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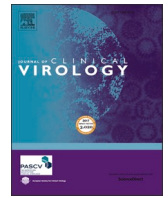
Changes in enterovirus epidemiology after easing of lockdown measures

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ABSTRACT

Introduction: Public health measures aimed at controlling transmission of SARS-CoV-2, otherwise known as "lockdown" measures, had profound effects on circulation of non-SARS viruses, many of which decreased to very low levels. The interrupted transmission of these viruses may have lasting effects. Some of the influenza clades seem to have disappeared during this period, a phenomenon which is described as a "funnel effect". It is currently unknown if the lockdown measures had any effect on the diversity of circulating viruses, other than influenza. Enteroviruses are especially interesting in this context, as the clinical presentation of an infection with a particular enterovirus-type may be clade-dependent.
Methods and materials: Enteroviruses were detected in clinical materials using a 5′UTR-based detection PCR, and partial VP-1 sequences were obtained, using methods described before. All samples with EV detections from a large part of the Netherlands were included in the study. The samples originated from general practitioners, general hospitals, university hospitals and public health offices.
Results: Five EV-genotypes circulated in significant numbers before and after the lockdown, EV-D68, E-11, CV-A6, CV-B5 and CV-A2. All five genotypes showed decreased genetic diversity after the lockdown, and four indicate a significant number of sequences clustering together with a very high sequence homology. Moreover, children with E-11 and CV-B5 detections were significantly older after the lockdown than before.
Conclusions: The reduced enterovirus transmission in the Netherlands during the pandemic, seems to have led to a decrease in genetic diversity in the five most commonly detected enterovirus serotypes

1. Introduction

The coronavirus pandemic and the public health efforts to diminish transmission (colloquially referred to as "Lockdown") had a profound impact on non-SARS-CoV-2 virus circulation [1–3]. Transmission of respiratory viruses, including influenza decreased, resulting in a much lower winter peak of influenza cases in some countries in 2020/2021 [4]. After relaxation of lockdown measures, these viruses reappeared [5, 6]. Nevertheless, the months of reduced virus circulation appears to have had lasting effects. The Influenza B/Yamagata line has so far not been detected since April 2020, and several clades of Influenza A (H3N2) have ostensibly disappeared since the spring of 2020 [7].

It is possible that influenza viruses clustering in these missing clades return, but it is increasingly likely that the collective lockdown measures led to a funnel effect for influenzaviruses, resulting in recurrence of only a few clades and lineages with less genetic variability. The molecular epidemiology of viruses other than influenza and SARS-COV-2 has not yet been investigated.

Potential changes in both molecular epidemiology are of particular interest for enteroviruses (EVs). EVs form a diverse family, of which different genotypes and clades co-circulate. EVs infections are frequently asymptomatic, but they may also cause mild to life threatening disease. Some enterovirus species have been classically associated with specific clinical syndromes such as respiratory infections caused by some coxsackie B viruses, and neurological infections caused by some coxsackie A and echo viruses [8]. Nevertheless, clinical manifestations associated with particular genotypes may change over time for reasons that are thus far insufficiently understood. EV-D68, which was known for causing a bronchiolitis-like respiratory illness in children has become associated with acute flaccid myelitis (AFM) [9]. Cases of AFM have

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occurred following EV-D68 infections with viruses belonging to multiple clades [10], however recently a study in the USA showed that some polymorphisms in the BC and DE loops of VP-1 were associated with higher oxygen requirements in affected children [11]. Also, EV-A71, a cause of hand-foot-mouth disease, is increasingly associated with neurological infections and AFM [12]. Some studies indicate that neurological complications of EV-A71 are more prominent in particular clades [13], suggesting that changes in clinical presentation may occur because of polymorphisms or shifts in circulating clades.

In this study we investigated the circulation of all enterovirus genotypes in the North-Eastern part of the Netherlands before, during and after lockdown measures, between 2018 and April 2022. Samples from general practitioners, regional hospitals and two university hospitals were included, in order to investigate potential changes in circulation of genotypes and clades, but also to investigate potential changes in clinical presentation of these viruses.

