

Human parechovirus infection in young infants: strategies to improve detection and management

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Summary

Human parechovirus (HPeV) infections are the second most common cause of viral meningitis in children. HPeV's are most frequently seen in infants less than 90 days of age. Clinical manifestations include encephalitis, meningitis, myocarditis and sepsis syndrome, which can lead to significant neurodevelopmental sequelae in young infants. Molecular techniques, including polymerase chain reaction assays are the diagnostic tool of choice and have contributed to a marked increase in reported cases, along with an increasing awareness of the aetiological role of HPeV in infant disease. However, focused clinical and diagnostic investigations for HPeV in infants and the use of such information in management is variable due in part to the lack of robust incidence data and its range of clinical presentations. A better understanding of the pathogenesis and epidemiology of HPeV infections is required to inform an evidence-based approach to diagnosis and treatment in the future.

Search strategy and selection criteria

A PubMed search was undertaken for articles published from January 1966 to September 2017 with the terms "Human Parechovirus", "Parechovirus" and "prevalence" or "seroprevalence" or "incidence" or "clinical features" or "epidemiology" or "diagnosis" or "treatment." We also search reference lists of identified studies.

Introduction

Human parechoviruses (HPeVs) are small, non-enveloped RNA viruses in the *Parechovirus* genus within the *Picornaviridae* family(1). Transmission occurs primarily via the faeco-oral or respiratory routes (2). Most cases of HPeV infection occur in children and cause mild upper respiratory or gastrointestinal symptoms (2). Less common, severe clinical manifestations of primary HPeV infection have been increasingly recognised, including encephalitis, meningitis, myocarditis and sepsis syndrome (3–7). Recent data suggest that infants with parechovirus encephalitis may have normal short term outcomes but can develop long term neurodevelopmental sequelae, such as gross motor impairment, communicative delay, cerebral palsy and visual impairment (8,9), and these have been under-reported previously.

The extent to which molecular diagnostic methods, such as polymerase chain reaction (PCR) for HPeV are actually used is highly variable between hospitals, and may be restricted to particular patient age ranges, presence of abnormal CSF parameters or clinician request; many hospital laboratories do not offer testing for HPeV. It is therefore likely that a substantial number, and perhaps even the majority, of HPeV infections are undiagnosed in acute, symptomatic presentations. This review will consider recent advances in our understanding of HPeV disease in infants to improve recognition of the full spectrum of HPeV infection, describe the clinical burden of disease, inform appropriate testing, and prioritise areas of future research.

Epidemiology of HPeV infections

The true overall burden of HPeV infections is not known. Studies have been broadly restricted to describing the incidence and burden of HPeV within specific, narrowly defined cohorts such as single centres or during outbreaks (10–14). It is difficult to make meaningful comparisons between cohort studies as the threshold to test, diagnostic techniques used, samples collected, populations analysed and years studied all vary substantially. Thus the type of robust longitudinal data that can help inform future treatment and prevention studies are largely missing. To our knowledge, there are no published data on the burden of HPeV disease in low and middle income countries. Such data are required for the identification of vulnerable populations, understanding why specific HPeV types are associated with severe disease and quantifying the disease burden relative to other infectious causes of meningitis, encephalitis and sepsis.

There are 19 known HPeV types, but the majority of significant disease is in infants under 3 months of age caused by type 3 (18, 10) (16–20). HPeV1, HPeV4 and HPeV6 are associated with less severe disease than HPeV3 and most frequently cause mild gastroenteritis in children under two years of age (21–23). A longitudinal study collecting stool samples over 12 months in Norwegian children showed no association between acquiring HPeV1 and developing disease (24). A case-control study in China showed no difference in HPeV1 viral loads in children admitted to hospital with gastroenteritis and asymptomatic cases (25). These findings suggest that HPeV1 isolated in the stool of infants with gastroenteritis may be an “innocent bystander” and not always the cause of disease.

