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ORIGINAL ARTICLE

Parechovirus A in Hospitalized Children With Respiratory Tract Infections: A 10-Year-Long Study From Norway

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Background. The role of Parechovirus A (PeV-A) in hospitalized children with respiratory tract infections (RTIs) is unclear. We studied the occurrence and impact of PeV-A over 10 years.

Methods. Children from Sør-Trøndelag County, Norway, hospitalized with RTI and a comparison group of asymptomatic children admitted to elective surgery, were prospectively enrolled from 2006 to 2016. Nasopharyngeal aspirates were cultured and analyzed with polymerase chain reaction tests for PeV-A and 19 other pathogens. The cycle threshold levels of PeV-A were reported as measures of viral genomic loads. Parechovirus A-positive samples were genotyped by amplification and sequencing of the VP3/VP1 junction.

Results. Parechovirus A was detected in 8.8% (323/3689) patients with RTI and in 10.1% (45/444) of the children in the comparison group (P = .34). Parechovirus A genotyping (n = 188) revealed PeV-A1 (n = 121), PeV-A3 (n = 15), PeV-A5 (n = 6), and PeV-A6 (n = 46). Viral codetections occurred in 95% of patients and in 84% of the children in the comparison group (P = .016). In multivariable logistic regression analysis, RTI was unrelated to PeV-A genomic loads, adjusted for other viruses and covariates. Similar results were found for PeV-A1 and PeV-A6.

Conclusions. Parechovirus A and viral codetections were common in hospitalized children with RTI and asymptomatic children in a comparison group. Our findings suggest that PeV-A has a limited role in hospitalized children with RTI.

Key words. children; genotypes; parechovirus A; respiratory tract infection.

The species *Parechovirus A*, formerly known as human parechovirus, belongs to the genus *Parechovirus* within the Picornaviridae family. It consists of Parechovirus-A genotypes 1–19 (PeV-A 1-19). Parechoviruses A are single-stranded, positive-sense RNA viruses, classified in their own genus since 1999 after first being known as echovirus [1]. The role of PeV-A3 as causative agent in meningoencephalitis and sepsis-like disease in infants is firmly established [2–4]. Likewise, it has been shown that several genotypes may cause gastrointestinal disease [5, 6]. The role of PeV-A in respiratory tract infection (RTI) is, however, less certain [7]. Several studies have reported that PeV-A may be found in 1%–8% of upper airway samples from children with RTI [8, 9], but often respiratory syncytial virus (RSV) and other well-known pathogens have been detected simultaneously [8, 10, 11]. Seroprevalence studies have shown a rapid increase after 6 months of age in the rates of children with antibodies against PeV-A1 and PeV-A2 and to a lesser extent PeV-A5 and PeV-A6 [12], suggesting stimulation of the immune system after early exposure in most of the children. However, high seropositive rates may not prove whether these children developed respiratory infection, other infections due to PeV-A, or had an asymptomatic infection [13]. We have detected PeV-A without viral codetections in a group of children attending day care who were diagnosed with mild upper respiratory tract infections (URTI) [14]. However, there is no clear evidence supporting PeV-A as a cause of lower respiratory tract infection (LRTI) in children in need of hospitalization.

We have continuously enrolled children admitted to hospital with RTI and a comparison group of asymptomatic children admitted to elective surgery during a 10-year-long period and assessed the occurrence of PeV-A, distribution of PeV-A genotypes, and viral codetection rates in children with PeV-A. To address the question of whether PeV-A may be related to RTI in children, we compared PeV-A genomic loads and viral codetection rates in children with RTI and children in the comparison group.

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METHODS

Study Setting

This study was conducted at Children's Department, St. Olavs Hospital, Trondheim University Hospital, Norway, which is the only hospital for 59 000 <16-year-old children in Sør-Trøndelag County.

Study Population

Children admitted to the Pediatric Emergency Department with acute RTI from November 2006 to July 2016 were invited to participate. Parechovirus A-positive infants with sepsis-like disease without signs of RTI and children with other infections were not included. Children undergoing chemotherapy or immunosuppressive treatment were also excluded. All children had been referred to pediatric assessment after evaluation at the public Emergency Department or at general practitioners.

