International Journal of Infectious Diseases 142 (2024) 106998

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Short Communication

Increased circulation of echovirus 11 in the general population and hospital patients as elicited by the non-polio enterovirus laboratory-based sentinel surveillance in northern Italy, 2023

Laura Pellegrinelli[1], Cristina Galli[1], Federica Giardina[2], Guglielmo Ferrari[3], Sara Colonia Uceda Renteria[4], Ferruccio Ceriotti[4], Arlinda Seiti[1], Sandro Binda[1], Antonino Maria Guglielmo Pitrolo[3], Roberta Schiavo[5], Sergio Maria Ivano Malandrin[6], Annalisa Cavallero[6], Marco Arosio[7], Claudio Farina[7], Massimo Oggioni[8], Pierluigi Congedo[8], Danilo Cereda[9], Francesca Rovida[2,3], Antonio Piralla[3,#], Elena Pariani[1,#,*], Fausto Baldanti[2,3,#], Non-polio community-based surveillance group[†], Non-polio hospital-based surveillance group[‡]

[1] *Department of Biomedical Sciences for Health, University of Milan, Milan, Italy*
[2] *Department of Clinical, Surgical, Diagnostic and Pediatric Sciences, University of Pavia, Pavia, Italy*
[3] *Microbiology and Virology Department, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy*
[4] *Virology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy*
[5] *Microbiology Unit, Hospital Guglielmo da Saliceto, Piacenza, Italy*
[6] *Microbiology Unit, Fondazione IRCCS San Gerardo dei Tintori, Monza, Italy*
[7] *Microbiology and Virology Laboratory, ASST "Papa Giovanni XXIII", Bergamo, Italy*
[8] *S.S.D. Microbiologia, Dipartimento dei Servizi Diagnostici, ASST della Brianza, Vimercate, Italy*
[9] *Direzione Generale Welfare Regione Lombardia, Milano, Italy*

ARTICLE INFO

Article history:
Received 22 December 2023
Revised 4 March 2024
Accepted 4 March 2024

Keywords:
Echovirus 11
Enterovirus
Non-polio enterovirus
Laboratory-based surveillance
Community-based surveillance

ABSTRACT

Objectives: Following the alert of echovirus 11 (E-11) infection in neonates in EU/EEA Member States, we conducted an investigation of E-11 circulation by gathering data from community and hospital surveillance of enterovirus (EV) in northern Italy from 01 August 2021 to 30 June 2023.
Methods: Virological results of EVs were obtained from the regional sentinel surveillance database for influenza-like illness (ILI) in outpatients, and from the laboratory database of ten hospitals for inpatients with either respiratory or neurological symptoms. Molecular characterization of EVs was performed by sequence analysis of the VP1 gene.
Results: In our ILI series, the rate of EV-positive specimens showed an upward trend from the end of May 2023, culminating at the end of June, coinciding with an increase in EV-positive hospital cases. The E-11 identified belonged to the D5 genogroup and the majority (83%) were closely associated with the

* Corresponding author: Elena Pariani, Department of Biomedical Sciences for Health, University of Milan, Via Carlo Pascal 36, 20133, Milan, Italy.
E-mail address: elena.pariani@unimi.it (E. Pariani).

# These authors contributed equally to the work and share the last authorship.

† **Non-polio community-based surveillance group**: Sentinel physicians of RespiVirNet in Lombardy; Federica Attanasi, Marcello Tirani, Michela Viscardi (Direzione Generale Welfare Regione Lombardia, Milano).