Surveillance data from one region in the UK reported an increase in HPeV cases during 2016 compared with previous years despite using the same assay and clinical testing algorithm (26). The National Enterovirus Surveillance Study (NESS) is a passive surveillance system that collects anonymous clinical

and demographic data on EV and HPeV from 17 laboratories across the USA (27). During 2009 – 13, HPeV3 was the most commonly reported HPeV type and peaks occurred in summer months in even years as has been reported widely elsewhere (4,24,11,28–30). This a biennial cycle of infections is consistent with studies in Scotland and The Netherlands which showed similar peaks of HPeV3 infection in infants every two years (2,31). The reasons for this remain unclear. A four-year national laboratory surveillance study in Denmark analysed 6,817 specimens collected from 4,808 children with suspected enteroviral disease who had fever and signs suggestive of meningo-encephalitis and attended hospital or primary care physicians (22). In total, 202 (3%) specimens from 149 (3%) children were positive for HPeV. The overall median age of HPeV-infected children was 39 days (interquartile range [IQR] 21 – 71 days).

In 2013-2014, 183 infants with HPeV3 disease were identified over a four month period in New South Wales, Australia (16), highlighting the ability of HPeV to cause epidemic as well as the endemic disease previously described. Active enhanced surveillance mechanisms were developed after clinicians recognised clusters of cases. Surveillance included identifying cases at 3 sentinel sites (active surveillance), laboratories reporting positive HPeV specimens from sentinel/regional laboratories to a central laboratory (passive surveillance) and emergency departments recording number of febrile infants attending/admitted to the hospital (syndromic surveillance). Increased awareness to test HPeV at the earliest opportunity in febrile infants ensured that length of stay was reduced by 30% compared to before the outbreak and thus antimicrobials discontinued earlier.

National surveillance (laboratory reporting of all positive HPeV cases to the national communicable diseases database) of circulating HPeV is necessary to detect outbreaks, monitor novel circulating strains, inform current epidemiology and potentially inform future treatment trials. In the event that surveillance at national level detected an outbreak, active enhanced surveillance (case finding by clinician/laboratories at individual hospitals) should be performed in order to ensure early disease recognition.

Understanding disease patterns through host, viral and environmental factors

Multiple cohort studies have shown that the prevalence of HPeV3-associated sepsis-like illness and CNS infection (encephalitis and meningitis) are highest in infants aged less than 3 months (5,16,17,18). The UK-ChiMES (UK Childhood Meningitis and Encephalitis Study) is the most recent and largest study to assess the aetiology of meningitis and encephalitis in children the UK. An interim analysis from this study of >3,000 infants and children with suspected meningitis and/or encephalitis demonstrated that EV and HPeV were the most common causes of viral meningitis in the paediatric population (33).

Infections with HPeV3 are much less common than HPeV1 in women of child bearing age and the general adult population, based on blood and CSF microbiological sampling studies from Europe (4,35,36). Seroprevalence studies have reported antibodies against HPeV1 are present in approximately 30% of infants at the age of 1 year, 70% by the age of 2 years and 92 – 99% of adults (36,37). In contrast, the seroprevalence of HPeV3 is low in children (<2.7% at in infants less than 3 months) and only increases to 10% – 13% by adulthood (36). The absence of maternal antibodies that might limit the susceptibility and systemic spread of HPeV3 may potentially account for the high rates of severe disease observed in young infants. A recent study in Japan measured neutralising antibody titres to HPeVs in 175 cord blood samples from newborns, and in serum samples from neonates and

young infants with severe clinical manifestations related to HPeV3 infection (38). Although systematically higher seroprevalence of HPeV3 up to 60% was observed in the Japanese adult population than previously in Finland and the Netherlands (38,39,37,40), negative or low antibody titres (<1:16) were observed in all HPeV3-infected infants at disease onset, supporting the hypothesis that maternal antibodies may help protect young infants from developing HPeV disease.