Written consent was obtained from caregivers and from children >12 years old. Due to practical challenges, some children who had collected a nasopharyngeal sample were invited to participate in the study, by mail after discharge. They received information about the study, and their caregivers were given the opportunity to resist enrollment within 2 weeks.

We also recruited a non-matched comparison group consisting of children without URTI symptoms who were referred for elective surgery, consecutively between 2007 and 2015. Children undergoing ear, nose, and throat surgery were excluded.

Clinical Classification

Included children were examined and treated by physicians according to local guidelines. Clinical data including history, clinical status at admission, and information about the clinical course were registered by physicians and the research group from medical records and predefined forms. Caregivers recorded general information and details about the current disease in a questionnaire.

The participants were retrospectively divided into 2 groups: children with URTI and children with LRTI. Upper RTI was defined as rhinosinusitis, otitis media, pharyngitis, laryngitis, or tonsillitis without signs of LRTI. Lower RTI was diagnosed in the presence of dyspnea, lower airway obstruction, or infiltrates as shown on chest x-ray, with or without signs of URTI. We defined chronic disease as the presence of either asthma, epilepsy, cerebral palsy, immunodeficiency, congenital anomalies or congenital heart disease.

Caretakers of children in the comparison group answered a questionnaire, including symptoms of common cold or runny nose during the past 14 days or at present.

Laboratory Methods

Nasopharyngeal aspirates were collected at admission. A total of 3689 aspirates from referred children and 444 aspirates from children in the comparison group were analyzed for PeV-A (Supplementary Figure 1). From the 331 PeV-A-positive samples in the patient group, 8 cases were excluded due to missing clinical data. The aspirates were placed into a standard virus transport medium without antibiotics. Samples were analyzed using in-house TaqMan real-time polymerase chain reaction (PCR) assays and viral cultures. A total of 17 viruses were analyzed: PeV-A, RSV, human rhinovirus (HRV), human adenovirus (HAdV), human bocavirus (HBoV), influenza virus A/B (Flu A/B), parainfluenza virus 1–4 (PIV 1-4), human enterovirus (HEV), human coronavirus (OC43, 229E, NL63, and HKU1), and human metapneumovirus (HMPV). PCR analysis was also performed for *Bordetella pertussis*, *Chlamydia pneumoniae*, and *Mycoplasma pneumonia*. The PeV-A-PCR and the other screening tests were performed as described earlier [2, 15]. Results were reported with a cycle threshold (Ct) value and semiquantitative results were defined as high (Ct value < 28), medium (Ct value 28-35), or low (Ct value ≥35-40). A Ct value >40 was defined as a negative result. Negative samples were controlled for the presence of human DNA to verify adequate sampling technique.

Parechovirus A-positive samples were genotyped by amplification of the VP3/VP1 junction and sequencing of the PCR product. Nested PCR was performed with outer and inner primers as described by Harvala et al. [8]. Both PCRs were done in a 50-µL reaction containing 5 µL 10X PCR-buffer I and 0·5 µL Amplitaq Gold (5U/µL) (Applied Biosystems), 2·5 µL 1 mM dNTP (Invitrogen), and 0·6 µM of each primer. The first PCR with outer primers was performed with 10 µL cDNA as template and comprised 95°C (10 min), 40 cycles of 95°C (30 s), 50°C (30 s), and 72 °C (60 s) and a final extension at 72°C (5 min), and 1 µL of the PCR product was used as a template in the second PCR with the inner primers and run with the same parameters as the first PCR but with 35 cycles and 55°C annealing temperature. The final PCR product was visualized on E-gel 2% with SYBR safe (Invitrogen), and PCR products containing a 304-bp band were prepared for sequencing using Rapid PCR Cleanup Enzyme Set (New England BioLabs). Sequence reactions were performed with GenomeLab Dye Terminator Cycle Sequencing Start Kit (BeckmanCoulter/Sciex) in both directions according to the procedure by BeckmanCoulter but with reduced reaction mix (¼), adding of 1·5 µL Sequence Reaction Buffer and the concentration of inner primers increased to 0·48 µM due to their degenerated sequences. The sequence products were purified by ethanol precipitation and analyzed on Beckman Coulter CEQ8800 (Sciex) The Parechovirus genotype was achieved by BLAST-search in NCBI (National Center for Biotechnology Information).

