Presence of human non-polio enterovirus and parechovirus genotypes in an Amsterdam hospital in 2007 to 2011 compared to national and international published surveillance data: a comprehensive review

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Enteroviruses (EV) and human parechoviruses (HPeV) are endemic worldwide. These infections are a constant cause of hospitalisation and severe disease, predominantly in young children and infants. Coordinated monitoring and surveillance are crucial to control these infections. We have monitored EV and HPeV epidemiology in Amsterdam from 2007 to 2011 with real-time RT-PCR and direct genotyping, facilitating highly sensitive surveillance. Moreover, we conducted a literature survey of existing surveillance data for comparison. Only 14 studies were identified. While HPeV1 was most frequently detected in Amsterdam, EV-B viruses dominated nationally and internationally. Furthermore, the top 10 strains detected differed yearly and per study. However, detection and typing methods were too varied to allow direct comparison and comprehension of the worldwide distribution and circulation patterns of the different genotypes. This limited a direct response to anticipate peaks. Uniform European monitoring programmes are essential to aid prediction of outbreaks and disease management.

Introduction

Human enteroviruses (EV) and parechoviruses (HPeV) are widespread and circulate globally. They are associated with a wide array of clinical manifestations ranging from respiratory or gastrointestinal symptoms, to neonatal sepsis and infections of the central nervous system [1-4]. Due to intensive vaccination and surveillance programmes, poliovirus has almost been eradicated. Nevertheless, outbreaks of pathogenic non-polio EV (NPEV) types remain, as illustrated by the recent outbreaks of EV71 in Asia [5-7].

EV were traditionally classified as poliovirus (PV) and the collectively named non-polio EV (NPEV), consisting of Coxsackie A and B viruses (CAV and CBV) and echoviruses (E), based on their pathogenicity in animals and cell culture. Molecular characterisation has led to their reclassification into four EV species A–D and the detection of new EV types (numbered) (<http://www.picornastudygroup.com>). HPeV were previously known as members of the EV genus (HPeV1 and 2) because of their similar cytopathic effect in cell culture. Based on genetic differences to EV, they were later classified as their own genus, which now comprises 16 genotypes (<http://www.picornastudygroup.com>).

Surveillance of EV and HPeV has traditionally been based on culturing and serotyping [3,8-10] and is primarily directed towards poliovirus eradication [11]. Nowadays, RT-PCR targeting the conserved 5'UTR is the standard method for detection followed by sequencing of the capsid genes, in particular VP1, for typing [4,8,12,13]. We have shown previously that screening by RT-PCR and direct genotyping from stool is much more sensitive than virus culture and leads to better detection of HPeV and CAV [8]. Not only does this contribute to surveillance, but it allows for a better understanding of NPEV and HPeV circulation and pathogenicity, which is of increasing importance in patient management and therapy [14-17].

Here we describe an epidemiological survey of NPEV and HPeV types detected from stool and cerebrospinal fluid (CSF) samples from patients admitted to hospital in the period from 2007 through 2011 in Amsterdam. We compare our findings with what is known from published EV surveillance data.

Methods

NPEV and HPeV positive sample cohort from patients from a tertiary hospital in Amsterdam

CSF and stool samples were obtained from 2007 through 2011 from patients (2007–2008 data published in [8]). A total of 570 patients were found positive for a NPEV ($n=339$), HPeV ($n=196$), or both ($n=35$) by real-time RT-PCR in stool and/or CSF as described previously [18]. The median age of infection with NPEV and HPeV was 8.3 months (0–71.6 years) and 6.3 months (0–68.1 years), respectively. Positive stool samples were cultured and serotyped or were genotyped directly from stool and/or CSF (GenBank accession numbers: KC893345-KC893502, KC893504-KC893549) [19]. Sequences were analysed by Simmonics Sequence Editor (SSE) [20]. EV were characterised with the genotyping tool from the Dutch National Institute for Public Health and the Environment (RIVM) [21], and HPeV were characterised by phylogenetic analysis [8,19].

Literature survey

The goal of our literature survey was to find EV and HPeV surveillance studies giving an overview of clinical surveillance trends over the years, to enable a comparison with our data. EMBASE and PubMed searches, encompassing publications listed on 18 October 2013, were performed (Figure 1) using the terms ‘enterovirus’, ‘parechovirus’, ‘surveillance or epidemiology’ and ‘geographic locations’ (MeSH term added to expand

search), while excluding animal studies and using filters for English and Dutch language. This resulted in 1,679 studies published between 1960 and 2013. After removal of duplicates, we retrieved 1,065 studies. After exclusion of studies describing a single type or fewer than 200 isolates, studies describing specific outbreaks and studies on methodology, 135 studies remained for screening of the abstract. We defined the inclusion criteria for this literature survey as follows:

- studies describing surveillance programmes of a general but symptomatic population in any region,
- coverage of at least five years,
- description of NPEV/HPeV genotype prevalence,
- detection of at least 200 NPEV/HPeV isolates.

