The need for treatment against human parechoviruses: How, why and when?

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EXPERT REVIEWS

The need for treatment against human parechoviruses: how, why and when?

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Since 1999, human parechoviruses (HPeVs) have been classified as a separate group in the large and expanding family of *Picornaviridae*. In contrast to the well-established group of the human enteroviruses (HEVs), HPeVs have long been considered as irrelevant and have only been associated with mild disease manifestations in children. The identification of HPeV-3 in 2004 and its association with neonatal sepsis, refocused attention on this neglected group of viruses. Clinically HPeV infections may mimic HEV infections and are increasingly recognized as viral causes of sepsis-like illness and CNS infections in young children. Therapy is not available against HPeVs or HEVs. In this article, we will demonstrate that therapy against this group of picornaviruses is urgently needed and we will review the current knowledge of treatment options as well as the current developments in antiviral therapy against picornaviruses in the scope of treatment possibilities against HPeVs.

Keywords: antibodies • HPeV • human parechoviruses • IVIg • picornavirus • pleconaril • therapy • treatment

Human parechoviruses (HPeVs) show many resemblances to human enteroviruses (HEVs) with respect to genome structure, cytopathological effect (CPE) in cell culture and clinical manifestations [1–3]. Detection of HPeV-1 and -2, previously known as echovirus-22 and -23, used to be part of enterovirus diagnostics by virus culture, showing an indistinguishable CPE on the same cell lines. While the structure of the genomes of HPeV and HEV are very similar, the nucleotide sequences of the HPeVs are relatively distinct from the HEVs. Therefore, separate molecular techniques are necessary to detect HPeVs. The development of molecular methods has led to a rapid expansion of the group of HPeV that now contains 14 genotypes [4]. By comparison, the HEV group contains over 100 serotypes. Infections with HEV are very common and the clinical course is usually mild. Nevertheless, HEVs are also the major viral cause of CNS infections (e.g., meningitis, encephalitis and acute flaccid paralysis), as well as neonatal sepsis and myocarditis [5,6]. Neonatal sepsis caused by HEVs can be fatal [5,7–9], which is also illustrated by a recent alert for increased severe neonatal sepsis caused by coxsackievirus B1 in the USA [10]. Encephalitis by HEV is a rare condition but sequelae are reported at a high frequency in these patients [11]. In patients with a humoral immunodeficiency, HEV infections can manifest as chronic meningoencephalitis with continuing detectable HEV in cerebrospinal fluid (CSF) and ongoing clinical symptoms [6]. These clinically severe conditions warrant therapy to stop continuing viral replication, and possibly decrease disease burden and prevent complications. Despite substantial effort to develop safe and effective antiviral drugs against HEVs, there is currently no therapy available.

Similar to infections with HEV, infections with HPeV are very common and the clinical course is usually mild, but occasionally they are associated with neonatal sepsis-like illnesses and possibly even with sudden infant death [12–15]. In addition, as HPeVs have also been identified as a significant cause of viral CNS infections that may lead to severe sequelae [14,16,17], effective therapy against HPeV infections is imperative.

HPeV biology

Classification & biology

Human parechoviruses are single-stranded, positive-sense RNA viruses within the *Parechovirus* genus of the large *Picornaviridae* family. The *Picornaviridae* family currently consists of 13 genera: *Enterovirus*, *Parechovirus*, *Hepatovirus*, *Cosavirus*, *Kobuvirus*, *Aphthovirus*, *Erbovirus*, *Teschovirus*, *Cardiovirus*, *Tremovirus*, *Sapelovirus*,

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Avihepatovirus and *Senecavirus*. Furthermore, two new genera, *Klassevirus* and *Aquamovirus*, have recently been proposed (Figure 1A). The genus *Enterovirus* contains over 200 different virus types known to infect humans, include rhinoviruses (HRVs), echoviruses, coxsackie-A and -B viruses (CAV and CBV), polioviruses and numerically identified enterovirus 68–109. By contrast, all human hepatitis A viruses in the genus *Hepatovirus* belong to a single serotype and are responsible for acute hepatitis. The genus *Aphthovirus* includes seven foot-and-mouth disease virus (FMDV) serotypes, which are very important pathogens of cloven-hoofed animals worldwide.

The first two serologically distinct HPeV types were discovered over 50 years ago during a summer diarrhea outbreak in the USA [18]. These prototype strains were originally described as echovirus-22 and -23 in the *Enterovirus* genus; the clinical presentation – enterovirus-like CPE on virus isolation and nonpathogenicity in both mice and monkeys – led to their original designation as enteric cytopathic human orphan (echo)viruses. However, they were renamed as HPeVs and reclassified into their own genus in 1999 based on evident differences in genome organization and structure, divergence of encoded proteins and other biological properties [1,19]. Since this reclassification, a further 12 HPeV types (HPeV-3 to -14) have been identified (reviewed in [20]; Table 1). In addition, a close relative of HPeV, Ljungan virus (LV), has been classified as a separate parechovirus species. LV has been primarily isolated from rodents (Figure 1B) [21].

