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The epidemiology of non-polio enteroviruses: recent advances and outstanding questions

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Abstract

Purpose of review—There are over 100 serotypes of human enteroviruses, which cause a spectrum of illnesses, including meningitis, encephalitis, paralysis, myocarditis and rash. Increasing incidence of hand-foot-and-mouth disease in the Asia-Pacific and recent outbreaks of enterovirus-associated disease, such as severe respiratory illness in the United States in 2014, highlight the threat of these viruses to human health.

Recent findings—We describe recent outbreaks of human enteroviruses and summarise knowledge gaps regarding their burden, spectrum of diseases and epidemiology.

Summary—Reported outbreaks of respiratory, neurological, skin and eye diseases associated with human enteroviruses have increased in frequency and size in recent years. Improved molecular diagnostics and genetic sequence analysis are beginning to reveal the complex dynamics of individual serotypes and genotypes, and their contribution to these outbreaks. However, the biological mechanisms underlying their emergence and transmission dynamics remain elusive. They are likely to involve changes in the virus, such as fitness, antigenicity, virulence or tropism, and in the human population, such as levels of sanitation and of homo- and heterotypic immunity. Improvements in surveillance, serological surveys and detailed genetic and antigenic characterisation of viral populations would help to elucidate these mechanisms. This will be important for the design of outbreak control and vaccine development strategies.

Keywords

Enterovirus; epidemiology; hand-foot-and-mouth disease; outbreak; vaccine

Introduction

Enteroviruses (EVs, *Picornaviridae* family) are a genus of small, non-enveloped, single-stranded RNA viruses. They have high mutation rates and undergo frequent recombination. The genome region encoding their capsid protein VP1 (which contains the majority of neutralization epitopes) correlates well with serotypes and molecular methods use it for serotyping [1]. Molecular typing has largely replaced neutralization tests and is now

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considered the gold standard. More than 100 enterovirus serotypes infect humans, and are currently classified into four species, A to D, according to molecular and antigenic properties (Table 1, Figure 1). This large group of viruses includes polioviruses, coxsackieviruses A and B, echoviruses, and various serotypes identified more recently simply called “enterovirus” and sequentially numbered (starting at 68).

Most human enteroviruses enter the body via the alimentary or respiratory tract and spread by both fecal-oral and respiratory routes, although a few exceptions to the usual modes of transmission exist [2]. Rhinoviruses, which are closely related to enteroviruses, have recently been classified within the genus *Enterovirus*, but they have unique biological and physical properties that cause them to be restricted to the upper respiratory tract [2]. Rhinoviruses are beyond the scope of this review, as are polioviruses, which are the target of a major eradication initiative.

Enterovirus infections can result in a wide range of clinical diseases, from minor febrile illness and rash to severe, sometimes fatal, conditions, including meningitis, encephalitis, paralysis and myocarditis (Table 2). Although most infections are subclinical, sporadic or regular outbreaks of disease are common worldwide, and can lead to significant morbidity and mortality. In particular, non-polio enteroviruses are recognised as an emerging cause of neurological diseases, which more often arise as complications of mild infections. This has become more evident during recent years. For example, hand-foot-and-mouth disease (caused by EV-A71 and CV-A16, among others) has been increasing in incidence in the Asia-Pacific, and despite polio having been eliminated from most countries, the burden of acute flaccid paralysis (AFP) remains high, with many of those cases associated with non-polio enteroviruses [3].

Enteroviruses often show seasonal patterns of incidence in both temperate and tropical climates, although seasonality is typically more marked in the former, with infections more common in summer and early autumn [2]. Passive laboratory surveillance of clinical isolates reveals different patterns of circulation over time and by geographic location for individual serotypes. For example, surveillance in the USA [4] recorded large and periodic outbreaks followed by years of relative quiescence for some serotypes (e.g. echovirus 9 exhibits large outbreaks every 3–5 years), whilst other serotypes have endemic patterns with relatively stable circulation (e.g. coxsackievirus B4), and others irregular patterns (Figure 2).