‡ **Non-polio hospital-based surveillance group**: Stefania Paolucci (Microbiology and Virology Department, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy), Alessandro Borghesi, Stefano Ghirardello, Amelia Di Comite (Neonatal Intensive Care Unit, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy), Thomas Fojadelli (Pediatric Clinic, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy), Gian Luigi Marseglia (Department of Clinical, Surgical, Diagnostic and Pediatric Sciences, University of Pavia, Pavia, Italy, Pediatric Clinic, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy), Giuliana Lo Cascio (Microbiology Unit, Hospital Guglielmo da Saliceto, Piacenza, Italy); Davide Oggioni (S.S.D. Microbiologia, Dipartimento dei Servizi Diagnostici, ASST della Brianza, Vimercate, Italy); Valentina Sottili (Maternal and Child Department, Pediatrics Unit, ASST della Brianza, Vimercate, Italy); Giovanna Mangili, (Neonatal Intensive Care Unit, ASST "Papa Giovanni XXIII", Bergamo); Maria Teresa Sinelli (Neonatal Intensive Care Unit, Fondazione IRCCS San Gerardo dei Tintori); Fabio Sagradi (Laboratorio di Microbiologia e Virologia, ASST Cremona, Italy), Diana Fanti, Alice Nava (S.C. Microbiologia Clinica- ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy), Federica Novazzi, Nicasio Mancini (Department of Medicine and Surgery, University of Insubria, Varese, Italy, Laboratory of Microbiology, ASST Sette Laghi, Varese, Italy), Annapaola Callegaro (Laboratory Medicine Department, Asst Bergamo Est, Bergamo Italy).

https://doi.org/10.1016/j.ijid.2024.106998

Hospital-based surveillance
Phylogenetic analysis
General population
Severe infection

novel E-11 variant, first identified in severe neonatal infections in France since 2022. E-11 was identified sporadically in community cases until February 2023, when it was also found in hospitalized cases with a range of clinical manifestations. All E-11 cases were children, with 14 out of 24 cases identified through hospital surveillance. Of these cases, 60% were neonates, and 71% had severe clinical manifestations.
Conclusion: Baseline epidemiological data collected since 2021 through EV laboratory-based surveillance have rapidly tracked the E-11 variant since November 2022, alongside its transmission during the late spring of 2023.

1. Introduction

Since the initial report of enterovirus (EV), echovirus 11 (E-11) infection in neonates in France in May 2023 [1], further cases have been reported in Italy and other European countries. The World Health Organization (WHO), having evaluated the limited data available, considers the public health risk to be low although countries are advised to investigate cases (https://www.who.int/emergencies/disease-outbreak-news/item/2023-DON474). Given that non-polio EV infections are not considered notifiable infectious diseases, this investigation aims to examine the circulation of E-11 in Lombardy (northern Italy). Non-polio EV infections spread by the respiratory and oral-faecal routes and usually present with a prodrome of influenza-like symptoms or fever, which can range from mild and self-limited disease to severe and life-threatening manifestations, including myocarditis, sepsis, hepatitis, meningitis, encephalitis and acute flaccid myelitis/paralysis [2]. They play a significant role in respiratory infections, causing a range of respiratory symptoms such as cough, cold and difficulty breathing, and can also lead to respiratory complications, particularly in infants and young children [3]. Understanding their role is crucial for effective diagnosis, management and public health interventions, highlighting the importance of on-going surveillance and research to understand their impact on public health and to develop targeted prevention strategies.

The aim of this study was to conduct event-based surveillance of non-polio EV by examining the regional virological database for influenza-like illness (ILI) surveillance (community-based surveillance) and reviewing laboratory data from 10 hospitals, including inpatients with respiratory or systemic/neurological symptoms (hospital-based surveillance), from 01 August 2021 to 30 June 2023.

2. Materials and methods

To evaluate EV circulation within the community, EV detection results were obtained from the virological ILI database of Lombardy's regional reference laboratory, which is part of the Italian respiratory virus surveillance network (https://www.iss.it/en/respivirnet). This network relies on the voluntary participation of sentinel physicians who are tasked with collecting respiratory specimens to monitor respiratory viruses (https://www.iss.it/en/respivirnet), including the identification of EVs through a real-time RT-PCR assay [4].

Additionally, a hospital-based laboratory surveillance of EVs has been established in Lombardy since October 2021 to evaluate the molecular characteristics of EVs in inpatients hospitalized with respiratory or neurological symptoms. Samples from individuals who tested positive for EVs and were either admitted to the emergency department or hospitalized with respiratory or neurological symptoms in 10 hospitals in northern Italy were analysed.

