Human enteroviruses and parechoviruses: disease spectrum and need for treatment in young children

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Chapter 11

Differences in maternal antibody protection against human parechovirus types 1, 3 and 4 in young infants


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In preparation
Abstract

Background
Human parechovirus (HPeV) type 3 can cause severe disease in neonates and infants, while HPeV1 generally infects older children and elicits mild symptoms. In the adult population, seroprevalence of HPeV3 antibodies is low compared to HPeV1 and HPeV4. Our hypothesis is that lack of maternal neutralizing antibodies (nAbs) is a risk factor for severe disease in infants with HPeV infection.

Methods
This is a prospective case-control study of mother-child pairs. Cases were children <1 year with a proven HPeV infection (n=38) and their mothers. Controls were children of similar age suspected of a viral infection with a negative HPeV PCR (n=65) and their mothers. Presence of nAbs against HPeV1, -3 and -4 was evaluated by neutralization assays in mothers and children.

Results
HPeV3 infected children (n=22) were younger (median age 1 month) and had more severe disease than HPeV1 infected children (n=8, median age 4 months) and HPeV4 infected children (n=6, median age 5.5 months) (p<0.05); one case was infected with HPeV6 and in one cases HPeV could not be typed. Seroprevalence of HPeV1, HPeV3 and HPeV4 in all mothers (n=102) was 99%, 2% and 76% respectively, and did not differ between cases and controls. Mothers of HPeV infected children <3 months (87% HPeV3) had low or absent titers of specific nAbs against the infecting type. HPeV type-specific nAbs against the infecting type were absent in 3 HPeV1 infected and 8 HPeV3 infected case children, while 44% of 16 control children had aHPeV1 nAbs, 12.5% had aHPeV3 nAbs and 38% had aHPeV4 nAbs.

Conclusions
Our results suggest that specific nAbs are protective against severe HPeV infection in the first months of life, but lack in case of HPeV3. Although aHPeV3 nAbs are virtually absent in the population, HPeV3 is almost exclusively eliciting disease in young infants, suggesting a different as yet uncharacterized mechanism of infection and host response in which antibodies do not play a major role.
Introduction

Human parechoviruses (HPEVs) are associated with a wide array of clinical symptoms, ranging from respiratory and/or gastrointestinal disease to life-threatening meningitis/encephalitis, sepsis-like illness (SLI), myocarditis and infant death syndrome. Of the current 16 known HPEV types, HPEV1 and HPEV3 are most frequently detected. While HPEV1 mainly causes mild gastrointestinal and respiratory disease, HPEV3 is associated with neonatal meningitis/encephalitis and SLI. HPEV4 is the third most frequent found HPEV type in the Netherlands, and causes mild respiratory and gastrointestinal symptoms, but data on clinical symptoms are scarce. Recently 2 cases of SLI in HPEV4 infected children under 3 months of age were reported. Treatment options for severe HPEV infections are very limited (reviewed in Wildenbeest et al.). Intravenous immunoglobulin (IVIG) is given as treatment with various clinical outcomes. The rationale for the use of IVIG in HPEV infections is based on similarities between HPEV and human enteroviruses (EVs). These virus groups are structurally closely related, both belonging to the Picornaviridae family, eliciting similar clinical symptoms. In EV infections the humoral immune response is considered to play a major role in pathogenesis, as shown in patients with X-linked agammaglobulinemia who are at risk for severe and chronic EV infections. In this group of patients the beneficial effect of IVIG has been acknowledged. For neonates, a lack of maternal antibodies is considered a risk factor for developing severe EV infection. Maternal IgG antibodies are passed through the placenta during pregnancy and are considered to protect against infection in neonates and young children. The half-life of maternal IgG is around 20 days, so protective effects are most pronounced till the age of about 3-6 months. Upon treatment with IVIG, EV infected neonates had a significant higher titer of neutralizing antibodies (nAbs) and a shorter duration of viremia if IVIG contained a high antibody titer against the infecting EV type, although no significant differences in clinical outcome were seen. Since there are over 100 human EV types it is difficult to predict nAb titers and therefore effectivity of IVIG. Data on HPEV seroprevalence show that practically all healthy adults (92-99%) have obtained antibodies against HPEV1, whereas in Europe HPEV3 seroprevalence is only 10-13% in the adult population. Since HPEV3 infection is predominantly seen in children <3 months, this may be due to the absence of maternal nAbs against HPEV3, in contrast to children with HPEV1 infections, who should practically all be protected by maternal nAbs. Indeed, maternal nAbs seem to play a role in preventing HPEV1 infection during the first months of life, but data are missing for HPEV3 infections and the lesser pathogenic HPEV4. To assess the role of protective maternal nAbs in HPEV1, -3 and -4 infections, we conducted a prospective case-control study of mother-infant pairs. The aim of this study was to determine whether the absence of specific maternal nAbs is a risk factor in the acquisition of HPEV infection at a young age and whether the presence of maternal nAbs is protective against HPEV in vitro. This study will add useful information about the importance of nAbs
in the defense against HPeV infections in young children and subsequently the rationale for (specific) antibody therapy in HPeV and EV infection.