Based on these criteria, 69 studies were selected for full-text analysis. Two additional papers were found through cross-referencing. Studies based on environmental surveillance or on the non-symptomatic general population only were excluded. In case of overlap in time span and geographical area, the largest study was selected. Fourteen studies remained for inclusion (Figure 1).

Tables with NPEV/HPeV genotype prevalence were extracted from the publications. The percentages of each individual genotype rather than absolute numbers were used to form a top 10 list of most prevalent types during the entire period from 2007 to 2011,

FIGURE 1

Inclusion criteria for literature search on enterovirus and human parechovirus surveillance

^a enterovir*[ti] OR parechovirus*[ti] AND (surveillance[ti] OR epidemiol*[ti] OR “Geographic Locations”[Mesh]) AND (english[la] OR dutch[la]) NOT (animals NOT (animals and humans)).

FIGURE 2

Non-polio enteroviruses within species EV-A to EV-D and human parechovirus genotypes in (A) Amsterdam 2007–11 (n=241 and n=157, respectively) and (B) the Netherlands, 1996–2011 (n=8,396)

A. Viruses detected in this study (n=398)

B. Viruses detected in Van der Sanden et al. [11] (n=8,396)

CAV: Coxsackie A virus; CBV: Coxsackie B virus; E: echovirus; EV: enterovirus; HPeV: human parechovirus.

thereby ruling out any bias by over-representation of studies with a large sample size.

Results

Distribution of NPEV and HPeV types in patients from a tertiary hospital in Amsterdam

In total, 241 NPEV and 157 HPeV could be typed (64.4% and 68%, respectively) from 374 EV-positive and 231 HPeV-positive stool and/or CSF samples. Typing was more successful in stool samples (69%) than in CSF (35%). Overall, we detected 22 different EV-B types, accounting for 55 CBV strains and 123 echovirus strains (Figure 2A). In CSF, only NPEV strains from species EV-B were found. EV-B strains accounted for 178 (74%) of the NPEV strains found overall, and seven of them ranked among the top 10 NPEV/HPeV types found (E30, E25, CAV9, E6, E9, E7 and CBV3, Table 1). Over the five years from 2007 to 2011, circulation of these

types varied, with different types co-circulating each year (data not shown). Viruses of the EV-A species comprised 58 strains (nine types), while only four strains could be characterised as EV-C (CAV1 (n=3) and CAV24 (n=1)) and one virus as EV-D (EV68).

Overall, the different EV types were frequently (n=206, 85.5%) detected in children under the age of three years, with 46.8% (n=96) of those under the age of three months (Figure 3A). There was a clear difference in the distribution of the species. Members of species B were predominantly identified in children under the age of three months, while members of species A were identified in children aged six to 12 months.

HPeV1 was the dominant HPeV type (n=88, 56%) and ranked first place, accounting for almost a quarter of all combined NPEV/HPeV strains (22%) (Table 1). HPeV3 was the second dominant strain (n=28, 12%) followed

TABLE 1

Top 10 non-polio enteroviruses and human parechovirus in Amsterdam 2007–11, the Netherlands 1996–2011 and in the international literature, 1967–2010

	Amsterdam (%) (this study)	The Netherlands (%) [11]	International (mean %; range) ^a
1	HPeV1 (22.1)	E6 (7.6)	E30 (12.32; 0.00–35.76)
2	HPeV3 (7.0)	E11 (7.5)	E6 (6.70; 0.00–18.57)
3	HPeV4 (6.5)	E25 (7.4)	E9 (6.55; 0.00–20.36)
4	E30 (6.0)	E30 (7.3)	CBV5 (5.23; 1.81–9.17)
5	E25 (4.8)	CBV5 (7.2)	E11 (5.16; 0.00–12.42)
6	CAV9 (3.8)	CBV4 (6.5)	E4 (4.69; 0.00–38.90)
7	E6 (3.8)	HPeV1 (5.2)	CBV3 (4.43; 0.00–15.68)
8	E9 (3.8)	CAV9 (5.0)	CAV16 (4.27; 0.00–19.92)
9	E7 (3.3)	E7 (4.7)	CAV9 (3.46; 0.00–9.44)
10	CBV3 (2.8)	CBV2 (4.6)	E13 (3.36; 0.00–16.14)

CAV: Coxsackie A virus; CBV: Coxsackie B virus; E: echovirus; HPeV: human parechovirus.