Each sample that tested positive for EV-RNA was further tested by real-time RT-PCR to detect EV-D68 genome [5,6]. EV-RNA positive samples with a viral load of Ct < 33 underwent molecular characterization through sequencing of the VP1 gene [7], followed by nucleotide sequence similarity analysis using the Basic Local Alignment Search Tool BLAST® (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and the RIVM enterovirus genotyping tool (https://www.rivm.nl/mpf/typingtool/enterovirus/).

3. Results

During the study period, 3781 respiratory specimens from ILI cases were examined, of which 8.2% ($n = 309$) were positive for EV-RNA. EVs were detected every week from week 2021-40, when the study started, showing a pattern of circulation that resulted in two epidemic waves (Figure 1). The first wave started in week 2022-19 (weekly EV-positivity rate: 20%), peaked in week 2022-28 (50%) and subsided in week 2022-37 (10%). The second wave started in week 2023-21 (12.5%) and peaked in week 2023-25 with a positivity rate of 38.6%. During the hospital-based surveillance, 341 EV-positive patients were identified. The temporal distribution of EV-positive cases identified during hospital- and community-based surveillance overlapped (Figure 1). In total, 22 EV-positive individuals (22/341; 6.5%) were newborns, all of whom were identified in the hospital setting.

All respiratory specimens collected during ILI surveillance were tested routinely for other respiratory viruses, namely, SARS-CoV-2, influenza virus A/B, respiratory syncytial virus (RSV), metapneumovirus (MPV), rhinovirus, adenovirus (AdV) and parechovirus (PeV). The presence of viral co-infection was observed in 45% of EV-positive samples; in detail, rhinovirus and RSV were identified in 48.9% and 23% of EV-positive samples, respectively, while SARS-CoV-2 and influenza virus A were detected in 4.3% and 5.8% of EV-positive respiratory specimens. AdV was identified in 14.4% of EV-positive samples and MPV in 3.6%; no EV/PeV coinfection was identified. For 87.7% ($n = 299$) specimens collected during hospital-based surveillance, data of test for other respiratory viruses (namely, SARS-CoV-2, influenza virus A/B, RSV, MPV, rhinovirus, AdV) were available; in detail, rhinovirus and RSV were identified in 47.3% and 11.5% of EV-positive samples, respectively, while SARS-CoV-2 and influenza virus A were detected in 3.4% and 6.1% of EV-positive respiratory specimens. AdV was identified in 24.4% of EV-positive samples and MPV in 7.4%.

Considering E-11 cases, no other viruses were detected in clinical samples collected in the hospital setting, but AdV and rhinovirus were detected in two ILI cases of E-11 (Table 1).

Analysis of EV-D68 specific assay and EV sequencing results revealed that 294 (45,2%) strains were classified as an EV type, while 356 (54.8%) EVs remained untyped due to low viral load. Twenty distinct EV types belonging to all the four EV groups (A-D) were identified at different frequencies including: EV-D68 ($n = 146$),

