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

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Chapter 4

Prolonged shedding of human parechovirus in feces of young children after symptomatic infection

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Abstract

After symptomatic human parechovirus (HPeV) infection in infants, the duration of (mostly asymptomatic) shedding in feces was 2-24 weeks (median 58 days). HPeV Ct-value could neither differentiate between symptomatic disease and asymptomatic shedding nor between severe and mild disease as high Ct-values (indicating low viral loads) were observed in HPeV3 infected children with severe disease.
Introduction

Human parechoviruses (HPeV) are small non-enveloped, positive-sense, single-stranded RNA viruses and belong to the family of Picornaviridae. Today, 16 types are known of which HPeV type 1 and 3 are the most prevalent. HPeVs are closely related to human enteroviruses (EVs), giving similar symptoms ranging from mild gastrointestinal and respiratory disease to life-threatening infections like meningitis/encephalitis and sepsis-like illness (SLI) (reviewed in Harvala et al.1). In recent years molecular techniques became state-of-the-art in clinical diagnostic settings; HPeV specific real-time PCR was designed to detect HPeV in all kinds of patient materials.2,3 Like EVs, the main site of replication of HPeV is the gastrointestinal tract, and HPeV PCR from fecal samples is very sensitive for the diagnosis of HPeV infection, even in the absence of gastrointestinal symptoms.4 Longitudinal studies in cohorts of young children showed that both EV and HPeV can be detected for several months in feces of children.5-7 The clinical relevance and relation with symptoms of a positive PCR in feces for both EV and HPeV is yet unclear.

In this study the duration and extent of HPeV shedding in feces of infants after symptomatic HPeV infection is described. In addition, we compared the amount of virus detected in feces in children with severe and mild disease.

Methods

Subjects

This study is part of the PARMA (PARechovirus infections and Maternal Antibodies) study, a prospective multicenter case-control study to evaluate presence of maternal antibodies in young children with HPeV infection. Children under the age of 1 year with a proven HPeV infection and their mothers were defined as cases and were selected for this study. Data on clinical signs and symptoms of included children were collected from the patient’s files and discharge letters. A final diagnosis was made for every child based on available clinical data. Patients were divided in severe and mild disease. Mild disease was defined as gastroenteritis and/or respiratory tract infection, or non-localized symptoms of a viral infection without any signs of SLI. Severe disease was defined as meningitis/encephalitis and/or myocarditis/pericarditis and/or SLI. SLI was defined as signs of circulatory and/or respiratory dysfunction defined by tachycardia or bradycardia, low blood pressure and/or decreased saturation.
Duration of shedding
The parents of children with a positive HPeV PCR in feces were asked to collect a feces sample every 2 weeks until HPeV PCR in feces was negative. Clinical symptoms at the moment of sample collection were documented during telephone contacts and recorded.

Virus detection and genotyping
HPeV and EV real-time reverse transcription (RT-)PCR was performed on available samples (feces, CSF, blood, nasopharyngeal aspirate) as described earlier. The cycle threshold (Ct)-value was used as a semi-quantitative read-out. A Ct-value of 40 or more was considered negative. HPeV positive feces samples were genotyped by sequencing the complete VP1 region as described previously.

Results
Patient characteristics
In total 38 HPeV infected children were included in the study between 2008 and 2012. The median age was 2 months, ranging from 5 to 352 days. More boys than girls were included (ratio 1.5:1).

HPeV types
Feces samples were collected within one week after the start of symptoms. HPeV3 was the most frequently detected type (n=22, 58%), followed by HPeV1 (n=8, 21%) and HPeV4 (n=6, 16%). HPeV6 was detected in one child. In one child HPeV typing was not successful.
Co-infection with other microorganisms was detected in 40% of the children at time of first sampling. Another virus was found in samples of 12 children of which EV was the most frequent co-infecting virus (7 feces samples). Bacterial co-infection was found in 4 samples. Significantly more HPeV1 and HPeV4 infected children had a co-infection with another microorganism (respectively 75% and 83%) compared to HPeV3 (18%, p=0.000).

Duration of HPeV shedding
Of the parents of the 38 HPeV positive children, 30 agreed to collect feces every two weeks until HPeV PCR became negative. The parents of three children discontinued collecting follow-up feces. Of the remaining 27 children, 7 were positive for HPeV1, 16 for HPeV3, 3 for HPeV4 and 1 for HPeV6. The initial Ct-value in feces of these children varied between 16 and 30 (Fig. 1). The median duration until HPeV RNA became undetectable was 58 days (range 2-24 weeks). In general, the viral load decreased gradually over time (Fig. 1). A rise in viral load (a decrease in Ct-value of more than 5 between 2 time points) was seen in five patients. Two patients (P3-6 and P6-1, Fig. 1) acquired a new HPeV1 infection (one had diarrhea and the other was without symptoms). One patient (P1-3) experienced a novel
episode of diarrhea and was co-infected with adenovirus. One patient (P4-4) was co-infected with EV from week two onwards and did not have any symptoms. One patient (P1-5) was still infected with the same HPeV type and did not have any symptoms.

