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MAJOR ARTICLE

Epidemiological and Clinical Insights into the Enterovirus D68 Upsurge in Europe 2021–2022 and Emergence of Novel B3-Derived Lineages, ENPEN Multicentre Study

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Enterovirus D68 (EV-D68) infections are associated with severe respiratory disease and acute flaccid myelitis (AFM). The European Non-Polio Enterovirus Network (ENPEN) aimed to investigate the epidemiological and genetic characteristics of EV-D68 infections and its clinical impact during the fall-winter season of 2021–2022. From 19 European countries, 58 institutes reported 10 481 (6.8%) EV-positive samples of which 1004 (9.6%) were identified as EV-D68 (including 852 respiratory samples). Clinical data were reported for 969 cases; 78.9% of infections were reported in children (0–5 years); and 37.9% of cases were hospitalized. Acute respiratory distress was commonly noted (93.1%) followed by fever (49.4%). Neurological problems were observed in 6.4% of cases including 6 diagnosed with AFM. Phylodynamic/Nextstrain and phylogenetic analyses based on 694 sequences showed the emergence of 2 novel B3-derived lineages, with no regional clustering. In conclusion, we describe a large-scale European EV-D68 upsurge with severe clinical impact and the emergence of B3-derived lineages.

Keywords. enterovirus D68 (EV-D68); respiratory infection; nonpolio enterovirus (NPEV); European Non-Polio Enterovirus Network (ENPEN); acute flaccid myelitis (AFM).

Enterovirus D68 (EV-D68) primarily infects the human upper respiratory tract and is mainly associated with mild to moderately severe upper respiratory symptoms, including sore throat, cough, congestion, and fever. However, infections can also be associated with lower respiratory tract infections and severe neurological conditions such as meningitis, encephalitis, or acute flaccid myelitis (AFM) [1–3]. Children up to 5 years of age are most commonly affected and are at greatest risk of developing severe disease [4]. Nevertheless, severe forms of EV-D68 have also been observed in adults, especially in elderly, immunosuppressed, or those with other underlying clinical conditions [2, 5].

EV-D68 is a member of the species *Enterovirus D* in the genus *Enterovirus,* family Picornaviridae [6]. The genus *Enterovirus* comprises 9 species and more than 200 types. Molecular detection is the gold standard to diagnose EV-D68 infections, either by an EV-D68 specific reverse transcription polymerase chain reaction (RT-PCR) or by an EV-generic assay targeting the conserved 5′ untranslated region followed by genotyping of the genes encoding capsid proteins VP1 or VP4–VP2 [7].

Phylogenetic analysis of VP1 sequences enables the differentiation of EV-D68 strains into genotypes A through D. The B genotype is further divided into subgenotypes/clades B1, B2, and B3, while the A subgenotype/clade is divided into A1 and A2, whereas A2 has been further divided into D1 and D2 (also referred to as A2/D1 and A2/D2, respectively). The most common EV-D68 subgenotype/clade circulating worldwide is B3, followed by A2/D2 [8].

The first EV-D68 reported outbreaks of acute respiratory disease in Europe occurred between 2008 and 2010 in the Netherlands and Italy [9–11]. Prior to that, clinical EV-D68 cases were rarely reported [12]. Since 2008, the epidemiology of EV-D68 in Europe and North America has shown a biennial epidemic cycle [13], with infections occurring predominantly in early fall and winter [14–17]. From 2014 onwards, the biennial EV-D68 outbreaks have been accompanied by reports of AFM cases testing positive for EV-D68 in the United States where AFM is subject to enhanced surveillance (reviewed in [16, 18]). AFM cases associated with EV-D68 were first reported in Europe in 2014 [19–21]. In 2019, a disruption of the biennial cycle was noted when an upsurge of EV-D68 infections with 93 cases was reported in 5 European countries [22]. The study also identified 5 EV-D68–infected children with severe

neurological disease and the circulation of B3-derived clusters, designated US18, EU18, and EU19.

During 2020, the first year of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, no EV-D68 cases were reported in Europe. However, during the fall of 2021, when countries eased nonpharmaceutical coronavirus disease 2019 (COVID-19) interventions, a substantial rise in EV infections was detected, in part due to increased EV-D68 circulation in some countries [4, 23]. The aim of this study was to examine the epidemiological, clinical, and molecular characteristics of this EV-D68 upsurge in Europe.

METHODS

Data Collection and Analysis

An invitation to participate in the study was sent to members of the European Non-Poliovirus Enterovirus Network (ENPEN). ENPEN brings together specialists from different fields including clinical virology, neurological and pediatric infectious diseases, academic/molecular virology, epidemiology, and public health [12, 24].

Institutes provided information on the source of the samples, sample types screened, and detection and typing methodology (Supplementary Table 1). Epidemiological data on the total number of samples tested and the total number of EV and EV-D68–positive samples were collected and analyzed per month from January through December 2021. All participating institutes were coded using a 2-digit country code, followed by a sequential number (eg, XX99; Supplementary Table 1).