Percentages are based on the number of samples typed; Amsterdam n=398, the Netherlands n=8,398, international n=180,995.

^a The percentages of each individual genotype rather than absolute numbers were in each study were added up and used to form a top 10 list for the international studies, thereby ruling out any bias by over-representation of studies with a large sample size.

by HPeV4 (n=26, 11%) (Table 1). HPeV3 was the only type found in CSF while HPeV1 was much more prevalent in stool. While HPeV1 and HPeV4 circulated every year, HPeV3 only circulated in the even years, predominantly in the summer and almost exclusively in children younger than three months (Figure 3B).

Comparison of Amsterdam data with national surveillance data

A recently published paper by Van der Sanden et al. presents an overview of Dutch NPEV/HPeV surveillance data over the years 1996–2011 [11]. The standard method for the majority of the data was virus culture and serotyping from stool samples. When analysing this set of national data, (Figure 2B), it was observed that the top 10 isolated viruses were similar to our data (Figure 2A), with six of 10 types found in both lists (Table 1). The contribution of the Amsterdam data to the nation-wide data is ca 5–10%. An untyped HPeV outbreak occurred in the Netherlands in 2010. Based on the seasonal and biannually distribution known for HPeV3, the untyped HPeV most probably represented HPeV3 [11]. The detection rates for EV in that study were low, with 2.87% EV-A, 0.49% EV-C and 0.06% EV-D (Figure 2B) [11].

Overall, our results were in line with the national data. More EV-A and HPeV strains could be detected and typed by the use of supplementary molecular methods.

Trends in published NPEV and HPeV surveillance data derived from a systematic literature survey and comparison with the Amsterdam data

Fourteen studies were selected describing NPEV surveillance from 1967 to 2011 in several European countries, Japan, South Africa, Taiwan, Tunisia and the United States (US) (Table 2) [2,22–34]. Across all studies, a total of 186,930 NPEV/HPeV were found, of which 180,995 NPEV and HPeV isolates were typed. The studies differed with respect to data collection, sample types and isolation methods, varying from virus culture to real-time RT-PCR and direct genotyping. Therefore, no significant conclusions could be drawn, but several trends were observed.

Age distribution

Percentages of 30–74% of all isolates were derived from children younger than five years, with up to 47% coming from patients younger than three months [2]. In our study, the vast majority of infections occurred in children under the age of three years with, respectively, 25% and 37% of NPEV and HPeV isolates found in children younger than three months. Moreover, one study found a significant association between neonatal (age under one month) enteroviral infection and higher mortality (11.5% vs 2.5–5.1%), specifically related to CBV4 and E9 [35]. A difference in the predominant EV types among neonates and older children was also observed: E11, CBV2 and CBV5 were most prevalent in neonates, while E30, E9 and E11 were most prevalent in the older age group [28].

Sample types

The NPEV and HPeV types were predominately detected from stool and therefore indicated the circulation of types rather than a direct clinical association. Interestingly, the Asian studies show throat swabs to be the most frequent sample taken [29,33,34].

Furthermore, 56% of all echoviruses in a study in Scotland came from CSF samples but only 28 and 34% of CAV and CBV enteroviruses [25]. This is in line with our observation that echoviruses were frequently found in CSF.

NPEV circulation

All analysed studies, including our data and national data, showed a seasonal pattern for NPEV circulation with a distinct peak in summer. The genotypes isolated in each study are summarised in Table 2, while Table 1 lists the 10 most frequently found genotypes. E30 was the most prevalent type internationally. Least prevalent were EV86, CAV12 and EV70. While most EV types were found at least once, CAV11, CAV19 and CAV22 were never seen. Similar to our data from Amsterdam, the composition of co-circulating types differed in the years studied.

Our data were similar to international data in that EV-B viruses, in particular echoviruses, were most frequently

FIGURE 3

Age distribution of non-polio enterovirus species EV-A to EV-D and human parechovirus genotypes, Amsterdam, 2007–2011 (n=398)

A. Age distribution for the four EV species and HPeV identified in this study (n=398)

B. Age distribution for the six HPeV genotypes identified in this study (n=157)

EV: enterovirus; HPeV: human parechovirus.