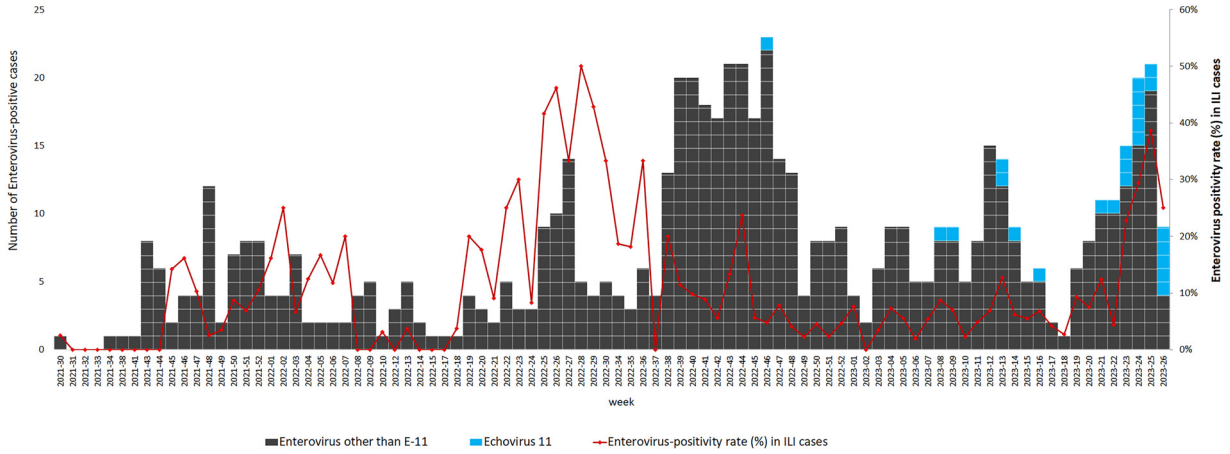

Figure 1. Weekly positivity rate of EV in community surveillance (ILI series) and number of EV-positive cases and E-11 positive cases in Lombardy, northern Italy from week 2021-30 to week 2023-26.

Table 1
Demographic, clinical and molecular characterization of E-11 cases identified in Lombardy, northern Italy from week 2021-30 to week 2023-26. The following abbreviations are used: NPS (nasal-pharyngeal swab), NPA (nasal-pharyngeal aspirate), CSF (cerebral spinal fluid), and BAL (brocho-alveolar lavage).

E-11 cases	Surveillance setting	Clinical manifestation	Type of specimen	Age	Week of sample collection	Weekly EV-positivity rate	E-11 genogroup	Detection of other viruses in clinical sample
1	Community	ILI	NPS	8 months	2022-46	0.2%	D5	None
2	Community	ILI	NPS	2 years	2023-08	8.7%	D5	None
3	Community	ILI	NPS	2 years	2023-09	7.0%	D5	None
4	Community	ILI	NPS	3 years	2023-13	12.8%	D5	None
5	Community	ILI	NPS	2 years	2023-13	12.8%	D5	Rhinovirus
6	Hospital	Fever	NPS	10 months	2023-14	6.1%	D5	None
7	Hospital	Fulminant hepatitis [2]	Blood	4 days	2023-16	6.9%	D5	None
8	Hospital	Fulminant hepatitis [2]	Blood	6 days	2023-16	6.9%	D5	None
9	Hospital	Fever, hyporeactivity, mild hypotonia	NPS	7 days	2023-21	12.5%	D5	None
10	Hospital	Acute otitis	NPA	5 years	2023-22	4.3%	D5	None
11	Community	ILI	NPA	10 years	2023-23	22.7%	D5	Adenovirus
12	Hospital	Encephalitis	CSF and faeces	6 years	2023-23	22.7%	D5	None
13	Hospital	Acute hepatitis	Blood	18 days	2023-23	22.7%	D5	None
14	Community	ILI	NPA	4 years	2023-24	29.4%	D5	None
15	Hospital	Fever and gastroenteritis	NPS	45 days	2023-24	29.4%	D5	None
16	Hospital	Fever and gastroenteritis	NPS	22 days	2023-24	29.4%	D5	None
17	Hospital	Respiratory distress	BAL	4 months	2023-24	29.4%	D5	None
18	Hospital	Asymptomatic	Faeces	6 months	2023-24	29.4%	D5	None
19	Hospital	Hyporeactivity, mild hypotonia, mild apnoea	NPS	3 days	2023-25	25.0%	D5	None
20	Hospital	Fever	NPS	10 days	2023-25	25.0%	D5	None
21	Community	ILI	NPS	3 years	2023-26	38.6%	D5	None
22	Community	ILI	NPS	9 years	2023-26	38.6%	D5	None
23	Community	ILI	NPS	4 years	2023-26	38.6%	D5	None
24	Hospital	Meningitis	NPS and CSF	10 years	2023-26	38.6%	D5	None

coxsackievirus (CV) A6 ($n = 27$), echovirus (E) 11 ($n = 24$), CV-A4 ($n = 15$), CV-B5 ($n = 15$), CV-B4 ($n = 12$), CV-A16 ($n = 10$), CV-B2 ($n = 7$), E-3 ($n = 7$), E-18 ($n = 7$), CV-A5 ($n = 5$), EV-C105 ($n = 4$), CV-B3 ($n = 4$), CV-A9 ($n = 3$), CV-A2 ($n = 2$), E-25 ($n = 2$), CV-A10 ($n = 1$), EV-A71 ($n = 1$), CV-A21 ($n = 1$), EV-C109 ($n = 1$).

