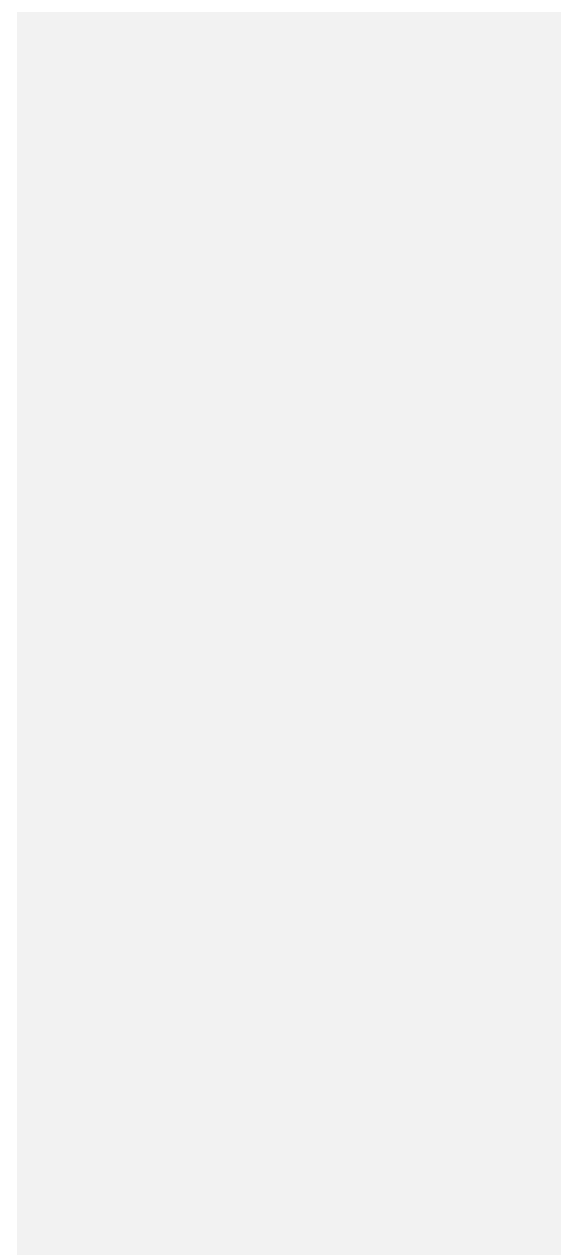


Supplementary Table S1(with M&M references)

		Epidemiological data - 2021					Clinical records - 2021/2022		Material and Methods				
		Enterovirus (EV)			EV-D68		EV-D68						
Country	Institute codes	Samples tested (n)	Positive samples (n)	EV positive detection (%)	EV-D68 positive samples (n)	EV-D68/ EV relative proportion (%)	Clinical cases (2021)	Clinical cases (2022)	EV, sample type	% respiratory samples tested	Source of samples (*)	Detection assay (**)	Sequencing assay (**)
Austria	AT01	381	2	0.5%	1	50.0%	1		Various	69.7%	EV clinical surveillance	<u>EV-PCR</u> All samples: AnDiaTec EV RT-qPCR testkit <u>EV-D68 PCR</u> (respiratory samples) [1]	<u>VP1 sequencing</u> (in house unpublished)

Belgium	BE01	10,661	547	5.1%	80	14.6%	80	Various	100.0%	EV clinical surveillance	<u>EV-PCR and EV-D68</u> Feces: Gastro Intestinal- microarray assay with EV-target (in house PCR); Respiratory samples: respiratory microarray with EV-target besides a specific EV-D68 target (both in house PCRs); Blood/punctates: in house EV-PCR; CSF: FilmArray with EV-target (commercial)	Not Available
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	BE02	614	491	80.0%	20	4.1%	18	Respiratory samples Feces Cerebrospinal fluid (CSF)	100.0%	EV clinical surveillance	EV-PCR [2]	<u>VP1 sequencing [2]</u> <u>VP1 sequencing (in-house, unpublished)</u> <u>VP4-VP2 sequencing [3]</u>
Bulgaria	BG01	399	13	3.3%	0	NA	0	Respiratory samples Feces CSF	2.0%	AFM/AFP surveillance AFP surveillance and partial surveillance on CNS infections	<u>Cell culture</u> <u>Pan-EV (in-house real-time RT-PCR)</u>	Not applicable
Czechia	CZ01	465	120	25.8%	0	NA	0	Various	40.0%	EV clinical surveillance	<u>EV PCR commercial (Gene Proof)</u>	<u>VP1 sequencing (in house unpublished)</u>
Germany	DE01	1,235	58	4.7%	-	-	0	Respiratory samples	0.2%	EV clinical surveillance	<u>EV PCR [4]</u>	<u>VP1 sequencing [5]</u>