**Methods**

**Study design**

This study is a prospective multicenter case-control study of mother-child pairs in the Netherlands. The Medical Ethical Committee of the AMC approved the study protocol. Written informed consent was obtained from the parents.

Our hypothesis was that maternal HPeV type-specific Abs would protect against HPeV infection in infants. A power analysis calculated that to verify this hypothesis in a case-control setting, 36 cases of mother-child pairs (22 HPeV3 infected cases and 14 HPeV1/HPeV4 infected cases) had to be included, with 2 controls per case resulting in a power of 93-95%. Based on published data, we assumed that 90% of the control mothers have nAbs against HPeV1. The hypothesis that maternal antibodies could protect against infection would be supported if less than 32% of the case mothers have nAbs. Since HPeV3 seroprevalence data were unknown at the time of the study design, it was estimated (based on prevalence data of HPeV3) that 50% of the control mothers would have aHPeV3 nAbs. To support our hypothesis, nAbs should be detectable in less than 8% of the case mothers. Mother-child cases consisted of children under the age of 1 year with a proven HPeV infection (defined as positive HPeV PCR in any clinical sample) and their biological mothers. Control mother-child pairs were children of similar age undergoing identical diagnostic tests, but with either a proven EV infection or without either EV or HPeV infection, with their biological mothers. Patients <1 year with clinical suspicion of a viral infection with HPeV and/or EV of whom diagnostic virological tests were obtained, were eligible for inclusion in the study. Patients were included between July 2008 and November 2012 in the following hospitals in the Netherlands: Academic Medical Center (AMC), Amsterdam (n=62), Amstelland Hospital, Amstelveen (n=12), Zuwe Hofpoort Hospital, Woerden (n=20), Meander Medical Center, Amersfoort (n=7), University Medical Center Utrecht (UMCU), Utrecht (n=1) and Free University Medical Center, Amsterdam (n=1).

Within 1 week after sampling for routine diagnostic virological tests, stool and blood samples were collected from mothers of the study participants. Results from routine virological tests on stool, blood, cerebrospinal fluid (CSF) and nasopharyngeal aspirate (NPA) were recorded. Exclusion criteria were prematurity before a gestational age of 34 weeks and maternal use of IVIG during pregnancy.

Demographics, data on presence and duration of clinical symptoms, use of antibiotics, the presence and site of isolation of other microorganisms and diagnosis at discharge were recorded from the patient’s files and discharge letters.
Virus detection

Stool, CSF, blood and NPA were tested in respectively 99%, 39%, 17% and 24% of the children (Table 1). HPeV and EV double infections in stool samples were included in the HPeV group (6 children), unless EV was detected as the only pathogen in CSF or blood (1 child). HPeV and EV real-time reverse transcription (RT-) PCR based on the 5' untranslated region (UTR) was performed on available samples (stool, CSF, blood, NPA) as described previously. A cycle threshold (Ct)-value of 40 or more was considered negative. HPeV positive stool samples were genotyped by sequencing the complete VP1 region.