Enterovirus surveillance relies on passive, voluntary, laboratory-based systems, where participating laboratories report enterovirus detection to a reference centre (e.g. in the USA, the UK, the Netherlands, France [4,5,6,7]). Although enterovirus surveillance may in theory provide information relevant to the detection of outbreaks and changing patterns of infection, the burden of disease and the emergence of new strains, in practice these systems suffer a number of important biases. First, most enterovirus-associated diseases are not notifiable (except AFP), such that only a small proportion of all cases of a clinical illness are tested. Second, methods for virus identification have changed during the last decade (cell culture has been superseded by molecular methods). Third, an increasing number of enterovirus-positive specimens remain untyped because many laboratories do not perform molecular typing and samples may not be submitted to reference centres with this capacity.

Fourth, laboratory methods used to test specimens are not standardised (e.g. use of different primers). Finally, in a context of an outbreak, the number of reports is likely to increase due to enhanced surveillance.

Recent outbreaks of disease, such as hand-foot-and-mouth disease (HFMD) in the Asia-Pacific and the outbreak of severe respiratory illnesses (caused by EV-D68) last year in the USA [8,9], have raised basic and applied questions about the mechanisms determining their emergence and transmission dynamics. Here, we review the epidemiology of recent outbreaks, progress towards the development of vaccines to control such outbreaks, and outstanding research questions that are important from a public health perspective.

Outbreak of severe respiratory illness in the USA in 2014

Enterovirus D68 (EV-D68) has biological features similar to rhinoviruses (e.g. acid lability and optimal growth at 33°C [10]) and has long been exclusively associated with respiratory diseases. First isolated in 1962 from four children with pneumonia and bronchiolitis in California [11], only sporadic cases were reported until the late 2000s. In the USA, only 26 cases were notified between 1970 and 2005 [4].

During the last decade, several clusters of relatively small size (no more than 40 cases) of respiratory illnesses associated with EV-D68 occurred in Asia [12,13,14,15], Europe [12,16,17] and the USA [12,18], mostly affecting young children aged 0–4 years. The clinical respiratory manifestations ranged from mild to severe, sometimes fatal. Most clusters showed an atypical late seasonality compared to other enteroviruses with a peak in autumn, instead of summer [12]. The apparent increase in the number of detections was first thought to be related to improvements in detection techniques, since older tests could not detect EV-D68 or misidentified it as a rhinovirus [12]. However, retrospectively tested samples from the Netherlands and Japan confirmed the increase [13,16], which was also accompanied by an expansion in genetic diversity in the late 2000s [16,19]. Currently, three co-circulating clades of EV-D68 have been described, which probably diversified during the mid to late 1990s [13,16,19]. These clades have recently been shown to have different antigenic properties [20*].

A number of biological mechanisms have been hypothesized for the observed global increase in EV-D68 detections over the last decade. For example, amino acid changes in the VP1 compared to the Fermon reference strain that have resulted in altered antigenic properties may have permitted immune escape [16,20*]. Alternatively, EV-D68 may have become more virulent as a result of nucleotide deletions in the 5' untranslated region [19].

In 2014, the largest outbreak to date of severe respiratory illness associated with EV-D68 occurred in the USA. The first cases were reported in August 2014 from Missouri and Illinois, and by January 2015 more than 1,150 cases across the country had been confirmed [8,9]. Of note, almost all the cases were children and a considerable proportion had a previous history of asthma or wheezing [8,9].

