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REVIEW

Global epidemiology of nonpolio enteroviruses causing severe neurological complications: A systematic review and meta-analysis

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Summary

Enteroviruses are RNA viruses found as commensals in the human gut and respiratory system, which may cause a wide spectrum of disease. Enteroviruses may cause severe neurologic complications including acute flaccid paralysis (AFP) and encephalitis and are the most commonly diagnosed agents of viral meningitis. Outbreaks of more severe disease are often associated with particular genotypes, such as enterovirus-A71 causing rhombencephalitis and AFP. There are more than 300 described genotypes of human enterovirus, with overlaps in clinical phenotypes between genotypes, and uncertainty about which genotypes are more prevalent in neurological manifestations.

A systematic review of observational studies was conducted to evaluate the most prevalent enterovirus genotypes causing AFP, encephalitis, and meningitis. The genotyping methods and sampling sites were compiled as secondary outcomes. Sources included MEDLINE, Embase (publications until January 2019), and references selected from included studies. Meta-analyses using a random effects model were performed to calculate the pooled proportion of enterovirus genotypes in each disease.

Ninety-six publications met the eligibility criteria, comprising 3779 AFP cases, 1140 encephalitis cases, and 32 810 meningitis cases. Enterovirus-A71 was most frequently associated with AFP (pooled proportion 0.12, 95% CI, 0.05-0.20) and encephalitis (0.77, 95% CI, 0.61-0.91). Echovirus 30 (0.35, 95% CI, 0.27-0.42) was the most predominant genotype in meningitis cases. Genotypes were most commonly determined using VP1 RT- reverse transcription-polymerase chain reaction, and most samples assessed were cerebrospinal fluid.

With the emergence of enteroviruses as an increasing cause of neurological diseases, surveillance and testing need to increase to identify the aetiology of the most common and most severe disorders.

KEYWORDS

acute flaccid paralysis, encephalitis, enterovirus, meningitis, systematic review

Abbreviations: EV, enterovirus; AFP, acute flaccid paralysis; CNS, central nervous system; NPEV, nonpolio enterovirus; EV-D68, enterovirus-D68; EV-A71, enterovirus-A71; RT-PCR, reverse transcription-polymerase chain reaction; 5′UTR, 5′ untranslated region; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analysis; WHO, World Health Organisation; CEBM, Centre for Evidence-based Medicine; CDC, The Centre for Disease Control and Prevention; AFM, acute flaccid myelitis.

1 | INTRODUCTION

Human enteroviruses (EVs) are a genus of the *Picornaviridae* family and responsible for a number of neurological conditions including acute flaccid paralysis (AFP), encephalitis, and meningitis.[1] They are divided into four subgroups in humans based on their pathogenesis and host range: *Enteroviruses A* through *D*.[2] Polioviruses are classified as part of the *Enterovirus C* species and have a well-defined pathogenicity in the human central nervous system (CNS). However, due to the success of poliovirus vaccination programs, there has been near-global eradication of clinical poliomyelitis.[3] The first nonpolio enterovirus (NPEV) was discovered in 1948 when Dalldorf et al inoculated newborn mice with faecal suspensions from suspected polio patients.[4] The inoculation resulted in paralysis of the mice, and the causative agent differed from poliovirus. This initially unclassified virus was deemed the first member of the group A coxsackie viruses. Since then there have been more than 300 genotypes of human NPEV identified using new methods of molecular detection.[5]

Many studies focus on identifying the species and genotype of NPEV that is implicated in an outbreak. For example, outbreaks of aseptic meningitis in Europe within the last decade have been associated with echovirus 30.[6] In comparison, enterovirus-D68 (EV-D68) is thought to be linked to an upsurge of AFP cases across Northern Europe and the United States in 2016.[7] Enterovirus-A71 (EV-A71) is known to be particularly neurovirulent and associated with fatalities, particularly within the Asia-Pacific region.[8] Typically, EV-A71 presents with hand, foot, and mouth disease, which is a benign and self-limiting condition. However, EV-A71 can also cause neurological infections such as those discussed in this review, which can then have a severe impact.[8] For example, outbreaks of EV-A71 in Singapore resulted in closure of schools to prevent ongoing transmission.[9] Despite the apparent predominance of certain EV genotypes in these diseases, the associations between different EV genotypes have not been statistically compared in meta-analyses for AFP, encephalitis, and meningitis.

In response to the potential for EVs to inflict severe neurological disease, the World Health Organization (WHO) considered NPEVs in the WHO Research and Development Blueprint for the first time in 2018.[10] NPEVs were deemed to require further research and development after being classified as a major public health threat. This systematic review is timely given this update, and also because of the ongoing development of vaccines. Phase III trials of an inactivated vaccine against EV-A71 in Taiwan have demonstrated seroprotection persisting for 2 years in most participants.[11]

NPEVs have been detected in different sites during episodes of neurological conditions. In order to make a definitive diagnosis of viral encephalitis or meningitis, CNS involvement needs to be confirmed, such as by detection of virus in the cerebrospinal fluid (CSF).[12] However, diagnosis may also be made when EVs are detected from other sites including stool, serum, and throat swabs.[13] Knowledge of which sampling site is most commonly assessed and which sites are more commonly positive may assist in the improvement of sampling guidelines.