The HPeV genome is approximately 7300 bases in length, and encodes a single polyprotein flanked by 5′ and 3′ untranslated regions (UTRs; Figure 2A). RNA released into the cell on virus entry is directly translated into a long polyprotein, which is subsequently cleaved by the viral protease (3C) into three structural proteins (VP0, VP1 and VP3) and seven nonstructural proteins (2A–2C and 3A–3D). The RNA-dependent RNA polymerase (3D) copies genomic RNA to make a template from which genomic and mRNA transcripts can be generated. In addition to a role in formation of membrane-associated replication complexes [22], 2C protein shows NTPase activity and binds RNA [23]. Without direct observational data, it can only be inferred from comparison with better characterized picornaviruses that 3B (VPg) is likely to be attached to the 5′ end of the genomic RNA and has a functional role in initiation of HPeV transcription. In most picornaviruses, there are four structural proteins (VP1–4) that form the virus nucleocapsid (Figure 2B), but the maturation cleavage of VP0 into VP2 and VP4 does not appear in parechoviruses (Figure 2A) [24]. However, the external appearance of HPeV particles has recently been shown by cryoelectron microscopy and image reconstruction and has proved consistent with the external appearance of other picornaviruses, most closely resembling FMDV in the *Aphthovirus* genus [25]. The mechanism by which the large VP0 protein is released or externalized during the life cycle of HPeV is currently unknown. Also, in contrast to other picornaviruses, the predominant antigenic sites of HPeV have been mapped to the N-terminal region of the VP0 protein, in a region that is not found to be antigenic in any other genera [26]. Antiserum against a synthetic peptide representing this region showed neutralizing activity. In addition, peptide antiserum against the C-terminal region of HPeV-1 VP1 protein, which contains the arginine-glycine-aspartic acid (RGD) motif, was also neutralizing. Neutralizing antibodies are thought to be critical in the control of picornavirus infections: it was demonstrated a long time ago that paralytic poliomyelitis follows the viremia and that neutralizing antibodies can prevent a poliomyelitis disease [27]. However, no data on whether neutralizing antibodies are elicited during HPeV infections, or their role in preventing disease and conferring immunity, have been obtained.

Human parechovirus replication is dependent on sequence elements and RNA secondary structures at the 5′UTR (and likely 3′ UTR) of the genome. Specifically, a long hairpin stem-loop and pseudoknot interaction with a downstream RNA structure in the 5′UTR have been shown to be required for HPeV replication [28]. Much of the rest of the 5′UTR forms a complex RNA structure with a demonstrated role as an internal ribosomal entry site (IRES [29]). This directs ribosomal binding to a position close to an internal methionine codon (position 710 in the HPeV-1 Harris strain) from which translation commences. IRES-mediated translation is found in all picornaviruses, although the structure of the IRES varies considerable between genera. The HPeV IRES is classified as type 2, structurally similar to those found in *Aphthovirus*, *Cardiovirus* and *Erbovirus* genera [30].

Human parechoviruses differ from most other picornaviruses by not shutting off host cell protein synthesis during replication. In HEVs this is principally achieved by cleavage of the eIF-4G subunit of the cap-binding complex by the 2A protein. This provides many picornaviruses with a replication advantage in preventing normal cap-dependent translation of cellular RNA, while enabling IRES-dependent translation to proceed. However, the parechovirus 2A protein is unlikely to possess a protease activity; indeed, its binding to the 3′ end of the HPeV genome suggest its vital role in virus replication [2].

Receptor interactions & possible determinants for pathogenesis

Human parechovirus types 1, 2, 4, 5 and 6 contain an RGD motif in the C-terminus of VP1 that is utilized by several other viruses for their attachment to cell surface integrins. Among picornaviruses, these include FMDV, CAV9 and echovirus 9 (E9; Barty strain). The RGD motif in HPeV-1 is functional, as demonstrated by blocking experiments with RGD-containing peptides and monoclonal antibodies against αv-integrins, suggesting that αvβ3 and αvβ6 integrins play an important role in the early stages of HPeV-1 infection [24,31–33]. It has been confirmed recently that the binding of both αvβ3 and αvβ6 integrins to HPeV-1 involves the RGD motif in VP1 [25]. Furthermore, similar to CAV9, αvβ6 integrin has shown to be a high-affinity receptor for HPeV-1, whereas αvβ3 integrin exhibits lower affinity [34]. Although a few occasional HPeV-1 variants without the RGD motif have been identified [35], the vast majority of clinical isolates possess this motif. Furthermore, the experimental deletion of the RGD motif from the Harris strain of HPeV-1 was lethal, underscoring its importance in