Demographic and clinical data were reported in aggregated format or pseudonymized manner and included cases from 2021 and 2022 (until February; Table 1 and Table 2). The data collected is summarized in Figure 1.

EV-D68 Phylodynamic and Phylogenetic Analysis

Sequences were processed for phylodynamic analysis with the Nextstrain augur pipeline to show the time-related divergence [15, 22, 25]. Briefly, the pipeline combines EV-D68 sequences from this study and those publicly available EV-D68 sequences from National Center for Biotechnology Information GenBank (via ViPR). The lattter were randomly subsampled down to 240 sequences per country per year in order to reduce disparity in representation between countries with different levels of sequencing. Sequences were then aligned using IQTree, and a time-resolved phylogeny was produced with Treetime, along which ancestral sequences were reconstructed also using Treetime (both programs as implemented in Nextstrain). EV-D68 subgenotypes/clades were assigned using mutational markers on the phylogeny. Sequence analysis included metadata on country, sample type, date of collection, and age groups. Three analyses were performed based on the length of sequence available: (1) >300 bp, which included the partial VP1 sequences >300 bp, the near-complete VP1 sequences >700 bp, and the complete genomes (n = 692); (2) >700 bp, which included the near-complete VP1 sequences >700 bp and the complete genomes (n = 210); and (3) only complete genomes (n = 82) (Figure 1). Near-complete VP1 sequences and complete genome sequences were used to achieve a higher phylogenetic resolution. In the VP1 analyses, we included all available VP1 study sequences longer than 300 bp or 700 bp and sequences >700 bp extracted from GenBank on 21 September 2022 (n = 3740) (subsampled as described earlier) (Figure 1). In the full-genome analysis we included all available study sequences longer than 6 kbp and all available sequences longer than 6 kb from GenBank on 3 September 2022 (n = 976) (Figure 1). The code used to run the analysis is available at https://github.com/emmahodcroft/ev_d68_enpen2022.

To highlight the evolutionary divergence shown in Nextstrain, neighbor-joining trees (Jukes-Cantor corrected) using MEGA 7 software were constructed [26]. Mean pairwise (uncorrected *p* distances) distances between sequence groups were calculated using SSE software [27]. VP1 study sequences that were >80% complete between nucleotide positions 2501–2842 (numbering based on the Fermon prototype sequence, KU844179) were analyzed together with 3300 publicly available sequences between these positions (>90% complete) extracted from GenBank on 31 October 2022 (Figure 1). Complete genome sequences were analyzed together with 1025 publicly available complete genomes with known dates extracted from GenBank on 31 October 2022 (Figure 1).

GenBank Accession Numbers

Sequences were deposited in GenBank under the following accession numbers: OM811651-OM811652, OM831155-OM831207, ON006421-ON006422, OP267493-OP267535, OQ120627-OQ120631, OQ126930-OQ127239, OQ139546-OQ139558, OQ139565-OQ139630, OQ148174-OQ148361, OQ586762-OQ586804, OQ589870 and PP069743.

Ethical Statement and Privacy

Patients' privacy and confidentiality issues, according to General Data Protection Regulation, were managed in compliance with national/European legislation. Approval from an ethics committee and informed consent for virus screening was attained in accordance to participating institutes' regulations.

RESULTS

Detection Frequencies of EV and EV-D68

A total of 58 institutions from 19 European countries participated in this study (Table 1 and Supplementary Table 1). Tested samples ranged from respiratory (852 of 969 [88%]) to feces and cerebral spinal fluid, depending on institute/country and diagnostic/surveillance system. EV-D68 laboratory

Table 1. Enterovirus Testing and EV-D68 Detection in Participating European Countries From January 2021 and February 2022