Of the remaining patients, three elicited clinical symptoms after the initial HPeV diagnosis. One patient (P1-2) was admitted 3.5 weeks after initial HPeV diagnosis with diarrhea caused by an adenovirus infection. Two other patients (P3-13 and P1-7) experienced an episode of diarrhea respectively 2 and 9 weeks after initial HPeV diagnosis. PCR was negative for other gastrointestinal associated viruses in these patients.

There were no significant differences in duration of shedding between HPeV1, -3 and -4 positive children. There was no relation between age and duration of shedding nor between severity of disease and duration of shedding.

**Figure 1.** Duration of shedding and Ct-value in feces of HPeV1, HPeV4 and HPeV6 infected children (A) and HPeV3 infected children (B).
Disease severity and Ct-value
At diagnosis, the mean Ct-value in feces of HPeV3 infected children (mean 26.9 ± SD 3.8) was significantly higher than the mean Ct-value of HPeV1 infected children (mean 20.5 ± SD 2.8, p=0.000). Although significantly more HPeV3 infected children had severe disease (11/22 patients) compared to HPeV1 (2/8 patients) and HPeV4 (0/6 patients) infected children (p=0.03), the initial Ct-value in feces did not differ significantly between children with severe disease and mild disease in total and per HPeV type (Fig. 2). However, Ct-values of 30-35 were seen in two of 10 (20%) HPeV3 infected children with severe disease.

Figure 2. Initial Ct-value in feces of symptomatic HPeV infected children.

Discussion
In infants with symptomatic HPeV1, -3 and -4 infection, asymptomatic HPeV shedding in feces occurred for a prolonged period of time (up to 6 months) after initial infection. Most children had no (gastrointestinal) symptoms during the period of shedding. Ct-value (as semi-quantitative measurement of viral load) could not differentiate between symptomatic disease and post-infectious shedding, although in the majority of children a trend towards higher Ct-values/lower viral loads was seen over time.

Longitudinal studies (of several years) in Norway and Finland described detection of HPeV in stool samples up to 5 months in healthy children above the age of 3 months. In those studies mainly HPeV1 was reported and there was no association between occurrence of HPeV shedding and clinical symptoms.

In our study the majority of the children was younger than 3 months and infected with HPeV3, showing that post-infectious shedding also occurs in very young infants with HPeV3 infection.
The longevity of asymptomatic shedding of HPeV in feces makes it difficult to interpret a positive HPeV PCR result from feces, because a relation with clinical symptoms is not always present. Especially in HPeV1 and -4 infected children, a high rate of co-infection with other microorganisms, mainly viruses, was found. Thus, a positive HPeV PCR in feces must be interpreted with caution, taking into account other possible causative organisms and diseases. However, we and others showed that HPeV1 and especially HPeV3 are able to cause (severe) disease \(^4,9\) and that HPeV is not only an innocent bystander as was suggested in a recent study.\(^10\)

We found no significant difference in HPeV Ct-value in feces between symptomatic infection and asymptomatic post-infectious shedding. This confirms the results of earlier studies, in which high viral loads were found in asymptomatic children.\(^10,11\)

In addition, the initial Ct-value during symptomatic disease did not differ between children with mild and severe disease, but Ct-value in HPeV3 infected children was significantly higher as compared to HPeV1 and -4 infected children. In a previous study we found that the Ct-value in children with severe HPeV3 disease (mainly meningitis/encephalitis) was significantly higher than in HPeV3 infected children with other diagnoses.\(^4\) The lower viral load in feces can be a result of differences in cell tropism of HPeV3 as is suggested by Westerhuis \emph{et al.}\(^12\); HPeV3 strains which caused CNS symptoms in the patients they were derived from, showed better replication kinetics in neural cell lines. In addition, replication kinetics on one gastrointestinal cell line (Caco-2) were high for all HPeV3 strains, but low or absent on another gastrointestinal cell line (HT29) while replication for HPeV1 was high on both cell lines. These results have to be interpreted with caution since they are derived from \emph{in vitro} experiments with continuous growing cell lines, which do not necessarily represent gastrointestinal cells \emph{in vivo}. However, our results implicate that even high Ct-values in feces of HPeV3 infected children can be of clinical relevance and secondly that viral load in feces is not a good instrument for evaluating severity of infection.

In conclusion: After symptomatic infection, shedding of HPeV1, -3 and -4 in feces can occur for months in infants. Viral load (Ct-value) cannot differentiate between asymptomatic shedding and symptomatic infection nor between severe and mild disease.

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References