<table>
<thead>
<tr>
<th>Group</th>
<th>Feces pos*</th>
<th>CSF pos*</th>
<th>Blood pos*</th>
<th>NPA/throat swab pos*</th>
<th>Other microorganism (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPeV1</td>
<td>8/8</td>
<td>2/2</td>
<td>1/4</td>
<td>6/8 (75%)</td>
<td></td>
</tr>
<tr>
<td>HPeV3</td>
<td>22/22</td>
<td>9/9</td>
<td>4/4</td>
<td>4/22 (18%)</td>
<td></td>
</tr>
<tr>
<td>HPeV4</td>
<td>6/6</td>
<td>0/1</td>
<td>0/4</td>
<td>5/6 (83%)</td>
<td></td>
</tr>
<tr>
<td>HPeV6</td>
<td>1/1</td>
<td></td>
<td></td>
<td>0/1</td>
<td></td>
</tr>
<tr>
<td>Controls (EV pos or HPeV/EV neg)</td>
<td>28/64</td>
<td>8/23</td>
<td>6/10</td>
<td>3/14</td>
<td>20/65 (31%)</td>
</tr>
</tbody>
</table>

*PCR positive for HPeV (in HPeV group) or EV (in control group), displayed as number positive / tested material for HPeV/EV PCR.

Detection of neutralizing antibodies

Neutralizing antibodies against HPeV1, -3 and -4 in children and mothers were tested using an end point neutralization assay as previously described. If children received IVIG, only blood samples before IVIG treatment were evaluated. For the neutralization of HPeV3, Vero cells were used since HPeV3 was reported to replicate sufficiently in this cell line. All samples were tested with the HPeV3-150237 strain, while part was also tested with the HPeV3-51930 strain. For the neutralization assays of HPeV1 and -4 HT29 cells were used with the HPeV1 Harris strain and HPeV4-K251176 strain respectively. The reported antibody titer was defined as the highest serum dilution able to prevent cytopathic effect (CPE) formation. An antibody titer of 1:8 or more was considered positive. Each maternal sample was tested twice and the mean titer was used for analysis. The highest dilution was used in the event of one dilution difference between titers. The end result was reported as positive if one test yielded a low positive antibody titer (1:8 to 1:16) while the other test had a negative result (n=9). Reproducibility between the two independently performed tests was high, leading in 99.5% of the results in less than 2 dilution difference in antibody titers.
Statistical analysis
Data were analysed using SPSS for windows, version 20. Categorical variables were compared by means of chi-square test or Fisher’s exact test if expected numbers were too low. Differences between normally distributed continuous variables were compared using student-t test and one-way ANOVA. For unequally distributed continuous variables the Kruskall-Wallis and Mann-Whitney U test were used. Spearman’s rank correlation was used to test correlations. A p-value <0.05 was considered to be significant.

Results

General description of cases and controls
In total 103 mother-child pairs were included; 38 case (HPeV infected) children (including one twin) and their mothers and 65 control children (29 EV infected children and 36 HPeV/EV negative children) and their mothers. The cases were divided into children infected with HPeV3 (n=22, 58 %), HPeV1 (n=8, 21%), and HPeV4 (n=6, 16%); one child was infected with HPeV6 and one child with an undetermined HPeV type. These latter two cases were not included in the analyses.

Clinical characteristics of the case and control children are described in Table 2. There was no significant difference in epidemiological characteristics between cases and controls. SLI occurred more frequently in HPeV infected children than in controls. IVIG treatment was given to 3 patients because of severe disease (one HPeV positive and two HPeV negative children).