			Sampling and Testing Data					Clinical Records		
			EV 2021			EV-D68 2021		EV-D68 Total, 2021–2022		
Country	Country Code	Institute (n)	Samples Tested (n)	Positive Samples (n)	EV Positive Detection, %	Positive Samples (n)	EV-D68 % of EV	Clinical Cases (n)	AFM Cases/ Acute Myelitis (n)	Sequences (n)
Austria	AT	1	381	2	0.5	1	50.0	1	0	0
Belgium	BE	2	11 275	1038	9.2	100	9.6	98	0	18
Bulgaria	BG	1	399	13	3.3	0	NA	0	0	0
Croatia	HR	1	106	14	13.2	0	NA	0	0	0
Czechia	CZ	1	465	120	25.8	0	NA	0	0	0
Denmark	DK	1	NR	431	NA[c]	17	3.9	12[d]	0	11
Finland	FI	1	176[b]	NA[b]	NA[b]	0	NA[b]	0	0	0
France	FR	10	28 237	1921	6.8	156	8.2	153[d,e]	1	132
Germany	DE	4[a]	6064	149	2.5	5	3.4	5	1	5
Hungary	HU	1	360	6	1.7	0	NA	0	0	0
Ireland	IE	1	10 075	1964	19.5	14	0.7	16[d]	0	16
Italy	IT	3	5296	415	7.8	20	4.8	24[d]	0	8
Netherlands	NL	11	18 258	1004	5.5	106	10.6	105	2	97
Norway	NO	3	8289	205	2.5	32	11.7	15[d]	0	1
Portugal	PT	1	1040	5	0.5	0	NA	3[d,f]	0	2
Slovenia	SI	3	5629	163	2.9	0	NA	0	0	0
Spain	ES	8[a]	39 738	1194	3.0	248	20.5	253[d]	1	249
Sweden	SE	2	6122	84	1.4	11	13.1	11	1	1
United Kingdom	UK	3	11 533	1753	15.2	294	16.8	273[d]	0	204
Total		58	153 443	10 481	6.8	1004	9.5	969	6	744

Abbreviations: AFM, acute flaccid myelitis; EV, enterovirus; NA, not applicable; NR, not reported.

[a]Contributor reported for other institutes.

[b]Finland only reported on tested samples for EV-D68.

[c]Denmark did not report on the total samples tested.

[d]Countries reporting EV-D68 cases in 2022.

[e]French institute reporting EV-D68 cases only in 2022.

[f]Portuguese institute only reporting EV-D68 cases in 2022.

confirmation was mostly based on respiratory samples. Of the institutes that reported the sample type information (n = 49), respiratory samples comprised 66% of the samples tested (Supplementary Table 1). Testing more than 150 000 samples revealed a total of 10 481 EV-positive samples (6.8%) from 1 January through 31 December 2021. Of the EV-positive samples, 1004 were confirmed as EV-D68–positive (9.6%). Large differences in the number of samples tested and proportions of EV and EV-D68–positive samples were observed among countries/reporting institutes (Table 1 and Supplementary Table 1, respectively). However, data could not be compared due to different catchment population and testing strategies. Most institutes tested multiple sample types, and higher proportions of respiratory samples tested did not reflect a higher proportion in EV-D68–positive detections. Notably, the 2 institutes that only performed testing on cerebrospinal fluid (CSF) samples did not report any EV-D68 cases.

Seasonality of EV and EV-D68

The number of samples tested for EV remained similar throughout the first 8 months of 2021 (average 10 000 tests/month) during the period when an increasing number and proportion of EV-positive samples were observed (Figure 2). The first EV-D68–positive sample was detected in June 2021 and EV-D68–positive samples were sporadically detected from June through August 2021. From September 2021 onwards, the number of samples tested for EV increased accompanying a higher number of EV-positive samples. During this time, the number of EV-D68–positive samples increased exponentially and reached a peak in October 2021 (405 of 1004, 40%).

Clinical Characteristics of EV-D68 Cases

Clinical data were reported by 41 institutes (13 countries) on 969 EV-D68 cases (Figure 1, Table 1, and Supplementary Table 1). Most EV-D68 cases were identified by testing a respiratory sample (n = 852, sample type known for 870 cases; 98%) whereas fecal (n = 16), vesicle (unknown origin; n = 1), or plasma (n = 1) samples were positive in the remaining cases. Most

Table 2. Demographic and Clinical Characteristics of EV-D68 Cases Reported by 14 European Countries From January 2021 Through February 2022 (n = 968)

Characteristic	EV-D68 Cases, No. (%)
Demographic characteristics	
Age group	
0–2 mo	79 (8.2)
3–23 mo	288 (29.7)
3–12 mo	215 (22.2)
13–23 mo	73 (7.5)
2–5 y	398 (41.1)
6–15 y	101 (10.4)
16–25 y	14 (1.4)
26–45 y	34 (3.5)
46–65 y	24 (2.5)
> 65 y	17 (1.8)
Unknown	14 (1.4)
Total	969
Sex	
Male	524 (54.08)
Female	361 (37.25)
Unknown	84 (8.67)
Clinical information	
Symptoms, data reported for	
Any symptom reported	668 (68.9)
Respiratory	622 (93.1)
Fever	330 (49.4)
Enteric	99 (14.8)
Neurological[a]	43 (6.4)
Rash	28 (4.2)
Coinfections	
Any coinfection reported	241 (24.9)
Rhinovirus	115 (47.7)
Adenovirus	45 (18.7)
RSV	33 (13.7)
CoV (OC43, 229E, and SARS-CoV-2)	12 (5.0)
Clinical history and hospital information	
Preexisting condition[b]	249[c] (25.7)
Hospitalized	369 (38.1)
Hospital stay, average days	2.8
Intensive care unit admission	61 (16.5)

Abbreviations: CoV, coronavirus; RSV, respiratory syncytial virus.

