European Journal of Clinical Microbiology & Infectious Diseases (2025) 44:197–207
https://doi.org/10.1007/s10096-024-04970-0

BRIEF REPORT

Incidence, clinical manifestations and characterization of Enterovirus in the last decade (2014–2023) in Asturias (Spain). Effect of the SARS-CoV-2 pandemic

Susana Rojo-Alba[1] · José María González-Alba[1] · Zulema Pérez Martínez[1] · Cristina Ochoa Varela[1] · Maria Agustina Alonso Álvarez[2] · Pablo Rubianes Fernández[3] · Mercedes Rodríguez Pérez[1] · Estibaliz Garrido García[2] · José Antonio Boga[1] · Santiago Melón García[1] · Marta Elena Álvarez-Argüelles[1]

Received: 27 September 2024 / Accepted: 22 October 2024 / Published online: 15 November 2024

Abstract

Enteroviruses (EVs) are a large group of genotypes that cause a variety of pathologies, some of them very serious. This study analyzed the last 10 years (2014–2023) of EVs diagnosis and classification. In 166,674 samples collected, EVs were found in 9,535 (5.7%) by rt-RT-PCR, and 332 (3.5%) were classified by Sanger methods. Symptoms were analyzed in 7623 cases. EVs were found in 5718/63,829 (8.9%) before, 1384/42,373 (3.3%) during and 2433/60,472 (4%) after the Covid pandemic ($p < 0.0001$), and in 7249/69,700 (10.4%) children under 6 years and in 2286/96,974 (2.35%) in oldest ($p < 0.0001$). The positive rate of EVs was high but decreased during the Covid period. In the youngest children EVs-A (associated with exantematohous disorders as well as respiratory manifestations and febrile syndromes) was most common, while EVs-B (frequent in neurological symptoms) was most common in children aged 6–15 years and EVs-D (associated to respiratory manifestations) in adults.

Keywords Enterovirus · Genotyping · Epidemiology · Surveillance

✉ Marta Elena Álvarez-Argüelles
martaealvarez@gmail.com

Susana Rojo-Alba
ssnrj4@gmail.com

José María González-Alba
jmgonzalba@yahoo.es

Zulema Pérez Martínez
zperezmrtnz@gmail.com

Cristina Ochoa Varela
crisochoavarela@gmail.com

Maria Agustina Alonso Álvarez
maruchi.al.al@gmail.com

Pablo Rubianes Fernández
pablo.rubianes@sespa.es

Mercedes Rodríguez Pérez
mercedes.rodriguezp@sespa.es

Estibaliz Garrido García
estibaliz.garrido@sespa.es

José Antonio Boga
joseantonio.boga@sespa.es

Santiago Melón García
santiago.melon@sespa.es

[1] Microbiology Department, Instituto de Investigación Sanitaria del Principado de Asturias, Hospital Universitario Central de Asturias, Avn Roma s/n, Oviedo 33011, Spain

[2] Pediatric Department, Hospital Universitario Central de Asturias, Avn Roma s/n, Oviedo 33011, Spain

[3] Emergency Department, Hospital Universitario Central de Asturias, Avn Roma s/n, Oviedo 33011, Spain

Introduction

Enteroviruses (EVs) are among the most prevalent viruses infecting humans world-wide [1–3]. They can be responsible for a common cold, meningitis, encephalitis, paralysis, and even death [1]. Currently classified into 116 serologically distinct EV types, which can be assigned into four genetically distinct species A to D [4]. Species Enterovirus B contains more than half of all know and they are the most common cause of aseptic meningitis worldwide, which mainly affects young children and is an important cause of hospitalization. However, only four types of EVs D have been defined. These include the EV-D68 and EV-A71 most commonly causes respiratory illness and hand, foot, and mouth disease (HFMD), respectively. Sometimes they are linked to acute flaccid paralysis (AFP) [3]. The relationship between type and clinical symptoms has recently been updated [3, 5].

Although there is no effective antiviral treatment available for EV, detection and identification of infections are vital for informing other treatment options, supportive care and prognosis of affected individuals [4].

It is well established that prevalent EV types are constantly evolving both temporally and spatially and typing assays must be robust enough to detect and characterize all EV subspecies to reflect this changing epidemiology. However, the effect of epidemiological changes on current genotyping methodologies remains unknown [6]. Molecular techniques such as reverse transcriptase PCR (RT-PCR) and subsequent nucleotide sequencing have gained significant importance. Sequence homology search in GenBank and phylogenetic analysis are the methodologies preferentially used to sort EV [1].

Here, we conducted a study to detect EV circulating in clinical samples in Asturias from 2014 to 2023. The aim of this study is to know the positive rate, associated-diseases and distribution of EV and to enrich the data of epidemiological molecular studies.

Table 1 Primers and probes used for 5'UTR fragment detection and sequencing

RT-qPCR	Sense	GCCCCTGAATGYGGCTAA
	Antisense	GAIACYTGWGCICCCAT
	Antisense	ATTGTCACCATAAGCAGCCA
	probe-MGB	ACTTTGGGTGTCCGTGTT
Sequencing		GAIACYTGWGCICCCAT
	Ex2	CCTTTGTRCGCCTGTTTTA
	In3	CCTTTGTRCGCCTGTTTTA
	In4	ATTGTCACCATAAGCAGCCA

Materials and methods

Samples

From 2014 to 2023, 166,674 samples were collected to determine the etiology of acute respiratory infection and related symptoms: 63,829 before SARS-CoV-2 pandemic (2014-19), 42,373 during (2020–2021) and 60,472 after (2022-23). The samples were collected at the Universitary Central Hospital of Asturias and analyzed in the Laboratory of Virology of this hospital.