									Feces CSF				
	DE02	2,856	82	2.9%	2	2.4%	2		Respiratory samples	100.0%	ILI/ARI surveillance	<u>EV PCR</u> [6]	<u>VP1 sequencing</u> [7]
	DE03	1,973	9	0.5%	1	11.1%	1		Various	73.0%	Not available	<u>EV-PCR</u> [8], commercial RT- PCR (FTD (HRV/EV), FilmArray) <u>EV-D68 PCR</u> [1]	<u>VP1 sequencing</u> [5, 7]
	DE04	Not reported	Not reported	Not reported	2	Not reported	2		Feces CSF	Not available	EV clinical surveillance	<u>EV-D86 PCR</u> [1]	VP1 sequencing [7]
Denmark	DK01	Not reported	431	Not reported	17	3.9%	11	1	Various	Not available	ILI surveillance and EV clinical surveillance	<u>EV-PCR</u> [9] <u>EV-D68 PCR</u> [10]	<u>VP1 sequencing</u> [7] [11] <u>VP2 sequencing</u> [12]
Spain	ES01	24,332	831	3.4%	175	21.1%	175	5	Respiratory samples	100.0%	EV clinical surveillance	<u>RV16-PCR</u> RV16 Allplex Respiratory Panel	<u>VP1 sequencing</u> [7]

											(Seegene) or <u>EV/HPEV PCR</u> RealCycle EV/hPeV detection (Progenie)	
ES02	6,255	127	2.0%	20	15.8%	20	Various	80.0%	ARI surveillance	<u>EV-PCR Allplex™</u> Respiratory Panel Assays; Pan-EV [13]	<u>VP4/VP2 sequencing</u> [14] <u>VP1 sequencing</u> [15, 16]	
ES03	1,891	14	0.7%	0	0.0%	0	Respiratory samples Feces CSF	2.3%	AFP and EV clinical surveillance	<u>cell culture</u> all samples <u>EV PCR, EV/HRV</u> <u>PCR</u> All resp samples negative for other respiratory viruses, CSF and faeces from neurological or	<u>VP1 sequencing</u> [7]	

												cutaneous illnesses: FilmArray
ES04	3,635	75	2.1%	3	4.0%	1	2	Various	65.0%	EV clinical surveillance	<u>EV-PCR</u> Enterovirus & Paraechovirus - Realcyler Progenie molecular and respiratory virus Fast Track Siemens <u>EV-PCR</u> _Filmarray (Rhino/EV), Seegene (Enterovirus + Rhinovirus)	<u>VP1 sequencing (at ES05) [17, 18]</u>

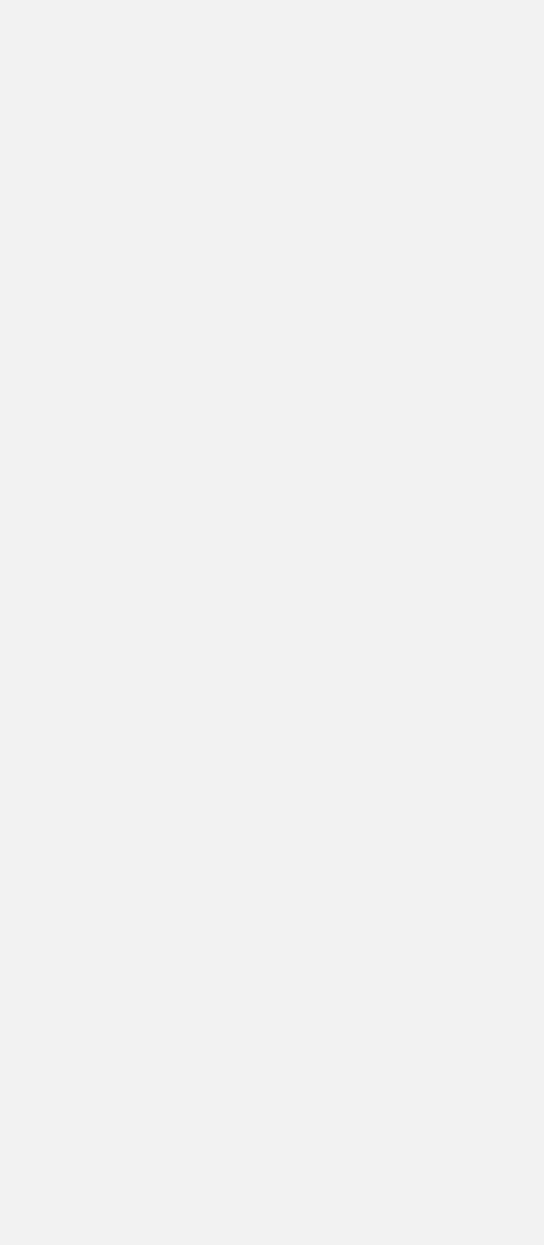
Con formato: Neerlandés (Países Bajos)

