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INTRODUCTION

Human non-polio Enterovirus (EV) and human Parechovirus (HPeV) are both viruses that belong to the family of the *Picornaviridae*. They are small (~30 nm), non-enveloped single-strand ribonucleic acid (RNA) viruses. *Picornaviridae* are classified into 29 genera. The genus *Enterovirus* is divided into 12 species (EV A-H, EV-J and Rhinovirus A-C) of which only EV A, B, C and D are found in humans. EV-A, EV-B, EV-C and EV-D consist of 25, 63, 23 and 5 types, respectively. The genus *Parechovirus* is divided into 2 species (Parechovirus A and B). The species Parechovirus A, which infects humans, consists of 16 types, HPeV-1 to 16. Parechovirus B (formerly named *Ljungan virus*), which infects rodents, consists of Ljungan virus types 1–4.\(^1\)\(^2\)

EV and HPeV are frequent causes of infection in humans, especially children. The clinical spectrum of their infections varies from fever to severe systemic disease, including central nervous system (CNS) infection such as meningitis.\(^3\)\(^6\) Reverse-transcriptase real-time quantitative polymerase chain reaction (RT-qPCR) is currently considered the gold standard for detecting both EV and HPeV RNA in different body specimens, particularly in feces, throat swabs, cerebrospinal fluid, blood and urine.\(^7\)

There is presently no effective or approved available antiviral drug treatment against EV and HPeV infections. Treatment is limited to supportive care only.\(^8\) Advances have been made towards the development of a vaccine against EV-A type 71 (EV-71). Three recent phase-3 clinical trials of inactivated monovalent EV-71 vaccines manufactured in China were found to have high efficacy (80.4–97.4%) against EV-71 in infants and young children. Two of these vaccines, licensed in China since December 2015, are expected to be available soon in China.\(^9\)\(^11\)

Little is known about the long-term outcome of EV and HPeV infection in children. In recent years, several reports indicate neurodevelopmental delay in children after an EV CNS infection. However, most of these studies involved only children infected with EV-71 during epidemics in Taiwan and China.\(^12\)\(^16\) Indeed in these countries, infections with EV-71 occur frequently, but in Western countries most of the EV infections are caused by other genotypes and not by EV-71. Furthermore, studies on long-term neurodevelopmental outcomes in children with EV CNS infection included only low numbers of children and their results were inconclusive.\(^17\)\(^19\) In addition, the only studies available in the literature on neurodevelopmental outcome after HPeV infection, involved low powered studies in selected patient populations (below 2 months of age and mostly admitted to a tertiary intensive care unit).\(^17\)\(^19\)\(^21\)
AIMS OF THIS THESIS

In the studies described in this thesis, we give an overview of the clinical spectrum of EV and HPeV infections in children. In addition, we compare the diagnostic values of available methods for diagnosing EV and HPeV infection in different body specimens. Finally, we report on the long term prognosis (motor function and neurocognitive development and behavior) of EV and HPeV infections in children.

STUDY DESIGN

This study was performed in different stages. Firstly, we performed two retrospective studies. One to describe the clinical spectrum of EV infections in children, with a focus on the children with EV meningitis, and another one to compare the diagnostic values of two molecular assays (the GeneXpert Enterovirus Assay (GXEA) and an EV-specific laboratory-developed reverse-transcriptase quantitative real-time PCR (RT-qPCR). Secondly, we performed a prospective multicentre study in two phases (Figure 1). In the epidemiological phase, we described the clinical features, not only of infections caused by different EV genotypes, but also of those caused by different HPeV genotypes. In addition, we compared the detection rates of EV and HPeV infection using RT-qPCR and viral culture in different body specimens. In the follow-up phase of the prospective multicenter study, we compared both the motor function and neurocognitive development and behavior of children with EV or HPeV meningitis with those children with EV or HPeV elsewhere in the body, e.g. in their gastrointestinal or respiratory tract or systemic illness (sick control), and with those children without EV or HPeV infection (healthy control).