Traditionally, virus isolation and serology neutralization methods were used for diagnosis of EVs, though these were time-consuming and labour-intensive.[14] Conventional immunological diagnosis methods have been shown to be less specific than molecular assays based on reverse transcription-polymerase chain reaction (RT-PCR) targeting various genomic regions.[15] The optimal target for EV detection and identification used to date has been VP1, which is a capsid protein located at the virion surface.[16] Two other structural proteins, VP4 and VP2, have also been used for EV identification, though with less success than VP1.[16,17] However, before these structural proteins were identified as targets, highly conserved sequences in the 5′ untranslated region (5′UTR) were amplified for EV RT-PCR and genotypes imputed using sequencing. This method was later suggested to be more suitable for detection of the presence of an NPEV rather than genotyping.[17] Given the proposed differences in sensitivity and efficacy of these targets, we aimed to obtain an estimate of which genotyping methods and targets are more prevalent across the selected studies.

Previous reviews in this area have either focused on a single neurological complication or have been limited geographically to one country or region.[18-21] The objective of this review was to conduct a systematic search of the published literature to ascertain which NPEV genotypes were globally most often diagnosed in cases of neurological complications. Secondary objectives included recording the sampling site and method of genotyping for these data.

2 | METHODS

2.1 | Literature search

Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines were followed in the reporting of this review.[22] The completed PRISMA checklist is provided in Table S1.

Two reviewers (S. S. and S. S. B.) conducted a systematic search for observational studies describing cases of NPEV AFP, encephalitis, or meningitis, which identified specific genotypes of EVs. Databases searched were MEDLINE and Embase from each database's date of inception to 29 January 2019. The database search strategy was created with a focus on terms for the virus and the terms for genotype. These were combined with terms for any of the targeted diseases—AFP, encephalitis, or meningitis. A combination of key words and relevant subject headings specific to each database were also included and listed in the full search strategy for MEDLINE in Table S2. No further limits were applied. Finally, the reference lists of the chosen articles were screened manually to obtain more articles after title and abstract exclusions.

2.2 | Inclusion criteria

Included studies were observational studies or surveillance reports concerning patients diagnosed with AFP, encephalitis or meningitis where specific genotypes of NPEV were identified from any sample

site. No requirements were specified for the method of diagnosis used; thus, each condition was recorded as reported by the author(s).

2.3 | Exclusion criteria

Exclusion criteria were as follows: (1) studies not presenting original data or those in the form of letters, conference abstracts, and comments; (2) studies that presented data in aggregate formats; (3) studies that reported fewer than 10 NPEV samples or that genotyped less than 50% of the NPEV-positive samples, and (4) studies limited to high-risk populations such as immunodeficient patients. Where we found multiple publications from one study, we selected only the paper with the longest follow-up time or the largest sample size.

2.4 | Data abstraction

2.4.1 | Study selection

One author (S. S.) initially removed duplicate studies from the results of the search. Two authors (S. S. and S. S. B.) then independently screened the titles and abstracts of the remaining studies. This was done with blinding of authors and journal titles, using a Microsoft Excel workbook specifically designed for screening.[23] Items for which there was disagreement were discussed by the two screeners and resolved by consensus. The full texts of the eligible studies were then obtained and divided into English and non-English studies. Attempts were made to find translations of non-English studies, but where this was not possible, the non-English papers were not included in the analysis. These studies are outlined in Table S3 with data extracted from the abstracts. Authors of the English studies unable to be obtained in full were contacted to request the manuscript. The remaining English studies were assessed for eligibility with reasons for exclusion detailed in Figure 1.

2.4.2 | Data extraction and quality assessment

We separated the final 96 studies into the three neurological complications of interest: AFP,[24-42] encephalitis,[43-63] and meningitis.[26,48,56,58,64-119] The "encephalitis" category also encompassed cases of rhombencephalitis, meningoencephalitis, and studies that did not distinguish between meningitis and encephalitis. As well as those patients explicitly diagnosed with AFP, patients diagnosed as having "myelitis" were included in the "AFP" category. Four studies included participants in two of the complication categories and were thus extracted separately in each category.[26,48,56,58]

The following five pieces of descriptive data were extracted into three separate Microsoft Excel workbooks for each neurological condition: (a) the citation of the study, (b) the country and the region designated by WHO that the study was conducted in, (c) the duration of the study, (d) whether the study was prospective or retrospective, and (e) whether the data were sourced from surveillance or hospital records.

The primary outcome was documented as the total number of NPEV positive samples, which were genotyped, including nontypable samples and those that were only typed into species or not reported. This was further divided into the number of samples allocated to each genotype. If two methods of genotyping were used generating different results, the one method with the higher number of successful genotypes identified was included. If multiple specimens were positive for the same viral genotype from one patient, they were recorded as one case. Data were extracted from figures using WebPlotDigitizer where needed.[120] The following five secondary outcomes were collected along with the descriptive data and primary outcomes in the Microsoft Excel workbooks: (a) the site of sampling, (b) the age of participants, (c) the method of genotyping used, (d) the method of diagnosis of the condition, and (e) whether the source of material genotyped was isolated virus or nucleic acid direct from clinical sample.