	ES05 (Epi. reporter for ES07, ES12)	2,417	135	5.6%	44	32.6%						EV-PCR Filmarray (Rhino/EV), Seegene (Enterovirus + Rhinovirus).	VP1 sequencing [17, 18]
	ES06	1,208	12	1.0%	6	50.0%	6	Respiratory samples	100.0%	ILI/ARI surveillance	EV/RV PCR [19] EV-D68 PCR [20]	VP1 sequencing [1] [18]	
	ES07						31	4				VP1 sequencing (at ES05) [17, 18]	
	ES12						9					VP1 sequencing (at ES05) [17, 18]	
Finland	FI02	176	0	0.0%	0	NA	0	Respiratory samples	NA	ILI/ARI I surveillance	EV-D68 PCR [1]	VP1 sequencing [7]	

France	FR01	5,452	1,000	18.3%	43	4.3%	43	Various	57.9%	EV clinical surveillance	<u>EV-PCR</u> all samples on demand except respiratory samples Commercial assay EV/RV PCR Respiratory samples: commercial assay EV-D68 PCR8 [1]	<u>VP4-VP2 sequencing</u> [21] <u>VP1 sequencing</u> [7] (generic), [22] (EV-B specific), [23] (EV-A specific) <u>VP1 sequencing</u> [24] <u>complete genome</u> [25]
	FR02	10,156	257	2.5%	74	28.8%	71	Various	20.0%	EV clinical surveillance	<u>EV-PCR</u> samples on demand: Enterovirus-r-gene assay, bioMérieux CSF: Biofire multiplex <u>EV-D68 PCR</u> all respiratory samples from	<u>VP4/VP2 sequencing</u> [14, 26, 27] <u>VP1 sequencing</u> [7, 15, 28]

											children < 5 years [20]	
FR03	5,623	96	1.7%	18	18.8%	18	Various	71.0%	EV clinical surveillance	EV-PCR Pan-EV in-house RT-PCR and Respiratory multiplex PCR (Fastrack)	VP1 sequencing (in house protocol based on [7, 20])	
FR04	194	15	7.7%	0	0.0%	0	Cerebral Spinal Fluid	0.0%	EV clinical surveillance	EV-PCR Enterovirus-rgene assay, bioMérieux	Not Applicable - typing assay performed at FR02	
FR05	696	46	6.6%	2	4.4%	2	Various	Not Available	EV clinical surveillance	EV-PCR RIDAGENE enterovirus Rbiopharm	Not Applicable - typing assay performed at FR02	
FR06	2,127	46	2.2%	2	4.4%	1	Various	72.0%	EV clinical surveillance	CSF: Enterovirus- rgene assay, bioMérieux or	Not Applicable - typing assay performed at FR02	

											BioFire FilmArray Meningitis/Encephalitis panel, bioMérieux ; Respiratory samples: AnyPlex II RV16, Seegene ; Other: Enterovirus- rgene assay, bioMérieux	
FR07	1,323	100	7.6%	14	14.0%	14	Various	3.3%	EV clinical surveillance	EV-PCR Commercial- RealStar Enterovirus RT- PCR kit (Altona Diagnostics) and Allplex™ Respiratory Panel Assays (Seegene)	Not Applicable - typing assay performed at FR02	



