Chapter 1

General introduction


The Picornaviridae

Picornaviruses are among the most prevalent viruses in humans and animals. In humans they are able to cause a wide variety of disease ranging from the common cold to life threatening infections like myocarditis and meningoencephalitis.

Picornaviruses are small, non-enveloped single-stranded RNA viruses. The Picornaviridae family nowadays consists of 26 genera of which 7 genera are known to infect humans: Enterovirus, Parechovirus, Hepatovirus, Cardiovirus, Cosavirus, Kobuvirus and Salivirus.\(^1\) Enteroviruses, hepatoviruses and parechoviruses are the most prevalent and clinically relevant picornaviruses in humans. The Hepatovirus genus consists of only one species and one serotype that can infect humans (hepatitis A virus (HAV)), giving symptoms of (self-limiting) acute hepatitis. Vaccination with an inactivated vaccine is highly efficacious in preventing clinical disease. In addition, passive immunisation with HAV specific immunoglobulins is available for young children and as post-exposure prophylaxis.\(^2\)

The Enterovirus genus consists of several human species (human enterovirus (EV) A-D, human rhinovirus (HRV) A-C) and multiple (sero)types.\(^1\) The disease spectrum varies widely from asymptomatic or mild disease to severe infections like meningoencephalitis and myocarditis. Most infections are self-limiting, but in the case of severe infection, treatment options are very limited since there is currently no effective anti-enteroviral drug available. Except for poliovirus (species EV-C) no vaccine is available. The Parechovirus genus contains two species: Ljungan virus and human parechovirus (HPeV). Ljungan virus consists of 4 serotypes and was first detected in bank voles.\(^3\) Ljungan virus is also frequently seen in rodents.\(^4\) Although a relation with disease in foetuses and infants was suggested,\(^5,6\) this remains controversial and has never been proven.\(^7\) The HPeV species is only found in primates and now consists of 16 types.\(^1\) The disease spectrum is similar to that of EV infections, although HPeV infection is almost exclusively seen in young children. No antiviral drugs or vaccines are available.

Genome structure of enterovirus and parechovirus

Both EV and HPeV have a positive sense, single-stranded RNA genome consisting of around 7400 nucleotides (Figure 1).\(^8\) A 5’untranslated region (5’UTR) of ~700 nucleotides precedes the single open reading frame of ~6600 nucleotides encoding a single polyprotein. This is followed by a small 3’UTR of 70-80 nucleotides and a poly(A)tail. The polyprotein consists of three regions (P1-P3). P1 encodes the structural region and is cleaved in the viral capsid proteins VP0, VP1 and VP3. The P2 and P3 regions encode the non-structural proteins 2A-C and 3A-D, which are involved in replication and host-cell interaction functions. In EVs the VP0 capsid protein is cleaved into VP4 and VP2 during maturation, resulting in 4 structural proteins. In HPeVs the VP0 capsid protein is not cleaved, resulting in only 3 structural proteins. The VP proteins form an icosahedral capsid of ~30 nm (Figure 1).
Disease spectrum and classification

Enterovirus

One of the most well-known human EVs is poliovirus (3 types), which was discovered in the 1940s (reviewed in Melnick et al.⁹). Subsequently the species Coxsackievirus (CV), later divided in CV-A and CV-B, was described for the first time in 1948.¹⁰ With newer techniques the species Enteric Cytopathogenetic Human Orphan (ECHO) virus was added. Since 1974 newly identified human EVs were no longer classified in the above-mentioned species, but were assigned a number.¹¹ With the development and widespread use of molecular techniques, the old classification was not adequate anymore and types were reclassified into the species A-D (Table 1).¹

