The 3-dimensional play of human parechovirus infection; Cell, virus and antibody

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Summary and General Discussion
Summary

HPeVs are widespread pathogens, infecting mainly young children worldwide. Transmission of HPeVs seems to occur via the fecal-oral route and most HPeV infections are mild, associated with respiratory tract infection and gastro-enteritis. HPeV infections are linked to a variety of severe diseases such as sepsis-like illness, myocarditis, encephalitis, meningitis and paralysis, most pronouncedly caused by the genotype HPeV3. Ever since HPeV3 was described for the first time in 2004, several reports showed association of HPeV3 with severe disease, and HPeV3 being the predominant type detected in CSF, showing the importance of HPeVs in CNS infections. Additionally children infected with HPeV3 are significantly younger than HPeV1 infected children. Not only with respect to clinical features but also with respect to biological features HPeV3 differs from the other genotypes known. HPeV3 is more difficult to propagate in cell culture, and HPeV3 lacks the arginine-glycine-aspartic acid (RGD) motif, which has been shown to be critical for integrin binding of HPeV1, and is an important antigenic site of HPeV1. In this thesis the differences shown between HPeV3 and the other genotypes is further investigated.

HPeV cell tropism

Several studies showed difficulties with HPeV detection in cell culture, because of only a low induction of cytopathic effect (CPE). In Chapter 2 we showed the different replication kinetics of the culturable HPeV genotypes 1 to 6 on a set of commonly used cell lines measuring viral copies/pcr with real time PCR. Replication monitored by PCR showed that growth of HPeV genotypes 1 to 6 was supported by most of the cell lines tested. By comparing replication kinetics, measured by PCR, and the CPE, we showed that viral replication could be measured before CPE appeared in the infected cell line, while sometimes CPE did not occur at all. In three of the nine cell lines (Vero, RD99 and A549) all six prototypes could be propagated. All HPeV1 to 6 genotypes showed replication with high viral titers on the RD99 cell line, but CPE was hardly seen. The combination of HT29 and Vero cells is suitable to detect all culturable HPeV types by CPE, where viral replication could be detected within 3 days. HPeV3 was shown to replicate on all cell lines except HT29.

For several EV genotypes, differences in disease manifestations and severity have been related to differences in cell tropism. In Chapter 3 we explored this for HPeVs by comparing the replication kinetics of HPeV1 and HPeV3 strains isolated from patients with known clinical symptoms in different continuous cells lines. Using real time PCR to detect replication, the HPeV3 strains showed better replication kinetics on the neural cell line SH-SY-5Y. Virus strains isolated from 3 out of 4 patients with CNS involvement showed better replication on the neural cell line SH-SY-5Y, reaching up to 3-fold higher viral titers,
than viruses from patients without CNS involvement. However this association could not be shown for the HPeV1 strains isolated from patients with severe symptoms. Additionally we could not link the strains isolated from patients with mild respiratory or gastrointestinal symptoms to replication in the lung carcinoma (A549) or in the colon carcinoma cell lines (HT29 and Caco-2). All the cell lines used in the previous studies are continuously growing cell lines obtained from tumours that do not necessarily represent the tissue from which they originated. In Chapter 4 the Human Airway Epithelial cells (HAE) was introduced as a primary cell culture system for HPeVs. After infection with the different HPeV1-6 genotypes no recognizable CPE was visible in the HAE cells. Efficient replication was shown for HPeV1, 2 and 3, while the replication of HPeV4, 5 and 6 was very low or absent. HAE cells isolated from different donors were infected with HPeV1 and 3 and in four out of five donors efficient viral replication could be shown, with faster replication of HPeV3. For the first time we were able to propagate two of the more recently discovered HPeVs, namely HPeV9 and HPeV14 which both lack the RGD motif. Therefore the HAE cell culture system provides a good tool to further study tropism and pathogenesis of the different HPeVs, including the previously unculturable RGD-less HPeVs.