Differences in age and disease severity between HPeV types
HPeV3 infected children were significantly younger (median age 1 month) than HPeV1 and HPeV4 infected children (median age 4 months (p=0.001) and 5.5 months (p=0.012), respectively) (Figure 1A). Of the HPeV3 infected children, 82% (18/22) were <3 months of age, and 41% were neonates (<28 days old). HPeV1 and -4 infections did not occur in neonates. Only 13% (1/8) of the HPeV1 infections and 33% (2/6) of the HPeV4 infections occurred in infants <3 months. Severe infection was diagnosed in 50% (11/22) of HPeV3 infected children (all <3 months), compared to 14% of HPeV1 (2/8, one <3 months) and HPeV4 (0/6) infected children (p=0.039). Children with severe disease were significantly younger (median 21,5 days (IQR 10-75 days) than children with mild disease (median 102 days, IQR 41-192 days, p=0.003).
Table 2. Characteristics of cases (HPeV positive) and controls.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cases</th>
<th>Controls</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of children</td>
<td>38</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>Male/female</td>
<td>1.5:1</td>
<td>1.95:1</td>
<td>0.57</td>
</tr>
<tr>
<td>Median age in days (range)</td>
<td>66.5 (5-352)</td>
<td>53 (0-350)</td>
<td>0.81</td>
</tr>
<tr>
<td>Underlying disease (%)</td>
<td>7 (18%)</td>
<td>13 (20%)</td>
<td>0.85</td>
</tr>
<tr>
<td>Premature (%)</td>
<td>3 (8%)</td>
<td>7 (11%)</td>
<td>0.63</td>
</tr>
<tr>
<td>Neonate (%)</td>
<td>10 (26%)</td>
<td>24 (26%)</td>
<td>0.99</td>
</tr>
<tr>
<td>Median duration of hospitalisation in days (range)</td>
<td>5 (0-83)*</td>
<td>5 (0-195)*</td>
<td>0.89</td>
</tr>
<tr>
<td>Clinical symptoms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fever</td>
<td>28 (74%)</td>
<td>52 (80%)</td>
<td>0.46</td>
</tr>
<tr>
<td>Sepsis-like illness</td>
<td>13 (34%)</td>
<td>11 (17%)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>CNS symptoms</td>
<td>24 (63%)</td>
<td>35 (54%)</td>
<td>0.36</td>
</tr>
<tr>
<td>Gastrointestinal symptoms</td>
<td>27 (71%)</td>
<td>37 (58%)</td>
<td>0.18</td>
</tr>
<tr>
<td>Respiratory symptoms</td>
<td>13 (34%)</td>
<td>14 (22%)</td>
<td>0.16</td>
</tr>
<tr>
<td>Skin symptoms</td>
<td>12 (32%)</td>
<td>17 (27%)</td>
<td>0.62</td>
</tr>
<tr>
<td>Other micro-organisms found (%)</td>
<td>13 (34%)</td>
<td>20 (31%)</td>
<td>0.72</td>
</tr>
<tr>
<td>Use of antibiotics (%)</td>
<td>22 (58%)</td>
<td>41 (63%)</td>
<td>0.60</td>
</tr>
<tr>
<td>Median duration of antibiotic treatment in days (range)</td>
<td>3 (1-21)</td>
<td>3 (1-42)</td>
<td>0.58</td>
</tr>
<tr>
<td>IVIG treatment</td>
<td>1 (3%)</td>
<td>2 (3%)</td>
<td>0.90</td>
</tr>
<tr>
<td>Mean age mother in years (+/-SD)</td>
<td>31.6 (+/-4.9)</td>
<td>31 (+/-5.0)</td>
<td>0.55</td>
</tr>
<tr>
<td>Symptoms mother</td>
<td>8 (23%)</td>
<td>13 (21%)</td>
<td>0.83</td>
</tr>
<tr>
<td>Feces pos mother/total tested</td>
<td>7/24 (29%)</td>
<td>8/38 (21%)</td>
<td></td>
</tr>
</tbody>
</table>

CNS, central nervous system; IVIG, intravenous immunoglobulin.
*documented in 32 children, #documented in 60 children.

HPeV1, HPeV3 and HPeV4 neutralizing antibodies in mothers of cases and controls

HPeV1 nAbs were detected in 99% (101/102), HPeV3 in 2% (2/102) and HPeV4 in 76% (78/102) of the mothers, including both the mothers of cases and controls. No significant differences in seroprevalence against HPeV1, -3 and -4 between mothers of cases and controls were found (Figure 2A).
Figure 1. Relation between age, disease severity and maternal neutralizing antibody (nAb) titers in HPeV infected children. A. Relation between age and disease severity per HPeV type. B. Severity and age of infection (open symbols <3 months of age, closed symbols >3 months of age) in relation to maternal nAb titers against the infecting HPeV type for HPeV1, -3 and -4 infected children.
Maternal antibody protection against HPeV type 1, 3 and 4