[a]Reported neurological symptoms included headache, dizziness and agitation, seizures, encephalitis, meningitis, acute myelitis, and acute flaccid myelitis/acute flaccid paralysis.

[b]Reported preexisting conditions were asthma, congenital malformations, epilepsy, prematurity, and cancer.

[c]There were 235 patients with comorbidities displaying respiratory signs.

infections were reported in children between 2 and 5 years of age (41.1%) followed by children between 3 and 12 months of age (22.2%) (Table 2). In total, 79% (n = 765) of EV-D68 cases were in the age group 0–5 years (median age of 2.9 years, range from new-borns to 93 years). More than half of the cases were male (54%). Detailed clinical information was available for 668 EV-D68 cases showing respiratory distress as the predominant symptom (93.1%). The second most common clinical sign was fever, being reported in approximately half of these cases.

As shown in Table 2, coinfections were reported for 241 of 969 (24.9%) of EV-D68 cases, of which almost half were also infected with human rhinovirus. More than 2 coinfecting viruses were reported in 69 cases (28.6%).

Of 969 EV-D68 cases, 369 (38.1%) were hospitalized between 0.5 and 136 days (interquartile range, 0.5–3 days). A total of 249 individuals with EV-D68 infection (25.7%) had known underlying medical conditions, for example prematurity, congenital malformations, asthma, and different cancer types (Table 2).

Neurological conditions were identified in 43 patients (6.4%), most of which were in the age group 0–5 years (n = 34; 79%). The neurological problems ranged from headache, dizziness, and agitation to seizures. One case was reported with encephalitis (8 years of age with comorbidities) and 4 cases were diagnosed with meningitis (up to 5 months of age). AFM was reported in 6 children: 5 cases up to 5 years of age and 1 in an older child (6–15 years of age). Patients with neurological disorders typically showed respiratory distress (32 of 43; 74.4%), fever (26 of 43; 60.5%), enteric symptoms (12 of 43; 27.9%), or rash (7 of 43, 16.3%); and 37.2% (16 of 43) had an underlying medical condition. Twenty-eight of 43 patients had severe neurological disorders requiring intensive care unit (ICU) admission, 82.1% (23 of 28) being between 0 and 5 years of age and 39.3% (11/28) with known comorbidities.

Phylodynamics and Divergence of EV-D68 Strains

To investigate the circulation of EV-D68 strains, in this study we included a maximum of 694 sequences of the received 744 sequences for analysis (Figure 1). A majority of the strains were reported by submitting institutes as B3, and 8 strains as A2/D2. The B3 strains were detected throughout the study period. The A2/D2 strains were detected from October through December 2021 (data not shown). Figure 3 shows a screenshot of the Nextstrain build of the 300-bp VP1 EV-D68 sequences over time, accessible at https://nextstrain.org/community/enterovirus-phylo/evd68-2022/vp1-300. All of the Nextstrain runs are available at https://github.com/enterovirus-phylo/evd68-2022. The phylogenetic tree showed a temporal ladder-like evolution and the emergence of 2 novel B3-derived lineages, designated lineage 1 and lineage 2 for this study. These patterns are visible in both of the VP1 analyses and the full-genome analysis.

The divergence between these lineages was estimated to be 4.2% based on the complete genome (5.2% based on the partial VP1). Lineage 1 descended from B3 strains reported in 2019 (previously designated US18 with the 2019–2020 upsurge) and was detected across Europe (Figure 4) predominantly in the United Kingdom, Netherlands, France, and Spain from August 2021 throughout January 2022. For the 4 AFM cases with available sequence data available, all fell into the B3-derived lineage 1.