<table>
<thead>
<tr>
<th>Enterovirus A</th>
<th>Enterovirus B</th>
<th>Enterovirus C</th>
<th>Enterovirus D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poliovirus</td>
<td>1-3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coxsackie A virus</td>
<td>2-8, 10, 12, 14, 16</td>
<td>9</td>
<td>1, 11, 13, 17, 19-22, 24</td>
</tr>
<tr>
<td>Coxsackie B virus</td>
<td>1-6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ECHO virus</td>
<td>1-7, 9, 11-21, 24-27, 29-33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enterovirus</td>
<td>71, 76, 89-91, 114, 119-121</td>
<td>69, 73-75, 77-88, 93, 97, 98, 100, 101, 106, 107, 111</td>
<td>95, 96, 99, 102, 104, 105, 109, 113, 116-118</td>
</tr>
</tbody>
</table>
Poliovirus has been associated with large outbreaks of acute flaccid paralysis with an impressive morbidity and mortality in especially children. It is the only human EV against which an effective vaccine has been available since the 1950s, resulting in an almost worldwide eradication of poliovirus. However, despite the efforts of the Global Polio Eradication Initiative that was started in 1988 by the World Health Assembly, with the aim to eradicate polio using the live attenuated oral polio vaccine (OPV), polio is still endemic in 3 countries (Afghanistan, Nigeria and Pakistan). In addition to local distribution problems related to political instability and suspicion of the local population about the purpose of vaccination, the emergence of vaccine-associated paralytic poliomyelitis as a result of genetic reversion to neurovirulent strains is another challenge. Prolonged circulation of circulating vaccine-derived polioviruses (cVDPVs) in areas with low vaccination coverage together with prolonged shedding of vaccine derived poliovirus in people with an impaired humoral immunity (immune-deficiency related vaccine-derived polioviruses (iVDPVs)) are other obstacles making global polio eradication more difficult to achieve. This was the reason for the World Health Organisation together with the Centers for Disease Control and Prevention to recommend a role for anti-polio viral agents in the combat against poliomyelitis. These agents (preferably two agents administered simultaneously at least to prevent emergence of resistance) can be used to treat cases of acute poliomyelitis, to eradicate persistent shedding and circulation of cVDPVs and iVDPVs, and can be used in outbreaks as prophylaxis of exposed individuals.

The non-polio EVs consist of more than 100 types causing a wide range of symptoms, from asymptomatic to mild respiratory and/or gastrointestinal infection, and more severe disease such as hand, foot and mouth disease (HFMD), meningitis, encephalitis, acute flaccid paralysis, pericarditis, myocarditis, hepatitis, pleurodynia and neonatal disseminated EV infection (reviewed in Tapparel et al.). Although the different serotypes can overlap in the spectrum of disease, specific types can be related to specific disease. For example, CV-B is often associated with myopericarditis; echoviruses and CV-B are associated with meningitis and CV-A frequently causes HFMD. The recently emerged EV71 mainly causes self-limiting HFMD, but may progress to severe neurologic disease like acute flaccid paralysis and brainstem encephalitis with cardiorespiratory dysfunction. Central nervous system (CNS) complications typically occur in (young) children. Since the late 1990s several outbreaks of massive EV71 infections with brainstem encephalitis and associated pulmonary edema caused hundreds of deaths in the Asian Pacific region (reviewed in Ooi et al.). This has led to major efforts to find anti-enteroviral drugs and/or an effective vaccine, since neither were available. Recently, a phase III clinical trial with an inactivated (alum adjuvated) EV71 vaccine was conducted in China with promising results.