Wherever possible, an analysis of the clinical presentation of patients with EV infection was carried out after consultation of medical records.

EV characterization was performed in 620 samples.

Laboratory diagnosis

The samples were divided into two aliquots according to laboratory protocols. The first (1 ml) was used for conventional monolayer cell culture (MRC-5 and mix of LLC-MK2, A549 and Hep-2), while the second (500 µl) was used for viral nucleic acid detection.

Nucleic acids extraction and virus detection

Nucleic acids were extracted and purified by using the automated nucleic acid purifier MagNA Pure96 (Roche Diagnostics SL, Swizertland) following manufacturer´s instructions. Extracted nucleic acids were resuspended in a final volume of 100 µl.

EV genome was detected and quantified by a multiplex real-time reverse transcription polymerase chain reaction (rt-RT-PCR) for Picornavirus using type-specific primer pairs and MGB probes (ThermoFisher) (Table 1), and the TaqMan Fast 1-Step Master Mix (Life technologies, CA). The rt-RT-PCR was performed with 5 µl of extracted nucleic acids in a final volume of 10 µl as follows: 50º/10', 95º/7', 45 cycles of 95º/5" and 60º/33".

In addition, the human ß-globin gene was quantified in each sample in order to evaluate sample quality and to calculate normalized viral load in copies/10^3 cells.

EVs characterization

For genotypic characterization, 620 extracted under 25 Ct in -RT-qPCR were randomly chosen and genotyped by Sanger sequencing method. A fragment of approximately 700 bp of the 5′ untranslated region (5′UTR) was amplified (Table 1). PCR products were analyzed by agarose gel electrophoresis, extracted by using Montage DNA Gel Extraction Kit (Millipore, USA) and sequenced with Big Dye Terminator v1.1

Cycle Sequencing Kit (Applied Biosystems, USA) supplemented with inner primers using an ABI 3130 genetic analyzer (Applied Biosystems, USA).

Phylogenetic reconstructions

Nucleotide sequences were translated and aligned using the MUSCLE algorithm implemented in MEGA. Sequences (97 to 549 nucleotides) generated in this work have been deposited in GenBank with the following accession numbers: PP530551-PP530882.

Sequences of each type obtained by the Basic Local Alignment Search Tool (BLAST) were used to identify the type (Supplementary File 1). Phylogenetic trees were constructed using ModelFinder, tree reconstruction and ultrafast bootstrap (1000 replicates) with IQ-TREE 2.1.3. The best-fit nucleotide substitution model GTR + F + G4 was identified according to Bayesian information criterion. Bootstrap values were estimated using the SH test and ultrafast bootstrap with 1000 replicates. The types of 332 sequences could be identified because they were grouped in a single monophyletic clade with previously typed viral sequences. Demographic data (age and sex) from those patients are in Supplementary File 2.

Diversity ($D = 1 - \sum f2$) of EV genotypes was analyzed over time, a measure of variability that takes into account the frequencies (f) of all types.

Statistical analyses

Statistical tests (One-way Analysis of Variance)were performed using GraphPad InStat v.3 (GraphPad Software, USA). Tests were considered significant if the p value was less than 0.05.

Results

Positive rate and genotypes of EV

Of the 166,674 samples tested over 10 years, 9,535 (5.7%) were positive, with variation between years. Of these, the symptoms of 7,623 infectious processes in 6,856 patients (with a difference of, at least 3 months between processes) were studied (Table 2; Figs. 1 and 2).

The positivity rate ranged from 8.9% (5718 samples) before Covid to 3.3% (1384 samples) in the Covid period and 4% (2433) after Covid ($p < 0.0001$).

EV were detected in 7249 (10.4%) children younger than 6 years, 1027 (4.5%) 6–14 years and 1259 (1.7%) older than 14 years ($p < 0.0001$).

The 7,623 known infectious processes were diagnosed in 6,988 upper respiratory tract swabs, 289 stool sample, 193 cerebrospinal fluid samples 71 lower respiratory tract samples, 58 skin swabs and 24 other samples (blood, biopsies, pleural fluid). The results of viral load by specimen and the association with syndromes in respiratory samples are shown in Table 3; Fig. 3. There are statistically significant differences between LRS, URS, Stool and skin swabs, CSF and other(One-way Analysis of Variance, $p < 0.05$). There are statistically significant differences between LRM, other and the rest(One-way Analysis of Variance, $p < 0.05$).

On the other hand, of the 640 samples sequenced 332 (3.4% of positive samples) could be typed, representing 4 species and 22 different types: 6 types A, 14 types B, 1 type C and 1 type D. The rest of the sequenced samples could not be typed since the sequenced fragment could not be assigned to a single type. Figure 4 shows the phylogenetic relationships of the sequenced strains in relation to reference strains, and the relationship of type and syndromes.

FS: febril syndrome; ED: Exanthematous disorders; NS: neurological symptoms; GI: gastrointestinal symptoms; LRM: lower respiratory manifestations; URM: upper respiratory manifestations.

It should be noted that EV species A was found in 37 (25.9%) of the exanthematous diseases, representing 72% (37/52) of this pathology. On the other hand, EV species B was found in 9 (10%) of the neurological manifestations, representing 64% (9/14) of this pathology. An EV species D was found in 23 (79.3%) cases of respiratory symptoms, in 13 of which caused lower respiratory tract diseases.