Figure 2. Neutralizing antibody (nAb) titers against HPeV1, -3 and -4 in mothers of cases and controls. 
A. NAb titers against HPeV1, -3 and -4 in the total group of included case and control mothers. B. NAb titers against infecting HPeV types in mothers of cases compared to mothers of controls of same age (2 controls per case, median and IQR).
Because of the significant difference in age of children infected with different HPeV types, we compared the nAb titers against the infecting type of the mothers of the cases with age-matched controls, thereby dividing the case group into groups infected with HPeV1, -3 or -4. As shown in Figure 2B, there was neither any significant difference in type-specific nAb titers against the infecting type between mothers of HPeV1 infected cases (median 1:128, IQR 1:32-1:512) and non-HPeV infected controls (median 1:128, IQR 1:64-1:512), nor for mothers of HPeV3 infected cases (median <1:8 (neg)) and controls (median <1:8 (neg)), or for mothers of HPeV4 infected cases (median 1:256, IQR 1:32-1:512) and controls (median 1:32, IQR <1:8 (neg)-1:32). Thus, HPeV1 and -4 seroprevalence in mothers was generally high, while maternal nAbs against HPeV3 were nearly absent in both case and control mothers, therefore protection of maternal nAbs against HPeV infection could not be proven in our case-control setting including children up to 1 year of age. In addition we related age and severity of disease of HPeV infected children to nAb titers in their mothers; low or absent nAb titers against the infecting HPeV type were found in mothers of children with severe disease and in mothers of children <3 months of age (Figure 1B).

The role of maternal neutralizing antibodies in infants younger than 3 months
Since children older than 3 months of age are less likely to be protected by maternal Abs, we further investigated the protective role of maternal nAbs in neonates and infants <3 months of age. We detected significant correlations; for both HPeV1 and HPeV4 the maternal nAb titer correlated with the nAb titer in their children (n=17) (p<0.05, Figure 3). Titers in the children were generally lower. Correlations between maternal and infant HPeV3 titers could not be performed because of low HPeV3 seroprevalence.

Figure 3. Correlation neutralizing antibody titers of HPeV1 and HPeV4 between mothers and children <3 months of age.
The nAb titers of mothers of cases <3 months of age with HPeV1, -3 or -4 infection (n=21) were compared to nAb titers of mothers of HPeV negative controls <3 months (n=42, Figure 4). Of the 21 cases <3 months, the majority was infected with HPeV3 (n=18), two were infected with HPeV4, and only one child with HPeV1. The mother of the HPeV1 case <3 months had a lower aHPeV1 nAb titer of 1:16 compared to the median titer of 1:128 in mothers of controls <3 months (IQR 1:164-1:512). Mothers of all HPeV3 cases <3 months lacked aHPeV3 nAbs and only 1 of 42 mother of controls <3 months (2.4%) was HPeV3 positive (low titer of 1:8). Two HPeV4 cases <3 months were diagnosed, with aHPeV4 nAb titers of 1:32 and 1:64 in their mothers, as compared to similar nAb titers in mothers of controls <3 months (median: 1:64, IQR 1:8-1:256). Thus, mothers of HPeV infected cases <3 months generally showed absent or low nAb titers against the infecting type.

Figure 4. Type specific neutralizing antibody titers in mothers of cases and controls <3 months (median and IQR).

**Neutralizing antibodies against HPeV in children**

Serum samples from cases – irrespective of their age – were available in sufficient amounts for 8 HPeV3 infected cases, 3 HPeV1 infected cases and 16 control children for testing the presence of HPeV type-specific nAbs. Titers against the infecting HPeV type of the cases were compared with that of HPeV negative controls. In Figure 5 is shown that HPeV1 nAbs were absent in the 3 HPeV1 infected children while in the control group 44% had HPeV1 nAbs, but this was not statistically significant (p=0.26). No differences in nAbs against HPeV3 were seen between cases (0%) and controls (12.5%, p=0.54). In the control group 38% had nAbs against HPeV4, but no blood samples were available from the 6 HPeV4 infected children. Because of the age difference between HPeV1 and -3, we also compared age matched controls with the cases, showing no significant difference in seroprevalence. Although no
significant differences in seroprevalence could be found between cases and controls, none of the HPeV1 and -3 infected children had nAbs against the infecting serotype.