HRVs were first discovered in the 1950s as cause of the common cold. HRVs differ from the other EVs and HPeVs because they do not survive in an acid environment like the gastric acid fluids. Their main site of infection and replication is therefore not the gastrointestinal tract, but the respiratory tract. HRV-A and -B consist of respectively 74 and 25 types (Figure
2). HRV-C has only recently been discovered by molecular techniques as they could not be cultured within the standard cell culture settings.\textsuperscript{21} Nowadays at least 50 types are identified (Figure 2).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{hrv_tree.png}
\caption{Phylogenetic tree of human rhinovirus types. Reprinted from Knipe DM, Howley PM (ed), Fields virology, 6th ed. Wolters Kluwer Health/Lippincott Williams & Wilkins, Philadelphia, PA with permission from Wolthers Kluwer Health/Lippincott Williams & Wilkins.}
\end{figure}
HRV is the most common cause of upper respiratory tract infections (URTIs), found in more than half of the episodes of URTI.22 Recently HRVs are identified as the second most common cause of bronchiolitis in hospitalized young children 23 and are associated with severe lower respiratory tract infections (LRTIs) in children admitted to the intensive care unit.24 In addition, HRV-associated wheezing in the first 3 years of life is a risk factor for the development of asthma at the age of 6 years in a high risk cohort.25 Several studies reported associations between HRV-C and asthma exacerbations,26-28 lower respiratory tract infections29 and more severe disease,30 although other studies did not detect more severe disease in HRV-C infected children.31,32 Moreover, asymptomatic HRV infections are also frequently seen in young children,33,34

**Human parechovirus**

Human parechoviruses were first discovered in 1956 during a summer diarrhea outbreak in the USA.35 They were originally classified within the *Enterovirus* genus as ECHO virus 22 and 23. This was based on their biology in cell culture, exhibiting a similar cytopathogenic effect (CPE) as EVs, and their clinical presentation. With the introduction of molecular techniques, these viruses were reclassified as HPeV types 1 and 2 within the new genus *Parechovirus*.36,37 Almost half a century after the discovery of HPeV1 and 2, a third HPeV type was discovered in Japan38 and since then the number of HPeV types increased rapidly. Up to date there are 16 HPeV types known (Table 2).1

<table>
<thead>
<tr>
<th>Type</th>
<th>Strain</th>
<th>Origin</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPeV1A</td>
<td>Harris</td>
<td>Ohio, USA</td>
<td>Hyypia <em>et al.</em>, 1992</td>
</tr>
<tr>
<td>HPeV1B</td>
<td>BNI-788 St</td>
<td>Bonn, Germany</td>
<td>Baumgarte <em>et al.</em>, 2008</td>
</tr>
<tr>
<td>HPeV 2</td>
<td>Williamson</td>
<td>Ohio, USA</td>
<td>Ghazi <em>et al.</em>, 1998</td>
</tr>
<tr>
<td>HPeV 3</td>
<td>A308/99</td>
<td>Aichi, Japan</td>
<td>Ito <em>et al.</em>, 2004</td>
</tr>
<tr>
<td>HPeV 4</td>
<td>K251176-02</td>
<td>Amsterdam, the Netherlands</td>
<td>Benschop <em>et al.</em>, 2006</td>
</tr>
<tr>
<td>HPeV 5</td>
<td>CT86-6760</td>
<td>Connecticut, USA</td>
<td>Oberste <em>et al.</em>, 1998</td>
</tr>
<tr>
<td>HPeV 6</td>
<td>NI561-2000</td>
<td>Niigata, Japan</td>
<td>Watanabe <em>et al.</em>, 2007</td>
</tr>
<tr>
<td>HPeV 7</td>
<td>PAK5045</td>
<td>Badin, Pakistan</td>
<td>Li <em>et al.</em>, 2009</td>
</tr>
<tr>
<td>HPeV 8</td>
<td>BR/217/2006</td>
<td>Salvador, Brazil</td>
<td>Drexler <em>et al.</em>, 2009</td>
</tr>
<tr>
<td>HPeV 10</td>
<td>BAN2004-10903</td>
<td>Bangkok, Thailand</td>
<td>Oberste <em>et al.</em>, unpub.</td>
</tr>
<tr>
<td>HPeV 12</td>
<td>BAN2004-10904</td>
<td>Bangkok, Thailand</td>
<td>Oberste <em>et al.</em>, unpub.</td>
</tr>
<tr>
<td>HPeV 14</td>
<td>451564</td>
<td>Amsterdam, the Netherlands</td>
<td>Benschop <em>et al.</em>, 2008</td>
</tr>
<tr>
<td>HPeV 15</td>
<td>BAN-11614</td>
<td>Bangkok, Thailand</td>
<td>Oberste <em>et al.</em>, unpub.</td>
</tr>
<tr>
<td>HPeV 16</td>
<td>BAN-11615</td>
<td>Bangkok, Thailand</td>
<td>Oberste <em>et al.</em>, unpub.</td>
</tr>
</tbody>
</table>
Clinical symptoms of HPeV infections are generally similar to EV infections, ranging from mild respiratory and gastrointestinal disease to more severe disease like meningitis and sepsis-like illness (SLI). In earlier decades, when only HPeV1 and 2 were known, HPeV infections were considered of little clinical importance, even though occasionally severe disease was reported for HPeV such as acute flaccid paralysis, myocarditis, meningitis, encephalitis and encephalomyelitis.\textsuperscript{48-52}