The 332 samples genotyped correspond to 190 men and 142 women. These samples correspond to 276 (83%) children under 5 years, 33 (9%) children aged 6 to 15 years, 19 (7%) adults aged 16 to 70 years and 4 (1%) adults aged 70 years and over.

The results of genotype diversity over time and in relation to age and sex are shown in Table 4; Fig. 5.

Discussion

Enteroviruses are a large group of viruses that can cause serious health problems, such as meningitis in children [7]. Although they are most common in spring and summer, they can be isolated throughout the year. Typing EVs is important for studying the relationship between EV type and time of circulation and clinical syndrome, to find types or variants, and for epidemiological surveillance as the predominant enterovirus type varies from year to year [8–10].

In a recent meta-analysis report, the median global prevalence was established at 6.3% [3]. In this study, the positivity rate was 5.7%, in the same range as previously reported, but

Table 2 Indicence and associated-diseasses of human enteroviruses in the study period (2014–2023)

Years	2014	2015	2016	2017	2018	2019	Pre-Covid	2020	2021	Covid	2022	2023	Post-Covid
Positivity rate (n)	13.9% 2683	12.9% 5626	9.2% 10,831	10.7% 12,628	9.5% 13,242	5.4% 18,819	**8.9%**	1.3% 22,187	5.4% 20,186	**3,3%**	5.9% 25,784	2.6% 34,688	**4.0%**
< 6 yo	21.7% 1092	20.7% 2535	16.4% 4226	18.7% 5324	15.4% 6064	9.6% 7890	**15.2%**	3.5% 5895	7.7% 12,457	**6.3%**	9.3% 13,387	6.5% 10,830	**8.1%**
6–14 yo	11.8% 297	14.3% 701	8.5% 1329	8.3% 1535	9% 1571	4.6% 2165	**8.1%**	1.4% 3243	2.6% 2581	**1.9%**	3.7% 4705	2.6% 4796	**3.1%**
> 14 yo	7.7% 1294	4.4% 2390	3.6% 5276	4% 5769	3.1% 5607	1.8% 8764	**3.3%**	0.3% 13,049	1.3% 5148	**0.6%**	1.2% 7692	0.5% 19,062	**0.7%**
Symptoms	139	480	965	1135	1057	848		252	867		1173	707	Total 7623
Febrile Syndrome	57	128	185	280	247	261		79	244		419	250	2150
< 6 yo	40	103	136	221	197	222		64	225		362	212	1782
6–14 yo	7	12	19	20	25	17		8	11		51	27	197
> 14 yo	10	13	30	39	25	22		7	8		6	11	171
Exanthematous disorders	13	68	217	226	196	134		11	137		109	83	1194
< 6 yo	7	51	183	207	178	119		10	130		98	74	1057
6–14 yo	2	6	16	13	14	8		1	4		8	8	80
> 14 yo	4	11	18	6	4	7			3		3	1	57
Neurological	7	102	50	78	49	29		7	12		35	27	396
< 6 yo	4	47	24	40	30	15		6	11		26	15	218
6–14 yo		31	10	11	9	6		1			6	11	85
> 14 yo	3	24	16	27	10	8			1		3	1	93
Gastrointestinal		2	5	37	88	97		10	33		63	46	381
< 6 yo		1	4	29	67	79		6	28		49	37	300
6–14 yo				3	13	10		2	4		7	5	44
> 14 yo		1	1	5	8	8		2	1		7	4	37
Respiratory manifestation	55	173	490	495	465	318		141	439		537	300	3413
< 6 yo	23	94	314	335	318	193		83	368		418	201	2347
6–14 yo	4	16	62	51	39	30		26	32		62	35	357
> 14 yo	28	63	114	109	108	95		32	39		57	64	709
Other	7	7	18	19	12	9		4	2		10	1	89
< 6 yo	2	3	8	12	6	3		3	1		6		44
6–14 yo	1	2	3	3	2	2			1		2	1	17
> 14 yo	4	2	7	4	4	4		1			2		28