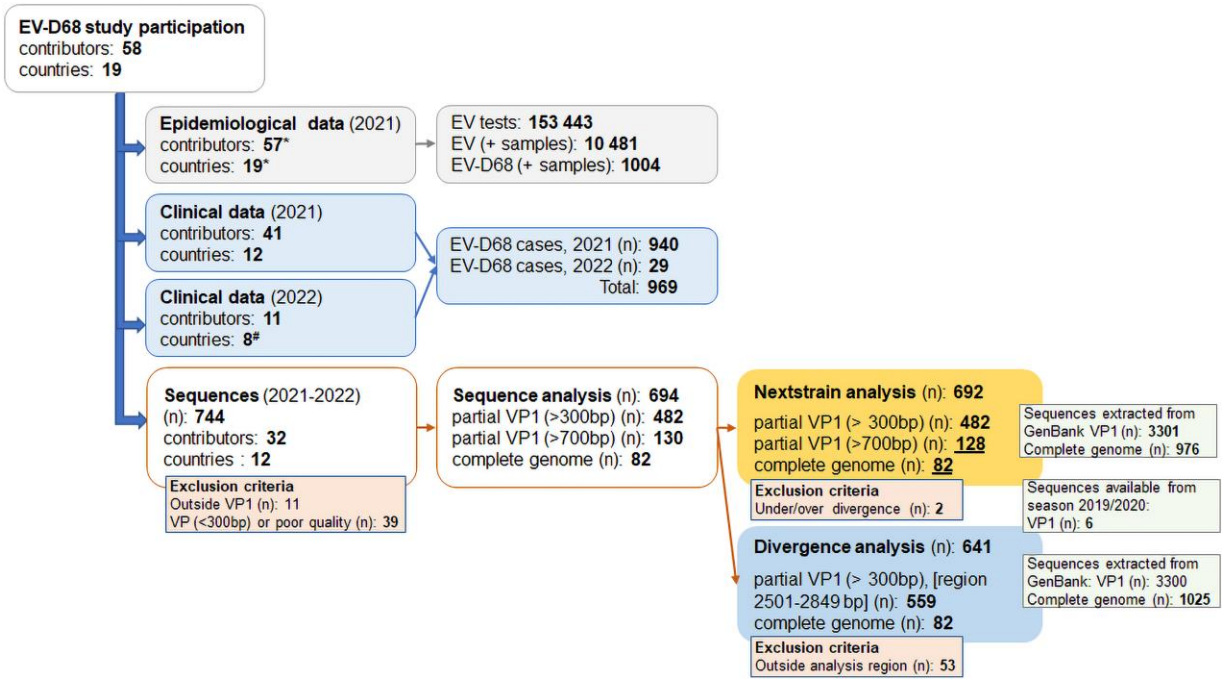

Figure 1. Diagram of the information collected: epidemiological data, clinical records, and sequence data with associated clinical metadata used for the analysis of the EV-D68 upsurge in Europe, 2021–2022. Sequence information is also represented separately for phylogenetic reconstruction (Nextstrain analysis) and divergence analysis. Publicly accessible sequences, extracted from Gen Bank, used for epidemiologic analysis are depicted in boxes alongside exclusion criteria. *Monthly testing data were received from 55 institutes, 18 countries; and monthly EV-positive data received from 57 institutes, 19 countries. #Two institutes only reported EV-D68 cases for 2022 (1 also representing the country cases).

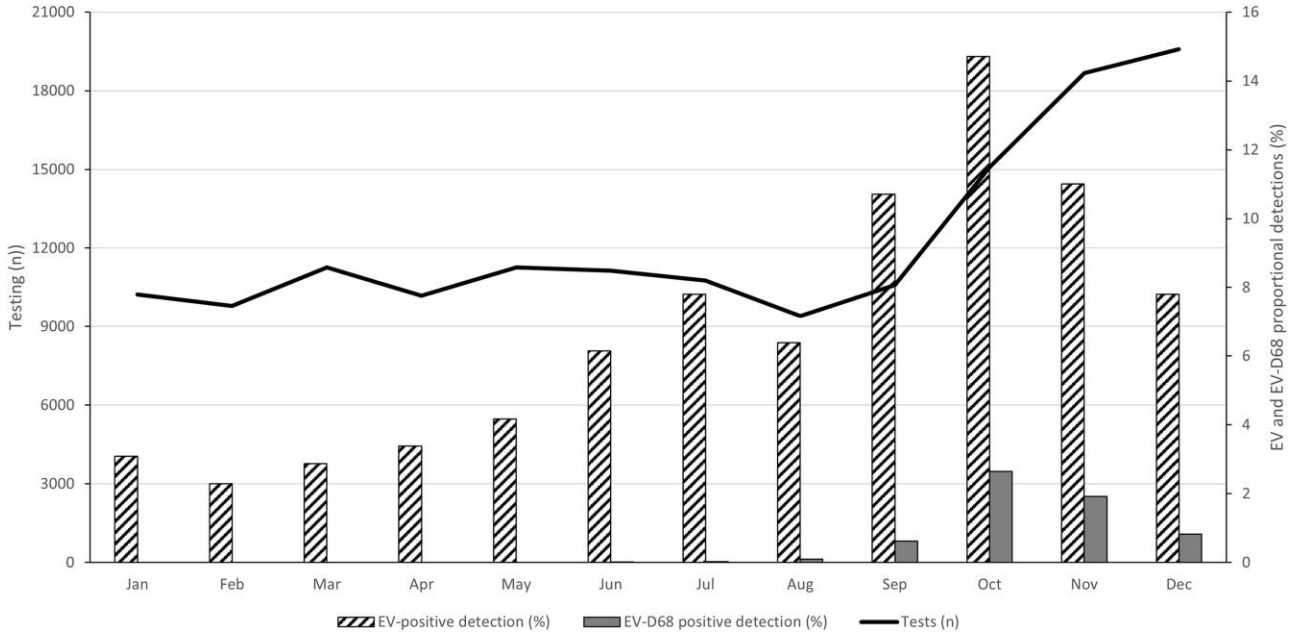

Figure 2. Proportion of samples found positive for EV (lined bars, % of EV-positive samples/number of samples tested) and EV-D68 (grey bars, % of EV-D68–positive samples/number of EV positive samples), and monthly totals of EV tests (line) reveal an increasing trend from September 2021 onwards. The highest numbers of EV and EV-D68 detections were observed in October 2021.