This perception of clinical presentations of HPeVs changed with the discovery of HPeV3.\textsuperscript{38} HPeV3 infections were predominantly associated with neonatal sepsis and CNS infection (meningitis and encephalitis).\textsuperscript{53-62} HPeV3 CNS infections account for approximately 3-17\% of cases of meningitis or encephalitis in young children under 3 months of age,\textsuperscript{44,54,63-66} ranking HPeV as the second dominant pathogen (after EV) of viral meningitis and encephalitis in young children.

HPeV1 is the most prevalent type, followed by HPeV3. HPeV4 is frequently found in stools,\textsuperscript{47,67-70} while HPeV6 seems to prevail as a secondary respiratory pathogen.\textsuperscript{71} Infections with HPeV2 and 5 are reported sporadically.\textsuperscript{57,67,72} HPeV4, 5 and 6 have mainly been associated with mild gastrointestinal and respiratory symptoms in children, often with an underlying illness,\textsuperscript{73} although recently 2 cases of neonatal sepsis caused by HPeV4 were described in Finland.\textsuperscript{74} Circulation patterns of the newly reported HPeV types 7–16 are yet to be determined.\textsuperscript{47,57,67}

While EVs generally affect individuals of all ages, more than 90\% of HPeV infections have been described in children younger than 5 years of age.\textsuperscript{40,47,67-81} Remarkably, the median age of children infected with HPeV3 is significantly lower than the median age of children infected with HPeV1,\textsuperscript{53} with the majority of HPeV3 infections occurring in neonates and children under the age of 2 months.\textsuperscript{44,47,54,58,61,63,65,82-84} This age difference in relation to the difference in disease severity between the two HPeV types suggests that neonates in comparison to older children might be less protected against HPeV3 infection. Most HPeV1 infections are presumed to occur within the first year of life, following the decline in circulating maternal antibodies. Seroprevalence data showed that 95-99\% of neonates were boosted with antibodies against HPeV1 which are most likely from maternal origin.\textsuperscript{85-89} This high HPeV1 seroprevalence suggests that the majority of infants are supposed to be protected from HPeV1 infection early in life via maternal antibodies. However, this may not always be the case as suggested by Ehrnst \textit{et al}.\textsuperscript{90} The HPeV1 seroprevalence decreases in the first 6 months of life, only to rapidly increase to 95\% in children older than 1-3 year.\textsuperscript{85,89} The low seropositivity from 6 months to 1-3 years is marked by an increase in infection frequencies among children in this age group.\textsuperscript{53,85,87-89}

For HPeV3, the seroprevalence is approximately 70\% among adults in Japan.\textsuperscript{38} The lowest seroprevalence rate (15\%) was seen in children between 7-12 months and steadily increased to 91\% in adolescence only to decline again to 56-87\% in adulthood. A recent study showed that seroprevalence among adults in Europe is only 10-13\%, and even lower in children (3\%).\textsuperscript{91} This is in contrast to what is seen for HPeV1 seroprevalence: >90\% of adults have
antibodies against HPeV.\textsuperscript{85,87,89,91} This may indicate that children are less protected through maternal antibodies specifically for HPeV3, explaining the young age and increased disease severity of HPeV3 infected children in comparison to HPeV1 infected children.