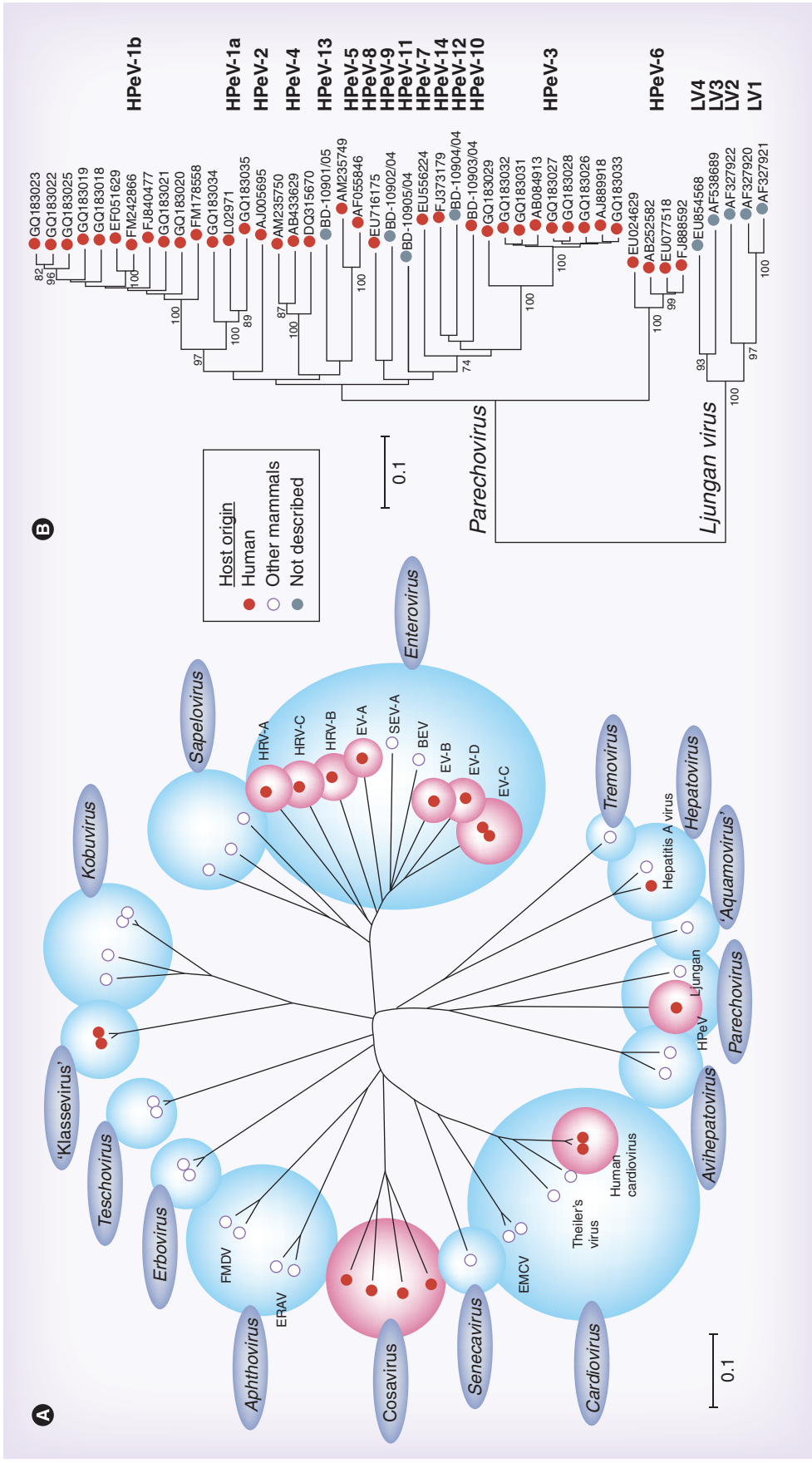

Figure 1. Evolutionary relationship of human parechovirus to other picornaviruses: classification into genera and species. (A) Evolutionary tree of picornaviruses showing its division into 13 designated and two proposed genera (*'Klassevirus'* and *'Aquamovirus'*). The tree was constructed by comparisons of amino acid sequences of the 3Dpol region of representative viruses from each designated genus and species (positions 5711–7252 in the prototype Harris genome [accession number L02971]). **(B)** Genetic heterogeneity within the *Parechovirus* genus: classification into two species. Comparison of nucleotide sequences from VP1 region (position 2336–3028) of all currently classified 14 HPeV types and four Ljungan virus types. Type designations are shown on the right.
BEV: Bovine enterovirus; EMCV: Encephalomyocarditis virus; ERAV: Equine rhinitis virus; EV: Enterovirus; FMDV: Foot and mouth disease virus; HPeV: Human parechovirus; HRV: Human rhinovirus; LV: Ljungan virus; SEV: Simian enterovirus.
Data from [4,20].

Table 1. Human parechovirus genotype reference strains and clinical association.

Genotype	Strain	Accession	Clinical association	Study (year)	Ref.
HPeV-1(A)	Harris	S45208	GIT and RT symptoms, bronchiolitis, pneumonitis, otitis media encephalitis†, paralysis†, myocarditis†	Hyypia *et al.* (1992)	[19]
HPeV-1(B)	BNI788st	EF051629		de Souza Luna *et al.* (2008)	[98]
HPeV-2	Williamson	AB084913, AJ889918	GIT and RT symptoms	Ghazi *et al.* (1998)	[99]
HPeV-3	A308/99, Can82853-01	AB084913, AJ889918	Neonatal sepsis, meningitis, encephalitis and paralysis†	Ito *et al.* (2004) Boivin *et al.* (2005)	[39] [13]
HPeV-4	K251176-02, T75-4077	DQ315670, AM235750	Fever and GIT and RT symptoms	Benschop *et al.* (2006) Al-Sunaidi *et al.* (2006)	[100] [101]
HPeV-5	CT86-6760, T92-15	AF055846, AM235749	Fever, GIT and RT symptoms, sepsis† and Reye's syndrome†	Oberste *et al.* (1998) Al-Sunaidi *et al.* (2006)	[102] [101]
HPeV-6	NII561-2000	AB252582	Fever, GIT and RT symptoms, paralysis† and Reye's syndrome†	Watanabe *et al.* (2007)	[47]
HPeV-7	PAK5045	EU556224		Li *et al.* (2009)	[60]
HPeV-8	BR/217/2012	EU716175	Enteritis	Drexler *et al.* (2009)	[59]
HPeV-9	BAN2004-10902			Oberste MS	[Unpublished Data]
HPeV-10	LK-106/LK-103	GQ402515/ GQ402516	Gastroenteritis	Pham *et al.* (2010)	[58]
HPeV-11	BAN2004-10905			Oberste MS	[Unpublished Data]
HPeV-12	BAN2004-10904			Oberste MS	[Unpublished Data]
HPeV-13	BAN2004-10901			Oberste MS	[Unpublished Data]
HPeV-14	451564	FJ373179		Benschop *et al.* (2008)	[35]

†Sporadically reported.
GIT: Gastrointestinal tract; HPeV: Human parechovirus; RT: Respiratory tract.

clinical pathogenicity of this virus [31]. By contrast, the RGD motif is important but not essential for the clinical pathogenesis of CAV9 and FMDV [36–38].

The receptors for the HPeV types that lack the RGD motif in VP1 (HPeV-3 and -7–14) are as yet unidentified [4,39]. How HPeV-3 (and other HPeVs without the RGD motif) enters the cell and whether this contributes to its more severe pathogenicity in humans in comparison to other HPeV types is currently unknown.

Possible animal models to study HPeV infections

Studies on the pathogenesis of HPeV infections have been very limited owing to the lack of suitable animal models. Newborn mice inoculated with HPeV-1 and HPeV-2 were only infrequently infected, while experimentally inoculated cynomolgus monkeys showed no neuropathological changes after the 30 day observation period [18]. Although an experimental mouse model has been developed to investigate the pathogenesis of the rodent-derived LV [40], it can be estimated that there are likely to be substantial differences in molecular pathogenesis between HPeVs and LVs. HPeVs and LVs are separate species within the genus *Parechoviruses*; they differ from each other genetically as much as, or more, than for example polioviruses do from HRVs. However, HPeV types 1 and 6 have recently been detected in the feces of monkeys with diarrhea [41]. The fecal samples were collected from farmed macaques in China. In this specific case, HPeV infection was suspected to have been transmitted from humans to monkeys and could, thus, potentially serve as an animal model to study the pathogenesis of HPeV infections.