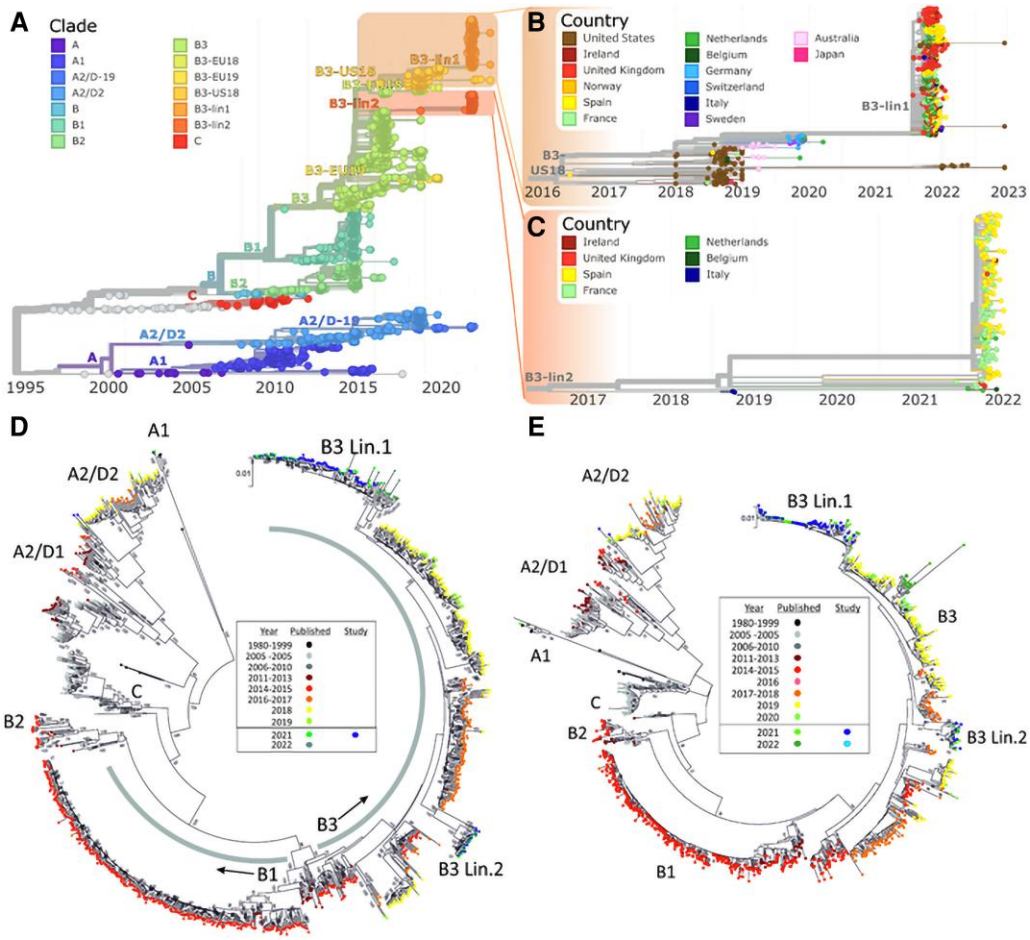

Figure 3. *A–C,* Phylodynamic analysis of EV-D68 with Nextstrain using 692 partial study VP1 sequences > 300 bp and 3307 publicly available VP1 sequences. *B* and *C,* Zoomed views of areas of the tree containing the 2 novel lineages. *D,* Neighbor-joining phylogenetic tree of complete genome from the study samples (n = 82) and 1025 sequences with data annotations from GenBank. *E,* Neighbor-joining phylogenetic tree of VP1 region sequences (positions 2501–2842, numbered using the Fermon prototype sequence, KU844179) from the study samples (n = 559) and 3306 sequences with data annotations from GenBank.

Lineage 2 was distantly related to a B3 sample from the 2016 outbreak in Europe and predominantly detected in southwestern European countries, such as Spain and France (Figure 4) from June 2021 through January 2022, with similar kinetics to lineage 1 variants (data not shown). Notably, lineage 2 showed a deletion at VP1:S143 not widely seen elsewhere on the phylogeny.