It has been proposed that differences may exist between HPeV types in their virulence (41,42). There is some virological support for such a hypothesis; HPeV3 replicates much more rapidly than HPeV1 *in vitro* in neuronal cells and this may equate to a more neuropathogenic phenotype *in vivo* (42). HPeV3 strains lack the arginine-glycine-glutamic acid sequence at the capsid protein that is used for virus entry and found in HPeV1. The use of an alternative entry receptor may thus modify the cellular tropism and neuropathogenicity of HPeV3 (41). A study of autopsies, in two preterm infants with HPeV3 infection who had significant periventricular white matter changes, showed that the infected cells were isolated to the meninges and smooth muscle of pulmonary vessels (43). The authors suggested that in the absence of infected periventricular cells the encephalitic change seen could be due to vascular compromise. A study characterising innate immune responses in 11 infants with HPeV meningitis showed that the majority of cytokines and chemokines remained in the control range due to early disruption of the interferon cascade (44). The dampened immune response seen in infants with HPeV meningitis may account for the lack of CSF pleocytosis seen in infants with HPeV meningitis.

A nationwide cohort linkage registry study on birth and sibling relationships in Denmark between 2009 and 2012 demonstrated a 9-fold increase in the likelihood of a younger sibling (under age of five years) contracting HPeV3 infection (but not other types of HPeV infection) compared with firstborn children (45). The authors demonstrated that the younger sibling was more likely to be affected by both HPeV3 and non-HPeV3 if there was a smaller age gap in siblings. This study suggests that older siblings play a significant role in transmission of HPeV to their younger siblings and appropriate infection control precautions are needed to prevent/reduce spread of the virus within households and in hospitals. Similar findings were noted during an outbreak of HPeV3 in Japan (46). This study included 43 young infants hospitalised with sepsis or sepsis-like illness with HPeV3 detected in CSF. Clinical data and stool samples were collected from close relatives. In total, 51% (22/43) of hospitalised cases had been in contact with family members who had symptoms indicative of viral infection (i.e. fever, diarrhoea and upper respiratory tract infection signs) and were tested positive for HPeV. Another recent case series describing an outbreak of HPeV on a neonatal intensive care unit (NICU) in Kansas reported that 11 out of 23 infected neonates had a symptomatic household contact (rash, gastrointestinal or respiratory symptoms) in the week prior to onset of symptoms (47). These findings should emphasise the importance of preventive measures, including hand hygiene, in household with young infants in order to prevent transmission of viral pathogens (48,49).

In summary, HPeV3 disease is most commonly seen in infants aged less than three months. This may result from host (e.g. lack of maternal antibody), viral (e.g. HPeV3 being a particularly neuropathogenic type) and environmental factors (e.g. presence of older siblings in the family). Understanding differences in HPeV1 and HPeV3 pathogenesis (i.e. receptor usage for HPeV3, infectivity in endothelial vascular cells using 3D models) may be necessary to inform any future treatment studies.

Clinical manifestations and outcomes of HPeV disease

HPeV infection causes a wide spectrum of clinical manifestations, most commonly fever, irritability, poor feeding, tachycardia and rash (Table 1). Multiple studies have shown that HPeV3 can cause severe sepsis like illness (5,13,50–52). Fever, reduced feeding, neurological symptoms (irritability and seizures) and rash are the commonest clinical symptoms in hospitalized children. Although leukopenia with spiking fever has been proposed as a specific feature associated with HPeV infection (10,53), no clinical features can clearly distinguish HPeV3 infection from other viral and bacterial causes of sepsis in young infants and hence a low threshold is needed for testing of blood and CSF samples for HPeV in unwell infants <6 months of age. Previous studies also emphasise that the absence of CSF pleocytosis is a common feature in HPeV meningo-encephalitis, so presence of raised CSF WBCs should not be used as a criterion to determine if HPeV testing should be performed. HPeV rarely causes clinical disease in adults but has been associated with myalgia in a small observational study in Japan, acute flaccid paralysis in a series of 6 patients in Jamaica and severe sepsis in an elderly Canadian lady (54–56).