Statistical Analysis

We defined background characteristics of patients and controls according to Table 1 and defined 4 epidemiological seasons per year: fall (September-November), winter (December-February), spring (March-May), and summer (June-August).

Table 1. Characteristics of Children with Respiratory Tract Infection (RTI) and Asymptomatic Children in a Comparison Group With and Without Parechovirus A (PeV-A)

	Children With PeV-A, No (%)[a]			Children Without PeV-A		
Characteristics	RTI (n = 323)	Comparison Group (n = 45)	*P*-value	RTI (*n* = 3366)	Comparison Group (n = 399)	*P*-value
Age, mo[b]	16.3 (9.7)	24.2 (16.8)	<.001	14.3 (22.6)	37.4 (44.8)	<.001
Age, mo						
<6	15 (5%)	0 (0%)		982 (29%)	22 (6%)	
6-11	54 (17%)	3 (6%)		507 (15%)	29 (7%)	
12-23	194 (60%)	19 (42%)	<.001	887 (26%)	80 (20%)	<.001
24-59	59 (18%)	20 (44%)		682 (20%)	159 (40%)	
>60	1 (0·3%)	3 (7%)		308 (9%)	109 (27%)	
Chronic disease[c]	50 (15%)	3 (7%)	.17	640 (19%)	28 (2%)	<.001
Premature birth[d]	43 (13%)[e]	6 (13%)	.98	459 (14%)	29 (8%)	<.001
Gender, male	198 (61%)	39 (87%)	.001	2001 (59%)	316 (79%)	<.001
Resp. symptoms						
Upper	95 (29%)	—	—	814 (25%)	—	—
Lower	228 (71%)	—	—	2419 (75%)	—	—
Season						
Sept-Nov	135 (42%)	16 (36%)		721 (21%)	136 (34%)	
Dec-Feb	118 (37%)	14 (31%)	.24	1264 (38%)	88 (22%)	<.001
Mar-May	55 (17%)	9 (20%)		993 (30%)	89 (22%)	
June-Aug	15 (5%)	6 (13%)		388 (12%)	86 (22%)	

[a]Data represent n (%) unless otherwise specified.
[b]Median, interquartile range.
[c]Chronic disease includes asthma, cerebral palsy, epilepsy, immunodeficiency, and heart disease.
[d]Gestational age <36 weeks.
[e]Missing data in 3 patients.

We used chi-square test, Fisher's exact test, Student's *t*-test, and Mann-Whitney *U*-test to compare data, as appropriate. To examine the role of PeV-A in RTI, we used multivariable logistic regression modeling. In these analyses, we estimated the odds ratios as effect measures with precision assessed by 95% confidence intervals. We used children with RTI vs children in the comparison group as a dependent variable, entered as the independent variable PeV-A Ct level categories (high, moderate, or low), and adjusted for the covariates age (months), sex (male, female), chronic disease (yes, no), premature birth (yes, no), and a compound variable defined as codetection of one or more severe viruses (RSV, HMPV, flu-A/B, and/or PIV 1-4) (yes, no). We also performed a multivariable analysis, including only children with RTI and asymptomatic children in the comparison group with PeV-A1 and PeV-A6. For all analyses, differences were considered statistically significant at $P < .05$. We used IBM SPSS Statistics 25 in the analysis.

Ethics

The study was approved by the Regional Committees for Medical and Health Research Ethics, Central Norway, in 2006 (No: 06/2289), 2008 (No: 08/2142), and 2012 (No: 12/1042).