Where necessary, authors of the selected studies were contacted via email to obtain missing data for the outcomes of interest. Authors of 4/17 (23.53%) studies that were contacted provided additional information.[31,82,107,114]

The quality of each selected article was scored according to the Centre for Evidence-Based Medicine (CEBM's) levels of evidence ver. 2011.[121] Disagreement in study appraisal was resolved by consensus.

2.5 | Data analysis

We conducted proportion meta-analyses with STATA 15.1 (StataCorp LP, College Station, TX, USA) using the "metaprop" command for the most common genotypes in each disease.[122] The pooled proportion was calculated with the total number of NPEV samples on which genotyping was performed as the denominator. The Freeman-Tukey variant of the arcsine transformation was applied to stabilize the variance of proportions.[123] A random effects model was employed, as there was significant heterogeneity anticipated given the nature of observational studies.[124] Confidence intervals (95% CI) were calculated using the DerSimonian and Laird method.[125] We calculated the I^2 statistic as a measure of the proportion of the overall variation that was attributable to between-study heterogeneity. Secondary outcomes were assessed using descriptive statistics generated through Microsoft Excel ver. 16.23 (*Microsoft* Corporation, Redmond, WA, USA). Due to the small number of studies in each arm of analysis, thorough subgroup analyses were not performed. However, we performed preliminary subgroup analyses of four AFP[33,37,39,41] and four encephalitis[49,51,56,62] papers, which genotyped nucleic acid direct from clinical sample, to assess for laboratory method bias.

3 | RESULTS

3.1 | Results of literature search

An adapted PRISMA flow diagram shows the process followed to select the studies used in this review (Figure 1).[22] The initial search yielded 1245 potential references, and three additional articles were

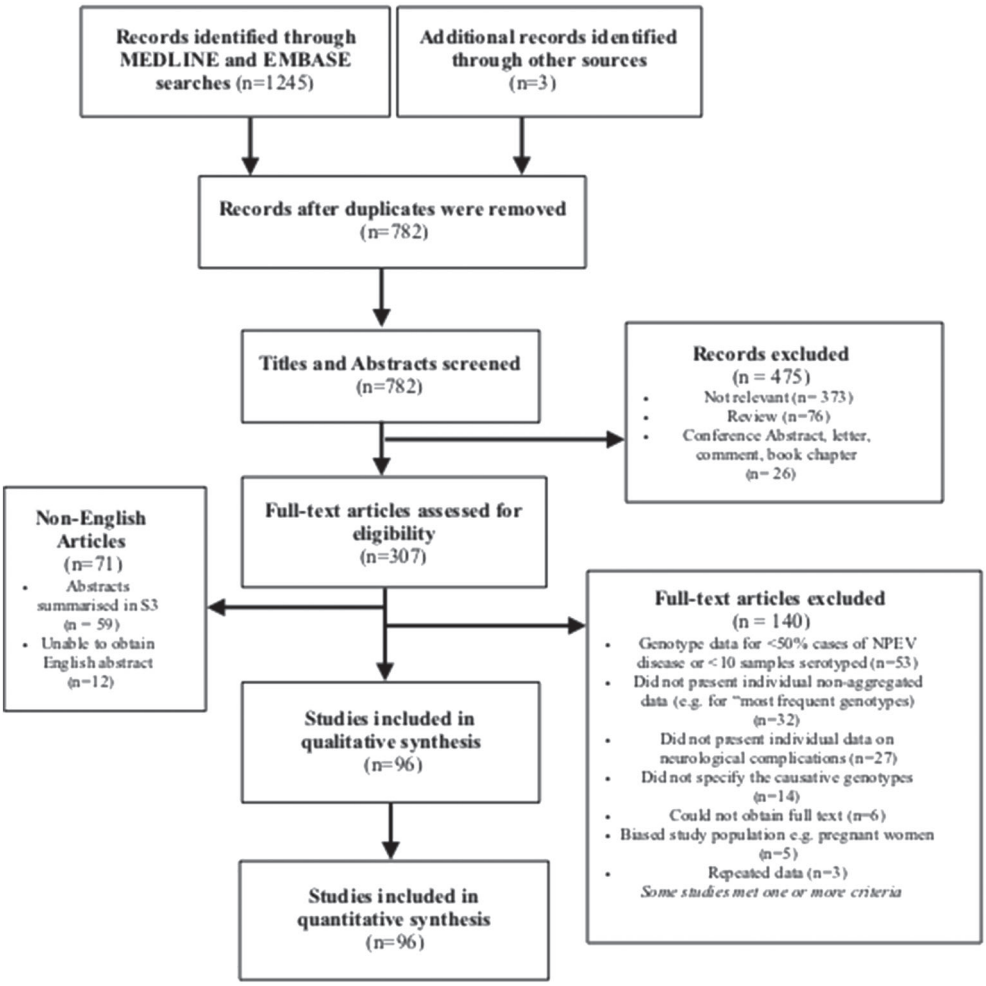

FIGURE 1 Search strategy for the identification of studies reporting which enterovirus (EV) genotypes were prevalent in acute flaccid paralysis, encephalitis, and meningitis. [a] "Not relevant" included studies focusing on diagnostics or therapeutics, studies of nonhuman EVs, evaluations of surveillance studies, studies of non-central nervous system manifestations, studies on polioviruses or other viruses, studies on environmental samples of EV exclusively, studies with coinfections of other viruses, papers on whole genome sequencing of particular strains, and single participant case studies

