, particularly during the epidemic seasons. The results also demonstrate the high frequency of HRVs in young children in general, which may lead to overestimation of their role in AOM if a representative control group is not analyzed at the same time.

Overall, our findings regarding the incidence of studied virus infections and their role in the development of AOM are in line with previous reports.[9, 14-18, 24] Of these three viruses, HRVs were detected most frequently, with 82.4% and 90.1% of the subjects testing positive for HRV at least once during the first year of life and by the age of 3 years, respectively. The incidence of HEV and HPeV infections was lower than that of HRV infections: by the age of 1 year HEV and HPeV was detected at least once in 18.2% and 29.2% of the participants, respectively, and 21.9% of the participants experienced at least one HEV infection by the age of 3 years. Even though HEV was less common and detected less frequently in samples taken within 2 weeks of an AOM episode than HRV or HPeV, HEV showed the strongest association with the development of AOM. This finding is supported by the results of a prospective, longitudinal cohort study using symptom-driven episodic sampling, which suggested that URI caused by HEV is complicated by AOM slightly more often than URI caused by HRV (34.4% vs. 30.4%).[4] On the other hand, a more recent prospective study among infants based on specimens collected monthly and during URI found HRV to be significantly associated with URI symptoms and AOM development, whereas HEV detection was associated with AOM development only marginally.[2] [9]

The incidence of HEV infections in our study was somewhat lower than that found in some other studies.[26] This may partly be explained by our sampling scheme: from the age of 1 year onwards,

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HEV detection was based solely on nasal swab samples that were collected quite infrequently (every 6 to 12 months), hence a part of the HEV infections may have been missed. On the other hand, our finding is in line with previous studies suggesting that HEV infections are less common in Finland than in most other European countries. [27]

Our findings regarding the incidence of HRV infections agree with previous studies, which have shown HRVs to be the most common cause of URIs in early childhood.[28] In the Finnish Otitis Media Cohort Study, 79-91% of the participating children had at least one HRV infection during the first 2 years of life, with more than 20% of the children having experienced their first HRV episode by the age of 6 months.[29] In a recent birth cohort study conducted in South-Western Finland, HRV was associated with 50% of AOM episodes diagnosed among children aged 0-2 years.[30] Similarly, in the previously mentioned study carried out among 0-12-month-old infants living in the United States, HRV was the most common virus detected during URI, and it was associated with the development of AOM, which complicated 27% of all URI episodes.[2] However, as described above, due to the high frequency of HRVs in children, the frequency of HRVs during AOM episodes should be compared to representative control group, and most previous studies have not made such comparisons.

One additional factor limiting the ability to draw conclusions from existing literature is the variability in the sensitivity and specificity of RT-PCR assays used to detect HRV and HEV infections, particularly since both HRVs and HEVs are amplified by the same commonly used primer pairs targeting the conserved 5-NCR of viral genomes. The RT-PCR assays used in the present study were specific and sensitive for these viruses, as verified repeatedly by best possible scores in external HRV and HEV quality control rounds (Quality Control for Molecular Diagnostics). These assays utilized primers and probes that were unique to each virus, and therefore no cross-amplification occurred between the viruses.

Previous studies have found that HPeV may also contribute to the development of AOM at least in some cases. [16-18] In this study, no significant association between HPeV and AOM was seen. This may be due to the relatively small size of our dataset. Our study found HEV and HPeV infections to be equally common during the first year of life, as 29% of the participants tested positive for HPeV at least once by the age of 12 months. This frequency is well in line with the results of two birth cohort studies previously conducted in Finland. [18][31]

Our study has some limitations. As all parental reports of AOM were counted as separate episodes regardless of the time period between consecutive reports, the incidence of AOM may have been overestimated. However, the incidence of AOM during the 3-year follow-up period (1 episode per person-year) agrees well with previous studies in which the incidence of AOM in pediatric populations has varied depending on the study setting and geographical location, ranging from 161 and 256 events per 1,000 person-years in Eastern and Western European countries, respectively, to the high incidence of 1,470-1,540 events per 1,000 person-years observed in Finland.[14, 32, 33]