**Diagnosis**

Classically, HPeVs and EVs can be diagnosed through cell culture isolation, usually involving monkey kidney cells and human fibroblasts.\textsuperscript{67,92} Other cell lines, such as the HT-29 (human colon adenocarcinoma), A549 (human lung carcinoma) and RD (rhabdomyosarcoma) cell lines can be used for culturing HPeV isolates as well.\textsuperscript{44,67,75,93} However, cell culture has its limitations and CPE produced by HPeVs is not significantly different from the CPE elicited by EVs resulting in misidentification of HPeVs as EVs in the laboratory settings in which specific serotyping is not readily available.\textsuperscript{53} This also explains the original classification of HPeVs as EVs.\textsuperscript{35} HPeV types other than HPeV1 and 2 cannot be serotyped because specific antibodies are not readily available (HPeV3-6), or because they cannot be cultured at all (HPeV7-14). HRVs are difficult to culture as well and grow best in human fetal embryonic lung fibroblast cell lines and certain HeLa cell clones.\textsuperscript{94,95} CPE appearance is very similar to EVs and can be distinguished by acid stability testing; HRVs are destabilized in an environment with low pH such as the gut while EVs are relatively resistant (reviewed in Jacobs et al.\textsuperscript{95}).

In recent years polymerase chain reaction (PCR) became the state-of-art test to detect EVs in different patient materials. Most real-time RT-PCRs target the 5’UTR region, which is highly conserved among all EVs and HRVs. A problem is cross-reactivity between HRV and EV, making differentiation sometimes difficult.\textsuperscript{95} PCR specific for EVs will fail to detect HPeVs because the targeted 5’UTR is too diverse between HPeVs and EVs.\textsuperscript{53,96-99} Therefore a separate real-time RT-PCR specifically targeting the 5’UTR of HPeVs has been developed and validated for HPeV detection in CSF, blood, stool and respiratory samples.\textsuperscript{40,47,81,100-103} Genotyping is increasingly used instead of serotyping to differentiate between the different species and types. By targeting the variable capsid region VP1 or VP1/VP3, HPeV and EV positive samples can be genotyped directly from clinical material.\textsuperscript{54,58,67,104} For genotyping of rhinoviruses the VP1 and/or VP4/VP2 region are commonly used.\textsuperscript{95,105}

**Transmission**

While HRV is thought to be mainly spread from person to person by aerosols,\textsuperscript{106} the transmission route of EV and HPeV is usually fecal-oral through direct person to person contact or through ingestion of contaminated food or water (indirect transmission). Surface water can get contaminated with HPeV and EV easily because these viruses are shed in high amounts in stools and concentrations remain relatively high, even in treated sewage
water. In addition, these viruses are able to persist in the environment for several weeks to months.\textsuperscript{107,108} Various outbreaks of recreationally associated waterborne disease by EVs have been reported (especially in children), but these are probably only the tip of the iceberg (reviewed in Sinclair \textit{et al.}\textsuperscript{109}).