obtained through hand searching of the bibliographies of included studies. There were 96 articles published since 1962 included in this systematic review. Four papers including data on two neurological complications were recorded for both complications, generating a total of 19 AFP studies, 21 encephalitis studies, and 60 meningitis studies. In total, there were 37 729 patients: 3779/37 729 (10.02%) AFP cases, 1140/37 729 (3.02%) encephalitis cases, and 32 810/37 729 (86.96%) meningitis cases. The majority of studies were from the Western Pacific Region (30/96, 31.25%) or the European Region (29/96, 30.2%).

A comparison of descriptive data for the three conditions is shown in Table S4. For AFP, 10 studies[24,31,34-36,38-42] used the WHO criteria as a method of diagnosis, one study[33] used separate criteria designated by a neurologist, and the remaining eight studies[25-30,32,37] did not report the criteria for diagnosis. Methods of diagnosis for cases of encephalitis and meningitis were not consistently reported; thus, these data were not collated.

Studies included in this review were eligible to be graded from levels 1b to 4 of CEBM's five levels. Of the 96 studies, 67 (69.79%) studies scored 2b or higher. The studies and corresponding CEBM levels are listed in Tables 1–3 according to the three neurological diseases.

3.2 | NPEV genotypes

The genotype distributions were analysed within each neurological complication and are presented in Tables 4–6. Each proportion should be considered as a pooled estimate of only those studies reporting the particular genotype. Hence, cumulative point estimates do not sum to 100%. Across AFP studies, EV-A71 was the most common genotype detected, yielding a pooled proportion of 0.12 (95% CI, 0.05-0.20). Other common genotypes were echovirus 13 (0.08, 95% CI, 0.05-0.12) and echovirus 11 (0.07, 95% CI, 0.05-0.09). For cases of encephalitis, EV-A71 was also the most frequent virus detected, (0.77, 95% CI, 0.61-0.91). This was followed by echovirus 30 (0.20, 95% CI, 0.03-0.44) and echovirus 18 (0.17, 95% CI, 0.00-0.53). In contrast, echovirus 30 was the most prominent genotype detected in meningitis patients (0.35, 95% CI, 0.27-0.42). After echovirus 30, echovirus 6 (0.13, 95% CI, 0.27-0.42) and echovirus 13 (0.13, 95% CI, 0.07-0.19) were most prevalent. The results of our preliminary subgroup analyses in AFP and encephalitis papers, which genotyped nucleic acid direct from clinical sample, demonstrated similar trends as above. Across both AFP (0.47, 95% CI, 0.02-0.95) and encephalitis (0.62, 95% CI, 0.19-0.96) studies, EV-A71 was the most predominant virus detected in these analyses.

TABLE 1 Characteristics of the studies included in the meta-analysis of AFP cases listed by WHO region

First Author, Year	Study Period	CEBM Level	Cases, n	Most Prevalent EV Genotype(s)
African Region				
Oyero, 2014[24]	N/A	3b	56	Echovirus 11
Sadeuh-Mba, 2013[25]	2008-2009	2b	146	Coxsackie virus B1
Eastern Mediterranean Region				
Bahri, 2005[26]	1992-2003	3b	54	Coxsackie virus B3, echovirus 6
Shaukat, 2013[27]	2010	1b	46	Echovirus 19
Angez, 2015[28]	2013	2b	215	Echovirus 19
Shaukat, 2012[29]	2009	3b	23	Echovirus 3, echovirus 11
Saeed, 2007[30]	2003	1b	474	Echovirus 6
Angez, 2017[31]	2003	2b	63	Coxsackie virus B5
European Region				
Wieczorek, 2017[32]	1999-2014	2b	16	Coxsackie virus B4
Region of the Americas				
Sejvar, 2016[33]	2014	2b	14	Enterovirus D68
South-East Asian Region				
Laxmivandana, 2013[34]	2009-2010	2b	422	Enterovirus-A76
Maan, 2013[35]	2010	2b	54	Echovirus 13
Rao, 2012[36]	2007-2009	3b	666	Enterovirus-A71
Western Pacific Region				
Zhou, 2016[37]	2010-2013	2b	26	Enterovirus-A71
Apostol, 2012[38]	1992-2008	2b	386	Coxsackie virus A24
Tang, 2014[39]	2006-2010	3b	98	Echovirus 13
Bingjun, 2008[40]	1997-2004	2b	195	Echovirus 13
Tao, 2014[41]	1988-2013	2b	792	Coxsackie virus B3
Kim, 2014[42]	2002-2011	2b	33	Enterovirus-A71

Abbreviations: AFP: acute flaccid paralysis; CEBM: Centre for Evidence-Based Medicine; EV: enterovirus; WHO: World Health Organisation.