RESULTS

From late 2006 to 2016, we detected PeV-A in 8.8% (323/3689) of the children with RTI and in 10.1% (45/444) of the children in the comparison group ($P = .34$). Among children with RTI, PeV-A was the fifth most common virus, after HRV (41%), RSV (30%), HEV (12%), and PIV 1-4 (10%). Children with RTI were younger than children in the comparison group (median age 16 vs 24 months, $P < .001$) (Table 1). In children with RTI, PeV-A appeared in all age groups, but the majority (60%) was 12–23 months old. In the comparison group, nearly 9 out of 10 were 12–59 months old, and only a few <12 months old (Table 1). There were more boys than girls in both groups (Table 1). The occurrence of chronic diseases was slightly higher among patients than controls (15% vs 7%), but the difference was not significant (Table 1). The rates of premature birth were 13% in both groups, which is a higher rate than generally in Central Norway (4% in 2006-2016) (Statistics Norway).

Annual PeV-A detection

Parechovirus A was detected every year from 2006 to 2016 in children with RTI and every year from 2007 to 2015 in the comparison group (Figure 1). The number of detections per year differed throughout the study period (Figure 1). No clear yearwise patterns were observed (Figure 1). In the year 2013, we observed a peak in detections in the comparison group that may be related to a higher inclusion rate (Figure 1).

Seasonal PeV-A detection

We observed a clear seasonal distribution and high numbers of PeV-A during fall and early winter and declining detection rates toward summer (Table 1, Figure 2). In the patient

Figure 1. Number of detections of Parechovirus A in children with respiratory tract infection and a comparison group enrolled at St. Olavs Hospital, Trondheim University Hospital, from 2006 to 2016.

group, 78% (253/323) detections were obtained between September and February, and only 4.6% (15/323) during the summer seasons. The comparison group showed similar seasonal distribution patterns with 67% (30/45) positive samples obtained from September to February. Children with PeV-A more often were enrolled in the study during the fall and less often during the spring, compared with PeV-A-negative children (Table 1).

PeV-A genotypes

In 58% (188/323) of the patient samples and 51% (23/45) of the samples from the comparison group, a PeV-A

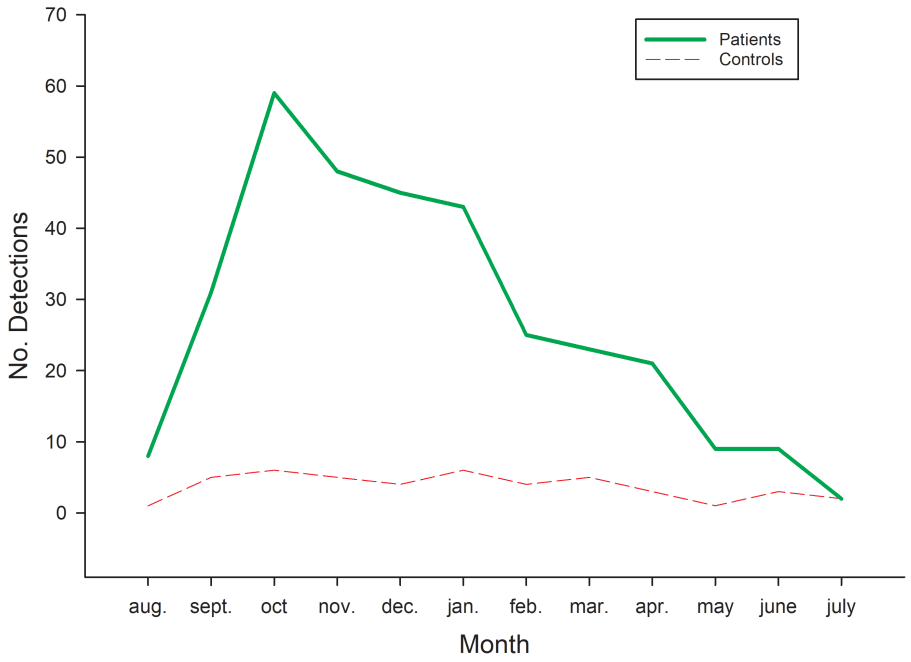

Figure 2. Monthly detections of Parechovirus A in children with respiratory tract infection and a comparison group enrolled at St. Olavs Hospital, Trondheim University Hospital, from 2006 to 2016.