To summarize, HPeV exhibits several distinct molecular features when compared with other picornaviruses. These include the lack of the maturation cleavage of the capsid protein VP0 to VP4 and VP2, a 5′UTR region resembling that of picornaviruses infecting animals and a unique nonstructural 2A protein. The structural differences in particular are relevant to the development of antiviral therapy for HPeV infections (Figure 2), but theoretically every step during the HPeV life cycle from receptor binding to the release of newly formed viral particles is a potential target for viral replication blockage.

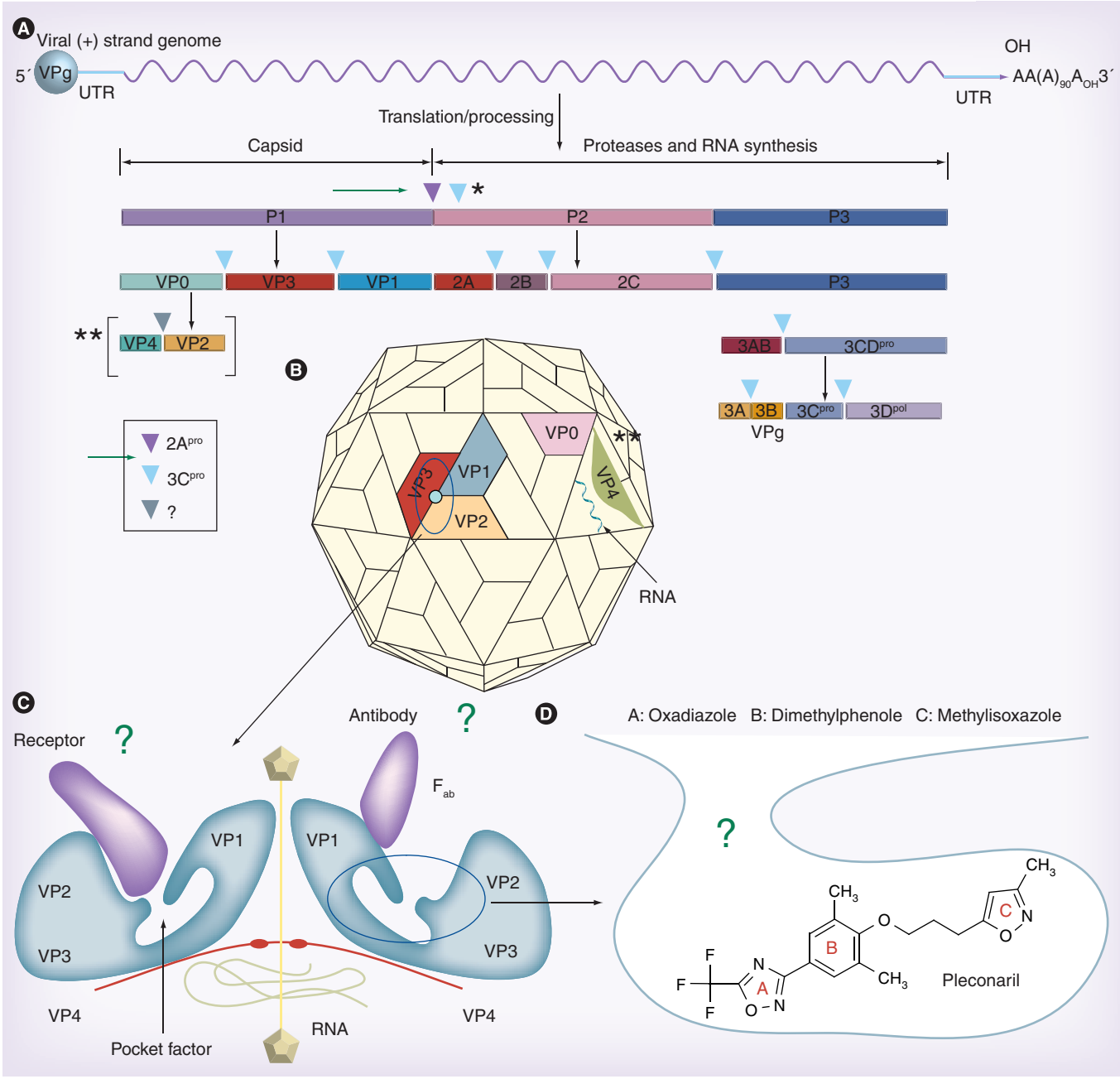

Figure 2. Capsid structure and genome organization of the human parechovirus and human enterovirus genomes with the main targets for antiviral therapy. (A) The viral genome, with the genome-linked protein VPg at the 5´-end, the 5´UTR, an open reading frame of approximately 7 kB, the 3´UTR, and the poly(A) tail. The coding region is divided into three regions: P1, the structural region encoding the capsid; P2 and P3, the nonstructural region encoding proteinases and polymerases, respectively. The large polyprotein is cleaved by viral proteinases. In human parechoviruses (HPeVs) only one protease, 3Cpro (*), is involved in processing. In human enteroviruses (HEVs) and human rhinovirus (HRV), P1 is cleaved from P2 by 2Apro, and an as yet unknown protease is responsible for cleavage of VP0 (in parenthesis, **). In HPeV, cleavage of VP0 does not occur. The green arrow indicates the two proteinases as the target for inhibition by protease inhibitors. **(B, C & D)** Viral capsid and the hydrophobic pocket. The capsid **(B)** consists of 60 protomers formed by the capsid proteins VP1 (blue), VP3 (red) and VP0 ** (pink) for HPeV, and VP2 (orange) and VP4 (green) for HEV and HRV. At the junctions of the capsid proteins lies a canyon with a hydrophobic pocket, important for receptor binding. **(C)** Model of HEV for how receptor binding can be blocked by neutralizing antibodies against the VP1 region; this is unknown for HPeV (green question mark). Pleconaril can bind inside the pocket of HEV **(D)** leading to increased stability of the capsid and conformational changes, thereby interfering with uncoating and cell entry.
VP: Viral protein.
Figures **(A)** and **(B)** are adapted from [103] with permission from ASM press license 2010. Figures **(C)** and **(D)** are adapted with permission from a figure courtesy of Dr M Schmidtke (Institute of Virology and Antiviral Therapy, Friedrich Schiller University, Jena, Germany).