A maculopapular rash is a common sign of HPeV infection. Shoji and colleagues showed that most infants with HPeV3 sepsis-like syndrome (12/15) developed an erythematous palmar–plantar rash within five days after the onset of fever (30). The rash was limited to the distal extremities and no other systemic rash present. A small retrospective study of children with meningitis and encephalitis in Germany showed that in 12 cases of HPeV was strongly associated ($p<0.0001$) in young infants with maculopapular rash and seizures (57). These data suggest that HPeV should be tested in febrile infants with a maculopapular rash.

HPeV has been associated with myocarditis in various case reports (58,59), but there are no robust cohort data describing how common cardiac sequelae are during HPeV infection. The published cases were aged 6 weeks, 5 months and 14 months old. Myocarditis should be considered a rare but serious event in acutely unwell.

Smaller studies have also shown that HPeV can cause white matter involvement of the brain (60). This can lead to physical and learning disabilities which has been shown to persist in one case until 7 years of age (11,61). A Dutch group showed that white matter abnormalities occurred in 9 out of 10 children who presented with seizures and had HPeV meningoencephalitis (based on positive CSF PCR). Results of MRI scans showed subcortical white matter changes which involved entire tracts of fibres (61). Normal neurodevelopmental outcomes occurred in 5 children and significant sequelae in the other 4 (cerebral palsy, learning disability, epilepsy and possible developmental abnormalities).

A recent prospective cohort study in Australia has shown the importance of close follow-up in children with HPeV meningo-encephalitis even with good clinical short term outcomes (8). In this study, 7/9 cases of confirmed HPeV encephalitis had white matter diffusion restriction on MRI (3 of whom had normal cranial ultrasound scans). Although only 3 had any sequelae noted at time of hospital discharge, by 12 months, 5 of the cases showed neurodevelopmental sequelae: 3 severe (2 cerebral palsy, 1 central visual impairment) and two had gross motor delay.

These are similar findings to other small sized cohort studies. Vergnano et al reported 10 out of 12 meningo-encephalitis cases in young infants had white matter changes and Khatami and colleagues reported 4 out of 11 had similar results (10,11). However, MRI was performed in sick infants who had clinical indications for neuroimaging. The degree of damage in more mild clinical cases is unknown, and it may be that some will have abnormalities which then may lead to more subtle deficits later.

These data suggest that MRI should be carefully considered in cases with HPeV meningo-encephalitis. Close follow up after discharge till 24 months is warranted to assess for neurodevelopmental sequelae. Long term sequelae in infants with sepsis like disease is unknown (10).

It is still unknown how many infants die as a consequence of HPeV disease because clinical samples are not being routinely taken and/or tested for HPeV (62,63). The largest study to specifically evaluate HPeV case-fatality rate reported that approximately 4% (18/426) of specimens taken from autopsies cases were due to HPeV (64). Most studies investigating deaths are case reports of infants with significant white matter abnormalities and HPeV3 infection (43,65). Histopathological findings from these case reports suggest that the virus disrupts blood vasculature through haemorrhage and thrombosis. Testing for HPeV should be performed routinely when investigating infectious causes of infant mortality.

HPeV infection should be highly suspected in unwell febrile infants less than six months of age. An MRI to assess white matter involvement should be performed in cases with suspected encephalitis, and they should be followed up till at least the age of two years. An MRI should also be considered in infants with neurological signs (e.g. irritability, lethargy, seizures). Infants with meningitis and no parenchymal involvement have been shown to have good clinical outcomes (66,67).

Improving diagnosis of parechovirus infections

A single HPeV gene is divided into regions coding for structural proteins, VP0, VP3 and VP1 that assemble to create the virus particle, and the replication-associated proteins such as the RNA polymerase (3D) and the protease (2C). Areas of the genome that are targeted by PCR for virus detection (5'UTR) and for virus typing (VP3/VP1) are indicated in figure 1.