**Immune response**

Most of what we know from picornavirus immunity is distilled from immunological studies with EV infections. In contrast with most viruses against which T cell dependent immune responses are of importance, an efficient host response against picornaviruses is considered to be mainly dependent on a proper humoral immune response with release of neutralizing antibodies (nAbs). After contact with an antigen, B lymphocytes are activated to form plasma cells. Plasma cells will subsequently produce antibodies which will neutralize the antigen. Part of the B cells will transform into memory cells. These memory cells can react quickly and release antibodies if the antigen is encountered again. The immunoglobulins produced by these plasma cells are mainly immunoglobulin G’s (IgGs). Maternal IgGs are transferred through the placenta, protecting neonates and young infants from infection. These maternal IgGs are of particular importance in protection against disease in the first 3-6 months of life. After 3-6 months maternal antibodies are waning and children have to rely on their own immune responses. The important role of the humoral immunity is underlined by the increased incidence of severe EV infections in patients with primary antibody deficiency (PID), such as X-linked agammaglobulinemia (XLA), in which chronic enteroviral meningoencephalitis (CEMA) is one of the most severe complications.\textsuperscript{110,111} Successful treatment with therapeutic immunoglobulin therapy (e.g. intravenous immunoglobulin (IVIG)) in PID patients with an EV meningoencephalitis provides additional evidence for an important role of nAbs for an adequate immune host response in severe EV infections. In addition, in neonates, lack of specific maternal EV antibodies is shown to be a risk factor for the development of severe illness.\textsuperscript{112} Knowledge of the host immune response to HPeV is in comparison to EV even more limited. In contrast to the evidence as described in the section above, there are no data available on the protective role of nAbs in HPeV infections. Seroprevalence of HPeV1 in adults is high (>95%) and HPeV1 infection in children is generally seen above the age of 6 months, suggesting that maternal nAbs protect young infants against HPeV1 infection.\textsuperscript{89} In addition, the lower seroprevalence of HPeV3 in adults\textsuperscript{38,91} in combination with the younger age at which HPeV3 infection occurs, might suggest a lack of maternal protection against HPeV3 in the early months of life.

The role of the innate immune response against \textit{Picornaviridae} was historically considered of no importance and received little attention in the field of immunological research. However, in recent years the importance of the innate immune response, especially Toll-like receptors...
(TLRs), against picornaviruses is more and more recognized. TLRs are transmembranic glycoproteins that are expressed on various cells types. There are 10 TLRs recognized in humans so far; TLR1, -2, -4, -5, -6 are expressed on the cell surface sensing mainly bacterial products while TLR3, -7, -8 and -9 are located intracellular in vesicles and are activated by intracellular nucleic acids. Once activated, TLRs induce inflammatory responses by enhancing the production of various cytokines (reviewed in Beutler et al. and Kemball et al.). TLR7 and TLR8 seem to be of importance in the immune response against EVs, HRVs and HPeVs. In addition TLR3 and TLR4 are triggered by CV-B infections, while TLR2 recognizes HRV6. The inflammation produced by enhanced expression of TLR8 seems to play a major role in the pathogenesis of dilated cardiomyopathy caused by CV-B. However, the exact mechanisms how TLRs and other parts of the innate immune system influence the host response against EVs and HPeVs remains to be elucidated.

**Treatment**

There is no antiviral treatment against EVs and HPeVs currently available. Despite decades of research on anti-picornavirus medication, none of the drugs was licensed for use in patients. Most effort was made to find a drug against EVs. Only pleconaril was tested in phase III clinical trials. Pleconaril inhibits viral replication by integration into the hydrophobic pocket inside the viral capsid. As a result, the virus capsid is rigidified and in several cases the uncoating and binding of the virus to the host cell are interrupted. The hydrophobic pocket is relatively well preserved among EVs and HRVs, resulting in a broad-spectrum anti-enteroviral and anti-rhinoviral activity of pleconaril. However, EV71 is not susceptible for pleconaril. Since the capsid of HPeVs is different, suggesting that the hydrophobic pocket differs from that of EVs, it is not likely that pleconaril has any activity against HPeV. The US Food and Drug Administration (FDA) rejected pleconaril for the treatment of common cold because of the risk of side effects. Meanwhile pleconaril was used on compassionate use basis in immunocompromised patients with severe or chronic enteroviral infections with various outcomes. The drug was never licensed for this indication. Now, the drug is no longer available, although 2 clinical trials were conducted recently; one trial studied the effect of pleconaril nasal spray on the occurrence of rhinovirus associated common cold and asthma exacerbations in children >6 years and adults (NCT00394914). The other study was a double-blind, placebo-controlled, virologic efficacy trial of pleconaril as treatment for neonates with enteroviral sepsis syndrome (NCT00031512). The results of both these trials have not been published yet.