Twenty-four cases of E-11 were identified (24/294: 8.2%). All E-11 cases were children aged between 3 days and 10 years. Out of these, 10 cases were identified within community surveillance and 14 within hospital surveillance. Overall, 14 (58.3%) E-11 cases had mild infection (ILI, fever, or acute otitis) and all were children aged between 22 days and 10 years. Ten cases (41.7%) had severe clinical manifestations, of which six were in neonates. Overall, of the 22 EV-positive neonates, 6 (27.3%) were E-11 cases, 4 (18.2%) were CV-B cases, 1 was CV-A16 (4.5%) and 1 was E-9 (4.5%).

As shown in Table 1, aside from the two cases of fulminant hepatitis [8], a wide range of symptoms were recorded, making the clinical diagnosis of E-11 infection highly unpredictable based on the observation of symptoms alone.

E-11 was sporadically detected in November 2022 (one case) and February 2023 (two cases), with an additional three cases detected in April 2023. Subsequently, the number of E-11 cases increased, with 16 cases recorded in May/June 2023.

Our analysis of the VP1 gene from 24 E-11 sequences identified that all belonged to genogroup D, lineage D5, similarly to other E-11 strains recently reported in France [1]. The phylogenetic tree (Figure S1) included 23 out of 24 E-11 sequences (one was excluded due to its limited lenght) and showed that 19 study E-11 sequences (83%) segregated together (mean nucleotide identity: 98.5%). These sequences clustered with recent French E-11 strains ($N = 9$) [1], sharing a mean nucleotide identity of 98.6%. The other four E-11 study strains showed separate segregation. Four sequences clustered with E-11 strains identified in China in 2017 and 2019.

4. Discussion and conclusion

In light of the WHO advisement and risk assessment of severe E-11 infection in Europe and given the lack of on-going surveillance of EV infection in Italy, we performed event-based surveillance to assess the spread of E-11 in northern Italy. From the virological data gathered through community-based surveillance of ILI, we observed a low level of EV circulation from August 2021 to May 2022, followed by two epidemics. The first occurred from May to July 2022 and was caused by EV-D68. The second wave occurred from end of May to July 2023. E-11 was detected both in individuals with mild symptoms and in hospitalized patients with self-limited severe manifestations, ranging in age from 9 days to 10 years. It is noteworthy that E-11 was also sporadically identified from November 2022 to April 2023, but only in individuals with ILI. Furthermore, phylogenetic analysis of the VP1 gene showed that 83% of the E-11 strains grouped together and clustered with the French E-11 strains [1]. As previously noted, recombination of E-11 with other EV types may have led to the emergence and spread of novel variants with chimaeric genome structures [9]. This could explain the severe illness and epidemic nature of the strains observed in Italy in 2013 [10] and in France in 2022-2023 [1]. In contrast, data from the US national enterovirus surveillance system has reported a significantly higher mortality from E-11 infection in neonates compared to infants older than 1 month [11], similar to what has been recently observed in a multicentre retrospective cohort study [12]. Consistent with these findings, our E-11 cases were either children >1 year of age or neonates with no underlying medical conditions, likely resulting in a mild self-limited clinical manifestation.

There are a few drawbacks to this study. First, it was not possible to molecularly characterize all EV samples with respect to their viral load. In addition, the routinely used typing techniques based on the VP1 fragment provide only partial information on viral evolution and no information on recombination events. Finally, although it would be useful to have information on possible co-infections, it was not possible to obtain this information for all cases included in this study: a viral panel was evaluated in all respiratory samples from ILI cases, but different pathogen panels were used in the hospital setting.

In conclusion, our community-based sentinel laboratory surveillance has detected an increase in the incidence of E-11 in recent months. In addition, hospital-based surveillance has captured the clinical features and severity of E-11 in neonates during the outbreak caused by a new variant of E-11 initially identified in France.