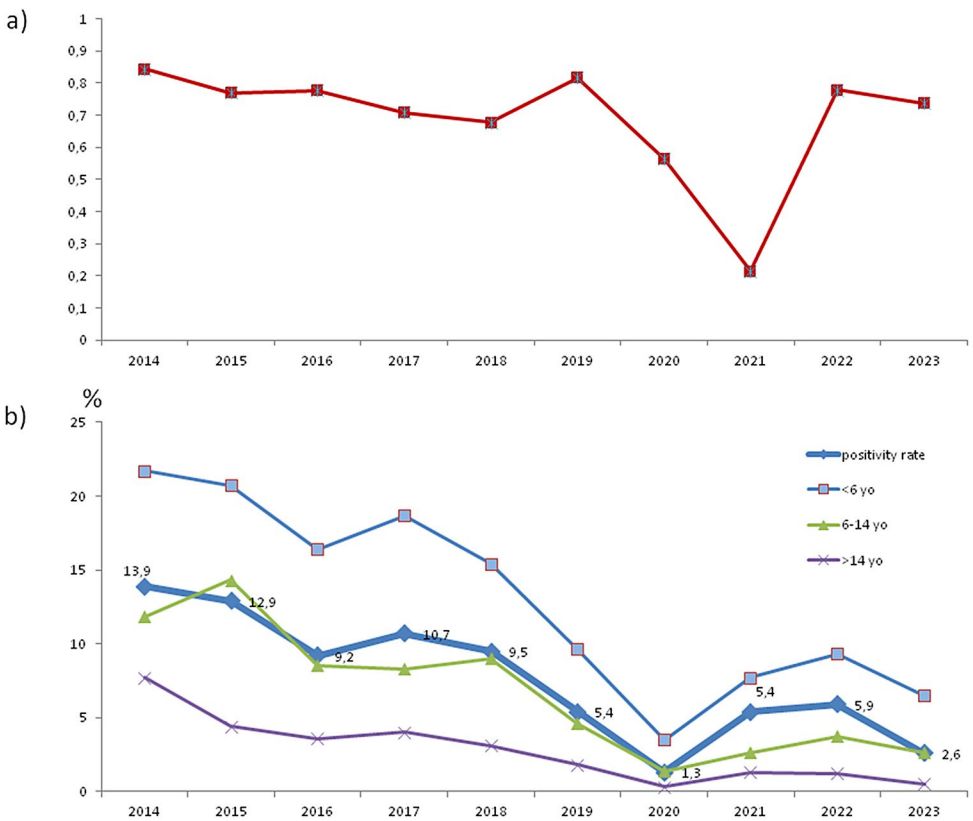

Fig. 1 - Diversity (**a**) and positivity rate of EV by year and age (**b**)

Fig. 2 Clinical disorders associated with EV over time (**a**), and by age (**b**). Resp M: respiratory manifestations; FS: febrile syndrome; ED: Exanthematous disorders; NS: neurological symptoms; GI: gastrointestinal symptoms

differences were observed in different time periods. Before the pandemic, the positivity rate was around 9%. Interestingly, in 2019, just before the emergence of SARS-CoV-2, the positivity rate was halved (5.4%), although it should be noted that twice as many samples were examined as in previous years. In 2020, it fell to 1.27%. Subsequently, the next two years reached again 5.5%. In the last year studied, the positivity rate decreased to 2.57%, but it should again be noted the large number of samples studied. These data show once again that the measures taken during the pandemic had an impact not only on the circulation of SARS-CoV-2, but also on all other viruses and pathogens [10–12].

Table 3 Viral load (log) in different samples and clinical syndromes

Type specimen	LRS	URS	Stools	Skin swabs	CSF	Other	
n	71	6988	346	58	256	24	
average	6.3 ± 1.75	6.35 ± 1.7	5.89 ± 1.4	4.99 ± 2.13	4.39 ± 1.2	4.79 ± 1.58	
range	1.3–9.68	1.3-14.58	2.7–9.68	1.8–9.8	1.3–9.68	2.6–8.9	
CI95%	5.9–6.7	6.6–6.7	6-6.3	5.6–6.7	5.1–5.7	5-6.5	
Symptoms	FS	ED	NS	GI	LRM	URM	Other
n	2114	1134	241	125	1642	1669	63
average	6.57 ± 1.72	6.56 ± 1.53	6.48 ± 1.61	6.6 ± 1.49	5.88 ± 1.73	6.41 ± 1.69	5.58 ± 1.81
range	1.32–14.58	1.36–10.29	1.43–10.41	3.11–9.56	1.32–13.32	1.38–14.59	1.56–9.58
CI95%	6.5–6.6	6.47–6.65	6.27–6.68	6.34–6.86	6.3–6.5	5.79–5.98	5.1-6

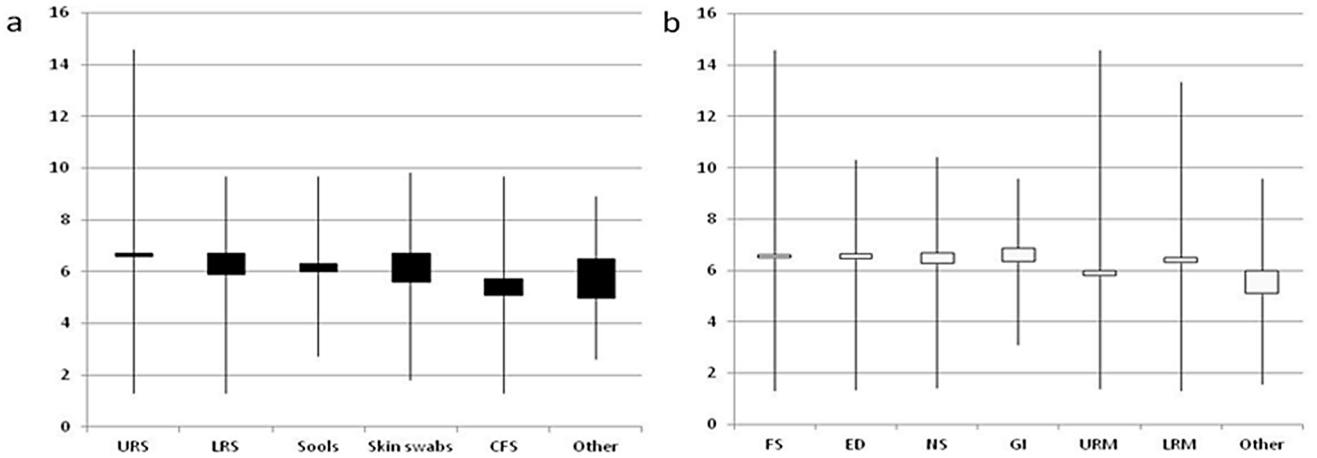

Fig. 3 Viral load by specimen (**a**) and in respiratory specimens by clinical syndromes (**b**).URS: upper respiratory samples, LRS: lower respiratory samples, CFS: cerebrospinal fluid samples. FS: febril syndrome; ED: Exanthematous disorders; NS: neurological symptoms; GI: gastrointestinal symptoms; LRM: lower respiratory manifestations; URM: upper respiratory manifestations