There have been several advances in developing more sensitive and rapid HPeV PCR tools (68–73). HPeV PCR methods target the 5' untranslated region (5'UTR) of HPeV genome as it is most conserved, whereas structural coding regions are used for typing (VP3/VP1, Figure 1) (74,75). The detection of HPeV in CSF sample is regarded as diagnostic of infection, whereas it is less clear if detection of HPeV in stool samples is associated with clinical disease (76,77). A Dutch group conducted a retrospective study investigating the correlation between positive PCR findings in stool and clinical symptoms (58). The authors showed that a positive HPeV3 stool sample was significantly associated with neurological symptoms, sepsis like illness and more commonly detected in infants less than 6 months than in older children. The same group showed that HPeV shedding in the faeces occurred up to 6 months after symptomatic infection (78). The viral load did not differentiate between severe or mild disease nor symptomatic or asymptomatic infection.

The uptake of RT-PCR assays to detect HPeV is variable across UK laboratories. Twenty-two out of 31 large paediatric centres in the UK taking part in the UK-ChiMES study varied in local practice regarding HPeV testing (Sadarangani M, unpublished data). In total, 40% (9/22) had no standard algorithm to test for HPeV and only tested samples on a case by case basis. Only a quarter (27%, 6/22) tested all CSF samples (regardless of CSF WCC). Just over a fifth (22%, 5/22) tested samples if requested by a clinician (highlighting the importance of awareness of the clinical signs associated with HPeV infections) and a further fifth (22%, 5/22) if there was any evidence of CSF pleocytosis (despite the number of published studies demonstrating the presence of severe HPeV infection in the absence of CSF pleocytosis).

Increased use of HPeV PCR testing has the potential to impact directly on clinical care of individual patients. A study in France compared the impact of PCR in managing patients during two separate outbreaks of echovirus 30 in 2000 and 2005 in a single tertiary unit (79). In 2000, cell cultures inoculated with CSF samples delivered a positive result in 10 days and negative result in 14 days at the earliest. PCR was used during the 2005 outbreak and reduced hospital length of stay from 5.4 days to 2.2 days, saving 322 000 Euros. The largest study evaluating the effect of an EV CSF PCR result on length of stay in infants under 90 days of life was conducted over 6 enteroviral seasons in a single tertiary US centre (80). The authors showed that there was 1.5 day decrease in length of stay and 33% reduction in antimicrobial use in cases who were EV positive in CSF.

Even without targeted treatment for HPeV infections, it could be anticipated that identifying infections in infants early can have a similar clinical and economic impact through, for example stopping unnecessary antimicrobial drugs, minimising extensive investigations and reducing the length of hospital stay. The costs of sensitive multiplex PCR assays continue to decline and results can be obtained within hours of receiving the sample in most diagnostic laboratories (68,81,82). It should be emphasised that there are multiple data which highlight that HPeV is routinely detected in CSF in the absence of pleocytosis (8,13,30,69,83–85). CSF samples should therefore be routinely tested for HPeV in infants aged under 6 months (where the clinical burden is highest) during an acute illness irrespective of CSF white cell count. Blood, stool and respiratory samples should also be considered for testing in this group in order to increase the diagnostic yield.

A positive diagnosis of HPeV infection would allow cessation of antibiotic therapy and may thereby facilitate earlier discharge from hospital (86). In addition, testing HPeV using PCR in blood samples of infants who are being evaluated for infection would increase the diagnostic yield as higher viral loads are seen in serum compared with CSF (51). A gradual shift in laboratory testing from pathogen specific (i.e. requiring a clinician to suspect and request a HPeV PCR) to syndromic (i.e. submitting samples for multiplex meningo-encephalitis and/or sepsis panels which include HPeV in a diagnostic array) will likely further improve detection. Increased testing of infant CSF and blood samples is required to better establish the true disease epidemiology and the full range of clinical syndromes, and will enable more targeted testing in the future.