3.3 | Secondary outcomes

All 96 studies reported method of typing and sampling site. These data are presented in Table 7 and Figure 2. The most commonly used method of genotyping was RT-PCR (80/96, 83.33%). The structural protein VP1 was the most utilized RT-PCR target (44/96 studies, 45.83%). Only one included study published in 1998 used virus isolation as a means of typing.[92] The majority of the samples were CSF alone or in addition to other samples (72/96 studies, 75.00%). Stool was the next most prevalent sample type after CSF with 18/96 studies (18.75%) relying on stool samples alone.

4 | DISCUSSION

In this review, the most common NPEV associated with AFP and encephalitis was EV-A71, and echovirus 30 was the most common NPEV associated with meningitis.

4.1 | AFP genotypes

The most commonly identified genotypes in AFP cases after EV-A71 were echovirus 13 and echovirus 11. This is consistent with results from another systematic review of AFP surveillance cases, which found that the most commonly associated genotypes were EV-A71 (7.9%) and echovirus 11 (5.7%).[18] In contrast, Suresh et al found that in case reports and case series, EV-D68 was the next most prevalent genotype after EV-A71. However, EV-D68 was not amongst the 10 most prevalent genotypes identified in the data presented here. This may be explained by our exclusion of case reports and case series involving less than 10 patients. Moreover, EV-D68 is most commonly detected in respiratory specimens, and as demonstrated in our review, most EV surveillance and outbreak programs used CSF or stool as analyte.[13] There have also been difficulties with EV-D68 detection due to cross-reactivity with rhinoviruses in various diagnostic settings.[126] Thus, both sampling and laboratory biases may have impacted the comparatively low proportion of EV-D68 in our dataset.

This affects the issues surrounding EV-D68 detection and causality in neurological syndromes. The Centers for Disease Control and Prevention (CDC) has reported a temporal association with EV-D68 respiratory illness and acute flaccid myelitis (AFM) cases in the United States, prompting ongoing surveillance.[127] However, the limited detection of EV-D68 in neurological specimens has led to

TABLE 2 Characteristics of the studies included in the meta-analysis of Encephalitis cases listed by WHO region

First Author, Year	Study Period	CEBM Level	Cases, n	Most Prevalent EV Genotype(s)
African Region				
Schoub, 1985[43]	1984	2b	10	Coxsackie virus B3
Eastern-Mediterranean Region				
Dalwai, 2009[44]	2003-2006	2b	29	Echovirus 9
European Region				
Casas-Alba, 2017[45]	2016	3b	50	Enterovirus-A71
Taravilla, 2019[46]	2016	2b	18	Enterovirus-A71
Papa, 2009[47]	2006-2007	3b	27	Echovirus 6
Region of the Americas				
Tavakoli, 2008[48]	2005-2006	2b	11	Coxsackie virus B3
South-East Asian Region				
Singh, 2016[49]	2011-2012	4	29	Echovirus 19
Kumar, 2011[50]	2008	3b	15	Coxsackie virus B5
Kumar, 2012[51]	2009-2010	1b	45	Echovirus 21
Kumar, 2013[52]	2009-2010	4	31	Coxsackie virus B5, echovirus 3, Echovirus 6
Western Pacific Region				
Zhang, 2013[53]	2002-2012	4	233	Echovirus 30
Wei, 2016[54]	2015	2b	26	Echovirus 30
He, 2013[55]	2010	1b	33	Enterovirus-A71
Huang, 2015[56]	2009-2013	2b	211	Enterovirus-A71
Ho, 1999[57]	1998	2b	39	Enterovirus-A71
Chen, 2018[58]	2013-2015	2b	97	Echovirus 18
Chen, 2010[59]	2008	2b	15	Enterovirus-A71
B'Krong, 2018[60]	1997-2010	1b	45	Enterovirus-A71
Ryu, 2010[61]	2008-2009	2b	41	Enterovirus-A89
Wang, 2015[62]	2012	2b	104	Enterovirus-A71
Yang, 2001[63]	1998-1999	3b	31	Enterovirus-A71

Abbreviations: CEBM: Centre for Evidence-Based Medicine; EV: enterovirus; WHO: World Health Organisation.

debate regarding the role of the genotype in causing disease.[128] Application of the Bradford-Hill criteria has supported a causal relationship between EV-D68 and AFM.[128] Moreover, in 2017, an experimental mouse model was inoculated with EV-D68, inducing a paralytic disease resembling AFM.[129] This model demonstrated all of Koch's postulates, supporting the hypothesis that EV-D68 can cause AFM. However, Koch's postulates have been revisited over time in light of developments in virus diagnostics. In postulates developed by Huebner, he considers the importance of tissue culture isolation; whereas Fredricks and Relman require sequence-based evidence for microbial causation.[130,131] This mouse model has yet to satisfy these modified postulates of microbial pathogenesis and thus requires further development to prove causality of EV-D68 and AFM. This review suggests the need for more detailed analysis of AFP cases where EV-D68 is detected. In addition, analysis of respiratory specimens in cases of AFP, as introduced by the CDC's surveillance, may allow for more sensitive detection of this genotype.[132]