Coinciding with the 2014 outbreak in the USA, there was an unusual number of reports of children who developed a sudden onset of acute weakness in arms or legs similar to that

caused by polioviruses; this is now referred to as acute flaccid myelitis (AFM) [21]. The first cluster was reported in Colorado, started in August 2014 and involved 12 children [22,23]. The total number of AFM reports by mid-April 2015 was 118 from 34 states in the USA and most cases were children <18 years of age [21,24]. Interestingly, during the two years preceding the EV-D68 outbreak, an unusual number (23 in total) of “polio-like” illness were also reported in California [25], among which some had a clinical and radiologic picture similar to the AFM cases described since August 2014 [25]. Most AFM patients reported a respiratory and/or febrile illness preceding the onset of the neurological symptoms [22,24] and some tested positive for EV-D68 in nasopharyngeal samples (5/11 in Colorado [22], 8/41 tested at CDC [24], 2/23 in California [25]). Although the virus has not been detected in the CSF of patients [22,24] (in fact, EV-D68 has only been isolated from CSF in two cases in the literature, once in 2005 [4] and once in 2008 [26]), these observations raised the question of an association between the outbreak of EV-D68 respiratory disease and those cases of neurologic illness. A new phylogenetic study has strengthened that putative association [27*]. It showed that EV-D68 strains from AFM cases belonged to a recent evolutionary cluster, clade B1, which emerged in 2010. The same study identified six single nucleotide polymorphisms (SNPs) specific to clade B1, some of which matched with SNPs in polioviruses and enterovirus D70 (which has previously been linked with AFP) and might have conferred neurovirulence to EV-D68 [27*].

Large-scale epidemics of acute haemorrhagic conjunctivitis

Two enteroviruses are the main etiological agents of explosive and large-scale outbreaks of acute haemorrhagic conjunctivitis (AHC): enterovirus D70 (EV-D70) and an antigenic variant of coxsackievirus A24 (CV-A24v). Both strains spread mainly via direct or indirect contact with eye secretions and both have been found in stool, suggesting that the fecal-oral route is also a mode of transmission [28,29]. A tropism of CV-A24v for the airways has also been suggested, which makes respiratory transmission also likely [30]. Although most cases resolve without sequelae after a 5–7 day course, microbial superinfection can occur and neurological complications such as “polio-like” paralysis have been reported during AHC outbreaks of EV-D70 [29,31]; however, the virus was not isolated from CSF [29,31] and cases were reported from settings where polio was still endemic, leaving the causal link uncertain.

The first documented outbreak of AHC due to EV-D70 occurred in Ghana in 1969 [32,33], and during 1970 and 1971 the virus spread globally, producing the first AHC pandemic and affecting millions of people [33,34]. Since the mid-1990s, no more outbreaks due to EV-D70 have been documented [35,36,37].

CV-A24v was first isolated in 1970 in Singapore [38] and remained restricted to South-East Asia and India until 1985. Since then, it has been responsible for outbreaks worldwide (e.g. Cuba 2008–2009 [28]; China 2010 [39]; India 2010 [40]; Japan 2011 [41]; Thailand 2014 [42]), including two pandemics: the first started in 1985 [43,44] and the second in 2002 [45]. Phylogenetic analyses of CV-A24v allowed the identification of four genotypes (GI to GIV): GI and GII included isolates from the 1970s, GIII from the late 1980s and early 1990s (i.e. including the first pandemic), and GIV from the second pandemic and others more recently

identified [28,39,40,42,46,47]. This suggests a turnover of CV-A24v genotypes: new genetic variants rapidly spread globally and then fade out to be replaced by new variants.

With some exceptions, outbreaks of AHC seem to principally affect tropical and subtropical regions during the hot, rainy seasons and some countries have experienced several outbreaks during the last decades (e.g. Singapore, Thailand, Brazil, China, India). A recent study from Thailand suggests that CV-A24v has been continuously circulating at least since 2002, producing large and periodic epidemics every 2–3 years interspersed with minor outbreaks and sporadic cases [42]. Older studies showed that infected individuals develop neutralizing antibodies that last only for a few years [48,49], which could explain the patterns observed in Thailand, where extensive outbreaks may occur when population immunity falls below a certain threshold.

The size of AHC outbreaks and their rapid spread impose a significant burden on primary healthcare systems and normal activities of affected countries may be partly paralyzed (e.g. schools, companies, sports) [50]. Because no treatment (either vaccines or antiviral drugs) is currently available, control measures are reduced to limiting the spread of the virus (e.g. encouraging hand-washing and increased hygiene, and alerts via radio and media channels).