DISCUSSION

In this study, we describe valuable information on the demographic and clinical features of nearly a thousand EV-D68 cases mostly reported in the fall and winter of 2021–2022 by 13 of 19 European countries participating in the study, and the emergence of novel B3-derived lineages. For the first time, the collection of denominator data, that is, the number of samples tested for EV and EV-D68 across Europe, allowed for a depiction of the proportion of both EV and EV-D68 infections found within different institutes in 2021. However, it should be noted that catchment population and testing strategies varied, and data could not be compared directly between institutes nor among countries. Nevertheless, the study revealed the importance of sharing data across Europe, which aimed to improve diagnostic awareness based on the increased circulation of EV-D68. It also enforced our understanding of the epidemiology and evolution of EV-D68. The study also revealed gaps in data comparability and the need for better harmonized medical and diagnostic practices.

Based on the biennial circulation pattern of EV-D68 previously recorded in Europe, an EV-D68 upsurge was expected in summer/fall of 2020. However, this 2-year cycle had already been disrupted by the previous EV-D68 upsurge in Europe in 2019 [22] and was disturbed further by COVID-19 nonpharmaceutical interventions. For EV-D68 (and other viral pathogens) the disruption in their circulation is also hypothesized to have led to a much larger EV-immune–naive cohort

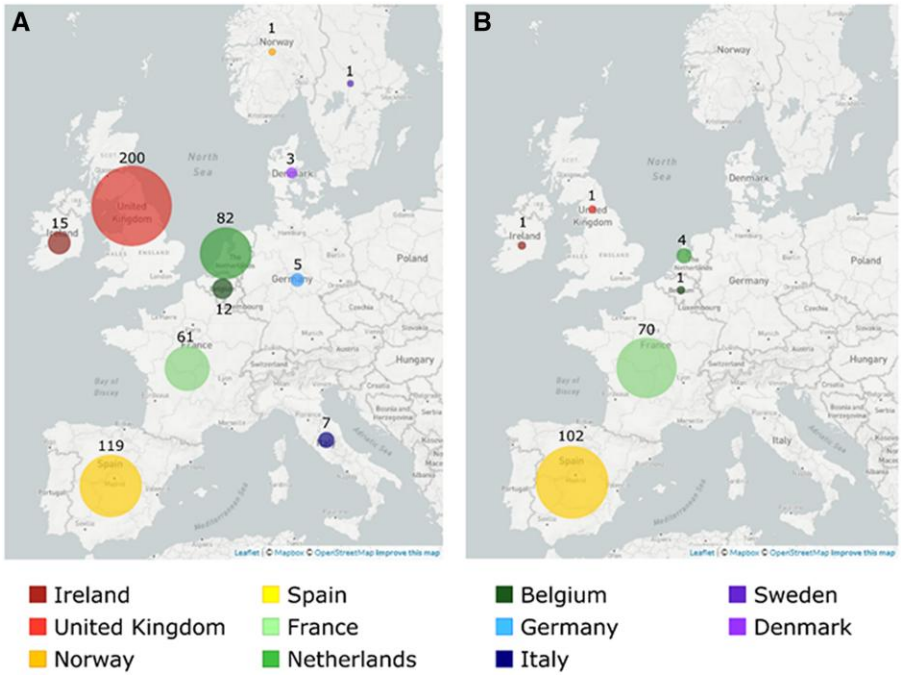

Figure 4. Maps showing the geographic distribution of the 2 novel EV-D68 B3-derived lineages, lineage 1 (*A*) and lineage 2 (*B*).

compared to the ones found in previous incidence cycles [15, 23, 28]. As a result, the easing of COVID-19 nonpharmaceutical interventions may have spurred new upsurges far greater than in previous cycles in Europe and beyond [4, 29–31]. Similarly, increased detections of other pathogens has been noted due to resumption of community circulation [32–34]. An additional contributory factor to the greater number of samples tested for EV and EV-D68 detections may have been the increased number of respiratory samples that were collected for syndromic testing on respiratory viruses, including SARS-CoV-2.

EV-D68 infections were predominantly associated with respiratory symptoms (93.1%), and nearly a quarter of EV-D68 infections were in individuals with underlying medical conditions, which may have played a role in the severity of EV-D68 infections [5, 16, 35, 36]. Similar proportions of underlying medical conditions among EV-D68 infections related to severity were also reported in previous studies, albeit the populations studied were different [16]. Most cases were detected by diagnostic testing of respiratory samples, highlighting the importance of including respiratory samples in EV surveillance as EV-D68 RNA is rarely detected in fecal and CSF samples, even in patients with neurological disease [7, 37]. It is noteworthy that rhinovirus was the predominant coinfection, which was also seen in other studies [36, 38]. It should be considered which underlying medical conditions and coinfections are to be included in the data collection to best promote standardization and further implementation in future EV-D68 studies [7, 12].