4.2 | Encephalitis genotypes

The most common EV genotypes identified in cases of encephalitis were EV-A71, echovirus 30, and echovirus 18. This is consistent with findings from the California Encephalitis Project in 1998 to 2005, which is one of few large-scale surveillance studies compiling encephalitis incidence data.[133] In California, EVA-71 in particular was associated with severe illness, with two of the four fatalities in the surveillance being attributed to EVA-71. This aligns with results from our study demonstrating that EVA-71 was proportionally higher in encephalitis rather than the typically less severe meningitis. At present, there are limitations to studying the genetic determinants of the genotype's neurovirulence. For example, there are restrictions concerning existing animal models, as humans are the only natural host for wild-type EV-A71 infection.[134] A recent study successfully infected NOD-*scid IL2Rγ*$^{-/-}$ (NSG) mice with human immune systems, allowing insights into the human immune response to EV-A71 infection.[135] This may provide a platform to evaluate EV-A71 therapies in the future.

TABLE 3 Characteristics of the studies included in the meta-analysis of Meningitis cases listed by WHO region

First Author, Year	Study Period	CEBM Level	Cases, n	Most Prevalent EV Genotype(s)
African Region				
Smuts, 2018[64]	2015-2016	3b	29	Coxsackie virus A9
Wolfaardt, 2014[65]	2010-2011	3b	30	Echovirus 4
Eastern Mediterranean Region				
Othman, 2016[66]	2011-2013	2b	21	Echovirus 4
Bahri, 2005[26]	1992-2003	2b	19	Coxsackie virus B5
Meqdam, 2002[67]	1999	2b	32	Echovirus 9
Dalwai, 2010[68]	2003-2006	2b	92	Echovirus 9
Richter, 2006[69]	2000-2002	2b	218	Echovirus 30
European Region				
Vollbach, 2015[70]	1998-2008	2b	14	Echovirus 30
Milia, 2013[71]	2012	3b	10	Echovirus 30
Braunova, 2019[72]	2012-2016	2b	67	Echovirus 30
Nougairede, 2014[73]	2013	2b	119	Echovirus 30
Volle, 2014[74]	2008-2012	1b	156	Echovirus 30
Chomel, 2003[75]	2000	2b	116	Echovirus 13
Bottner, 2002[76]	2000	3b	30	Echovirus 13
Logotheti, 2009[77]	2007	2b	46	Echovirus 4
Frantzidou, 2007[78]	2005	3b	11	Echovirus 5
Cabrerizo, 2008[79]	2006	2b	116	Echovirus 30
Trallero, 2003[80]	2000	2b	538	Echovirus 30
Ortner, 2009[81]	1999-2007	2b	107	Echovirus 30
Wieczorek, 2015[82]	2011-2014	2b	189	Echovirus 6
Druyts-Voets, 1997[83]	1980-1994	2b	790	Echovirus 30
Brunel, 2008[84]	2005	3b	51	Echovirus 30
Sensoy, 2009[85]	1999-2004	2b	104	Echovirus 30
Dumaidi, 2006[86]	2003-2005	3b	14	Echovirus 11
Siafakas, 2004[87]	2001	2b	34	Echovirus 6
Sojka, 2011[88]	2005-2009	2b	100	Coxsackie virus B4
Thoelen, 2004[89]	1999-2002	2b	342	Echovirus 30
Cordey, 2017[90]	2013-2015	2b	65	Echovirus 30
Holmes, 2016[91]	2008, 2011-2014	3b	163	Echovirus 30
Atkinson, 1998[92]	1975-1994	2b	24120	Echovirus 11
Trallero, 2010[93]	1982-2007	2b	1544	Echovirus 30
Rudolph, 2017[94]	2008, 2013	3b	196	Echovirus 30
Region of the Americas				
Berlin, 1993[95]	1986-1990	1b	161	Coxsackie virus B5
Julian, 2003[96]	2001	2b	43	Echovirus 13
Dagan, 1988[97]	1982-1983	2b	55	Echovirus 30
Karzon, 1962[98]	1955	2b	127	Echovirus 6
Tavakoli, 2008[48]	2005-2006	2b	39	Coxsackie virus B5
Peci, 2014[99]	2005-2011	2b	583	Coxsackie virus A9
Luchs, 2008[100]	2004	3b	23	Echovirus 6

(Continues)

TABLE 3 (Continued)