FR08	752	32	4.3%	1	3.1%	1		Various	0.0%	EV clinical surveillance	EV-PCR Enterovirus-r gene assay, bioMérieux	Not Applicable - typing assay performed at FR02
FR09	1,017	102	10.0%	0	0.0%	0	1	Various	8.0%	EV clinical surveillance	EV-PCR Commercial Enterovirus RT-PCR kit (Elitech MGB Kit) performed on Elitech Ingenius. Commercial multiplex RT-PCR kit (Seegene Respiratory full panel) for respiratory samples	Not Applicable - typing assay performed at FR02
FR10	897	227	25.3%	2	0.9%	2		Various	100.0%	EV clinical surveillance	EV/RV-PCR [19]	Not Applicable - typing assay performed at FR01

Croatia	HR01	106	14	13.2%	0	0.0%	0		Respiratory samples Blood/serum	22.9%	EV clinical surveillance and EV-D68 laboratory surveillance	<u>EV-PCR</u> Ausdiagnostics RV panel 16 well, FilmArray (Biofire Diagnostics) <u>EV-D68 PCR</u> Only EV PCR positive samples [1], modified probe	Light Diagnostics Pan-Enterovirus Reagents and Blends, Millipore, USA
Hungary	HU01	360	6	1.7%	0	0.0%	0		Various	24.3%	AFP and EV clinical surveillance	<u>EV/RV-PCR</u> [29]	<u>VP1 sequencing</u> [7]
Ireland (Rep)	IE01	10,075	1,964	19.5%	14	0.7%	14	2	Various	100.0%	EV clinical surveillance	EV/RV-PCR CSF and blood EV: Extracted on EasyMAG, tested using Altona PCR kits Respiratory samples EV testing:	<u>VP1 sequencing</u> [7]

											NxTAG Luminex Respiratory Panel Respiratory samples D68 testing: In-house assay Stool for EV testing: In-house assay	
Italy	IT01	998	44	4.4%	8	18.2%	8	Respiratory samples	100.0%	ILI/ARI surveillance and AFP surveillance	<u>EV/HPEV-PCR</u> [30] <u>EV-D68 PCR</u> [10, 20]	<u>VP1 sequencing</u> [7, 14, 31] <u>VP4-VP2 sequencing</u> [3, 14] <u>VP3-VP1 sequencing</u> [32]
	IT02	2,141	325	15.2%	1	0.3%	1	Various	67.3%	ILI/ARI surveillance	<u>EV-PCR</u> [33] <u>EV-D68 PCR</u> [10]	<u>VP1 sequencing</u> [7, 14, 31] <u>VP4-VP2 sequencing</u> [3, 14]

											when positive to discriminate EV/RV <u>EV-D68 assay</u> (at NL02) [1, 35]	
NL02	635	41	6.5%	11	26.8%	11	Respiratory samples	100.0%	ILI/ARI surveillance	<u>EV-PCR</u> [1] <u>EV-D68 assay</u> (at NL02) [1, 14]	<u>VP1 sequencing</u> [7] <u>complete genome</u> (unpublished)	
NL03	937	132	14.1%	0	0.0%	0	Various	36.0%	EV clinical surveillance	<u>EV-PCR</u> [1] <u>EV-D68 assay</u> (at NL02) [1, 35]	<u>VP1 sequencing</u> [7] <u>complete genome</u> (unpublished)	
NL04	4,224	107	2.5%	24	22.4%	23	Various	85.0%	EV clinical surveillance	<u>EV-PCR</u> [1] <u>EV-D68 assay</u> (at NL02) [1, 35]	<u>VP1 sequencing</u> [7] <u>complete genome</u> (unpublished)	
NL05	1,995	97	4.9%	13	13.4%	13	Various	100.0%	EV clinical surveillance	<u>EV-PCR</u> [1] <u>EV-D68 assay</u> (at NL02) [1, 35]	<u>VP1 sequencing</u> [7] <u>complete genome</u> (unpublished)	
NL06	3,484	80	2.3%	5	6.3%	5	Various	46.0%	EV clinical surveillance	<u>EV-PCR</u> [1] <u>EV-D68 assay</u> (at NL02) [1, 35]	<u>VP1 sequencing</u> [20] <u>complete genome</u> (unpublished)	