The sampling scheme and diagnostic methods applied might be seen as another potential limitation of our study. On the other hand, regular sampling eliminated biases which could have been introduced by symptom-driven episodic sampling. In any case, as viruses were detected by RT-PCR from samples collected at pre-defined 1-12-month intervals, a part of infections was probably missed due to relatively short shedding of the virus. However, the excretion period of HPeV is relatively long ranging from 2-24 weeks, and monthly collected stool samples have been suggested to be a fairly good method for detecting HPeV infections.[31, 34] Hence, we can assume that our study provides a good estimate for the incidence of HPeV infections among Finnish infants. The shedding periods for HRV and HEV, on the other hand, are thought to be shorter, lasting up to 5-6 weeks for HRV and 2-3 weeks for HEV in NS samples, although longer excretion periods for HEV in stool samples have also been reported.[26, 35] While stool specimens have been considered the classical sample type for HEVs and HPeVs replicating primarily in the intestinal and oropharyngeal mucosa, also HRVs have been detected frequently in stool samples.[36-38] A more frequent

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sampling scheme, including stool samples also after 1 year of age or additional use of serological methods might have provided a more accurate estimate of the incidence of HRV and HEV infections during the second and third years of life.

Since viruses have been observed to play a significant role in the development of AOM, preventing viral URIs would be beneficial. A systematic review suggested that the influenza vaccine may reduce the incidence of AOM in infants and small children.[39] Our study and previous reports indicate that picornaviruses should also be targeted to prevent AOM in young children. Indeed, our previous study implied that the oral polio vaccine (OPV), a live attenuated enterovirus vaccine, may reduce the incidence of AOM by providing cross-protection from non-polio HEVs and other picornaviruses that have been associated with AOM.[40] While vaccines targeting certain HEVs have already been developed, and currently new HEV vaccines are under clinical development [41], attempts to produce an HRV vaccine have failed.[42, 43] However, recent studies from animal models suggest that it might be possible to develop a multivalent HRV vaccine that can induce immunity against several different HRV types.[44] Altogether, the development of vaccines against AOM-associated picornaviruses remains an important goal in future research due to the immense medical and health-economic burden caused by AOM.

CONCLUSIONS

This population-based birth cohort study supports previous observations, suggesting that HRVs and HEVs contribute to the development of AOM in young children. HEV showed the strongest association with the development of AOM. These results suggest that antiviral strategies targeted at picornaviruses might be beneficial in the prevention of AOM in this age group.

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Table 1. The demographic characteristics of the subjects and their impact on the mean number of acute otitis media (AOM) episodes during follow-up.

Characteristic	n (%)	Mean no. of AOM episodes	P-value [a]
Sex			
Male	169 (52.2)	3.0	0.454
Female	155 (47.8)	2.7	
First-born child			
Yes	170 (52.5)	2.6	0.045
No	154 (47.5)	3.2	
Siblings			
≥ 1	200 (61.7)	3.0	0.119
None	124 (38.2)	2.6	
Exclusive breastfeeding at 3 months of age			
Yes	178 (66.9)	3.1	0.192
No	88 (33.1)	2.6	
Duration of breastfeeding ≥ 6 months			
Yes	226 (73.6)	3.1	0.053
No	81 (26.4)	2.6	
Use of pacifier			
Yes	200 (73.3)	3.1	0.802
No	73 (26.7)	2.9	

Parental smoking			
Yes	61 (19.4)	2.9	
No	253 (80.6)	2.9	0.567
Pet living mostly indoors			
Yes	121 (38.1)	3.3	
No	197 (61.9)	2.6	0.039
Age at starting in daycare <12 months			
Yes	37 (15.7)	3.1	
No	198 (84.3)	3.4	0.308
Age at starting in daycare <18 months			
Yes	128 (54.5)	3.6	
No	107 (45.5)	2.9	0.167
Age at starting in daycare <24 months			
Yes	181 (77.0)	3.5	
No	54 (23.0)	2.7	0.222
Living environment			
Urban	260 (82.8)	2.9	
Rural	35 (11.1)	3.0	0.745
Both	19 (6.1)	3.1	

[a] Wilcoxon rank sum test

Table 2. The association between different background factors and the proportion of virus positive samples (stool and nasal swab samples combined for HRV and HEV) during follow-up.