Hand-foot-and-mouth disease in the Asia-Pacific region

Hand-foot-and-mouth disease (HFMD) is a common, typically mild and self-limiting illness that mainly affects young children under 5 years of age (clinical manifestations include fever, a rash with blisters on hands, feet and buttocks, and vesicles in the mouth). The most common etiological agents are enterovirus A71 (EV-A71) and coxsackievirus A16 (CV-A16). Approximately 30% of hospitalised HFMD patients with EV-A71 infection develop more severe disease including neurological symptoms (e.g. aseptic meningitis, encephalitis, acute flaccid paralysis) and cardiopulmonary complications [51,52], which in some cases can be fatal. These severe outcomes are very unusual with CV-A16 [51]. EV-A71 is currently considered the most neurotropic enterovirus beyond polioviruses and there is a considerable literature on it (for reviews see [53,54,55]).

Only small outbreaks and sporadic cases of EV-A71 were reported throughout the world between the early 1970s and the late 1990s, with the exception of two large outbreaks, one in Bulgaria in 1975 [56] and one in Hungary in 1978 [57], both associated with a high mortality. During the last 10–20 years, major outbreaks of HFMD have occurred in the Asia-Pacific, becoming an important public health problem. This has led to increasing attention from health authorities, declaration of HFMD as a notifiable disease and the introduction of case-based surveillance systems [58]. Unprecedented outbreaks occurred in 1997 in Sarawak, Malaysia (at least 34 deaths [59,60]) and in 1998 in Taiwan (total number of cases estimated at around 1.5 million, >120,000 cases reported, among which >400 had severe diseases and 78 died [60,61]). Large-scale outbreaks have subsequently been reported in Malaysia, Taiwan, Singapore, China, Hong Kong, Japan, Republic of Korea, Vietnam and Cambodia. In China, from 2008 to 2014, >10 million cases of HFMD and >3,000 deaths were reported [62], with 80% of severe cases and 93% of fatal cases due to EV-A71 [63**].

Although outbreaks tend to occur in warmer months, significant geographical variability in the epidemic patterns of HFMD has been observed. A study based on surveillance data from China showed that the periodicity of the outbreaks and the timing of the peak varied with latitude [63**]: northern provinces had one peak in June, (as observed in Japan [64]), whereas southern provinces had two peaks, around May and October (as observed in the South of Vietnam [65]). Other countries, however, such as Hong Kong and Singapore, have observed changes over time in the periodicity of the peaks [66,67,68,69]. Although seasonal variation has been suggested to be associated with climatic factors [63**], other factors, like changes in the circulating and predominant serotypes (some of which could circulate or peak earlier than others) could also be involved (e.g. in Singapore, changes in the dominant serotype coincided with changes in the annual number of peaks [66]).

Temporal changes in the circulating and predominant serotypes have been observed in many places. For example, EV-A71 seems to have a periodicity of 3–4 years in Japan [64], and 3 years in Malaysia [70], whereas in China it circulated widely every year from 2008 to 2014 [62,63**]. Coxsackievirus A6 (CV-A6) has become a predominant serotype in many places during the last 2 to 5 years, often replacing EV-A71 and CV-A16 as the main cause of HFMD: e.g. Japan [64], Singapore [67,68], and different regions in China [71,72,73,74,75,76]. In a few places, coxsackievirus A10 (CV-A10) has also been recently reported as a predominant serotype [77,78]. Some of the CV-A6 and CV-A10 strains currently circulating in the Asia-Pacific are likely to have originated in Europe, where they caused outbreaks of atypical HFMD (e.g. associated with nail shedding [79,80]).

Concerns that large-scale outbreaks of HFMD may occur in India have arisen, mainly because of the close proximity to China and other conditions that could favour the rapid spread of the virus, including high population density and poor hygiene and sanitation [81]. Nevertheless, only small-scale outbreaks have occurred to date. Why the disease seems to be mainly restricted to the Asia-Pacific remains unknown.