2. Methods

2.1. Study design and data sources

This retrospective observational study used laboratory surveillance data from January 2018 until March 2022 for EVs. Four clinical microbiological laboratories in the North and East of the Netherlands participated in the study. Two laboratories are based in university medical centers with pediatric wards and pediatric intensive care units (Radboud university medical center in Nijmegen, and the University Medical Center Groningen (where also the sequencing took place), and two serving non-university hospitals with general pediatrics wards, as well as general practitioners and public health offices (Certe Leeuwarden; Certe Groningen) All EV detections with sufficient viral load (Ct <31) were selected for sequencing. The test platforms for detection of EV are described by Poelman et al. [14], and included laboratory developed PCR tests as well as the FilmArray respiratory syndromic panel (Biomérieux, France) followed by a targeted 5′UTR based enterovirus PCR in case of a positive rhinovirus/enterovirus result

(Fig. 1). The clinical specimens ($n = 1200$, including feces, respiratory samples, blood, CSF and vesicular fluids) of patients from a large region of the Netherlands, were sent to the sequencing laboratory anonymously, only detailing the date of birth of each patient, as well as a description of the sample type (table S4). Only samples with a qPCR Ct<31 were selected for sequencing, and samples from the same patient within 21 days were considered part of the same illness episode. Cases of AFM were indicated. For the subset of genotypes that were detected in increased numbers after the lockdown, laboratories were asked to specify the main clinical symptom of the patient as one of the following categories: neurological infection, myocarditis, neonatal fever, respiratory infection, incidental finding.

Enterovirus detection and partial VP-1-sequencing was performed as previously described [14–17], (phylogenetic analysis details in Supplementary material), viral typing was performed using the Enterovirus typing tool [18].

2.2. Statistical analysis

Results of enterovirus circulation are presented as the mean of case detection per month, data was analyzed using SPSS (v.23) and visualized with GraphPad Prism 8.0.1 (GraphPad Software, USA). Chi-square tests, and two-tailed Mann-Whitney U tests and ANOVA were used to assess the statistical significance. Only p-values < 0.02 were considered significant.

Data availability

The partial VP1 sequences that were determined in this study ($n = 782$) have been deposited in the GenBank under the BioProject number: PRJNA967728.

Ethics statement

All experiments were performed in accordance with the guidelines of the Declaration of Helsinki and all samples were anonymized. A waiver
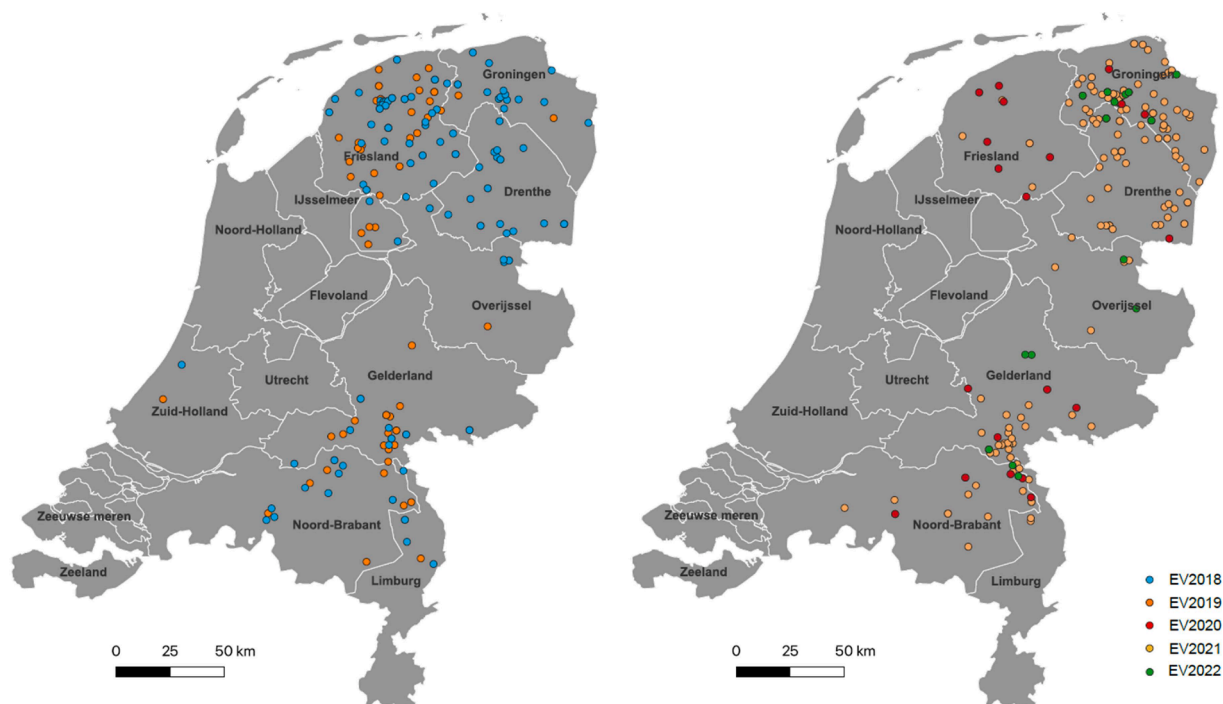