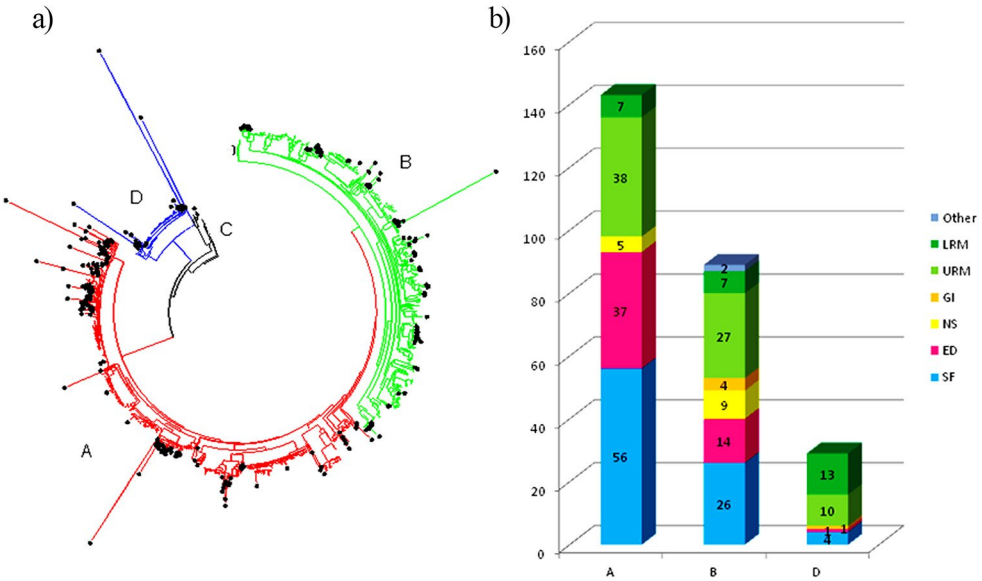

Fig. 4 (**a**) Phylogenetic tree of EVs from Asturias (bold dot) and reference EV of species A (red), species B (green), and species D (blue). (**b**) Number of EV from each species and symptoms

The age group with the highest positivity rate was, as expected, the youngest (10%) and decreased with increasing age (4.5% in children aged 6–14 years and 1.7% in adults) [7].

The most common species was A, followed by B, D and C, and it was associated to youngest children (under 6 years old). To characterise Enterovirus, 5'NTR has been used. Some reports recommend VP1 or other proteins as

Table 4 Indicence and diversity of human enteroviruses in the study period (2014–2023) by age and sex

Years		2014	2015	2016	2017	2018	2019	Pre-Covid	2020	2021	Covid	2022	2023	Post-Covid	Age	Sex
Inciden positivity rate (%)		13.9	12.9	9.2	10.7	9.5	5.4	**8.9**	1.3	5.4	**3,3**	5.9	2.6	**4.0**	< 6/6–15/>15	M/F
Genotypes															167/9/5	106/75
A	A2		1	2	1	4		**8**	11		**11**				17/1/1	13/6
(*n* = 181)	A4		2	2	10	2	19	**35**				1	3	**4**	36/2/1	21/18
	A6	1	15	14	19	5	6	**60**	4	38	**42**	1	4	**5**	98/6/3	62/45
	A8	2	1				1	**4**							4/-/-	3/1
	A10												2	**2**	2/-/-	2/-
	A16	1			1	1	5	**8**		2	**2**				10/-/-	5/5
															92/21/5	73/45
B	A9		18	1		2	2	**23**							21/1/1	14/9
(*n* = 118)	B1						1	**1**							1/-/-	-/1
	B2	1						**1**							1/-/-	1/-
	B3			4				**4**							4/-/-	2/2
	B5				1			**1**	1		**1**	1		**1**	-/1/2	2/1
	E2									2	**2**				2/-/-	1/1
	E6	1	3			4	6	**14**	1		**1**	1	1	**2**	11/6/-	10/7
	E7		4	2			11	**17**		1	**1**				14/4/-	11/7
	E9		2	1		1		**4**							2/2/-	2/2
	E11		4	12	1			**17**					8	**8**	20/4/1	15/10
	E13						4	**4**							3/1	3/1
	E18			1	10		1	**12**							10/2/-	1/1
	E25	1	1					**2**							2/-/-	1/1
	E30					1	1	**2**							1/1/-	2/-
C	A21	1						**1**							-/-/1	-/1
(*n* = 1)																
D	D68	3		2	1	23		**29**				2	1	**3**	17/3/12	11/21
(*n* = 32)																
Diversity		0,843	0,769	0,777	0,708	0,677	0,814	**0,880**	0,519	0,214	**0,473**	0,778	0,737	**0,803**		