HFMD vaccine development

Owing to the growing threat of HFMD in countries in the Asia-Pacific and the lack of effective antiviral therapies, efforts to develop an EV-A71 vaccine have recently been accelerated. The successful use of inactivated and live-attenuated poliovirus vaccines as part of the Global Polio Eradication Initiative emphasizes the potential to achieve safe and durable protective immunity against enterovirus infection. A variety of EV-A71 vaccine candidates have been evaluated in preclinical studies, including inactivated [82], live-attenuated [83], virus-like particle [84], subunit [85], and DNA vaccines [85]. Among these, formalin-inactivated whole-virus vaccines are the most advanced, and five candidates developed in mainland China, Taiwan and Singapore have entered clinical evaluation [86]. Recently, phase III randomized, double-blind, placebo-controlled trials were conducted in China for three of these vaccines [87,88,89]. Each trial involved the administration of two doses of alum-adjuvant inactivated EV-A71 vaccine or placebo at an interval of 28 days to more than 10,000 children aged 6–35 months [88,89] or 6–71 months [87]. Surveillance for EV-A71-associated disease (including HFMD, CNS disease and other EV-A71-associated sequelae) was then performed for up to 1 year. Each vaccine displayed an acceptable safety

profile, and protective efficacy against EV-A71-associated HFMD ranged between 90.0% and 97.4%, raising the possibility that an EV-A71 vaccine will soon be licensed in China.

Despite this recent progress, several hurdles to the introduction of a vaccine against HFMD remain. Co-administration studies are required to test for potential interactions with other vaccines included in the Expanded Program on Immunization, while further studies are merited to determine whether the vaccines are efficacious in children <6 months of age. The cross-protective efficacy of the EV-A71 vaccines is also uncertain. To date, the vaccines that have entered clinical evaluation have been based on locally dominant EV-A71 genotypes (C4 in China, B4 in Taiwan and B2 in Singapore). Although antibodies induced by a given EV-A71 strain exhibit cross-neutralizing activity against other genotypes [90], this may not extend to all other EV-A71 strains [91]. Moreover, vaccinating against EV-A71 tackles just one cause of HFMD. The EV-A71 vaccines showed no efficacy against HFMD caused by CV-A16 or other serotypes during the recent phase III trials [87,88]. In the longer term, the development of multivalent vaccines targeting EV-A71, CV-A16 and other enteroviruses associated with HFMD may be desirable. Preclinical studies of multivalent HFMD vaccines have commenced [92*].

Conclusion

Focusing on a few examples of disease outbreaks, we have illustrated various clinical syndromes caused by non-polio enteroviruses, the complexity of their circulation dynamics and their capacity to spread rapidly.

The mechanisms of emergence of outbreaks are not known and our understanding is limited by relatively weak surveillance systems, typically based on passive reporting by public health laboratory. A combination of virus-specific, population-level and other external factors is likely to be involved. The evolutionary dynamics of enteroviruses confer them with the capacity to experience rapid phenotypic changes, including transmissibility, antigenicity, virulence and tropism (i.e. spectrum of disease). At the population level, the number of non-immune individuals needs to be above a certain threshold for an outbreak to be triggered, and depends on the number of births and the serological status of the population. The immune status of the population at a given time is related, among others, to the previous exposure of that population to the same or a related virus, the cross-reactivity between different genogroups of a given serotype (and between different serotypes), and the decay of neutralizing antibodies over time. Finally, climatic and environmental variables seem to correlate with the peaks of incidence of disease (e.g. HFMD), which suggests that they might affect the survival of the virus outside the host or the efficiency of transmission, which might differ among serotypes depending on their transmission routes.

Through genome sequencing, molecular epidemiology is helping to track the temporal and spatial circulation of different strains and determine their origin and geographical range. Large-scale genetic analyses have revealed the evolutionary and epidemiological dynamics of some serotypes. Distinct phylogenetic patterns have been described for different serotypes or even for different clades within a serotype (e.g. coxsackievirus B5 [93]). In some cases, ladder-like trees similar to influenza A have been obtained, consistent with continuous

selection for new antigenic variants (e.g. echovirus 9 [94]). In other cases, co-circulation of several lineages has been observed, suggesting different selective forces or greater geographic isolation (e.g. echovirus 6 [95]). It has also been reported that peaks in incidence of some serotypes have been preceded by the emergence of recombinant groups (e.g. echovirus 9 [94]).