HPeV infections & their clinical relevance

Epidemiology

By detection of antibodies to HPeV, 90% of children have been shown to be infected with at least one HPeV type by 2 years of age [42,43]. This is confirmed by observations over a time-span of 30 years showing that 60% of 580 HPeV isolates originated from children under 1 year of age [44]. Similarly, surveillance data in the USA between 1983 and 2005 revealed that 73% of 456 HPeV-1 infections and 68% of 34 HPeV-2 infections occurred under the age of 1 year [45], while HPeV-1 and HPeV-3 infections in The Netherlands have been recorded almost exclusively in children under 3 years of age [35]. High incidence of HPeV infections in this age group was also reported in a longitudinal community-based study from Norway, where 11.3% of 1941 fecal samples were HPeV-positive [46]. Interestingly, there are only a few reports in the literature of HPeV infections in individuals over the age of 10 years [35,47–49]. Overall, 90% of HPeV infections have been described in children younger than 5 years of age [35,48,50–52], while HEVs generally affect individuals of all ages.

Many HEV surveillance programs still report HPeV-1 and -2 as echovirus-22 and -23. HPeV-1 is one of the most commonly occurring genotypes when both HPeV and HEV circulation are considered [53,54], circulating throughout the year but less frequently detected during summer [12,55]. After HPeV-1, HPeV-3 and -6 are the next most frequently detected, depending on the year of isolation and method of screening [35,47,51,52]. HPeV-3 has a biannual circulation pattern and is most frequently found in the summer of even years [14,35,55], although this specific circulation pattern might be different in other regions of the world [47]. Infections with HPeV-2 are reported sporadically [55,56], while circulation patterns of the newly reported HPeV types 4, 5 and 7–14 are yet to be determined.

Clinical relevance of HPeV infections

High seroprevalence of HPeV-1 in children and adults indicates that HPeV infections are extremely common and mild or even subclinical, already occurring at a young age. HPeV-1 and -2 were first identified in children suffering from diarrhea [18]. Occasionally, severe conditions such as encephalitis, paralysis and myocarditis were attributed to HPeV-1 infections (reviewed in [2,20]), while infections with HPeV-2 could only be associated with milder symptoms [56].

Historically, the HPeVs were not considered as separated viral pathogenic entities because they were detected in virus culture designed for HEV diagnostics. With the emergence of molecular techniques HPeVs were no longer diagnosed since PCRs designed for HEV detection were unable to detect HPeVs. With the discovery of the HPeV-3 in 2004 [39], the view on HPeVs changed dramatically, and a new wave of publications increased the knowledge about different HPeV types and their clinical significance (reviewed in [2,4,20,57]).

Mild disease: gastrointestinal & respiratory infections

Early reports identified echovirus 22 by cell culture or an increase in numbers of neutralizing antibodies in children with gastrointestinal symptoms or respiratory infections (reviewed in [2,20]). WHO data showed that 29% of 581 reported cases of HPeV-1 infections were from patients with gastroenteritis and 26% from patients with respiratory infections [44]. A retrospective investigation of 109 Swedish children with HPeV-1 infection demonstrated diarrhea as the most common clinical finding (32% of cases) followed by respiratory symptoms (13%). HPeV-2 infections have been described in small nosocomial outbreaks of gastroenteritis [56].

Recent studies report high frequencies (11.6–16%) of HPeV detection in stool samples from children with acute diarrhea [51,58]. In addition, the newly identified HPeV-8 and -10 were first isolated from children with enteritis [59,60].

Human parechoviruses have been linked to a respiratory disease outbreak on a neonatal unit [4] and associations with upper respiratory tract infections as well as bronchiolits, pneumonitis and otitis media have been described [47,48]. In a longitudinal follow-up study carried out by Tauriainen *et al.* otitis media and cough were clearly found to be associated with HPeV-1 infections [61]. Conversely, in a screen of 3844 respiratory samples collected in 2007, HPeVs were only detected in 1.2% of the samples [52]. HPeV-1 and -6 have been reported as the most frequently identified types in respiratory specimens [52]. Although HPeV-3 has been reported in association with respiratory disease as well, detection of this type in respiratory samples is scarce [47,48,52].

Although HPeVs have been diagnosed in a variety of clinical conditions, the detection of HPeV in stool and respiratory samples is not always associated with clinical symptoms. A recent longitudinal study in stool samples from infants showed 11.3% positive for HPeV, irrespective of presence or absence of clinical symptoms [46]. In addition, in the follow-up study carried out by Tauriainen *et al.* no clear association between HPeV-1 infection and gastroenteritis could be found [61]. In addition, approximately 40% of the cases in which HPeV was detected in respiratory specimens originated from children without respiratory symptoms [52]. Indeed, HPeV infections are highly prevalent in children and shedding of viral particles or nucleic acid may occur for weeks [Wildenbeest JG *et al.*, Unpublished Data]. Detection of HPeV may therefore represent asymptomatic carriage, even lasting weeks after (symptomatic) infection.

One may assume that the gastrointestinal and respiratory tracts are the primary replication sites of HPeVs. Infection with HPeVs will therefore lead to detection of virus from these sites independent of the presence or nature of clinical symptoms.

CNS infections, neonatal sepsis & other disease associations

Human parechovirus-1 infections were occasionally associated with encephalitis and paralysis [49,50,62], but less frequently than other echovirus infections [1,44]. Recent reports from The Netherlands, UK and USA have now associated HPeV-3 infection with neonatal sepsis and CNS infections [12,14,17,63]. When HPeV-3 was first characterized, it was isolated from a 1-year-old Japanese girl suffering from transient paralysis [39]. Immediately thereafter, three additional HPeV-3 infections were found in Canadian neonates with neonatal sepsis [13]. The marked clinical

difference between HPeV-3 and the two previously known types was initially observed in a Dutch study involving 37 children with an HPeV-1 or -3 infection [12]. Neonatal sepsis was found in 70% of the HPeV-3-infected children and in only 8% of the children infected with HPeV-1. In 50% of the children infected with HPeV-3, CNS-associated symptoms were reported. In comparison to HPeV-1 infections, infections with HPeV-3 were associated with more severe symptoms and with a younger age [12,55].