Declarations of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding

This research was partially supported by EU funding within the Next Generation EU-MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases (Project no. PE00000007, INF-ACT) and by funding within Centre for Disease Prevention and Control (CCM) of the Italian Ministry of Health (SURVEID Project, program 2022).

Ethical statement

Ethical approval was not required for this study because the study was part of the routine activities of the Italian respiratory virus surveillance network (RespiVirNet) and the routine management and treatment of patients.

Author contributions

LP, AS, CG conducted virological investigation of ILI cases; SCUR, FC, RS, SMIM, AC, MA, CF, MO, PC conducted virological investigation of hospitalized cases; CG, FG, GF, AMGP carried out phylogenetic analyses, EP, DC, FR, SB retrieved epidemiological data, LP, EP, AP, FB wrote the first draft of the manuscript. AP, EP, FB revised the manuscript and supervise. All authors were involved in the study group on surveillance of enterovirus infections, contributed to the manuscript and approved the final version.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2024.106998.

References

[1] Grapin M, Mirand A, Pinquier D, Basset A, Bendavid M, Bisseux M, et al. Severe and fatal neonatal infections linked to a new variant of echovirus 11, France, July 2022 to April 2023. *Euro Surveill* 2023;**28**:2300253.
[2] Fischer TK, Simmonds P, Harvala H. The importance of enterovirus surveillance in a post-polio world. *Lancet Infect Dis* 2022;**22**(1):e35–40. doi:10.1016/S1473-3099(20)30852-5.
[3] Imamura T, Oshitani H. Global reemergence of enterovirus D68 as an important pathogen for acute respiratory infections. *Rev Med Virol* 2015;**25**(2):102–14. doi:10.1002/rmv.1820.
[4] Galli C, Pellegrinelli L, Bubba L, Primache V, Anselmi G, Delbue S, et al. When the COVID-19 pandemic surges during influenza season: lessons learnt from the sentinel laboratory-based surveillance of influenza-like illness in Lombardy during the 2019-2020 season. *Viruses* 2021;**13**:695.
[5] Piralla A, Girello A, Premoli M, Baldanti F. A new real-time reverse transcription-PCR assay for detection of human enterovirus 68 in respiratory samples. *J Clin Microbiol* 2015;**53**:1725–6.
[6] Poelman R, Schuffenecker I, Van Leer-Buter C, Josset L, Niesters HG, Lina B, et al. European surveillance for enterovirus D68 during the emerging North-American outbreak in 2014. *J Clin Virol* 2015;**71**:1–9.
[7] Nix WA, Oberste MS, Pallansch MA. Sensitive, seminested PCR amplification of VP1 sequences for direct identification of all enterovirus serotypes from original clinical specimens. *J Clin Microbiol* 2006;**44**:2698–704.
[8] Piralla A, Mojoli F, Pellegrinelli L, Ceriotti F, Valzano A, Grasselli G, et al. Impact of SARS-CoV-2 omicron and delta variants in patients requiring intensive care unit (ICU) admission for COVID-19, Northern Italy, December 2021 to January 2022. *Respir Med Res* 2023;**83**:100990.
[9] Chu PY, Tyan YC, Chen YS, Chen HL, Lu PL, Chen YH, et al. Transmission and demographic dynamics of coxsackievirus B1. *PLoS One* 2015;**10**:e0129272.
[10] Rueca M, Lanini S, Giombini E, Messina F, Castilletti C, Ippolito G, et al. Detection of recombinant breakpoint in the genome of human enterovirus E11 strain associated with a fatal nosocomial outbreak. *Virol J* 2022;**19**:97.
[11] Khetsuriani N, Lamonte-Fowlkes A, Oberst S, Pallansch MACenters for Disease Control and Prevention. Enterovirus surveillance—United States, 1970-2005. *MMWR Surveill Summ* 2006;**55**:1–20.
[12] Wang P, Xu Y, Liu M, Li H, Wang H, Liu Y, et al. Risk factors and early markers for echovirus type 11 associated haemorrhage-hepatitis syndrome in neonates, a retrospective cohort study. *Front Pediatr* 2023;**11**:1063558.