A total of 43 EV-D68 patients (6.4%) displayed neurological disorders, and half of them were diagnosed with seizures, encephalitis, meningitis, or AFM, providing further evidence for a potentially neurovirulent property of this virus [39]. Although data are reported on only 6 AFM cases with confirmed EV-D68 infection, it was noted that for several other patients with similar paralytic clinical presentations laboratory testing for EV-D68 was not performed or failed due to the delayed onset of neurological symptoms (unpublished data; [40]). This could have resulted from inappropriate sampling for EV-D68 testing or clinical presentations that were not identified as AFM due to lack of clinical awareness. Furthermore, AFM initially starts with a respiratory prodromal phase and samples may not have been collected at an appropriate time before the onset of AFM [41]. Inappropriate sampling and diagnosis can lead to underdiagnosing and underreporting [42, 43] and thus, clear guidelines on how to diagnose AFM are required.

Of the reported clinical data, over 38% EV-D68 cases required hospitalization and 17% of them at ICU level, revealing a substantial utilization of health care resources from EV-D68 infections in our study population, especially in young children. These results are consistent with previous studies [36]. The high proportions of hospitalization can be the result of a sampling bias due to testing strategy. Thus, standardized surveillance that includes the general asymptomatic population is essential to estimate the true burden of disease. The EV-D68 hospitalization rate is concordant with that associated

with influenza virus infections (34%) [31], and ICU admission rates due to Respiratory Syncytial Virus infections resemble those of EV-D68 (both 17%) [44].

By comparing the 2021–2022 sequence data to previous EV-D68 strains, we were able to detect a similar stepwise diversification of EV-D68 as observed with other viruses [45–47]. In this study, the majority of the sequences encompassed partial VP1, as most institutes use the assay developed by Nix and colleagues [48].

Despite the interruption in EV-D68 circulation during the COVID-19 pandemic, EV-D68 evolution continued, resulting in the emergence of 2 novel postpandemic B3-derived lineages. Lineage 1 clearly originated from the prepandemic strains designated as B3-US18 by Midgley et al [22]. In that study, 2 other clusters/lineages were observed and designated as B3-EU18 and B3-EU19 [15, 22]. These were not observed in 2021–2022, and may no longer be circulating, or circulating only at very low levels.

In contrast, another B3-derived lineage (lineage 2) was identified in 2021 and is less clearly linked to recent outbreaks, with a common ancestor in 2016. This highlights the need for more comprehensive surveillance of EV-D68 to better understand where such lineages may have circulated before becoming widespread. To track whether these novel lineages persist in the coming years or are replaced by other novel variants, vigilant monitoring and rapid molecular characterization shared among institutes is required.

This study has a number of limitations, particularly related to differences in surveillance systems without a uniform case definition and sampling strategy, and differences in screening and typing methods across institutes. In addition, not all institutes were able to provide detailed monthly testing data. Clinical records were incomplete in some cases due to different reasons such as General Data Protection Regulation (GDPR) or constraints of the reporting systems used by the institutes. The varied and incomplete reporting may have led to biased data, therefore we propose the standardization of data collection with comprehensive reporting to better determine the disease burden of EV-D68 infections. Finally, EV-D68 samples are generally only collected from symptomatic and hospitalized individuals, which most likely do not fully reflect the demographics or overall geographic distribution. Despite these limitations, the epidemiological and clinical data show the considerable disease burden of this infection, especially in younger children, as well its reemergence and continued evolution during and after the alleviation of nonpharmaceutical interventions in the wake of the SARS-CoV-2 pandemic. With globalization and human connectedness, pathogenic agents are also constantly on the move and continue to evolve. EV-D68 should be considered in the differential diagnosis, especially when attending children with respiratory and/or neurological symptoms. It is our understanding that timely diagnosis could improve medical handling, in particular when neurological signs are present. Concurrently, standardizing sampling, clinical and viral diagnosis, and typing requirements would all provide better data, which can then contribute towards the improved understanding of the clinical and public health impact of EV-D68 and other enteroviruses [7, 12].

CONCLUSION

This study substantiates and extends the previous description of an upsurge in EV-D68 infections in September 2021 [23], which raised awareness of EV-D68–associated respiratory and neurological infections in many countries and led to enhanced vigilance. The data shown in this study underline the clinical and public health impact of EV-D68. The observation of rapid genetic diversification of EV-D68 into novel B3-derived lineages emphasizes the value of continued phylogenetic monitoring of EV-D68 and calls for further genomic analyses to investigate potential strain-associated differences in neuropathogenicity suspected in previous outbreaks [1, 2]. Overall, we highlight the need for the implementation of a mandatory and harmonized pan-European EV surveillance system.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Author contributions. K. B., H. H., and T. K. F. initiated the study. K. B. and M. S. collected and analyzed the data, wrote the first draft, prepared the tables and figures, and edited the final manuscript. E. H., K. B., and P. S. analyzed the sequence data. E. H., H. H., P. S., and T. K. F. helped with drafting the manuscript. All other authors were responsible for diagnostics, testing, and data collection at partner sites, provided and checked data, critically reviewed and edited the manuscript, and have accepted the final version of the manuscript.

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