Human parechoviruses can be detected as the second most prevalent virus in CSF samples from children [14,17]. By real-time reverse transcriptase-PCR, HPeV was detected in 4.2% of 761 CSF samples from children under the age of 5 years (median age: 1.2 months) [17]. A total of 75% of the HPeV-positive children presented with sepsis-like illness, whereas symptoms of CNS infection were reported in 16% of these children. Other reported clinical symptoms were gastrointestinal symptoms (39%), respiratory symptoms (36%) and rash (17%). Although in this study HPeV typing from CSF was not performed, the incidence of HPeV positivity in CSF followed the same biannual cycle as noted previously for HPeV-3 from fecal samples [12,55], suggesting HPeV-3 to be the predominant type to infect the CNS.

In a study from Scotland, comprising 1575 CSF samples from all age groups obtained in 2006–2008, HPeV was detected in 2.6% of the patients, with the highest frequency in 2008 (7.2%) exceeding that of HEVs [14]. All positive samples originated from infants less than 3 months of age with suspected sepsis or pyrexia. Molecular typing of these CSF samples revealed all infections to be due to HPeV-3.

Neonates with HPeV encephalitis exhibit similar clinical symptoms to children with encephalitis caused by HEV infection, the most frequent signs being fever, seizures, irritability, rash and feeding problems [3]. Pleocytosis is found only in a minority of the CSF samples from children with either HEV or HPeV infections, while protein and glucose levels remained normal in all HPeV cases. Normal CSF findings can therefore be misleading when diagnosing neonatal HPeV infection. From the same group, data reported that in ten out of 14 children diagnosed with encephalitis over the last 10 years, HPeV could be detected, mostly typed as HPeV-3 [16]. These were all newborn infants presenting with seizures, fever and rash.

Extensive white matter abnormalities with unfavorable neurodevelopmental outcome have been reported in relation to HPeV-3 encephalitis [16]. HPeV-3 has recently also been identified as a cause of neonatal hepatitis-coagulopathy syndrome [63,64] and even infant death [15].

A number of case reports and small studies propose associations of HPeV with a wide range of other diseases, including myocarditis, hemolytic uremic syndrome, and necrotising enterocolitis (HPeV-1), myositis (HPeV-3), lymphadenitis (HPeV-4) and Reye's syndrome, an acute, noninflammatory encephalopathy with hepatic dysfunction and fatty infiltration (HPeV-5 and -6) [20,47,65]. Further studies are needed to confirm these disease associations with HPeV infection.

In conclusion, the clinical spectrum of HPeV infections ranges from asymptomatic infections or mild disease to severe disease symptoms mostly found in young children. In particular, HPeV-3 appears to display a variety of serious clinical presentations including neonatal sepsis, meningitis, encephalitis and hepatitis, and is probably more common than previously anticipated.

Treatment of picornaviruses: limited options

Successful vaccines have been developed against poliovirus, hepatitis A virus and FMDV viruses from three different genera within the *Picornavirus* family.

Vaccination against poliomyelitis has been successful in eradication of the poliovirus from most parts of the world. However, despite huge efforts by the WHO to eradicate poliovirus worldwide, in 2010 poliovirus is still circulating in India, Afghanistan, Pakistan and Nigeria [66]. It has been suggested that additional antiviral therapy is needed in the polio eradication strategy [67]. Despite long-term efforts, development of antiviral therapy against picornaviruses has not yet been successful and treatment options for human picornaviruses, such as HEVs, HRVs as well as HPeVs, are limited.

As illustrated in a recent review on the prognosis of neonates with HEV myocarditis, supportive treatment and administration of intravenous immunoglobulin (IVIg) currently are the only options, with mortality rates of aproximately 30% in these conditions [68]. Until mid-2000, the drug pleconaril was occasionally used to treat patients with severe HEV infections. Here, we will review the backgrounds and effects of these treatments against HEV infections to understand potential treatment options against HPeV infection.

IVIg & maternal antibodies in HEV treatment

Neonates are particularly at risk for severe picornavirus infections. Their immune system is not yet fully developed and maternal antibodies derived before birth and during breast feeding play an important role in their host defense. Mothers of neonates with a severe HEV infection frequently had a history of a viral illness preceding or immediately following delivery [5]. In neonates with severe HEV infection, the maternal titers of neutralizing antibodies against the specific HEV serotype were detectable, but generally low, suggesting that a lack of specific maternal antibodies is a risk factor for the development of severe illness [69].

Another group at risk for severe or chronic HEV infections are patients with primary or secondary immune deficiencies and especially those patients with hypo- or agammaglobulinemia, indicating that a proper humoral immune response is important for HEV clearance [6]. In both groups, lack of (specific) antibodies is associated with severe or chronic infection. This is the rationale to use IVIg as a treatment for severe HEV infections.

In neonates, IVIg was used in severe meningoencephalitis, sepsis, hepatitis and/or myocarditis with various clinical outcomes. In the only blinded randomized controlled study, 16 neonates with a proven HEV infection were enrolled [69]. Only the neonates (n = 5) who had received IVIg with a neutralizing antibody titer of greater than 1:800 against their causative HEV were able to clear the HEV. However, the study was too small to show statistically significant differences and no effect on clinical outcomes could be found.

Administration of maternal plasma to severely ill HEV-infected infants has been advocated early in infection [9,70], although it is of note that maternal serum does not always contain high antibody titers against the infecting strain [69].

The reviews of Crennan *et al.* and Misbah *et al.* describe the effect of IVIg on chronic enteroviral infections in patients with primary immunodeficiencies [71,72]. The use of high-dose IVIg and/or intrathecal immunoglobulins demonstrated variable beneficial effect in patients with chronic enteroviral meningitis in agammaglobulinemia (CEMA). However, the therapeutic efficacy of IVIg in HEV infections has not yet been proven.