genotype could be determined. Mean PeV-A Ct values in non-genotyped samples were higher compared with genotyped (35.6 vs 30.9, $P < .001$). We detected 4 genotypes: genotypes 1, 3, 5, and 6 (Figure 3). In the patient group, the majority had either PeV-A1 (64%, 121/188) or PeV-A6 (24%, 46/188). Parechovirus A3 was detected in 8% (15/188) and PeV-A5 in 3% (5/188) (Table 2). Parechovirus A1 and PeV-A6 were detected almost every year, except 1 year without PeV-A6, and with the highest detection rates from September to February. They were also detected mainly in children aged 12–23 months old (Table 2). Because of small numbers, firm patterns for PeV-A3 and PeV-A5 could not be established, but there were some indications that PeV-A3 may have been detected more frequently during spring and summer. In addition, PeV-A3 was the most prevalent in children <6 months of age. Only one infant with PeV-A3 had sepsis-like disease in addition to RTI.

The same PeV-A genotypes, except PeV-A5, were detected in the comparison group: PeV-A1 39% (9/23), PeV-A3 22% (5/23), PeV-A5 0% (0/23), and PeV-A6 39% (9/23).

Diagnoses in Children With PeV-A and Relations to Viral Codetections

Among 323 children with PeV-A and RTI, 29% (95/323) were diagnosed with URTI, and 71% (228/323) had LRTI. Only 5% (16/323) had a single PeV-A detection, and 95% (307/323) had viral codetections. URTI was more common among those with single PeV-A compared with those with codetections (75% [12/16] vs 27% [83/307], $P < .001$).

In the comparison group, 16% (7/45) had single PeV-A and 84% (38/45) had viral codetections. Single PeV-A detection was more frequent in the comparison group than in the patient group (16% vs 5%, $P = .014$).

Codetected Viruses

Human rhinovirus was the most common codetected virus in the patient group (57%, 183/323) and in the comparison group (51%, 23/45). RSV was more frequently detected among children with RTI than children in the comparison group (26%, 84/323, vs 4%, 2/45) (Supplementary Table 1), whereas detection rates for HBoV, PIV, HAdV, HCoV, HMPV, and flu A/B did not differ between the groups (Supplementary Table 1).

Relations Between RTIs, PeV-A Genomic Loads, and Viral Codetection Rates

There was no difference in mean PeV-A Ct values between the patient group with RTI and the comparison group (32.8 vs 33.2).

In multivariate logistic regression modeling, RTI was not associated with PeV-A genomic load categories, but the presence of RSV, Flu A/B, PIV-1–4, and HMPV was strongly associated with RTI (Table 3). In analyses including PeV-A1 and PeV-A6, similar results were found (Table 3).

DISCUSSION

Main Findings

In a hospital study recruiting children admitted with RTI from the Sør-Trøndelag County, Norway, over a 10-year-long period, we found that PeV-A is a common finding in the airways of children. We detected PeV-A in 1 out of 10 and with similar frequency in children with RTI and a comparison group of children admitted to elective surgery, and codetections rates were very high in both groups. Hence, we found no evidence that PeV-A may have a role in children admitted to hospital with RTI.

Seasonal Variations of PeV-A and PeV-A Genotypes

Our data verify that PeV-A appears during long periods every year among children referred to a hospital with RTI. The seasonal distribution of PeV-A had a characteristic pattern with high numbers every year during a 5-month-long period from September to February. Our findings resemble the seasonal distribution patterns found in studies from Hong Kong [10], Finland [11], Italy [9], Japan [16], and Australia [17]. However, in a study from Scotland, Harvala et al. [8] reported that PeV-A mainly appeared between July and December.