First Author, Year	Study Period	CEBM Level	Cases, n	Most Prevalent EV Genotype(s)
South-East Asian Region				
Kumar, 2013[101]	2009-2010	2b	51	Echovirus 32
Lee, 2007[102]	2005	3b	24	Coxsackie virus B5
Kim, 2012[103]	2008	3b	287	Echovirus 30
Joo, 2005[104]	1995-1999	3b	12	Coxsackie virus B1, echovirus 6, echovirus 30
Baek, 2011[105]	2008-2009	2b	16	Coxsackie virus B1
Hyeon, 2013[106]	1999-2011	2b	1063	Echovirus 30
Western Pacific Region				
Momoki, 2009[107]	2004-2008	2b	24	Coxsackie virus B5
Cui, 2010[108]	2005	3b	15	Coxsackie virus A9
Liu, 2014[109]	2009	3b	16	Coxsackie virus B5
Kao, 2003[110]	2001	3b	17	Echovirus 30
Zhu, 2016[111]	2009-2010	2b	85	Echovirus 9
Xiao, 2013[112]	2012	2b	38	Echovirus 30
Tao, 2014[113]	2006-2012	2b	84	Echovirus 30
Kaida, 2004[114]	2001-2002	3b	36	Echovirus 13
Hsu, 2011[115]	2008	2b	19	Echovirus 30
Chen, 2013[116]	2009	2b	17	Coxsackie virus B5
Huang, 2015[56]	2009-2013	2b	30	Enterovirus-A71
Chen, 2018[58]	2013-2015	2b	54	Echovirus 18
Zhao, 2005[117]	2003	2b	18	Echovirus 30
Huang, 2003[118]	2000	2b	75	Echovirus 33
Papadakis, 2014[119]	2007-2012	2b	315	Echovirus 6

Abbreviations: CEBM: Centre for Evidence-Based Medicine; EV: enterovirus; WHO: World Health Organisation.

TABLE 4 Proportion of NPEV genotypes reported in the analyzed AFP cases

Genotype	Studies, n	Proportion (95% CI)	I^2
Enterovirus-A71	11	0.12 (0.05-0.20)	96.62
Echovirus 13	12	0.08 (0.05-0.12)	90.17
Echovirus 11	12	0.07 (0.05-0.09)	78.9
Echovirus 6	12	0.06 (0.04-0.09)	87.94
Coxsackie virus B3	11	0.05 (0.03-0.09)	85.32
Coxsackie virus A24	7	0.05 (0.01-0.09)	92.96
Echovirus 7	12	0.04 (0.03-0.05)	59.77
Coxsackie virus B5	10	0.04 (0.02-0.06)	72.06
Echovirus 14	10	0.04 (0.02-0.06)	77.19
Echovirus 19	9	0.04 (0.02-0.06)	81.42
Other	18	0.6 (0.52-0.67)	94.16

Abbreviations: AFP: acute flaccid paralysis; NPEV: nonpolio enterovirus.

TABLE 5 Proportion of NPEV genotypes reported in the analyzed encephalitis cases

Genotype	Studies, n	Proportion (95% CI)	I^2
Enterovirus-A71	10	0.77 (0.61-0.91)	93.45
Echovirus 30	5	0.20 (0.03-0.44)	95.72
Echovirus 18	3	0.17 (0.00-0.53)	93.8
Echovirus 6	6	0.13 (0.06-0.22)	77.82
Coxsackie virus B5	10	0.11 (0.05-0.18)	71.78
Other	20	0.53 (0.34-0.71)	97.26

Abbreviation: NPEV: nonpolio enterovirus.

4.3 | Meningitis genotypes

The most common EV genotypes identified in cases of meningitis were echovirus 30, echovirus 6, and echovirus 13. Echovirus 30 has been responsible for numerous outbreaks in continents including Asia[103,112,117] and Europe,[71,73,81] indicating an active global circulation of this genotype. The predominance of echovirus 30 in cases of viral meningitis has not been entirely explained, with research continually being done into its mechanism of disease. In particular, Bernit et al suggested that echovirus 30 transmission may not be strictly faecal-oral as other NPEVs are, but this theory is yet to be substantiated.[136] Consequently, as with EV-A71, the reasons for the neurovirulence of echovirus 30 have yet to be comprehensively ascertained. Our review thus confirms the importance of research into specific virulence factors and treatments in each of these prevalent genotypes.

4.4 | Genotyping method

The most common method of genotyping used was RT-PCR. The structural protein VP1 was the target for RT-PCR amplification in

TABLE 6 Proportion of NPEV genotypes reported in the analyzed meningitis cases

Genotype	Studies, n	Proportion, 95% CI	I^2
Echovirus 30	41	0.35 (0.27-0.42)	98.74
Echovirus 6	36	0.16 (0.11-0.21)	96.37
Echovirus 13	23	0.13 (0.07-0.19)	96.95
Coxsackie virus A7	2	0.10 (0.10-0.10)	0.00
Echovirus 9	27	0.07 (0.05-0.09)	89.41
Coxsackie virus A9	22	0.07 (0.04-0.10)	90.14
Echovirus 11	20	0.07 (0.03-0.11)	98.25
Echovirus 18	16	0.06 (0.03-0.10)	92.55
Echovirus 7	12	0.03 (0.01-0.06)	98.04
Echovirus 19	3	0.03 (0.00-0.14)	98.92
Other	54	0.47 (0.42-0.53)	97.65

Abbreviation: NPEV: nonpolio enterovirus.

TABLE 7 Methods of EV genotyping across all 96 studies

Method of genotyping	Number of studies
VP1 RT-PCR	44/96; 45.83%
MN assay and VP1 RT-PCR	12/96; 12.50%
MN assay	10/96; 10.42%
5′UTR RT-PCR and VP1 RT-PCR	8/96; 8.33%
5′UTR RT-PCR	5/96; 5.21%
IFA	6/96; 6.25%
VP1 RT-PCR and VP4 RT-PCR	3/96; 3.13%
MN assay, VP1 RT-PCR and 5′UTR RT-PCR	2/96; 2.08%
RT-PCR unspecified target	2/96; 2.08%
IFA,VP1 RT-PCR and 5′UTR RT-PCR	1/96; 1.04%
VP2 RT-PCR	1/96; 1.04%
VP4/2 RT-PCR	1/96; 1.04%
Virus isolation	1/96; 1.04%

Abbreviations: EV: enterovirus; IFA: immunofluorescence assay; MN: microneutralization; RT-PCR: reverse transcription-polymerase chain reaction.