The capsid inhibitor pleconaril for treating HRV & HEV

Drugs with capsid-inhibiting properties have been demonstrated to be the most promising in the treatment of picornavirus infections. Of these, pleconaril has been evaluated most extensively in clinical trials.

Pleconaril integrates within a hydrophobic pocket inside the viral capsid, leading to increased stability and compression of the viral capsid (Figure 2D). As a result, uncoating and binding of picornavirus to the host cell and of viral RNA are interrupted [73,74]. The hydrophobic pocket is relatively well preserved among HEVs and HRVs, resulting in a broad-spectrum anti-enteroviral and anti-rhinoviral activity [73]. Effectiveness of pleconaril was first shown in the treatment of colds due to picornaviruses in adults [75] and the efficacy of pleconaril has been summarized in several reviews [6,74,76]. The effect of pleconaril in neonates with severe HEV infection varied [77] and effects on recovery of HEV meningitis was minor [78]. Pleconaril has been used as treatment on a compassionate-use basis in patients with immunodeficiencies and severe HEV infections, very often in combination with IVIg. In a group of 17 immunoglobulin-deficient patients with CEMA treated with pleconaril for 7–10 days, 12 patients (75%) showed a clinical response to therapy [77]. Further support for the benefit of pleconaril in immunocompromised patients is anecdotal [79,80], while cases with fatal outcome also have been described [81]. In our hospital, a child with CEMA with an echovirus-13 infection cleared the virus from the CSF after treatment with pleconaril and IVIg [Wildenbeest JG *et al.*, Manuscript in Preparation].

Later investigations revealed that pleconaril induces hepatic cytochrome P450 3A enzymes, leading to menstrual irregularities and therefore risk of unplanned pregnancy in women who used oral contraceptives. This, and other concerns about possible drug interactions and resistance resulted in the rejection by the US FDA in 2002 of use of pleconaril as a treatment for the common cold [76]. Thereafter, production of pleconaril was abandoned and the drug is no longer available [74].

In summary, although the efficacy of pleconaril could not inconclusively be demonstrated for all indications, it was the only antiviral compound ever to be available for treatment of severe HEV infections. Based on the structure of pleconaril, other capsid-inhibiting compounds are being developed with the emphasis of activity against EV-71, which was resistant to pleconaril [82–84]. In addition, compounds targeting the protease are being designed such as 3C protease inhibitors against EV-71 (Figure 2A) [76,85].

Expert commentary: the need for development of anti-HPeV therapy

Current options

The need for therapy against HEVs and HRVs has been emphasized in numerous reviews and studies over the last decade [6,74,76]. HPeV infections can be severe and even life-threatening, indicating a need for treatment. So far, no systematic data are available on HPeV treatment. In a case report describing a twin with neonatal sepsis and hepatitis infected with HPeV-3, one child received IVIg and subsequently recovered, while the other recovered having received acyclovir which does not have antipicornaviral activity [64].

If IVIg is given to neonates to reduce disease burden from HEV infection, it seems rational to give IVIg to severely ill neonates with HPeV infection as well. High antibody titers against the specific serotype might be needed for protection [69]. Neutralizing antibody titers in IVIg vary between batches [86] and geographic regions [87], but the high seroprevalences of HPeV-1 and -3 in adults would suggest IVIg to contain high titers of neutralizing antibodies against these HPeV types.

Another option that should be explored for treatment of HEV and HPeV infections is the use of monoclonal antibodies. New approaches to rapidly generating human monoclonal antibodies have been successful in the development of monoclonal antibodies against influenza viruses [88] and respiratory syncytial virus [89]. For HEV, protective antibodies are presumably neutralizing type-specific antibodies against the VP1 capsid protein; therefore, monoclonal antibodies against HEV will not exhibit broad cross-neutralizing capacity, as recently described for influenza virus. This is a problem when considering generating monoclonal antibodies for the treatment of HEV infections, with over 100 serotypes and multiple serotypes circulating at the same time without a clear type-dependent disease association. By contrast, for HPeV this approach could be feasible. The HPeV group is much smaller and neutralizing antibodies elicited against VP0 showed cross-reactivity [26]. Furthermore, HPeV-3 stands out for its association with more severe disease, making it an ideal target for monoclonal antibody neutralization.

Although there is circumstantial evidence for protection of antibodies against severe disease in HEV infections, this has never been shown for HPeVs and is questioned by Ehrnst and Eriksson [50]. They observed that, despite the presence of maternal antibodies in almost all mothers in their study, symptomatic infection with HPeV-1 occurred in infants that still should have maternal antibodies present. In addition, symptomatic HPeV-3 infection in infants occurs at a very young age, arguing against maternal protection by antibodies, although one could argue that infants with severe HPeV-3 infections were all born from HPeV-3 seronegative mothers. Therefore, the potential of antibodies to protect against or to reduce symptomatic HPeV infection still needs to be determined. Currently, the antibody approach seems to be the only one available for treatment of HPeV infections.

Potential options

Theoretically, every step in the viral life cycle is a potential target for developing antiviral therapy. An extensive overview of compounds that can inhibit picornavirus replication is given by de

Palma *et al.* [76]. Although this review is quite recent, no data can be found on compounds that can potentially inhibit HPeVs. The most promising candidates for antipicornaviral therapy propagated in the literature are capsid inhibitors and 3C protease inhibitors (Figure 2) [76,82,83,85].