Figure 5. Prevalence of type specific neutralizing antibody titers in case and control children.

Maternal HPeV infection and symptoms
Stool samples of 24 case mothers were available for HPeV PCR testing; 7 (30%) were positive for HPeV (4 HPeV3, 1 HPeV1 and 2 undetermined HPeV types). Only one HPeV3 infected mother, had clinical symptoms (of diarrhea), the rest was asymptomatic. However, four of the HPeV3 infected children of these infected mothers had severe disease as neonates (SLI, meningitis and encephalitis).

Discussion
This is the first case-control study on the protective effect of maternal nAbs against HPeV infections in young children. As only a few HPeV genotypes are commonly circulating in the general population, this virus group should be suitable to study the effect of maternal antibodies in this study design. In contrast, the closely related group of EVs has never been studied in a case-control setting.

Neutralizing antibodies against HPeV1 were found in almost all mothers (99%), while nAbs against HPeV3 were found in only 2% of the mothers. NAbs against HPeV4 were found in 76% of the mothers. Although we could not find significant differences in the presence and titers of nAbs against HPeV1, -3 and -4 between the groups of case and control mothers (because of presence of aHPeV1 nAbs and absence of aHPeV3 nAbs in virtually all mothers),
our results do indicate that the presence of maternal HPeV nAbs protect against (severe) disease in infants. This is illustrated by the fact that HPeV1 infection did not occur at young age. Nearly all infants <3 months of age were infected with HPeV3, with their mothers lacking nAbs. HPeV3 infected children were significantly younger and had more severe disease than HPeV1 and -4 infected children who were in general older than 3 months of age, as reported in previous studies. In addition, in HPeV1 infected infants, clinical symptoms occurred only in the absence of (maternal) nAbs. Therefore our data indicate that young children benefit from maternal nAb protection against both HPeV1 and -4. Cross-neutralization of nAbs between HPeV3 and other HPeV types was not likely based on this and earlier data.

The high seroprevalences of HPeV1 (99%) and -4 (67%) found in mothers is in accordance with recent data in the general population of Dutch and Finnish adults. Seroprevalence of HPeV3 was only 2% in our study, the lowest prevalence reported up to now. Low HPeV3 seroprevalence has been reported in the Finnish (13%) and Dutch (10%) adult population previously. The low seroprevalence of HPeV3 nAbs in our study is also reflected in low HPeV3 nAb titers in different batches of IVIG. IVIG contains IgGs from a pool of more than 1000 donors, and thus is a good representation of circulating nAbs in the general population. Our results further implicate that IVIG is not suitable as treatment in HPeV3 infection; this is in contrast to HPeV1, against which nAb titers in IVIG are high. In contrast, in the Japanese population a higher seroprevalence of 73% in adults was found. Accordingly, in the Japanese population HPeV3 infection in children is seen at an older age (mean age of 1 year) with predominantly mild gastrointestinal and respiratory symptoms, underscoring our hypothesis that maternal nAbs prevent (severe) disease during the first months of life. Although exact data are missing, the higher seroprevalence of HPeV3 in Japan could be due to longer circulation of HPeV3 in this population. The first HPeV3 strains in Europe have been reported in 1994. It is unclear whether circulation in the population was already occurring at that time, but evolutions studies suggest HPeV3 to circulate for more than 100 years.

The reported differences in HPeV3 seroprevalence could also be explained by differences in the techniques used. For this study, a standard neutralization assay based on CPE formation was used to determine the titer of nAbs. For HPeV1 and -4, complete inhibition of growth by absence of CPE formation could be found in the majority of cases. In general, HPeV3 grows slower in cell culture than HPeV1, and CPE is not easily recognized (unpublished data; own observations). Although slower growth was observed in our virus cultures, none of the serum samples was able to completely inhibit HPeV3 growth in vitro. To exclude the possibility of genetic drift as an explanation for lack of neutralization, two different virus strains, both closely related to the currently circulating HPeV3 strains, were used in the neutralization assay. In addition, in an earlier study we showed that the Japanese polyclonal specific antibody against HPeV3 could neutralize the Japanese strain, but not our European strains, suggesting differences between strains in nAb binding. However, the Japanese strain
was very similar to the European strains. The exact origin of this differences remains to be elucidated.