In our study, PeV-A1 and PeV-A6 were dominating, and both were detected every year during fall and winter time, apart for 1 year with absent PeV-A6. Only a few PeV-A5 cases were detected in the fall and wintertime. Other researchers have reported similar seasonal distribution for PeV-A1 [10, 18], but, to our knowledge, the epidemiology of PeV-A6 has not been studied previously. Parechovirus A3 mainly appeared during summertime, in accordance with findings in other studies [18–23].

Prevalence of PeV-A

Parechovirus A appeared in 8.8% of the children admitted to hospital with RTI and in 10.1% of hospital controls. These figures are higher than described in most of the previous studies. Harvala et al. [8] reported a PeV-A detection rate of 1.2% in airway samples from children with RTI and 2 other studies reported PeV-A in less than 2% of children under 2 years of age with RTI [9, 10]. A recent study from Switzerland demonstrated a higher PeV-A viremia rate of 5.9% in children with fever without a source [24]. We believe that our findings are likely to be representative, because there is good evidence from serological studies performed in Finland and the Netherlands that the majority of children have been exposed to PeV-A before 2 years of age [12]. A few studies have shown that PeV-A may be shed for more than 3 weeks and that might also account for the high detection rates in our study [25].

The Relation Between PeV-A and RTIs in Need of Hospitalization

The detection of 2 PeV-A genotypes PeV-A1 and PeV-A6 with characteristic epidemic patterns over a 10-year-long period in