As described in more detail earlier in this article, pleconaril was the most promising capsid-inhibiting compound with almost all criteria for a good antipicornaviral drug present: *in vitro* and *in vivo* activity and clinical activity shown for some patient groups, combined with a favorable safety profile. A major difference between HPeVs and HEV/HRV is that the capsid consists of three structural proteins rather than the four typically seen [2]. Therefore, capsid stability and infectivity must be differentially regulated in HPeVs. The 3D structure of HPeV does have similarity to some other picornaviruses (most closely to FMDV), despite very limited amino acid sequence identity [25]. The external appearance of HPeV-1 particles is much smoother than other picornaviruses, such as CAV9, most likely due to truncated surface loops of VP1. This could indicate that the hydrophobic pocket differs from that of HEVs, possibly preventing the activity of pleconaril. Indeed, data from our laboratory show that HPeV-1 and -3 are resistant against pleconaril [4] [Wolthers KC *et al.*, Unpublished Observation]. Interestingly, Holmberg *et al.* used pleconaril for treatment of mice and rats infected with LV, another member of the *Parechovirus* genus [90]. Minor inhibitory effects of pleconaril on LV in cell culture growth were described but a CPE inhibition test was not performed and IC_{50} values were not given.

Several capsid-inhibiting compounds are being developed against HRV and HEV, some with the emphasis on targeting the pleconaril-resistant CBV3 or HEV-71 [76,84]. Given the different structure of the HPeV viral capsid, these HEV/HRV capsid-inhibiting compounds may not inhibit HPeV capsid functionality, although this could easily be tested *in vitro*.

The protease 3Cpro is ubiquitous in the picornavirus family and for HPeVs it seems to be the only protease [1]. For the 3C protease inhibitor rupintrivir, antiviral activity was shown in a human experimental HRV challenge trial, where disease severity and viral load were reduced [76]; however, no reduction of these parameters could be found in naturally infected patients, and the clinical development of rupintrivir was halted. Despite this, rupintrivir was recently promoted for treatment of severe EV-71 infections [91]. The 3C protease inhibitor compounds have a broad antipicornaviral activity [76]. It is uncertain whether rupintrivir would have had any inhibitory effect on HPeVs; although the genome structure of HPeVs is similar to HEVs, the genome variability is extensive, and despite conservation of the 3CD regions, structure and function of the HPeV 3C protease might differ from other picornaviruses.

Human parechoviruses can be cultured *in vitro* on standard cell lines; HPeV-1 and -2 can easily be propagated on many cell lines used in the laboratory, while HPeV-3 grows slower and only on a limited amount of cell lines. Thus, susceptibility of HPeVs to different antiviral compounds can be tested *in vitro* just as well as for HEVs and HRVs. Antiviral effect and cytotoxicity of pleconaril and related compounds have been studied for CVB3 by measuring cell viability and inhibition of cytopathic effect in cell culture [84]. Cell culture models are also used in high-throughput screening of antiviral compounds as recently presented [92,93]. Including HPeV-1 as the prototype HPeV, and HPeV-3 as the most pathogenic HPeV type in these screenings would be a step further in development of an anti-HPeV treatment.

Highly speculative options

In a recent study, ribavirin was shown to have *in vitro* and *in vivo* effectivity against EV-71 in a mouse model and it was suggested that ribavirin could be a potential drug for EV-71 [94]. Of note, Holmberg *et al.* used ribavirin in addition to pleconaril in the treatment of rats and mice infected with LV [90]. Ribavirin is a nucleoside analogue with broad-spectrum antiviral activity, currently used to treat patients infected with hepatitis C and occasionally in patients with Lassa fever. Ribavirin acts by different mechanisms to inhibit virus replication. One mechanism of action described for poliovirus is lethal mutagenesis, which is the loss of infectivity with an increase in mutation rate [95]. Passaging poliovirus in the presence of ribavirin leads to a viral population resistant to ribavirin, but these viruses are less adaptable, making them more susceptible to other antiviral drugs. This would be a challenging approach for combination therapy, but the mutagenicity of ribavirin will also make it difficult to get the drug approved for use in human picornavirus infection.

Recent investigations explore the role of RNA interference in inhibiting replication of picornaviruses such as EV-71 and CAV-21 [96,97]. By targeting virus gene regions or host factors critical for viral replication by small interfering RNAs, virus replication can be suppressed, indicating that this is a promising approach for developing antivirals. At this time, RNAi seems much more a tool that could be applied for research on HPeVs than for treatment development.

Five-year view

Compared with HEVs, less is known about receptor use, replication pathways, viral pathogenesis or virus–host interactions of HPeVs [1,2,4]. More research is needed to elucidate the specific characteristics of this clinically relevant group of viruses and to develop treatment strategies. In the meantime, the HPeVs should be included in the ongoing search for antiviral compounds against picornaviruses. Although the withdrawal of pleconaril has been a major setback for the treatment of picornavirus infections, many promising compounds are designed and tested against several picornaviruses. However, it may take years before this will lead to a candidate drug that can be tested in the clinic. If such a compound does not have a broad-spectrum activity against picornaviruses, including the HPeVs, than for the latter group, development of an antiviral compound may take much longer. Antibody-based therapies therefore seem to be the most feasible as a short-term option for treating HPeV infections.

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Key issues

- The group of human parechoviruses (HPeVs) belong to the family of *Picornaviridae* and are closely related to the well-known group of the human enteroviruses (HEVs), a large group of viral pathogens that comprise the most common causes of human infections.
- The clinical profile of HPeVs overlaps with that of HEVs, ranging from mild febrile disease to neonatal sepsis, CNS infections and hepatitis.
- The HPeV group contains 14 HPeV genotypes, of which HPeV-3 is associated with neonatal sepsis and CNS infections in younger children compared with infections with other HPeV types.
- No antiviral treatment is currently available against HPeVs or HEVs.
- Intravenous Immunoglobulin could be of help for treatment of severe HPeV infections; intravenous immunoglobulin is sometimes used to treat severe HEV infections, based on the observation that lack of serotype-specific antibodies can lead to severe or chronic HEV infection.
- Development of specific HPeV antibodies for treatment of HPeV infections could be a feasible approach for the near future.
- Capsid inhibitors are currently under development for antipicornaviral therapy, of which pleconaril is the best studied. Pleconaril will most likely not have antiviral activity against HPeVs; and also, this drug is no longer available.
- The two most promising approaches to development of antipicornaviral therapy are capsid inhibition and protease inhibition. Compounds in development should be tested for antiviral activity against HPeVs.

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