**HPeV Ab neutralization**

We hypothesized that the age difference between children infected with HPeV1 and HPeV3 could be due to lack of the maternal Ab protection against HPeV3 in the first 6 months of life. High seroprevalence have been described for HPeV1 and 2, however, seroprevalence data on HPeV3 in the adult population are only available from Japan. Therefore we conducted a seroprevalence study in Chapter 5. Here we described the nAb positivity against HPeV1-6 genotypes in 554 sera obtained from Finnish and Dutch individuals. High nAb positivity was found for HPeV1 and HPeV2 (86%-99%) in the total adult study population in both countries. The high nAb positivity against HPeV2 (86% and 95%) is difficult to comprehend because HPeV2 viruses have been detected very rarely over the last decades in Finland and the Netherlands. We speculate that the high HPeV2 seropositivity could indicate HPeV cross-neutralization and thus cross-protection among the different genotypes. Chapter 5 also described the first seroprevalence data on HPeV4-6. Seroprevalence of HPeV4 was similar in both populations (60% in Finland and 62% in the Netherlands) whereas the seroprevalence of HPeV5 (75% vs. 35%; p<0.001) and HPeV6 (74% vs. 57%; p=0.04) was significantly higher in the Dutch adult population compared to the Finnish, which is in line with the prevalence of the viruses found in these countries. As expected the Ab positivity increases with age; for HPeV1 more than half of the children with age up to 5 years were already HPeV1 nAb positive. In contrast, we observed a very low nAb positivity against HPeV3, 4% in Finland and 8% in the Netherlands. This is seemingly in discordance with the high frequency of HPeV3 detection in both countries. One explanation could be a
very recent introduction of HPeV3 in the population. However, in Chapter 2 we already showed that the polyclonal Ab against HPeV3 we obtained from Japan failed as well to neutralize HPeV3 infection in vitro, while neutralization of HPeV1, 2, 4, 5 was efficient with their respective polyclonal Ab. And in Chapter 3, IVIg (IgGs from >1000 donors) manufactured between 2005-2010 was shown to contain high neutralizing Ab titers against HPeV1, but hardly neutralized HPeV3 strains. Moreover, very low neutralizing Ab titers were found in serum of two HPeV3-infected donors at time of infection as well as one year post infection, while high neutralizing Ab titers against HPeV1 could be detected in these donors.

To further investigate virus neutralization, in Chapter 6 we generated a polyclonal rabbit IgG mixture by rabbit immunization with HPeV1, as well as two HPeV1 specific monoclonal Abs, AM18 and AM28. The mAbs were generated by screening culture supernatants of antibody producing B cell cultures obtained from adult donors for direct neutralization of HPeV1. Both polyclonal and monoclonal Abs showed specific HPeV1 neutralization, but also neutralization of HPeV2. In addition, HPeV1 specific mAb AM18 showed cross-neutralization against HPeV4, 5 and 6, and even against the HEV CAV9. This Ab cross-reactivity seems to be restricted to strains containing the RGD motif at the C-terminus of the capsid protein VP1. A VP1 specific ELISA confirmed that the AM18 indeed bound the capsid protein of HPeV1, 2 and 4. In contrast, the HPeV1 specific mAb AM28, which neutralized HPeV1 much more efficiently than AM18, showed no cross-reactivity with the other HPeVs or HEVs and did not react with any of the capsid proteins, suggesting a non-linear epitope for this Ab. To identify the non-linear epitope for mAb AM28, Cryo-EM studies were performed on HPeV1 viral particles, labelled with AM28-Fabs (Chapter 7). We showed that the AM28 Fab indeed did not overlap with the integrin binding site, which is the binding site for AM18. The antibody footprint was found to be across two protomers from adjacent pentamers in the capsid recognizing both VP0 and VP3. The conformation recognized by AM28 requires two adjacent protomers from two different pentamers, one contributing VP0, the other contributing VP3. Three-dimensional reconstruction and pseudo-atomic model fitting that AM28 recognizes quaternary epitopes on the capsid composed of VP0 and VP3 loops from neighbouring pentamers. Reconstruction suggests that the AM28 Ab staples the neighbouring pentamers together thereby stabilizing the whole capsid, preventing uncoating. In Chapter 8 the generation and characterization of aHPeV3 Abs is described. A rabbit polyclonal aHPeV3-HARLAN Ab and the three different purified mAbs AM15, AM17 and AM18 were generated with the same techniques as in Chapter 7. The B cell cultures for generation of HPeV3 mAbs were isolated from healthy donors, who experienced a HPeV3 infection in the past, and specificity for HPeV3 was tested by capacity to bind HPeV3. The polyclonal Ab was able to bind all 6 genotypes, but
specifically neutralized HPeV3 although at very high concentrations of Abs. The mAbs all showed specific binding to HPeV3, but showed no binding to the different capsid proteins, indicating a non-linear epitope. None of the three mAbs could neutralize HPeV3. Structural studies can give good insights into the differences in receptor usage and Ab binding of HPeV1 and HPeV3. This will lead us to a better understanding of the pathogenic difference between type 3 and other HPeV. With cryo-EM we resolved a high resolution of HPeV3 shown in Chapter 9. The HPeV3 structure has features similar to enteroviruses such as the presence of canyon around the 5-fold vertices and annulus below the vertices. Different from HPeV1, the HPeV3 viral particle has an open channel at the 5-fold vertices. By fitting homology models of HPeV3 into the EM density we have identified loops, which could be utilized as epitopes to generate therapeutic antibodies against the HPeV3 capsid.
General discussion