Therapeutic interventions in development

Manufacture of antivirals against RNA viruses needs to take into account their high mutation rate (87). There is currently no approved antiviral therapy for treatment of HPeV disease and no phase 2 or 3 treatment trials in progress. Pleconaril had been shown to inhibit the replication of some enterovirus types and rhinoviruses (88) by inhibiting the uncoating of viral RNA and progeny virions during EV replication. However, recent structural studies suggest that the binding site is blocked in HPeV3 (89). These findings have been supported by a clinical report that pleconaril has no effect on HPeV replication (90).

Intravenous Immunoglobulin (IVIG) has been shown to have some efficacy in treating severe disease, similar to its use against severe enterovirus infection in neonates, but data have been restricted to case reports and not controlled studies (91,92). A recent report showed some success in using IVIG in an infant with severe myocarditis caused by HPeV1 and dilated cardiomyopathy (93). The infant had an increase in HPeV1 antibody titers after treatment with IVIG which correlated with a marked

improvement in clinical presentation. Karelehto and colleagues examined the titers of anti-HPeV3 antibodies in Dutch and Japanese IVIG preparations and showed there was a decline in neutralisation efficiency in batches against clinical strains identified after 2005 (94). The authors suggested that the loss of neutralisation capacity may reflect the IgG obtained from adult plasma donors who have not met recent HPeV3 strains.

A Finnish group have developed two monoclonal antibodies which act against HPeV1 (95). The antibodies function by preventing aggregation and inhibiting RNA genome uncoating and replication in the cell. Most recently, a state of the art reconstructive design of the HPeV3 genome suggests that a highly ordered region of the virus may have a role as a treatment target for neutralising antibodies (89). Antibody based therapies are a potentially feasible option to treat HPeV as they have restricted sequences. However, to date no HPeV3 monoclonal antibody has been developed which could be used to help treat the commonest disease causing type in young infants.

Summary

HPeVs are an increasingly recognised cause of morbidity in infants. This is likely due to the introduction of highly sensitive molecular techniques to detect virus and changes in disease epidemiology (variations in virulence or incidence of the virus in susceptible populations) as witnessed by recent outbreaks in Australia and increased rates of disease in the UK. Routine hygiene measures should be reinforced by healthcare workers to prevent transmission from adults and siblings to young infants less than 6 months. Clinicians should be aware of the potential aetiological role of HPeV3 in infant disease. In the evaluation of a febrile infant, CSF, blood, respiratory and stool samples should be submitted for HPeV testing. In turn, laboratories should routinely test for HPeV in CSF samples in infants less than 6 months. These measures will improve the diagnostic yield and improve identification. Detecting HPeV in affected infants should be encouraged as part of good antimicrobial stewardship, minimising unnecessary investigations and potentially reducing length of hospital stay thus being of clinical, economic and public health utility. Furthermore, recent Australian data have highlighted the importance of carefully following up affected infants with HPeV encephalitis in order to assess for neurodevelopmental sequelae and to consider a brain MRI. There are no antiviral/vaccine candidates in the pipeline, which is in part due to the lack of longitudinal data informing these trials. Active enhanced surveillance is essential to monitor outbreaks and monitor long term disease trends as well as contribute towards understanding of the clinical burden of HPeV infection. Future studies should better understand pathogenicity (e.g. which strains are responsible for causing outbreaks and severe disease), host immunity (e.g. which infants are most likely to develop disease) and antiviral candidates.

Contributors

S.K and M.S conceptualised the paper and conducted the literature review and devised table 1. S.K wrote the first draft of the paper. P.S and H.H devised figure 1. All authors reviewed and approved the final version of the manuscript.