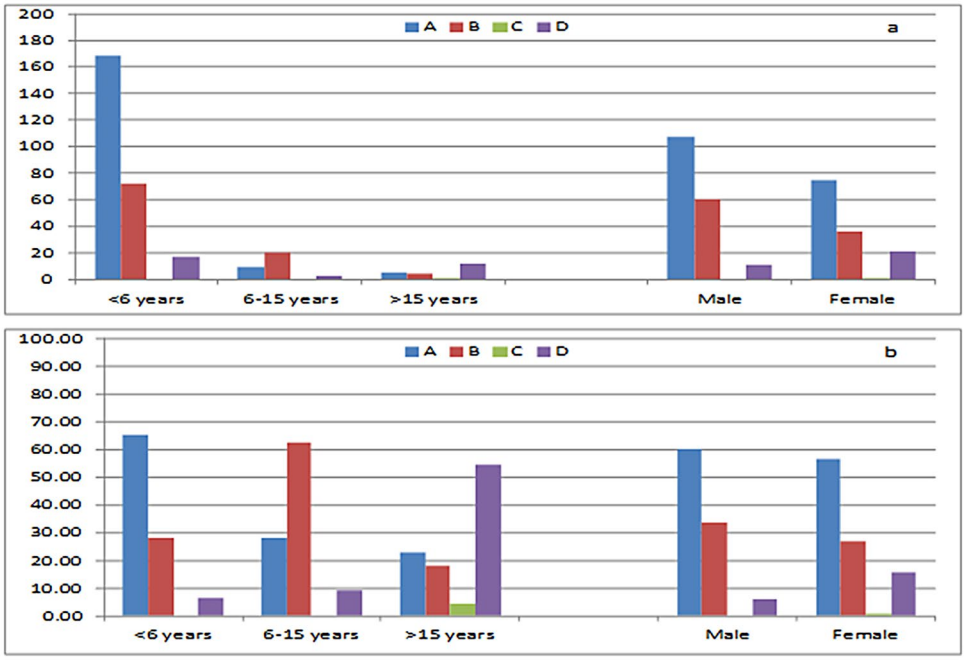

Fig. 5 Types of EV by number (**a**) and proportion (**b**) according age and sex

more specific, but in a recent report the species concordance between both fragments was 92% [14].

Generally, EV-A are mainly associated with herpangina and hand-foot-and-mouth disease (HFMD) and EV-B with herpangina and viral meningitis or encephalitis [15].A large study in Spain describing the epidemiology of EV infections from 2006 to 2020, identified 85% belonged to species A [16].This was expected as classically, the HFMD was associated with EV-A, principally CVA16 and EV-A71. Since 2009, the increased detection of CVA6 was already observed, displacing CVA16 as the main cause of HFMD. As in other neighboring countries, CVA6 emerged in Spain and is now considered an endemic serotype, being one of the five most frequent EV that circulate every year. In general, according to a recent meta-analysis by Brouwer et al., CVA6 is the most prevalent EV globally [3].CVA10 is another EV-A which began to be detected and associated with HFMD in the same period of time, both in Spain and in other countries. Although it is detected every year, it does so at low levels [16].

In our series, the predominant type in Asturias was CVA6 (32%) leaving CVA16 but less than 3%, and CVA10 did not get 1%. These data support the replacement of CV-A16, which during 2010 was the most frequently detected type in other studies, with CV-A6. And it was observed that CV-A6 was the unique EV found every year. CVA10 began to be detected in the last year, although this type, together with CV-A6, was one of the main causative agents of the outbreaks described during 2008–2010 in Finland, France and Spain [17]. Some publications describe the strong ability of CVA6 to produce HFMD in adults but in our sample only 3% were over the age of 15 [3].

On the other hand, while CV-A4 (12%) and CV-A2 (6%) were the most common and were found almost every year, CV-A8 was less frequently detected (1%), as in other studies [16, 18].

EVs species A was present in two out of three exanthematous disorders, but it was also associated with febrile syndromes and respiratory manifestations. It was less common in neurological syndromes.

In a recent meta-analysis cited above, EV-B types were the most common worldwide, with types CVA9, CVB1-5 and several echoviruses being particularly prevalent, and with echovirus 30 being the most common. They have been identified in children under one year of age and are characterized by severe disease with high mortality. In our sample, EV-B is only the second most common (35.6%) and was associated with children aged 6–14 years (63.6%), indicating small differences compared to the others [3, 16–21].

Although EV species B was also associated with respiratory manifestations and febrile syndromes, it was proportionally more common in neurological syndromes than other genotypes, representing the 64% of these cases.

The most common types were CVA9 and echoviruses E6, E7, E11 and E18, all among the most frequent. In Spain, E6 and E11 are among the ten most frequently reported [20].and EV13, considered rare in Spain and worldwide, was reported in 1% [21]. However, EV-30, long described as a cause of aseptic meningitis worldwide, is frequently detected in the Spanish territory, but was found in only 0,6% [19].

It is worth noting the difference observed before and after the SARS-CoV-2 pandemic. Before 2020, there were up to 13 types with higher positive rate, whereas after the pandemic only B5, E6 and E11 were found. It is likely that more types will be found over time.

CVA21 was the only EV type C reported in our sample and only 1case, EV-C were rarely found in studies conducted in Europe and were mostly found almost exclusively in stool samples [3].It should be noted that in this study 98% of the samples were from the respiratory tract.

Of the four EV-D types, EV-D68 is the only one found and represents a high positive rate. Analysis of EV-positive specimens collected from April 2014 to December 2018 from Spanish hospitalized patients with respiratory illnesses confirmed the presence of EV-D68 in almost half of the total characterized EV. It is known to cause predominantly respiratory disease, as it is noted in this study [3]. As previously reported, most of the EV-D68 infections were detected in young children. However, adult patients were also infected, half of them over the age of sixty [23, 24].In this study, EV-D68 was the fourth most common type found (10%) and thirty-eight% of patients were older than 14 years old. Furthermore, EV-D68 accounted more than half of the EVs found in this group. EV-D68 has also been associated with severe neurological cases, indicating the need for better surveillance of this EV [17].

As mentioned above, viral respiratory infections were strongly reduced during the most stringent public health measures to control SARS-CoV-2 transmission in 2020, but EVs re-emerged rapidly after they were relaxed [24].These measures adopted during the pandemic also reduced viral diversity. Up to 20 different types circulated before the pandemic, but only seven after.

This decrease in diversity was mainly observed in species B EV, as mentioned above.