	HRV			HEV			HPeV		
Characteristic	No. of samples	Positive samples (%)	P-value [a]	No. of samples	Positive samples (%)	P-value [a]	No. of samples	Positive samples (%)	P-value [a]
Sex									
Male	2210	485 (21.9)		2210	51 (2.3)		1532	83 (5.4)	
			0.164			0.330			0.394
Female	2038	411 (20.2)		2038	57 (2.8)		1320	62 (4.7)	
First-born child									
Yes	2305	374 (16.2)		2305	50 (2.2)		1526	95 (6.2)	
			<0.001			0.097			0.004
No	1943	522 (26.9)		1943	58 (3.0)		1326	50 (3.8)	
Siblings									
>1	2604	628 (24.1)	<0.001	2604	69 (2.6)	0.618	1768	72 (4.1)	0.002

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	N	n (%)	p	N	n (%)	p	N	n (%)	p
None	1644	268 (16.3)		1644	39 (2.4)		1084	73 (6.7)	
Exclusive breastfeeding at 3 months of age									
Yes	2475	539 (21.8)	0.858	2475	63 (2.5)	0.492	1684	90 (5.3)	0.766
No	1040	223 (21.4)		1040	31 (3.0)		693	39 (5.6)	
Duration of breastfeeding >6 months									
Yes	3077	657 (21.4)	0.559	3077	79 (2.6)	0.731	2074	105 (5.1)	0.545
No	980	200 (20.4)		980	27 (2.8)		653	37 (5.7)	
Use of pacifier									
Yes	2690	558 (20.7)	0.854	2690	68 (2.5)	1.000	1791	97 (5.4)	1.000
No	976	205 (21.0)		976	24 (2.5)		661	35 (5.3)	
Parental smoking									
Yes	822	160 (19.5)	0.232	822	23 (2.8)	0.714	546	33 (6.0)	0.332
No	3324	713 (21.5)		3324	85 (2.6)		2236	111 (5.0)	
Pet living mostly indoors									
Yes	1583	322 (20.3)	0.458	1583	44 (2.8)	0.546	1050	53 (5.0)	1.000

	HRV N	HRV n (%)	p	HEV N	HEV n (%)	p	HPeV N	HPeV n (%)	p
No	2619	559 (21.3)		2619	64 (2.4)		1772	90 (5.1)	
Age at starting in daycare <12 months									
Yes	664	143 (21.5)	0.518	664	30 (4.5)	<0.001	449	28 (6.2)	0.487
No	2543	518 (20.4)		2543	50 (2.0)		1695	91 (5.4)	
Age at starting in daycare <18 months									
Yes	1869	401 (21.5)	0.170	1869	55 (2.9)	0.066	1263	70 (5.5)	1.000
No	1338	260 (19.4)		1338	25 (1.9)		881	49 (5.6)	
Age at starting in daycare <24 months									
Yes	2488	503 (20.2)	0.320	2488	67 (2.7)	0.221	1674	87 (5.2)	0.173
No	719	158 (22.0)		719	13 (1.8)		470	32 (6.8)	
Living environment									
Urban	3407	705 (20.7)	0.148	3407	88 (2.6)	0.145	2278	124 (5.4)	0.032
Rural or both	731	169 (23.1)		731	12 (1.6)		508	16 (3.1)	

Abbreviations: HRV, human rhinovirus; HEV, human enterovirus; HPeV, human parechovirus

[a] Fisher's exact test

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Table 3. The mean number of virus-positive nasal swab and stool samples among children with or without at least one acute otitis media (AOM) episode by the age of one year.

Sample type and virus	≥1 AOM Mean	No AOM Mean	P-value [a]
Nasal swab	No. of children = 95	No. of children = 144	
HRV	0.5	0.5	0.498
HEV	<0.1	<0.1	0.695
Stool	No. of children = 129	No. of children = 186	
HRV	2.3	1.6	<0.001
HEV	0.4	0.2	0.038
HPeV	0.4	0.5	0.934
Nasal swab and/or stool	No. of children = 132	No. of children = 192	
HRV	2.6	1.9	0.002
HEV	0.4	0.2	0.039

Abbreviations: HRV, human rhinovirus; HEV, human enterovirus; HPeV, human parechovirus

[a] Mann-Whitney U-test

Table 4. The proportion of virus-positive samples obtained from participants with and without acute otitis media (AOM) within 2 weeks before or after giving samples and the association between virus-positivity and AOM.