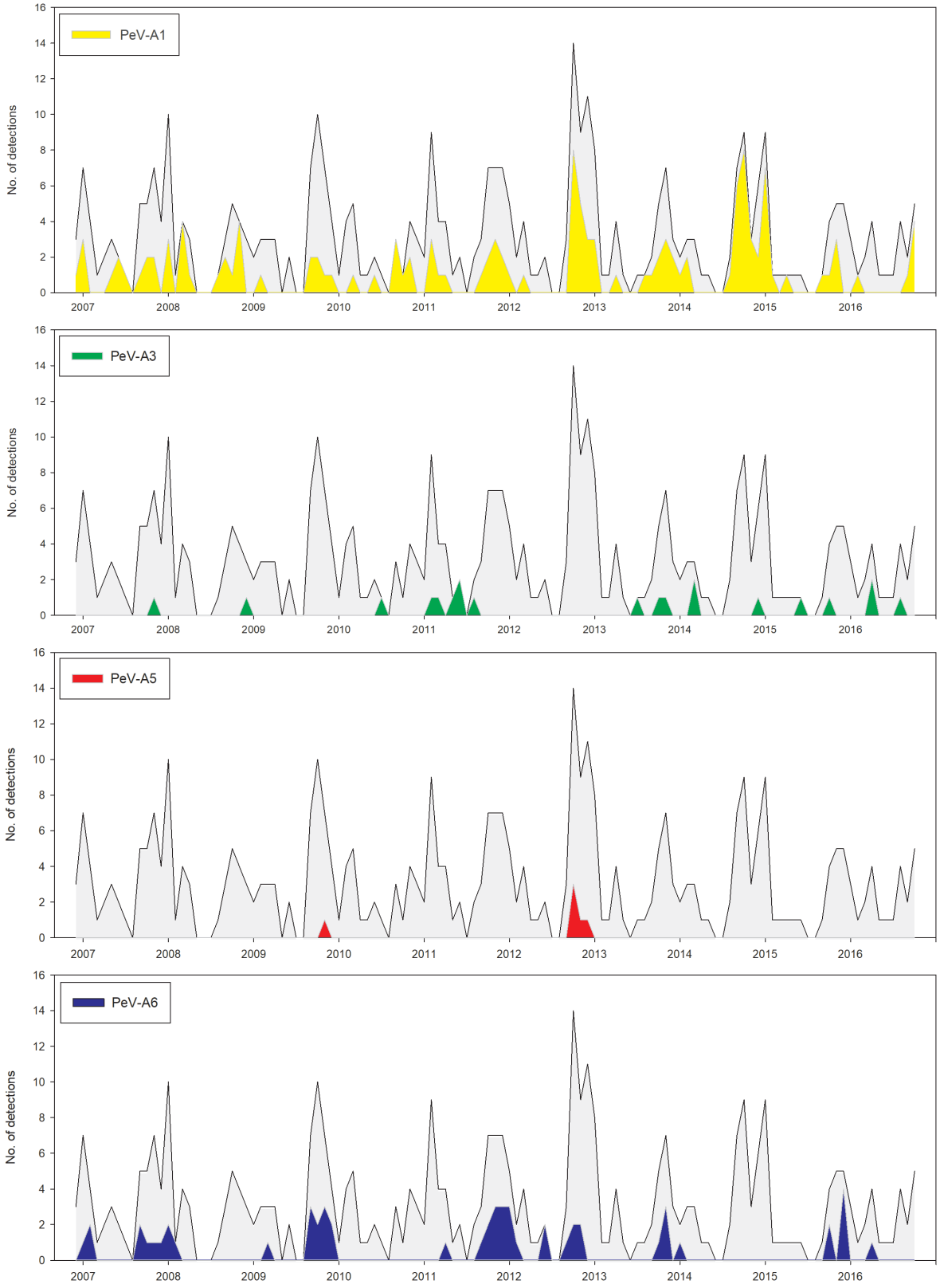

Figure 3. Monthly detections of Parechovirus A genotypes in children with respiratory tract infection and a comparison group enrolled from 2006 to 2016. Gray shade represents the total number of detections of Parechovirus A.

Table 2. Parechovirus A (PeV-A) Genotype Distributions in 188 Children With Respiratory Tract Infection (RTI)

	PeV-A Genotypes, No (%)[a]			
	Genotype 1 (n = 121)	Genotype 3 (n = 15)	Genotype 5 (n = 6)	Genotype 6 (n = 46)
Age, mo				
<6	5 (4%)	5 (33%)	1 (17%)	2 (4%)
6-11	24 (20%)	3 (20%)	1 (17%)	6 (13%)
12-23	81 (67%)	4 (27%)	3 (50%)	27 (59%)
24 to >60	11 (9%)	3 (20%)	1 (17%)	11 (24%)
Season				
Sept-Nov	71 (59%)	3 (20%)	5 (83%)	25 (54%)
Dec-Febr	34 (28%)	2 (13%)	1 (17%)	17 (37%)
March-May	11 (9%)	6 (40%)	0	3 (7%)
June-Aug	5 (4%)	4 (27%)	0	1 (2%)
Diagnosis				
Upper RTI	39 (32%)	5 (33%)	4 (67%)	13 (28%)
Combined RTI	63 (52%)	8 (53%)	1 (17%)	29 (63%)
Lower RTI	19 (16%)	2 (13%)	1 (17%)	4 (9%)

[a]Data represent n (%)

hospitalized children with RTI might from an epidemiological perspective suggest causal relations. However, this is opposed by our findings of a high detection rate of PeV-A in the comparison group and high viral codetection rates in all PeV-A-positive children. Based on the assumption that acute viral infection usually is associated with high viral genomic loads, we compared PeV-A genomic load categories in patients with RTI and asymptomatic children in multivariable analysis. Controlling for the presence of other respiratory viruses, no association was found. Several studies have previously reported the detection of PeV-A in children with RTI and influenza-like diseases, but often with low prevalence and frequent viral codetections, and only a few compared with controls. Researchers from Hong Kong have reported single PeV-A detection in a group of children outside the hospital with URTI symptoms [10], and we found that PeV-A may have a role in URTI in children attending day care [14].