Unfortunately, molecular analyses alone do not allow the biological mechanisms determining the patterns of circulation of individual serotypes to be elucidated, and in particular, the role of immunity remains poorly understood. However, molecular epidemiological studies combined with investigations of antigenic changes and serological surveys have the potential to provide novel insights towards better understanding the occurrence of outbreaks. This would also allow elucidation of the mechanisms acting across genogroups or serotypes, which could explain the periodic changes in predominant variants. For instance, one study with isolates of EV-A71 from Taiwan showed using phylogenetic analyses that every large outbreak between 1998 and 2008 was associated with a genotypic change, alternating between B and C (B1, C2, B4, C4, B5), and then using antigenic cartography showed that the predominant strain in every major outbreak was antigenically distinct from the predominant strain in the previous outbreak [96].

Understanding the contribution of strain- or serotype-specific and heterotypic population immunity to the patterns of circulation of non-polio enteroviruses may help explain and predict outbreaks of disease. Ultimately this may lead to improved vaccination strategies, including the selection of appropriate vaccine strains.

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* of special interest

** of outstanding interest

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Key points

- Non-polio enteroviruses are an emerging cause of neurological diseases, predominantly arising as complications of mild, self-limiting infections.
- The full spectrum of illnesses caused by individual serotypes remains unclear; e.g. EV-D68 has long been associated with respiratory diseases but is now suspected to also be responsible for acute flaccid myelitis.
- Some enteroviruses spread worldwide very quickly, as illustrated by the pandemics of acute haemorrhagic conjunctivitis caused by CV-A24v, with a rapid turnover of genetic variants that emerge, spread globally and then vanish being replaced by new variants.
- Patterns of circulation of individual serotypes, including changes in the predominant ones, are complex and how serotype-specific and heterotypic immunity affects those is not well understood; e.g. why have EV-A71 and CV-A16 been replaced by CV-A6 as the main causative agent of HFMD in several countries in recent years?
- Three inactivated candidate vaccines against EV-A71 have completed phase III trials in China and could be licensed soon.