Human Parechovirus infection: bowel or brain, that’s the question?

HPeVs have been shown to be important pathogens for humans throughout the world. HPeV1 is the most frequently detected genotype of all EVs and HPeVs in stool samples, and seem to be more often associated with mild gastro-intestinal symptoms compared to EVs. HPeV3 was even identified as the most common picornavirus type detected in CSF in the UK (1). The transmission route of HPeVs is supposed to be fecal-oral, and the main site of replication for HPeVs is thought to be the gut. The route to CNS infection remains unknown. Two essential factors in invasion of the CNS, and thus severe disease, are cell tropism and the immune response against HPeVs. Previous studies showed that HPeV3 differs from the other genotypes and is significantly more associated with severe symptoms (2–8). HPeV3 was thought to be only clinical relevant in infants, whereas more recent reports showed symptomatic HPeV3 outbreaks among adults in Japan (9). This knowledge indicates a great need for antiviral treatment, as there is no treatment against HPeVs available at the moment. In our studies we show the importance of cell tropism and Ab protection in the pathogenesis of HPeV infection.

HPeV cell tropism, implications for pathogenesis

The differences in pathogenesis between HPeV3 and the other genotypes could be explained by different receptor use and thus different cell tropism. For HPeV1 it has been shown that it utilizes the vibronectin αvβ3 and αvβ6 integrins as its receptors, via the RGD motif (10–12). The receptor and the receptor binding motif for HPeV3 remains unknown. Given the lack of the RGD motif in HPeV3, it is likely that HPeV3 uses a different receptor than HPeV1. Comparing the different growth characteristics of HPeV1-6 and different HPeV1 and HPeV3 strains isolated from patients with known clinical outcome, we could not show a clear relation between pathogenicity of HPeVs in different standard cell lines tested. We showed that 3 out of 4 virus strains isolated from patients with CNS involvement showed better replication on the neural cell line SH-SY-5Y, reaching up to 3-fold higher viral titres, than viruses from patients without CNS involvement. But for HPeV1 a correlation between in vitro replication dynamics and disease severity could not be found (Chapter 3). Additionally we could not link the strains isolated from patients with mild symptoms to replication in the lung carcinoma (A549) nor in the colon carcinoma cell lines (HT29 and Caco-2). For CAV9 and E9 a relationship between clinical manifestations and growth characteristics in vitro has been suggested, which could even be linked to the presence of the RGD motif. For CAV9 and E9 it has been shown in mice, that lack of the RGD motif was related to a lower pathogenicity (13–15). However, not all E9 strains isolated from patients with paralysis were able to cause paralysis in mice, showing the same inconsistencies as in
our studies. (14). This link between higher pathogenicity and presence of the RGD motif does not seem to be the case for HPeVs, where the RGD-lacking HPeV3 is significantly more associated with severe symptoms compared to the other genotypes. Possibly HPeV3 uses a different RGD-independent pathway than shown for CAV9 and E9. Previous studies show that the RGD-lacking CAV9 was not able to infect the A549 cell line, which can be efficiently infected by HPeV3 (16, Chapter 2).