detected (n=140,078; 74.9% of total EV/HPeV) (Table 2) [2,22,23,25–28,30,32]. Similarly, 10–30% of all virus isolates were CBV strains [19,23,26,27,32]. E30, among the top 10 types in our data, was observed as being the most prevalent type in the analysed literature. While six of our top 10 NPEV strains were also represented in the top 10 internationally, none of the 14 studies showed E25 dominance (Table 1), which is in sharp contrast to the E25 dominance observed in our data. This type came 16th in the international ranking. The literature survey further showed that EV-B viruses were frequently responsible for outbreaks throughout the years. E30 activity in the US between 1975 and 2005 was always associated with a new genetic lineage [28]. E6, E13 and E30 caused widespread outbreaks in 2000 and 2001 in Austria, Germany, Iceland, Kosovo, the Netherlands and the United Kingdom, [23,36,37]. Continued E13 outbreaks were seen in 2004 and 2006 in

several European countries and the US, causing severe meningitis [11,24,32,38]. E13 had rarely detected been in England, Wales and the US before the large outbreak in 2000 [24]. Other viruses of the EV-B species responsible for outbreaks throughout the years were CAV9, E4, E6, E9, E11 CBV4 and CBV5 [11,23,24,32,38].

The second dominant EV species in our data set and internationally was EV-A (13.1% of all NPEV/HPeV). However, EV-A was the most dominant species in two of three Asian studies (35.4–71.0% of all NPEV/HPeV) [29,33,34] where major outbreaks were primarily caused by CAV16 and EV71, types known to cause hand, foot and mouth disease. The most notable EV71 outbreak occurred in 1998 in Asia, where EV71 was implicated in a large number of fatal cases of encephalitis [39]. Most EV-A types were seen to circulate at low rates throughout the years both in our data and Dutch

TABLE 2

Number of isolated enteroviruses and human parechoviruses, percentages of typed isolates and characteristics of studies included in the literature review

Location	Time span	Isolation method	Sample type ^a	Total (n) NPEV/ HPeV	EV-A (%)	EV-B (%)	EV-C (%)	EV-D (%)	HPeV (%)	Reference
Amsterdam	2007–2011	PCR genotyping	Stool	398	14.6	41.7	1.1	0.5	41.7	This study
The Netherlands	1996–2011	Virus culture / serotyping (since 2007 also PCR genotyping)	Stool	13,952	2.9	52.8	0.5	0.1	2.9	Van der Sanden [11]
Scotland	2005–2010	PCR genotyping	CSF	232	2.1	60.1	0	0	13.7	Harvala [2]
Japan	2004–2008	Virus culture / serotyping and PCR genotyping	Throat swab	241	71.0	14.1	0	0	ND	Momoki [30]
Spain	1998–2007	Virus culture / serotyping and PCR genotyping	CSF	2,572	1.4	93.8	0.7	0	0 ^b	Trallero [33]
United States	1970–2005	Virus culture / serotyping and PCR genotyping	No data	49,637	3.1	94.8	0.3	0.1	0 ^b	Khetsuriani [29]
Taiwan	2000–2005	Virus culture / serotyping and PCR genotyping	Throat swab	12,052	48.1	22.0	0.7	0	ND	Tseng [34]
Germany	2000–2005	Virus culture / serotyping and PCR genotyping	Stool	674	5.6	83.8	0.3	0	0	Roth [31]
France	2000–2004	Virus culture / serotyping and PCR genotyping	Stool	2,754	0.7	95.0	0.4	0	0.6	Antona [24]
Tunisia	1992–2003	Virus culture / serotyping	Stool	236	0	90.3	8.1	0	0.8	Bahri [27]
Spain	1988–1997	Virus culture / serotyping	CSF	727	1.0	98.6	0	0	0.4	Trallero [32]
Belgium	1980–1994	Virus culture / serotyping	Stool	3,333	4.0	89.5	1.3	0	5.2	Druyts-Voets [28]
England and Wales	1975–1994	Virus culture / serotyping	Stool	40,364	3.5	87.8	0.3	0	8.4	Maguire [25]
Japan	1981–1991	No data	Throat swab	28,570	35.0	62.0	0	1.0	1.0	Yamashita [35]
South Africa	1981–1989	Virus culture / serotyping	CSF	3,098 ^c	ND	66.9	ND	ND	ND	McIntyre [23]
Scotland	1967–1974	Virus culture / serotyping	No data	42,440	8.1	89.7	0	0	0.6	Grist [26]

CSF: cerebrospinal fluid; EV: enterovirus; HPeV: human parechovirus; ND: not done; NPEV: non-polio enterovirus.

^a Predominant sample type.

^b Without rounding 0.04%.

^c Enteroviruses untyped: 33%.

national data. In addition, no other major type-specific outbreaks of EV-A were seen.

Circulation of viruses of the EV-C and EV-D species was low in all studies, which is consistent with our data. On average, they accounted for less than 1% of the total detected NPEV/HPeV in the analysed studies.