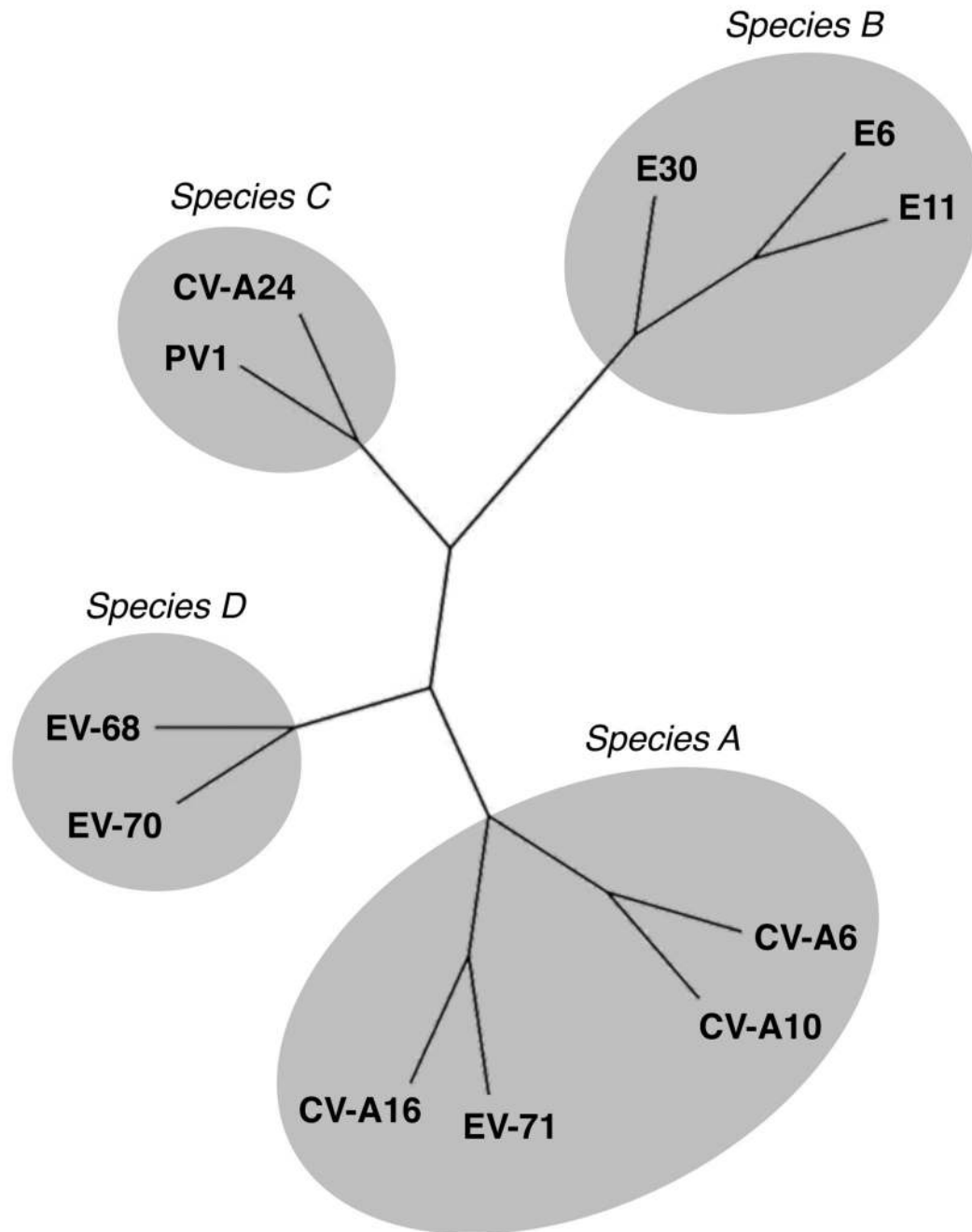


Figure 1.

Schematic representation of the phylogenetic relationship between representative serotypes of the four species of human enteroviruses based on VP1 sequences reported in GenBank. A multiple sequence alignment was generated using Clustal Omega. A Neighbour-Joining tree was then created from this alignment, and visualised using FigTree v1.4.2 (<http://tree.bio.ed.ac.uk/software/figtree/>). Branch lengths are not to scale. Accession numbers were as follows: CV-A6, AF081297; CV-A10, AF081300; CV-A24, AF081311; E6, AF081321; E11, AF081326; E30, AF081340; EV-68, AF081348; EV-71, JN168790; EV-70, D00820

(nucleotides 2425–3339); PV1, V01149 (nucleotides 2480–3385); and CV-A16, U05876 (nucleotides 2446–3336). CV-A: coxsackievirus A; E: echovirus; EV: enterovirus; PV: poliovirus.