Primary cell culture systems in which cells are used representing the tissue exposed to the viral infection provide a good alternative for pathogenicity studies. In this thesis we introduced the human airway epithelial as a primary cell culture system for HPeVs, as transmission of HPeVs is supposed to be oral-fecal, where the respiratory epithelium is the initial tissue exposed to HPeVs. We showed efficient replication of HPeV3 and we were the first to propagate HPeV9 and HPeV14, all three lacking the RGD motif. The replication of HPeVs in HAE suggests an important role of the respiratory epithelium in HPeV infections. For EV71 pathogenesis studies in rhesus monkeys different routes of infection were studied. Monkeys infected via the respiratory route showed more severe lesions in the CNS compared to monkeys infected via the digestive route (17). This route of infection in severe infections could also be the case for HPeV3, supported by the efficient replication in the HAE cells. In addition, the yet unidentified RGD-independent receptor should be expressed on the respiratory epithelium. Therefore, HAE provides a new tool for characterization of receptors for the RGD-lacking HPeVs. We found one donor in which replication of HPeV1 and HPeV3 was not detected (Chapter 4), indicating the importance of donor screening and selection for further pathogenesis and receptor studies. This lack of replication could be because of the missing (co-) receptor for viral binding or entry, and thus comparison of the different donors using micro-array could lead to identification of the unknown HPeV receptor in healthy human tissue.

The mechanism of entry of HPeVs remains unclear. Integrins are an important receptor for HPeV1 cell attachment as well for E1, CAV9 and E9. For these HEVs the integrins serve as a primary receptor, as co-receptors are needed for capsid alterations and thus infectious entry (16, 18–23). The usage of co-receptors and the internalization of virus is a complex phenomenon wherein a virus may use different mechanisms to enter different cell types. Possibly HPeV1 uses, next to the integrins as their primary receptor, one or more co-receptor(s) for entry into the cells. HPeV1 infection can be fully inhibited by blocking of the RGD motif, indicating that the RGD-integrin binding is essential for binding, but not necessarily also for entry. The first and only study on HPeV1 entry suggested the role of other molecules involved in entry, due to the lack of co-localization of HPeV1 and the integrin subunits during the entry process (11). In case of CAV9 the primary interaction
with the αvβ3 integrin is followed by utilizing the major histocompatibility complex class I (MHC-I), which is involved in the internalization of the virus. One subunit of the MHC-1 complex is the β2-microglobulin (β2M), used by CAV9 (12), as well as by E11 (24) and EV7 (25). Many HEVs use decay accelerating factor (DAF or CD55) as a cell surface receptor; EV7, EV70, CBV3, CBV5, and CAV21 (26–29), where CVB3 requires CAR as co-receptor, and CAV21 requires the intracellular adhesion molecule ICAM-1 for cell entry (28), both members of the Ig superfamily. In case of human rhinoviruses A and B, ICAM-1 seems to be to functioning as a single receptor involved in binding and entry (reviewed in (30)). As several HEVs use similar (entry) receptors, this knowledge provides a tool for the identification of (co-) receptors for HPeVs as they might use common receptors, by blocking the known receptors with Abs or peptides.