Sample type and virus	AOM (%) No. of samples		No AOM (%) No. of samples		OR (95% CI)	Adj. OR (95% CI) [a]	Adj. OR (GEE) (95% CI) [b]
Nasal swab							
HRV	101	28 (27.7)	1286	274 (21.3)	1.42 (0.90-2.23)	1.38 (0.88-2.18) [c]	1.36 (0.85-2.18) [c]
HEV	101	5 (5.0)	1286	20 (1.6)	3.30 (1.21-8.98)	3.05 (1.12-8.35) [c]	3.26 (1.31-8.12) [c]
Stool							
HRV	184	50 (27.2)	2677	544 (20.3)	1.46 (1.04-2.05)	1.57 (1.10-2.24) [d]	1.54 (1.04-2.30) [d]
HEV	184	12 (6.5)	2677	71 (2.7)	2.56 (1.36-4.81)	1.94 (1.01-3.72) [d]	2.05 (1.10-3.80) [d]
HPeV	184	15 (8.2)	2668	130 (4.9)	1.73 (0.99-3.02)	1.44 (0.81-2.56) [e]	1.45 (0.84-2.51) [e]

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Nasal swab and/or stool [f]							
HRV	227	63 (27.8)	3309	654 (19.8)	1.56 (1.15-2.11)	1.53 (1.12-2.09) [d]	1.40 (0.96-2.04) [d]
HEV	227	15 (6.6)	3309	79 (2.4)	2.89 (1.64-5.11)	2.15 (1.19-3.88) [d]	2.04 (1.06-3.91) [d]

Abbreviations: HRV, human rhinovirus; HEV, human enterovirus; HPeV, human parechovirus; GEE, generalized estimation equations

[a] Generalized linear model

[b] Generalized estimation equations

[c] The final model included virus positivity and having siblings.

[d] The final model included virus positivity, being firstborn and age at time of sampling.

[e] The final model included being firstborn and age at time of sampling. Virus positivity was added as an additional variable.

[f] Includes only samples obtained during the first 12 months of life

Figure 1. The proportion of virus positive samples and the number of acute otitis media (AOM) per month during the indicated follow-up periods.

Abbreviations: HRV, human rhinovirus; HEV, human enterovirus; HPeV, human parechovirus

Figure 2. The proportion of acute otitis media (AOM) episodes and human rhinovirus (HRV), human enterovirus (HEV) and human parechovirus (HPeV) RNA findings in nasal swab and/or stool samples detected each month, out of all AOM episodes and respective virus findings.