Young children seem to benefit from maternal nAbs against HPeV4, since the median age of infection is even higher than in HPeV1 infected children. However, HPeV4 seroprevalence in mothers is substantially lower than HPeV1 seroprevalence in adults. In our study, two children were infected with HPeV4 under the age of 3 months and none of the children had severe disease. However, SLI caused by HPeV4 has been previously described.

Several questions remain unanswered. Unlike EV infection, that occurs at all ages, HPeV infection is restricted almost exclusively to young children. For HPeV1 this can be explained by the high circulation rate in young children, and hence a high seroprevalence and protection rate against infection at adult age. For HPeV3, seroprevalence in adults is low, suggesting a lack of protection. However, HPeV3 infections have rarely been reported in adults. The only outbreak of symptomatic HPeV3 disease in adults was reported in Japan, where seroprevalence among adults is higher as compared to our data. In this study we showed that adults can be infected with HPeV3, although most infections remain asymptomatic. Thus, it is possible that other factors than the humoral immune responses are of significance and predominate in the host defence against HPeV3 infection. Cellular immunity may play an important role in the protection against HPeV infection, in particular against the HPeV3 serotype. Young infants are known to have an immature T cell response. For example, lower cellular immune responses were seen in EV71 infected patients with brainstem encephalitis and pulmonary edema compared to patients with uncomplicated EV71 infection. Little is known about T cell responses to HPeV infection. Another explanation for the predilection of HPeV3 for young infants could be that young children express specific receptors that enable HPeV3 to display a broad tissue tropism leading to systemic infection including CNS involvement, and that with advancing age this receptor disappears or becomes less expressed, protecting older children and adults from severe or symptomatic HPeV3 infection. Such changes in receptor expression have been shown in mouse models for toll-like receptor (TLR) 8, which is abundantly expressed in neurons and axons of the developing brain but is only marginally expressed in the adult brain. This enhanced expression of TLR8 in the developing brain might play a causal role in the development of white matter injury in neonates with HPeV3/EV encephalitis. The exact nature and extent of cellular immunity, mechanisms of entry and receptor use, however, have not been elucidated for HPeV3 or any of the other HPeVs.

Another question that remains unanswered is why only a low number of infants get symptomatic or severe HPeV3 disease when virtually none of them are protected by maternal nAbs. Most neonates and infants <3 months live relatively protected with low risks of contracting infections, compared to older children. After that age, infection does not cause disease as serious as observed in the very young neonates and infants. This can explain why HPeV3 infection is not as wide-spread as for example HPeV1 which is often causing symptoms when contracted at older age and maternal Abs have disappeared. In
fact, the presence of older siblings in the family is considered a risk factor for acquiring HPeV infection in infants.\textsuperscript{33} (Lodder et al., in preparation) Polymorphisms in TLRs as part of the innate immune system can also lead to differences in infectious disease susceptibility between individuals and between populations. Indeed, polymorphisms that lead to reduced TLR3 signaling were significant more seen in patients with EV myocarditis or dilated cardiomyopathy as compared to controls.\textsuperscript{34} TLR7 and TLR8 mediated responses are involved in the immune response against HPeV1,\textsuperscript{35} but the role of TLRs in HPeV3 infection is unclear. As suggested above, the enhanced expression of TLR8 in the developing brain might play a causal role in the pathogenesis of HPeV3 encephalitis in neonates.\textsuperscript{32}

In conclusion, our results suggest that (high titers of) maternal specific nAbs are protective against (severe) disease in young children with HPeV infection. These results support the use of specific antibody therapy in (severely) ill children with HPeV or EV infection. HPeV3 nAbs are virtually absent despite circulation of this virus in Europe for at least 20 years and HPeV3 is almost exclusively eliciting disease in young infants, suggesting a different yet unknown mechanism of infection and host response, warranting further research.

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References