Lack of direct HPeV3 Ab neutralization

For most HEVs it has been shown that antibodies play a major role in controlling viral spread and thus disease severity. Patients with a deficient humoral immune response, such as X-linked agammaglobulinemia, are at great risk of chronic enteroviral infections (31) where prolonged viral replication was shown (32, 33). In neonates it has been shown that the lack of maternal antibodies is a risk for severe disease (34). In our studies we could not detect HPeV3 protective antibody titers in two adult donors during and one year after infection. In addition, IVIG manufactured between 2005-2010 contained high neutralizing Ab titres against HPeV1, but could hardly neutralize HPeV3 strains. In contrast to our data on low HPeV3 seroprevalence in Finland and the Netherlands, Japan is the only country that reported high seroprevalence for HPeV3; this could be due to different methods of scoring Ab neutralization. In our studies we did see some inhibition of CPE in our serum neutralization assays (chapter 3), but full neutralization as seen for the other genotypes was absent. Scoring for full neutralization will lead to lower seroprevalence numbers, compared to scoring on inhibition. Although if Ab neutralization had been scored by 50% inhibition, this would still have lead too much lower (non-protective) titers for HPeV3 Abs compared to Ab titers found against the other genotypes in our studies. To further study neutralization of HPeV3 in vitro we isolated three different HPeV3 mAbs. These Abs were specific in HPeV3 viral binding, but we could still not detect efficient neutralization of viral infection. Taken together, all this seems to indicate that Ab protection against HPeV3 might fail, even in the presence of specific aHPeV3 Abs. This possibly indicates that the Abs are not able to efficiently reach the neutralizing epitope and thus cannot interfere with receptor binding. A highly speculative theory to explain this is the membrane hijacking recently shown for the picornavirus Hepatitis A (HAV) (35). HAV released from hepatocytes is cloaked in host cell derived membranes, thereby protecting the virions against antibody-mediated neutralization, while they are fully infectious. These enveloped HAV particles were only
found in plasma and not in faeces indicating that this membrane hijacking is cell type dependent. HAV replication is well established in the liver, indicating that the production of these enveloped viruses only occurs in the liver, from where the virus can spread via the blood protected from antibody neutralization. During viral replication in the intestine these enveloped viruses seem not to be produced as the virus isolation from faeces only reveals non-enveloped viruses. To further investigate this phenomenon the viral supernatants from different (primary) cell lines including our HAE, need to be tested for virus presence in fractions of a gradient loaded with the supernatants, as the enveloped virus particles will appear in a different fraction based on size segregation. During our Cryo-EM studies we never detected any of these envelopes, which is possibly due to the purification protocol obtaining large amounts of high concentrated HPeV3, maybe losing or disrupting the membrane-containing viral particles. A second hypothesis to explain neutralization failure is that the Abs are not able to neutralize viral infection in *in vitro* experiments. For FMDV it has been shown that mAbs against conformational epitopes passively protect neonatal syngeneic (BALB/c) mice at dilutions that could not neutralize virus infection *in vitro*. This indicated that opsonisation and subsequent phagocytosis of the Ab-virus complex play a major role in the immune response against FMDV (36). Possibly this antiviral effect of Abs is as well the case for the immune response against HPeV3, where virus-Ab complexes activate complement proteins that bind to the virus, leading to opsonisation by phagocytes containing receptors for complement. Another possible mechanism could be antibody dependent cell-mediated cytotoxicity (ADCC). The antibody binds membrane surface antigens of the infected cells, inducing cell lysis mediated by natural killer cells or macrophages. Whether these Abs are able to protect against HPeV3 infection *in vivo*, animal models are needed for further research. It has been shown that the availability for animal models for HPeV infection seems to be limited. Newborn mice inoculated with HPeV1 and HPeV2 were only infrequently infected, and cynomolgus monkeys showed no neuropathological changes after 30 days of observation (37). However, HPeVs were detected in feces of monkeys with diarrhea (38), which suggests that they could serve as an infectious model for studying Ab protection of HPeV3.

The isolation of the highly neutralizing Abs against HPeV1 (AM18, AM28) does show the importance of direct neutralization of infection by the immune system for HPeVs. Both mAbs showed high cross-neutralization with HPeV2. This cross-neutralization indicates that HPeV1 and 2 share similar neutralizing epitopes, as discussed in chapter 7. The AM18 Ab was cross neutralizing HPeV2, 4, 5 and 6 infections, but not with HPeV3. We even detected cross reactivity between CAV9 and HPeV1 with the AM18 mAb, a feature unexpected in a single monoclonal antibody against a human picornavirus. This observed cross-reactivity of two monoclonal Abs gives a good perspective for the development of
cross-protective anti-HPeV treatment (as discussed below). This cross-reactivity of Abs against HPeV1 with HPeV2 could nicely explain why high seroprevalence against HPeV2 is found in the Netherlands and Finland (86-99%) (Westerhuis et al., 2013), where HPeV2 infections are hardly detected (39–41). Mapping the neutralizing epitopes of these Abs revealed that the AM18 was inhibiting viral infection directly by binding the integrin RGD motif, again showing the importance of the RGD motif in HPeV1 viral infection (Chapter 7). The AM28 did not show inhibition of viral infection by direct interaction with the RGD. Reconstruction suggests that the AM28 Ab staples the neighbouring pentamers together, thereby stabilizing the whole capsid. We showed that the HPeV1 labelled with AM28 raises the temperature for capsid destabilization, preventing uncoating of RNA from the capsid on cell entry. We identified several contributions from both VP0 and VP3 involved in the Fab binding by homology modelling. To further look into contribution of the specific residues in the different loops in vitro responsible for the neutralization, alanine scanning methods needs to be performed.