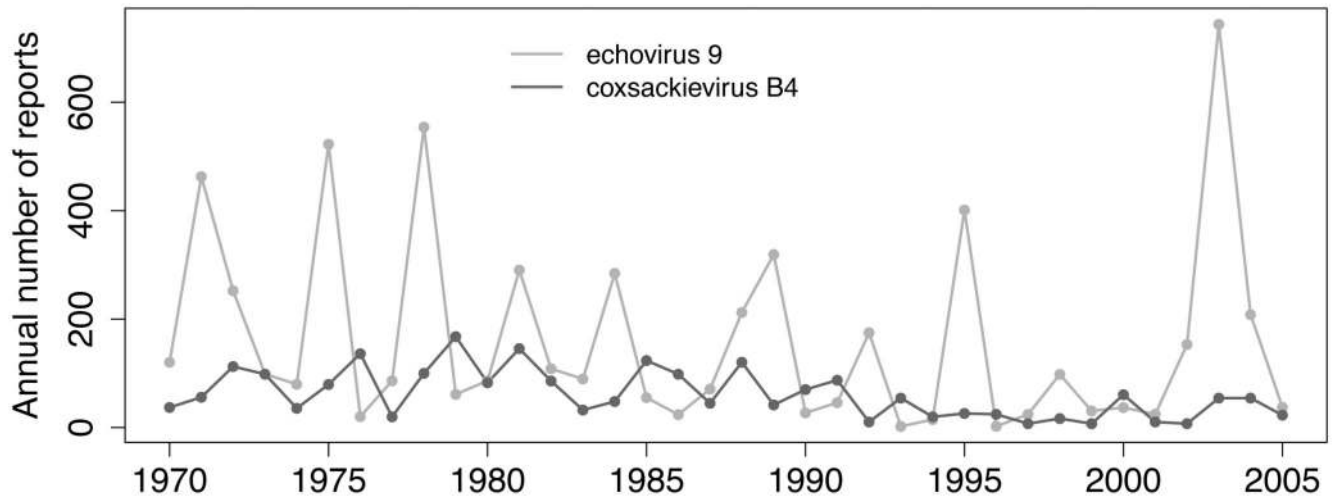


Figure 2. Number of annually reported echovirus 9 and coxsackievirus B4 detections in the United States. Based on data from [4].

Table 1
Human enterovirus serotypes. Updated from[2]

EV species	Serotypes
Species A	Coxsackievirus A 2–8, 10, 12, 14, 16, Enterovirus 71, 76, 89–91, 114, 119–121
Species B	Coxsackievirus A 9, Coxsackievirus B 1–6, Echovirus 1–7, 9, 11–21, 24–27, 29–33, Enterovirus 69, 73–75, 77–88, 93, 97, 98, 100, 101, 106, 107
Species C	Poliovirus 1–3, Coxsackievirus A 1, 11, 13, 17, 19–22, 24, Enterovirus 95, 96, 99, 102, 104, 105, 109, 116–118
Species D	Enterovirus 68, 70, 94, 111

Table 2
Diseases caused by enteroviruses. Based on data from [2]. CV-A: coxsackievirus A; CV-B: coxsackievirus B; EV: enterovirus; E: echovirus; PV: poliovirus

Disease	Serotypes commonly implicated
CNS infections	
<i>Acute flaccid paralysis</i>	PVs, EV-A71
<i>Aseptic meningitis</i>	PVs, CV-Bs, CV-A5, 7, 9, 16, E4, 6, 9, 11, 14, 16, 25, 30, 31, EV-A71
<i>Encephalitis</i>	PVs, E6, 9, 17, 21, CV-A2, 9, CV-B1, EV-A71
Heart disease <i>(acute and chronic myocarditis, dilated cardiomyopathy)</i>	
	CV-Bs
Eye infections	
<i>Acute haemorrhagic conjunctivitis</i>	CV-A24v, EV-D70
Skin rashes	
<i>Hand-foot-and-mouth disease</i>	CV-A 4–6, 9, 10, 16, CV-B2, 5, EV-A71
<i>Herpangina</i>	CV-As, CV-Bs, E6, 9, 11, 16, 17, 22, 25, EV-A71
Respiratory infections <i>(common cold, rhinitis, pharyngitis, pneumonia, bronchiolitis and others)</i>	
	CV-As, CV-Bs, EV-D68, Es
Muscle disease	
<i>Epidemic pleurodynia</i>	CV-Bs, CV-A4, 6, 9, 10, E1, 6, 9, 16, 19
<i>Acute and chronic inflammatory myopathies</i>	CVs (in experimental animals)
Pancreatitis	
	CV-Bs
Diarrhea	
	CV-As, Es
Undifferentiated febrile illness	
	PVs, CV-Bs
Neonatal infections <i>(including prenatal, natal and postnatal infections)</i>	
	CV-Bs, Es