On the opposite, CVA6 never disappeared and was the dominant type especially during the COVID epidemic (65%). The implementation of epidemic prevention measures did not eliminate the common type in Asturias. Given the serious and potentially life-threatening complications associated with hand-foot-and-mouth disease (in which CVA6 is widely implicated), the necessity of the prioritization of vaccine development is clear. Presently, inactivated vaccines demonstrate considerable efficacy, persistent immunogenicity and acceptable safety profiles within the vaccinated population [15].Our results suggest that only vaccines, including CV-A6, may be viable options for EV control in Asturias, and that the only approved EVA71 vaccines are irrelevant.

In 2022 situations returns to normality: prevention measures disappear and diversity will increase again. Types like A4, E11 and D68 reappear after easing the COVID-19 crisis isolation [25].

Factors that influence sequencing, such as viral load and sample quality, as well as the fact that PCR primers do not have the capacity to amplify all types, may explain the low number of sequenced samples. On the other hand, the sequenced fragment may not have sufficient resolution to identify a single type [26].

Due to the small sample size, the results may not accurately reflect the diversity or incidence circulating in this area, but given the importance of EV infection surveillance, it is a first insight and a stimulus for further studies where other regions of the virus can be studied to improve classification, and even attempt to study the whole genome by NGS methods, as was done in the SARS-CoV-2 pandemic.

Finally, analysis of viral load in respiratory exudates, the most common specimen obtained for aetiological diagnosis, demonstrated the validity of the specimen and the high rate of viral replication in each clinical presentation. Moreover, this high replication rate is maintained at every site of EV infection, even in cerebrospinal fluid, where the mean viral load reached almost 5 log copies/ml.

Conclusion

In summary, EV infection is frequent, especially in children. It can be observed that EVs-A species are associated with the very young, EVs-B with middle age and EV D with adults. Cov-A6 was the most common, even during the SARS-Cov-2 pandemic, when restrictive measures were taken and diversity decreased.

Recognition of EV-associated diseases will allow us to better assess the burden of EV disease, to monitor the emergence of new strains, and will also be necessary for the possible implementation of vaccination programs and therapeutic strategies.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10096-024-04970-0.

Acknowledgements Project partially supported by Grupin IDI/2021/000033.

Author contributions All authors contributed to the study conception, design, material preparation, data collection and analysis . The first draft of the manuscript was written by J.M.G.A. and S.M.G. and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Data availability Sequence data that support the findings of this

study have been deposited in GenBank with the accession number OR493270-OR493385.

Declarations

Ethical approval The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013) and has been approved by Hospital Universitario Central de Astuiras Ethical Committee (nº 2020.383). Our study is based on the use of remmants of samples used for routine diagnosis. The retrospective collection of the informed consent belonging to the collection of samples stored by the Mycrobiology Service of HUCA was complicated, so the Ethical committee was asked to exempt informed.

Competing interests The authors declare no competing interests.