**HPeV infection; the need for treatment**

In the previous years the clinical importance of HPeVs has been revealed. Currently fast diagnostics are available and the implementation of HPeV viral diagnostics is increasing among laboratories. Despite the fact that HPeV infections can be severe and life threatening, no antiviral treatment is available. The groups most at risk for severe HEV and HPeV infections are neonates as their immune response is not fully developed, and patients with a humoral immune deficiency (31, 34, 42, 43). In neonates an option for supportive treatment is the use of intravenous immunoglobulins (IVIg), while in patients with a humoral immune deficiency IVIg is already used as a replacement therapy. A pitfall of this treatment is that IVIg remains unspecific (31) and Igs against newly introduced or rare pathogens are not covered. We showed that the IVIg batches contained only a very low concentration of neutralizing Abs against HPeV3 in vitro, possibly resulting in failure of treatment. An option for antiviral treatment of HEV and HPeVs would be the use of specific monoclonal Abs. HPeV is a good candidate for development of mAbs due to the restricted group of genotypes. In this thesis we describe two good candidates for Ab treatment of HPeV1 infections (Chapter 6 and 7). One candidate even gives cross-neutralization to the other RGD containing genotypes. Unfortunately, the three mAbs specifically binding HPeV3 showed no in vitro neutralization of infectivity. Although we found some viral inhibition of HPeV3 infection, revealing that possibly nAbs against HPeV3 do exist, leaving a great challenge finding these Abs.

Although the different antigenic sites and the observed cross reactivity makes HPeVs a good target for the development of therapeutic human Abs, the lack of (cross-) neutralization of HPeV3 might be a pitfall. Next to Ab therapy other options would be capsid inhibitors
or replication inhibitors. A promising drug in HEV treatment was the capsid inhibitor pleconaril, showing clinical activity in some patient groups (reviewed in (44–46). However, even when the compound for some reason would return to the market, it will not be beneficial in the treatment of HPeVs since our group showed that HPeV1 and HPeV3 were resistant against pleconaril in vitro (47). Compounds with antiviral activity against HEV will not automatically possess antiviral activity against HPeVs. Although the genome structure looks similar to that from HEVs, the genome variety of HPeVs is extensively different. During the maturation cleavage of VP0 into VP4 and VP2 does not occur in HPeVs. Secondly, the viral capsid of HPeVs are shown to be much more smoother compared to HEVs ((12), Chapter 7, 9), containing different features of the capsid compared to HEVs. Therefore, capsid inhibitors against HEV will probably not inhibit HPeVs. Next to capsid inhibitors a wide range of processes in the viral life cycle are potential targets in the development of antiviral treatment. However in the non-structural proteins again differences between HEVs and HPeVs are shown. In the case of HPeVs 2A lacks the proteolytic activity seen in other picornaviruses; furthermore it seems that only one protease, 3Cpro, is involved in processing (48–50). Phylogenetic analyses of the 3Dpol region showed only a low degree of identity of HPeVs compared to other picornaviruses (48). For HEVs several compounds are describes showing broad picornaviral activity (46), although these compounds never have been tested for antiviral activity against HPeVs. Given the differences between HEVs and HPeVs it remains to be seen whether these compounds show antiviral activity against HPeVs.

In conclusion

HPeVs are among the most frequently detected picornaviruses in the world, and are nowadays accepted as an important viral agent causing severe CNS symptoms in neonates. These severe infections are most pronounced for HPeV3. In this thesis we showed that the lack of direct neutralization might play a role in these severe infection. Thereby we showed that the respiratory epithelium might play an important role in HPeV3 infection and entry. The first step towards more answers is the identification of the receptor binding epitopes and the receptor for HPeV3. We introduced a primary respiratory cell culture tool for HPeV infection expressing the RGD independent receptor for HPeVs, as culturing of the RGD-lacking HPeV types was successful. Together with blocking infection with viral HPeV3 peptides, available microarray techniques, and cryo-EM techniques, receptor identification should be feasible.

The development of cross-neutralizing HPeV mAbs displaying high neutralizing titers against several HPeVs shows promising results for monoclonal Ab based therapy, although this approach might not yet work against HPeV3 for which no highly neutralizing Abs
could be identified. This underlines the need for elucidating epitopes for binding and neutralization of HPeV3, as well as for further investment in developing anti-picornaviral therapy with broad antiviral activity, including activity against HPeVs.
References