	NL08	447	55	12.3%	1	1.8%	1		Various	20.0%	EV clinical surveillance	EV-PCR [1] EV-D68 assay (at NL02) [1, 35]	VP1 sequencing [7] complete genome (unpublished)
	NL09	257	34	13.2%	1	2.9%	1		Various	1.6%	EV clinical surveillance	EV-PCR [1] EV-D68 assay (at NL02) [1, 35]	VP1 sequencing [7] complete genome (unpublished)
	NL10	2,128	182	8.6%	36	19.8%	36		Various	66.0%	EV clinical surveillance	EV-PCR [1] EV-D68 assay (at NL02) [1, 35]	VP1 sequencing [7] complete genome (unpublished)
	NL11	543	58	10.7%	1	1.7%	1		Various	7.3%	EV clinical surveillance	EV-PCR [1] EV-D68 assay (at NL02) [1, 35]	VP1 sequencing [7] complete genome (unpublished)
	NL12	2,072	80	3.9%	8	10.0%	8		Various	63.0%	EV clinical surveillance	EV-PCR [1] EV-D68 assay (at NL02) [1, 35]	VP1 sequencing [7] complete genome (unpublished)
Norway	NO01	110	45	40.9%	14	31.1%	14	1	Various	Not available	AFP and EV clinical surveillance	Not available	Not available
	NO02	6,079	8	0.1%	8	100.0%	0		Various	84.0%	EV clinical surveillance	EV-PCR (in-house, pan-EV,	VP1 sequencing [(36), modified]

												unpublished) <u>EV-D68 PCR</u> ([36], modified)	
	NO03	2,100	152	7.2%	10	6.6%	0		Various	53.0%	EV clinical surveillance	<u>EV-PCR</u> ([37], modified)	in-house
Portugal	PT01	1,040	5	0.5%	0	0.0%	0	3	Respiratory samples	100.0%	ILI/ARI surveillance	<u>EV-PCR</u> Allplex - Seegene kit for respiratory viruses panel <u>EV-D68 PCR</u> [10, 20]	<u>VP1 sequencing</u> [7]
Sweden	SE01	6,082	52	0.9%	10	19.2%	10		Respiratory samples	100.0%	EV clinical surveillance	<u>EV-PCR</u> commercial Allplex respiratory panel (Seegene Inc.) <u>EV-D68 PCR</u> [10], modified	<u>VP1 sequencing</u> [7] <u>VP4-VP2 sequencing</u> [3]
	SE02	40	32	80.0%	1	3.1%	1		Respiratory samples	10.0%	EV clinical surveillance	<u>cell culture on EV positive feces samples</u>	Sanger sequencing

									Feces CSF				
Slovenia	SI01	633	40	6.3%	0	0.0%	0		Respiratory samples	100.0%	ILI/ARI surveillance	<u>EV-PCR</u> [8] <u>EV-D68 PCR</u> [10]	<u>VP1 sequencing</u> [7]
	SI02a	513	13	2.5%	0	0.0%	0		Cerebral Spinal Fluid	0.0%	EV clinical surveillance	<u>EV-PCR</u> FilmArray Meningitis/Encephalitis Panel (BioFire Diagnostics) or Xpert EV (Cepheid) <u>EV-D68 PCR</u> [38]	<u>VP1 sequencing</u> [7]
	SI02b	4,483	110	2.5%	0	0.0%	0		Respiratory samples	100%	EV clinical surveillance	<u>EV-PCR</u> Ausdiagnostics RV panel 16 well, FilmArray (Biofire Diagnostics)	<u>VP1 sequencing</u> [7]
United Kingdom	UK01	1,441	1,103	76.5%	209	19.0%	190	4	Various	Not available	EV clinical surveillance	<u>EV-PCR</u> in-house based on [39]	<u>VP1 sequencing</u> [7] (Sanger)

												<u>EV-D68 PCR</u> [1]	
UK03	3,413	184	5.4%	74	40.2%	66	2	1. Respiratory samples with clinical indication 2. Skin swab with clinical indication 3. All CSF 4. Blood with Clinical symptoms	60.0%	EV clinical surveillance	<u>EV/PEV-PCR, EV-D68-PCR, RV PCR</u> EV-D68 reflex PCR on positive samples (RV is a separate respirate RT-PCR)	Not Applicable - typing assay performed at UK01	
UK04	6,679	466	7.0%	11	2.4%	11	0	Various	63.0%	ILI/ARI surveillance	<u>EV-PCR</u> AusDiagnostics' Viral 8-well PCR and Respiratory Virus 16-plex	<u>VP1 sequencing</u> [7, 40, 41]	

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