References

1. Wollants E, Beller L, Beuselinck K et al (2019) A decade of enterovirus genetic diversity in Belgium. J Clin Virol 121:104205. https://doi.org/10.1016/j.jcv.2019.104205Epub 2019 Oct 25. PMID: 31722268
2. Liu BM, Mulkey SB, Campos JM, DeBiasi RL (2024) Laboratory diagnosis of CNS infections in children due to emerging and re-emerging neurotropic viruses. Pediatr Res 95(2):543–550. https://doi.org/10.1038/s41390-023-02930-6Epub 2023 Dec 2. PMID: 38042947
3. Brouwer L, Moreni G, Wolthers KC, Pajkrt D (2021) World-wide prevalence and genotype distribution of enteroviruses. Viruses 13:434. https://doi.org/10.3390/v13030434
4. Hayes A, Nguyen D, Andersson M et al (2020) A European multicentre evaluation of detection and typing methods for human enteroviruses and parechoviruses using RNA transcripts. J Med Virol 92(8):1065–1074. https://doi.org/10.1002/jmv.25659Epub 2020 Jan 17. PMID: 31883139; PMCID: PMC7496258
5. Machado RS, Tavares FN, Sousa IP (2024) Global landscape of coxsackieviruses in human health. Virus Res 6 de Abril De 344:199367
6. Howson-Wells HC, Winckles S, Aliker C et al (2020) Enterovirus subtyping in a routine UK laboratory setting between 2013 and 2017. J Clin Virol 132:104646. https://doi.org/10.1016/j.jcv.2020.104646Epub 2020 Sep 17. PMID: 32979770
7. Harvala H et al (2018) Recommendations for enterovirus diagnostics and characterisation within and beyond Europe. J Clin Virol. ;101:11–17. https://doi.org/10.1016/j.jcv.2018.01.008. Epub 2018 Feb 6. PMID: 29414181
8. Abedi GR, Watson JT, Nix WA, Oberste MS, Gerber SI (2018) Enterovirus and Parechovirus Surveillance - United States, 2014–2016. MMWR Morb Mortal Wkly Rep 67(18):515–518. https://doi.org/10.15585/mmwr.mm6718a2PMID: 29746455; PMCID: PMC5944979
9. Yang X, Cai S, Wu X, Zhan. Analysis of the Distribution Characteristics of Enterovirus Types Based on Environmental Surveillance from 2013 to 2021 in Fujian Province, China. Biosaf. Health.:5:240–249., Kim MJ, Lee JE, Kim KG et al (2023) Long-term sentinel surveillance of enteroviruses in Gwangju, South Korea, 2011–2020. Sci Rep. 2023;13(1):2798. doi: 10.1038/s41598-023-29461-8. PMID: 36797345; PMCID: PMC9933826
11. Rojo-Alba S, Martínez ZP, González-Alba JM et al (2024) Respiratory syncytial virus incidence and typing in the last six seasons in the north of Spain (Asturias). Genetic characterization during the SARS-CoV-2 pandemic. J Med Virol. ;96(3):e29499. https://doi.org/10.1002/jmv.29499. PMID: 38469919
12. Chow EJ, Uyeki TM, Chu HY (2023) The effects of the COVID-19 pandemic on community respiratory virus activity. Nat Rev Microbiol 21(3):195–210. https://doi.org/10.1038/s41579-022-00807-9Epub 2022 Oct 17. PMID: 36253478; PMCID: PMC9574826
13. Olsen SJ, Winn AK, Budd AP et al (2021) Changes in Influenza and other respiratory virus activity during the COVID-19 pandemic - United States, 2020–2021. MMWR Morb Mortal Wkly Rep 70(29):1013–1019. https://doi.org/10.15585/mmwr.mm7029a1PMID: 34292924; PMCID: PMC8297694
14. Gulholm T, Yeang M, Nguyen I, Andrews PI, Balgahom R, Beresford R et al (2022) Molecular typing of enteroviruses: comparing 5'UTR, VP1 and whole genome sequencing methods. Pathology 54(6):779–783 Epub 2022 Jun 21. PMID: 35738943
15. Itani TM, Chalapa VI, Slautin VN et al (2023) Non-polio Enterovirus Surveillance in the Ural Federal District and Western Siberia, 2022: is there a need for a vaccine? Vaccines (Basel) 11(10):1588. https://doi.org/10.3390/vaccines11101588PMID: 37896990; PMCID: PMC10610881
16. Calvo C, Ruiz-García M, Megias G, Valencia-Ramos J, Otero A, Cabrerizo M (2021) Surveillance for Enteroviruses Associated with Hand, Foot, and Mouth Disease, and other mucocutaneous symptoms in Spain, 2006–2020. Viruses 13(5):781. https://doi.org/10.3390/v13050781PMID: 33924875; PMCID: PMC8146579
17. Cabrerizo M, Tarragó D, Muñoz-Almagro C et al (2014) Molecular epidemiology of enterovirus 71, coxsackievirus A16 and A6 associated with hand, foot and mouth disease in Spain. Clin Microbiol Infect 20(3):O150–O156. https://doi.org/10.1111/1469-0691.12361Epub 2013 Sep 14. PMID: 24033818
18. González-Sanz R, Taravillo I, Reina J et al (2019) Enterovirus D68-associated respiratory and neurological illness in Spain, 2014–2018. Emerg Microbes Infect 8(1):1438–1444 PMID: 31571527; PMCID: PMC6781473
19. Del Cuerpo M, Gonzalez de Audicana J, Fernandez-Garcia MD et al (2022) Epidemiology of Echovirus 30 infections detected in a University Hospital in Catalonia, Spain, in 1995–2020. Microorganisms 10(3):592. https://doi.org/10.3390/microorganisms10030592PMID: 35336167; PMCID: PMC895514
20. Gámbaro F, Pérez AB, Agüera E et al (2021) Author Correction: Genomic surveillance of enterovirus associated with aseptic meningitis cases in southern Spain, 2015–2018. Sci Rep. ;11(1):22899. https://doi.org/10.1038/s41598-021-02194-2. Erratum for: Sci Rep. 2021;11(1):21523. PMID: 34795374; PMCID: PMC8602408
21. Avellón A, Casas I, Trallero G, Pérez C, Tenorio A, Palacios G (2003) Molecular analysis of echovirus 13 isolates and aseptic meningitis, Spain. Emerg Infect Dis 9(8):934–941. https:/

/doi.org/10.3201/eid0908.030080PMID: 12967490; PMCID: PMC3020609
22. Yang X, Duan L, Zhan W, Tang Y, Liang L, Xie J, Luo M (2023) Enterovirus B types cause severe infection in infants aged 0–3 months. Virol J 20(1):5. https://doi.org/10.1186/s12985-023-01965-9PMID: 36624466; PMCID: PMC9830867
23. Duval M, Mirand A, Lesens O et al (2021) Retrospective study of the upsurge of Enterovirus D68 Clade D1 among adults (2014–2018). Viruses 13(8):1607. https://doi.org/10.3390/v13081607PMID: 34452471; PMCID: PMC8402803.)
24. Schuffenecker I, Mirand A, Josset L et al (2016) Epidemiological and clinical characteristics of patients infected with enterovirus D68, France, July to December 2014. Euro Surveill 21(19). https://doi.org/10.2807/1560-7917.ES.2016.21.19.30226PMID: 27195770.)
24. Forero EL, Knoester M, Gard L et al (2023) Changes in enterovirus epidemiology after easing of lockdown measures. J Clin Virol 169:105617. https://doi.org/10.1016/j.jcv.2023.105617Epub 2023 Nov 10. PMID: 37977075
25. Benschop KS, Albert J, Anton A et al (2021) Re-emergence of enterovirus D68 in Europe after easing the COVID-19 lockdown, September 2021. Euro Surveill 26(45):2100998. https://doi.org/10.2807/1560-7917.ES.2021.26.45.2100998PMID: 34763750; PMCID: PMC8646978
26. Liu B, Universal, PCR Primers Are Critical for Direct Sequencing-Based Enterovirus Genotyping (2016) J Clin Microbiol 55(1):339–340. https://doi.org/10.1128/JCM.01801-16PMID: 28031445; PMCID: PMC5228251