44/96 studies (45.83%). This is in line with recent recommendations from the European NPEV Network that RT-PCR with VP1 should be used for diagnosis of EV.[17] After VP1, VP2, and VP4 are recommended as second-line targets of RT-PCR. The less frequent use of 5′UTR in our results may be explained by the more conservative nature of the 5′UTR in comparison to the VP1 gene among EV species.[137] There has also been more genetic recombination found in the 5′UTR region in comparison to the VP1 region.[138] Both these issues have been proposed to explain why a study conducted by Chiang et al demonstrated more false positives and mismatched genotyping associated with 5′UTR than VP1 sequencing.,[137,138] Moreover, the papers included in our review did not utilize Next Generation Sequencing methods. Currently, genome sequencing is a requirement for identification of new recombination events and recombinant EVs, although it is yet to be employed diagnostically on a large scale.[17] In the future, it is expected that sequencing methods will be readily used for diagnostic EV typing in surveillance and outbreaks, especially as improvements are made in cost and efficiency.[17]

4.5 | Limitations

The main benefit of performing this meta-analysis was the improved power and precision gained by pooling estimates of genotype prevalence from individual studies. Our review included studies from all six WHO regions, and 67.97% of included studies were rated 2b or higher according to the CEBM criteria.

Nonetheless, this review poses a number of limitations. Firstly, there is a risk of publication bias, due to the exclusion of both grey literature and conference abstracts that did not provide sufficient data for analysis. We attempted to minimize this risk through a comprehensive literature search strategy and secondary hand-searching of bibliographies from included studies. Secondly, the exclusion of non-English studies from the meta-analyses may lead to language-bias. To minimize this, we reviewed the non-English abstracts presented in Table S3. Furthermore, the reliance on reported clinical diagnoses of the neurological conditions may be associated with inaccuracies, as there is often clinical overlap between encephalitis and meningitis.

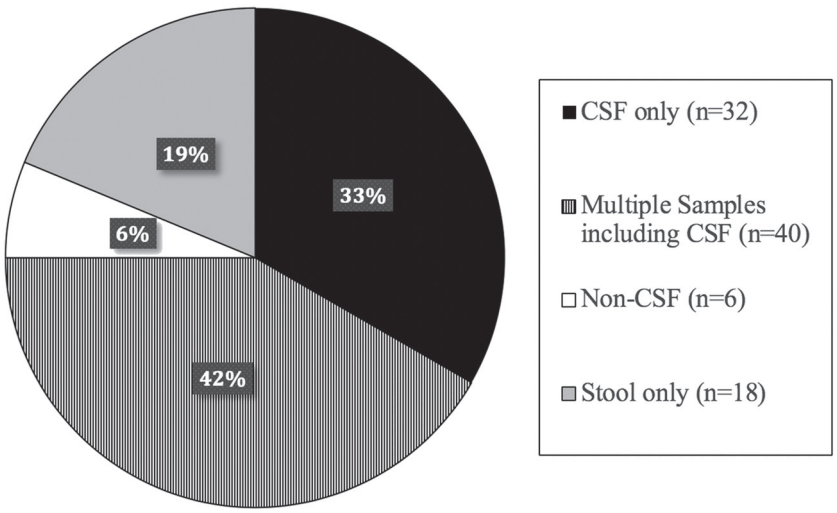

FIGURE 2 Site of sampling of specimens across all 96 studies

Finally, it is possible that other neurotropic EVs, which are less easily isolated or detected, may contribute to a greater degree than is apparent in our review. This could also be influenced by the source of material genotyped: isolated viruses or nucleic acid direct from clinical sample. We were not able to perform extensive subgroup analyses in this regard due to a restricted sample size, particularly in the isolated viruses arm of analysis. However, brief subgroup analyses of four AFP and four encephalitis papers, which genotyped nucleic acid direct from clinical sample, revealed similar trends to the global analysis. It is expected that more thorough diagnostic guidelines for EV neurological complications and advancement in Next Generation Sequencing will improve these limitations for future research in this subject area.

5 | CONCLUSIONS

This first meta-analysis of the global genotype distribution of EVs for AFP, encephalitis, and meningitis shows that the predominant EV genotypes differed between these conditions. Review demonstrated that the most common genotypes were EV-A71 for AFP and encephalitis, and echovirus 30 for meningitis. This epidemiological knowledge may assist in directing development in diagnostics and therapeutics for EV neurological infections. Given the potentially severe outcomes of such infections, attempts to characterize the neurovirulence of the different genotypes must continue. This research will be aided by development of Next Generation Sequencing techniques, more rigorous surveillance programs, and advances in EV treatments and vaccines.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to disclose.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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