Patient population and enrolment

Retrospective studies

In one retrospective study we described the clinical spectrum of EV infection in children less than 16 years of age. It was based on their hospital records and on the registry of the Laboratory for Microbiology for the diagnosis EV infection between 2003 and 2008. Clinical, laboratory and virological data of these children were analysed. In another retrospective study, we compared the diagnostic values of two molecular assays. We included patients of all ages, in which cerebrospinal fluid (CSF) was collected and tested for the presence of EV in the Laboratory for Microbiology registry between 2007 and 2009.
Prospective study – epidemiological phase

During the epidemiological phase, children below 16 years of age referred with fever or any other suspected symptom of viral infection to the pediatric departments of the participating two non-university teaching hospitals (St. Elisabeth Hospital, Tilburg and Amphia Hospital in Breda) and non-teaching hospital (Tweesteden Hospital, Tilburg) in the Netherlands, between 1st of March 2008 and 30th of September 2011, were eligible for inclusion. Children, who met the inclusion criteria and whose parent(s) or legal guardians signed a written informed consent, were invited to participate. At inclusion, standardized questionnaires were filled in by the treating pediatrician. This included questions on different clinical symptoms and findings of the physical examination. Following inclusion, nasopharyngeal, blood, urine and feces specimens were collected on which an EV and HPeV RT-qPCR was performed. In addition, viral culture was only performed on feces and nasopharyngeal specimens. If there was a clinical suspicion of meningitis, a lumbar puncture was performed.

Figure 1  Overview of the prospective multicenter study.
This figure gives an overview of the prospective multicenter study which contains an epidemiological and follow-up phase. In the epidemiological phase, we describe the clinical features of EV and HPeV infections and compare the detection of EV and HPeV infection using RT-qPCR and/or viral culture in different body specimens. In the follow-up phase we compared both the motor function and neurocognitive development and behavior of children with EV or HPeV meningitis with children with EV or HPeV elsewhere in the body (such as gastrointestinal, respiratory or generalized) (sick control) and children without EV or HPeV infection (healthy control).
and CSF specimen were collected for EV and HPeV RT-qPCR and viral culture, in addition to a bacterial culture. Children with any of the following criteria were excluded: age >16 years, detection of another viral, bacterial or fungal pathogen, if a non-infectious cause of the clinical symptoms was found or if parents did not give informed consent.

**Prospective study – follow-up phase**

Included children were invited for a follow-up visit at 6, 12, and 24 months after inclusion for the assessment of their motor and neurocognitive development and behavioral function.

Motor function was assessed with the Bayley Scales of Infant and Toddler Development 3 (BSID-III) for children between 24 and 42.5 months (at the time of follow up) and the Movement Assessment Battery for Children-2-NL (M-ABC-2-NL) for children older than 42.5 months. Neurocognitive and behavioral tests in children aged 0–5 years were performed with the Dutch version of the Wechsler Preschool and Primary Scale of Intelligence (WPPSI-III-NL) and the Child Behaviour Checklist (CBCL). Both the pediatric physical therapist and psychology master students, who performed respectively the motor function and neurocognitive and behavioral development tests, were blinded to the diagnostic groups to which the children belonged.

**OUTLINE OF THIS THESIS**

In PART 1 chapter 1, we give an introductory overview of the total spectrum of EV and HPeV infections in children, from historical, epidemiological and pathophysiological perspectives. In addition, we give an overview of the known diagnostic and treatment options, and neurocognitive outcomes of EV and HPeV infections in childhood.

In PART 2 we focus on describing the clinical symptoms of EV and HPeV infection in children. In chapter 2, we retrospectively report the results of a study on the clinical spectrum of disease in 149 children with EV infection and describe the clinical and laboratory features of children with EV meningitis and no CSF pleocytosis. In chapter 3, we prospectively analyzed the clinical symptoms associated with different EV and HPeV genotypes in 184 infected children.

PART 3 consists of studies on diagnostic aspects of EV and HPeV infections in children. In chapter 4, we retrospectively compared the diagnostic values of two molecular assays used for detection of EV RNA in cerebrospinal fluid of 232 patients. In addition, we prospectively
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compared the detection rates of EV and HPeV using RT-qPCR and/or viral culture in different body specimens of 285 children (chapter 5).

In PART 4 we report the results of follow-up studies of children after an EV or HPeV infection. We described the motor development (chapter 6) and neurocognitive development, as well as behavioral problems (chapter 7) in children with EV and HPeV CNS infection and compare this with children with EV and HPeV detected in other body locations and children without any infection.

PART 5 consists of the general discussion, conclusions and future perspectives (chapter 8) and a summary (chapter 9).

REFERENCES

1. Www.ictvonline.org/virusTaxonomy.asp.