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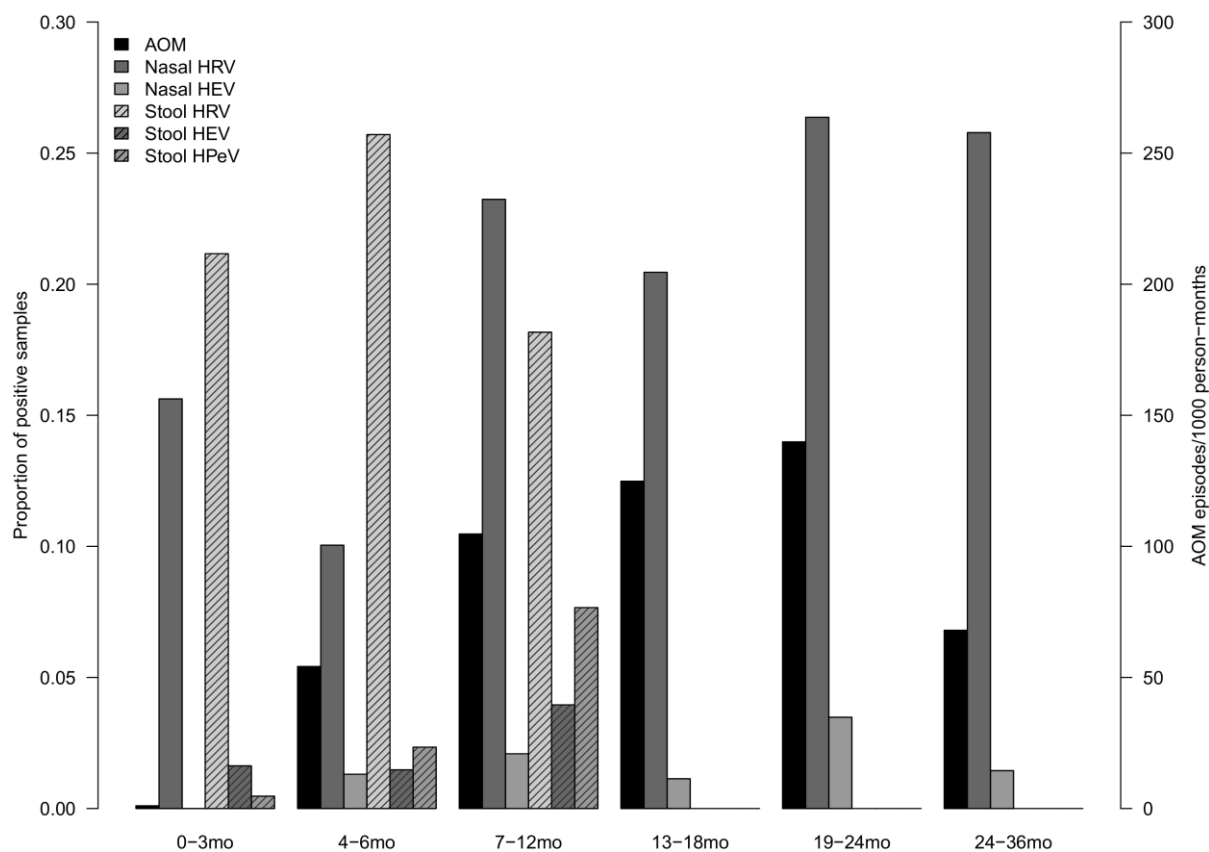
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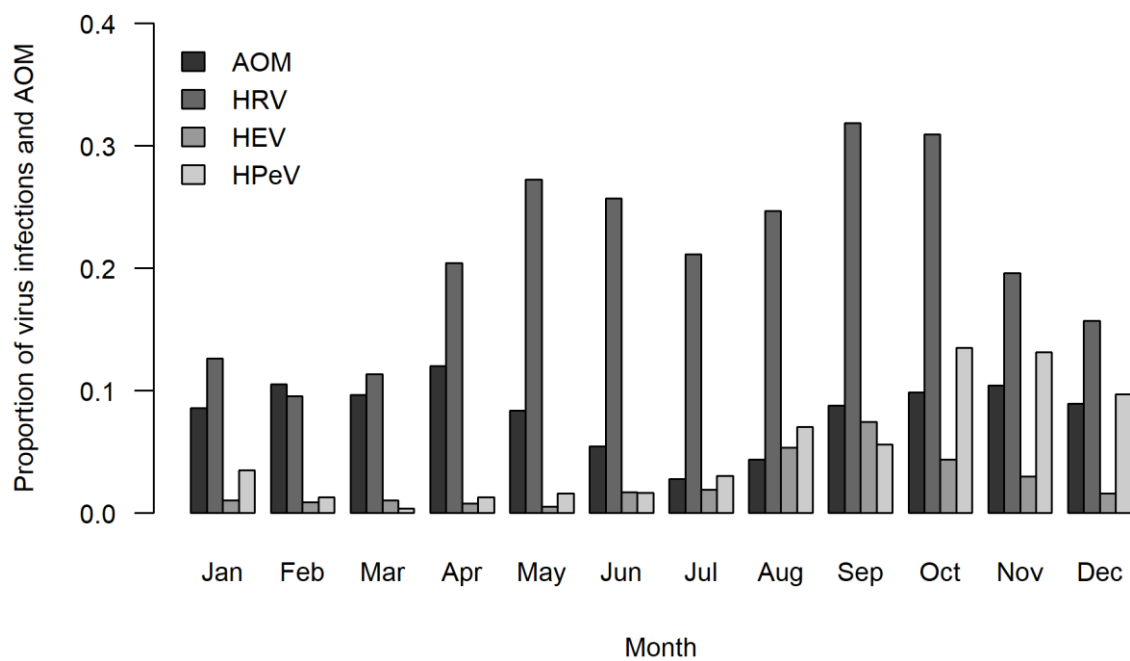
Figure 1

Figure 2