One may speculate why PeV-A appears in hospital controls, and simultaneously with other and more pathogenic viruses in hospitalized children with RTI, despite limited evidence for a role in RTI. Long-term shedding more than 3 weeks after previous infections with persistently low levels of residual nucleic acids may be one explanation [25]. This has also been described for other respiratory viruses such as HBoV [26]. Another explanation might be reactivation during new infections with

Table 3. Associations Between PeV-A Ct Levels and RTI, Comparing Children with RTI (n = 3689) and Asymptomatic Children in a Comparison Group (n = 444) in Logistic Regression Models

	Model A[a,c]		Model B[b,c]	
	OR	(95% CI)	OR	(95% CI)
PeV-A				
Ct <28	1.24	(0.50-3.11)	1.13	(0.42-3.04)
Ct 28-35	0.95	(0.55-1.63)	1.26	(0.60-2.64)
Ct >35	0.70	(0.41-1.20)	0.71	(0.22-2.26)
Codetected viruses	7.53	(5.32-10.65)	7.97	(5.51-11.52)
Age, months				
< 6	4.33	(2.62-7.16)	4.44	(2.68-7.37)
6-11	1.65	(1.06-2.56)	1.71	(1.08-2.69)
12-23 (reference)	..	..	..	..
24-59	0.31	(0.23-0.41)	0.33	(0.25-0.44)
≥60	0.19	(0.13-0.27)	0.20	(0.14-0.29)
Sex, male	0.32	(0.25-0.42)	0.34	(0.25-0.44)
Chronic disease[d]	6.92	(4.58-10.46)	6.83	(4.49-10.40)
Premature birth	1.53	(1.03-2.28)	1.49	(0.98-2.25)

Abbreviations: CI, confidence interval; OR, odds ratio; Ct, cycle threshold; PeV-A, Parechovirus A; RTI, respiratory tract infections.

[a]Model A includes PeV-A detections in children with RTI (n = 323) and in the comparison group (n = 45).

[b]Model B includes PeV-A genotypes 1 and 6 detections in children with RTI (n = 167) and in the comparison group (n = 23).

[c]Adjusting for codetected viruses (respiratory syncytial virus, influenza virus A and B, human metapneumovirus, and/or parainfluenza virus type 1-3) and age group, sex, chronic disease, and premature birth.

[d]Chronic disease includes asthma, cerebral palsy, epilepsy, immunodeficiency, and heart disease.

other viruses, as it has been shown for adenovirus [27], but it is unknown whether similar mechanisms exist for PeV-A. Asymptomatic PeV-A infection might also be a possible explanation in both groups.

Limitations

It is a strength of the study that children were included over a 10 year long period and were referred to the only pediatric department in Sør-Trøndelag County, Mid-Norway. Furthermore, the same in-house PCR tests and viral culture methods were used throughout the study, and we provide data on PeV-A genotype distributions. However, we were not able to genotype all positive samples. Several samples with high Ct value in the PEV-A test did not give a product in the VP3/VP1 junction, but it is unlikely that it influenced the patterns of genotype distributions. Viral genomic load estimations based on Ct values in single nasopharyngeal aspirate samples may not be reliable, but, in the present study, this method was used to compare the relative virus contents in infected and noninfected children, and not exact viral loads. The enrollment of a comparison group is a strength, and although ear-nose-throat surgery patients were avoided, the comparison group had higher rates of premorbid conditions, potentially associated with increased risk of infection. The comparison group, furthermore, was not matched for age, but age differences and other confounding variables were controlled for in the multivariable analyses.

Conclusions

Our 10-year-long study recruiting all children with RTI in need of hospitalization from an entire county in Mid-Norway shows that PeV-A appears frequently in children hospitalized with RTI and in a comparison group with asymptomatic children. Four PeV-A genotypes were detected, among which PeV-A1 and PeV-A6 dominated and appeared in characteristic seasonal patterns. Nearly all children with and without RTI and PeV-A had viral codetections. Our findings suggest that PeV-A has a limited role in RTI in hospitalized children.

Supplementary Data

Supplementary materials are available at *Journal of the Pediatric Infectious Diseases Society* online.

Notes

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Potential conflicts of interests. All authors declare that they have no commercial or other associations that might pose a conflict of interests.

All authors have submitted the ICMJE Form for Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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