The major problems with anti-enteroviral treatment are that the *Enterovirus* genus is very diverse with many serotypes, therefore a drug with broad-spectrum antiviral activity is needed. Furthermore, the mutation rate in picornaviruses is relatively high, resulting in a high risk of selecting drug resistant strains.
Nowadays supportive treatment and administration of IVIG are the only available options for treatment of severe EV and HPeV infections. IVIG is haphazardly given to neonates and children with severe disease like myocarditis to reduce disease burden from EV infection, although its efficacy has not been proven. A randomized trial in neonates indicated that IVIG with a high nAb titer against the infecting EV type resulted in faster clearance of viremia. However, no effect on clinical outcome was demonstrated (possibly due to the small sample size).\textsuperscript{125} The use of IVIG in EV71 outbreaks was evaluated retrospectively and showed a beneficial effect when given early in the course of the disease.\textsuperscript{126,127} This was supported by high titers of EV71 specific nAbs found in Chinese donors, although a randomized controlled trial was never conducted.\textsuperscript{128} Evaluation of effectivity of IVIG is also complicated by the observation that EV nAb titers in IVIG vary between batches produced in various geographic regions.\textsuperscript{128-130}

Outline of this thesis

The aim of this thesis is to describe the disease spectrum of picornavirus infections in children (including EV, HPeV and HRV) and to assess the clinical relevance of an infection detected in the era of new and sensitive molecular diagnostic tools (PCR) which are now widely routinely used in clinical settings. The second aim is to evaluate the need for treatment against these infections, the available treatment options and the role of neutralizing antibodies as potential therapeutic options.

Part one focuses on the clinical relevance of HPeV and HRV-C infections. In contrast to the EVs, which are well known to cause significant morbidity, these species have only recently been discovered and their disease spectrum is not yet fully established. Despite the increasing number of studies on disease caused by HPeV, the clinical relevance of HPeVs (especially in stool samples) is still under debate. In chapter 2 the prevalence of HRV infections in an unselected birth cohort is described and clinical symptoms are compared with HRV negative children and between HRV species A, B and C infected children. In chapter 3 the clinical relevance of a positive HPeV1 and 3 PCR in stool samples is discussed and differences in clinical characteristics between HPeV1 and 3 are described. In chapter 4 the duration of HPeV shedding in stools after symptomatic infection is described and related to viral load and clinical symptoms. The role of environmental (water) exposure in the occurrence of HPeV and EV infections is studied in chapter 5.

In part two treatment options for EV and HPeV infections are discussed. Chapter 6 gives an overview of (the lack of) treatment options for severe HPeV infections. This is compared with the available treatment options for severe EV infections. In chapter 7 treatment with pleconaril and IVIG in 2 patients with agammaglobulinemia and chronic enteroviral meningitis is described and compared to in vitro susceptibility of the EV types. Chapter 8 describes the characteristics of the natural pleconaril resistant echovirus 11 strain found
in chapter 7 and possible mechanisms how this resistance could have been evolved. The successful treatment with IVIG of an infant with HPeV1 associated dilated cardiomyopathy and relation with HPeV1 specific neutralizing antibody titers in IVIG is described in chapter 9. **Chapter 10** describes specific cell tropism and neutralization characteristics of HPeV1 and 3 and implications for therapy development. In **chapter 11** the relation between (severity of) HPeV infection in infants and maternal antibodies is studied as part of the PARMA-study (PARechovirusinfections and Maternal Antibodies study). The aim of this study was to provide a rationale for specific antibody therapy in severe HPeV and EV disease. In part three the results of this thesis are summarized (**chapter 12**) and discussed (**chapter 13**) and put in perspective of the current knowledge.
References