HPeV circulation

Despite HPeV dominance in our population, HPeV were only the third most common type after EV-B and EV-A internationally (2.4% of all isolated EV/HPeV). However, two of three studies using RT-PCR genotyping as the

main detection method, ranked HPeV types in their top five of most frequently isolated types [2,19].

HPeV circulates endemically in the States [28]. A seasonal pattern was identified for HPeV in Amsterdam and Scotland [2].

Discussion

This study described the clinical epidemiology of NPEV and HPeV types over a five-year period in an academic hospital in Amsterdam as identified by real-time RT-PCR and direct genotyping. We compared our

data with clinical surveillance data published across Europe, Japan, South Africa, Taiwan, Tunisia and the US, providing an overview of the complex circulation pattern of different NPEV and HPeV types over several decades [2,11,22-34]. Conclusions could not be drawn due to the heterogeneity of the available data, but several trends were observed.

In all studies including our own, NPEV/HPeV infections predominantly affected neonates (under the age of one month), with up to half of all infections identified in neonates. In addition, a higher mortality has been reported, in some cases related to specific types frequently found in neonates [28,35]. These comprise mostly EV-B types [19].

With respect to circulation of the NPEV/HPeV viruses, our study was representative for what is found nationally and internationally (Table 2). However, there were differences in the frequency of HPeV and CAV. While large outbreaks in one region can influence the distribution of viruses in other regions and the studied time periods differed, it seems likely that these differences are attributable to the difficulty of culturing these viruses on standard EV cell lines, since most international studies rely on data from virus culture. Furthermore, RT-PCR for HPeV is not routinely performed and therefore some studies do not detect HPeV (Table 2). Among the top 10 virus types identified in our study, the most common were EV-A viruses and HPeV. Studies using RT-PCR and direct genotyping generally detect a larger proportion of HPeV and EV-A viruses, arguing that these types may have been underreported in other studies based on cell culture and serotyping [2,19]. These observations do not apply for the Asian studies, where CAV16 and EV71 are the dominant types. The more pathogenic Asian subgenogroups have already been described in Europe [40-42]. Although more pathogenic genotypes may be overrepresented because diagnostics are more likely to be requested for severe cases, EV71 is not routinely monitored in Western countries leaving a void in the international surveillance of this type.

Interestingly, E25 was observed as one of the dominant NPEV types in our population and was among the top 10 types nationally. Before 2011, however, E25 had been seen less frequently in literature [26]. Most studies included here cover time periods earlier than our own data, which could explain the lack of E25 detection. The recent peak in E25 detection could be related to a genetic divergence in this type to which the population is not immune, as has been shown for other EV types [40,43].

Recently the US Centers for Disease and Control and Prevention reported an increase in EV-D68 cases with severe respiratory disease [44]. The US outbreak has led to increased awareness of the virus in European countries. EV-D68 was rare in our study between 2007 and 2011 (one case in 2010) and nationally (n=5, 0.1%)

between 1996 and 2011 [11]. However, EV-D68 did cause an increase in severe respiratory infections in the fall of 2010 in the Netherlands [45]. Because of the acid-sensitive phenotype, EV-D68 is rarely detected in stool, which could explain the lack of the virus in both studies which were for the most part based on stool isolations. Currently, an increase in the number of EV-D68 severe respiratory cases is reported through the general practitioner surveillance which monitors influenza-like illness and other acute respiratory infections and through the national enterovirus surveillance [46].

Data on yearly circulation patterns may be helpful in predicting such peaks in the future. However, the different time periods covered in the two studies prevented us from studying yearly prevalence and co-circulation of NPEV and HPeV.

Conclusion

This is the first study that has compared local data with national and international clinical surveillance data. Comparison and comprehension of the data proved difficult due to differences in techniques, samples collected and years studied. Coordination of data collection and standardisation of methods, as well as the design of easy-to-use databases for the collection of sequence data in combination with epidemiological data [47], are essential in order to elucidate epidemiology, calculate disease burden and improve outbreak management of individual types. First efforts to create such a coordinated environment have been made in the Netherlands with the creation of VIRO-TypeNed [47]. Uniform international data collection using of similar techniques would be a next step in international coordination.

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Conflict of interest

None declared.

Authors' contributions

V. Janes: literature survey and draft of the manuscript; R. Minnaar: PCR and genotyping experiments; G. Koen, H. van Eijk, and K. Dijkman-de Haan: culture and serotyping experiments; D. Pajkrt: critical review of the manuscript; K. Wolthers: draft and critical review of the manuscript; K. Benschop: data maintenance and analysis, draft and critical review of the manuscript.

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