Declaration of interests

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Study	Khatami A, Australia (10) (n = 118)	Vergnano S, UK (11) (n = 50)	Wildenbeest J, Holland (58) (n = 138)	Sharp J, USA (59) (n = 66)	Selvarangan R, USA (17) (n = 58)
Demographics	Inclusion: Retrospective clinical cohort <12 months over 4 months in 2013 across 5 hospitals during HPeV outbreak Age: 75% <60 days old Sex: 55% male Intensive Care Unit (ICU) admission: 25%	Inclusion: Retrospective clinical cohort <12 months during 2008-2012 in 3 hospitals Age: 48% <30 days old Sex: 56% male ICU admission: 50%	Inclusion: Universal molecular study in stool samples collected during 2004 – 2008 in 2 hospitals Age: 43% HPeV1 and 69% HPeV3 cases <6 months. Sex: 59% HPeV1 male, 65% HPeV3 male ICU admission: Unknown	Inclusion Universal molecular study in CSF samples collected over 5 months in 2009 in 1 hospital Age: 100% < 5 months Sex: 60% male ICU admission: 12%	Inclusion: Universal molecular study in CSF samples during 2006 – 2008 in tertiary centre Age: mean 6.6 weeks Sex: 70% male ICU admission: Unknown
Type	HPeV3 100%	Not tested	81% HPeV1, 19% HPeV3	77% HPeV3 (51/66 positive CSF specimens)	98% HPeV3 (52/53 positive CSF specimens)
Main clinical features at presentation (% of cases)	Tachycardia (98%) Fever (94%) Irritability (93%) Tachypnoea (91%) Poor feeding (70%) Poor perfusion (55%)	Poor feeding (96%) Fever (82%) Appeared unwell (74%) Irritability (64%) Mottling (54%)	HPeV 1: Gastrointestinal symptoms (97%) URTI 39% Fever 34% HPeV3: Gastrointestinal symptoms 80% Fever 53% skin symptoms 37%, Neurological symptoms 32%	Fever (91%) Irritability (91%)	Irritability (98%) Fever (95%) Non-specific rash (59%)
Major laboratory parameters at presentation (% of cases)	Absent CSF pleocytosis ¹ (96%) CRP <10mg/DI (69%) APTT>50 seconds/PT≥1.5 (54%) Blood WBC <1 × 10 ⁹ /L (19%) Platelets <150 × 10 ⁹ /L (10%) AST/ALT >100 U/L (33%)	Absent CSF pleocytosis (95%) Deranged clotting (95%, only 18 tested) CRP <10mg/DI (80%) AST/ALT >100 U/L (45%)	Not measured	Absent CSF pleocytosis (98%)	

Follow up after discharge	4 cases gross motor delay at 6 month follow up	1 infant died, developmental delay in 6/19 infants and 3 identifiable neurological sequelae till age of years old	Not measured	Not measured	Not measured
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Table 1: Clinical and laboratory features of the five most recent and largest HPeV clinical cohort studies

¹ Defined as CSF WCC >12/ μ l in a neonate aged less than 1 month

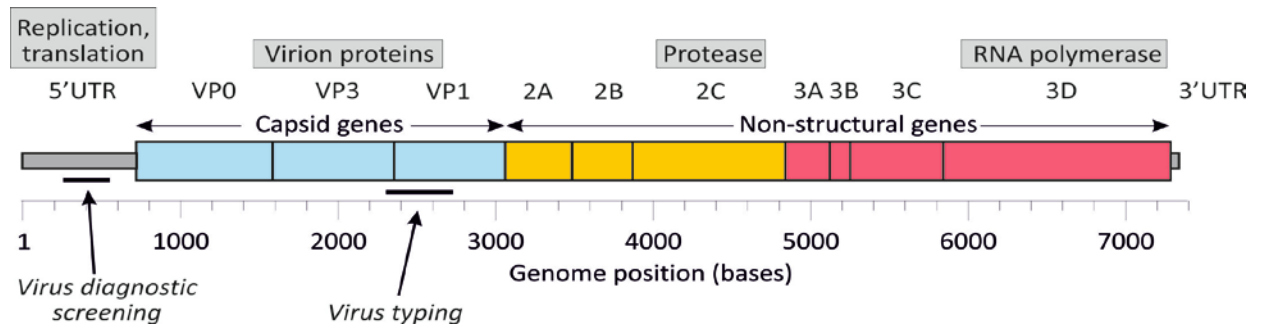


Figure 1: Diagrammatic view of the parechovirus genome