Fig. 1. Map showing geographical origin of individuals with enterovirus infections included in this study before the lockdown in 2018 (blue dots), 2019 (orange dots) (left image), and in 2020 (red dots), 2021 (yellow dots), and 2022 (green dots) (right image).

was obtained by the UMCG Ethics Committee: METc-2018/393 and METc-2021/143.

3. Results

3.1. Enterovirus detections

Enterovirus circulation diminished significantly during lockdown measures in the Netherlands. Detections decreased from 10.7 to 2.7 per month until December 31st' with no detections in April, June and July of 2020, and remained low until July 2021. EV-circulation usually reaches a peak in early fall in Europe, with co-circulation of multiple serotypes, however this peak was not observed in 2020 (Fig. 2a).

Between March 2020 and March 2022 the Netherlands went through different degrees of lockdown: from limited lockdown (23 March 202-31 May 2020) during which people were asked to diminish the out-of-home activities as much as possible [19], to restricting contacts to a very limited number, discontinuing in-person lessons in schools (15 December 2020) and a curfew (23 January – 27 April 2021). The periods of strict lockdown measures were interrupted by more lenient measures limited to only closure of cafés and restaurants. After schools reopened in spring of 2021, a peak in EV detections was observed (Fig. 2a).

3.2. Enterovirus detections per genotype

A total of 849 partial VP-1 sequences were obtained from clinical samples from 784 patients during the study period, identifying 40 circulating EV genotypes (Table 1). Fecal samples were most frequently selected for EV genotyping (62 % of samples), followed by respiratory samples (22.9 % of samples). A considerable percentage of EV detections

was non-typeable (351/1200, 29.2 %) (table S3), most commonly fecal samples (227/744, 30.5 %) and respiratory samples (83/275, 30.2 %) (table S4). The percentage of non-typeable detections did not change significantly during the study period. Nearly all detections took place during two observation periods: the first (pre-lockdown; $n = 425$ typed EV) from 2018 to March 2020, and the second (post-lockdown; $n = 326$ typed EV) from April 2021 to April 2022 (Fig. 2a and b).

Five EV-genotypes which circulated significantly before the pandemic, were not detected during the second observation period: CV-A16, CV-A9, E-13, E-25, and E-30.

Two EV-genotypes were more prominent after the lockdown: CV-B1 was not observed in the years before the pandemic, only appearing after the lockdown (13 detections). E-21 was detected infrequently before and during the lockdown (four detections each in 2019 and 2020). However, after relaxation of the measures, the virus was detected significantly more, i.e. 37 detections in 2021 and one in 2022.

Five genotypes were detected in significant numbers (>10 times) during both observation periods: EV-D68 ($n = 103$), E-11 ($n = 91$), CV-A6 ($n = 72$), CV-B5 ($n = 46$), and CV-A2 ($n = 41$). Detections of each of these viruses occurred over a period of several months, with peaks during the summer and fall prior to the pandemic and for a longer time after the lockdown measures were eased (Fig. 2b).

3.3. Clinical and phylogenetic features of the two EV-genotypes (CV-B1 and E-21) which surged after the lockdown

CV-B1 and E-21 were more frequently detected after the lockdown than before, and both viruses caused significant morbidity. Especially CV-B1, which was detected 13 times after the lockdown, was associated with severe illness. The virus was found to cause neonatal fever four
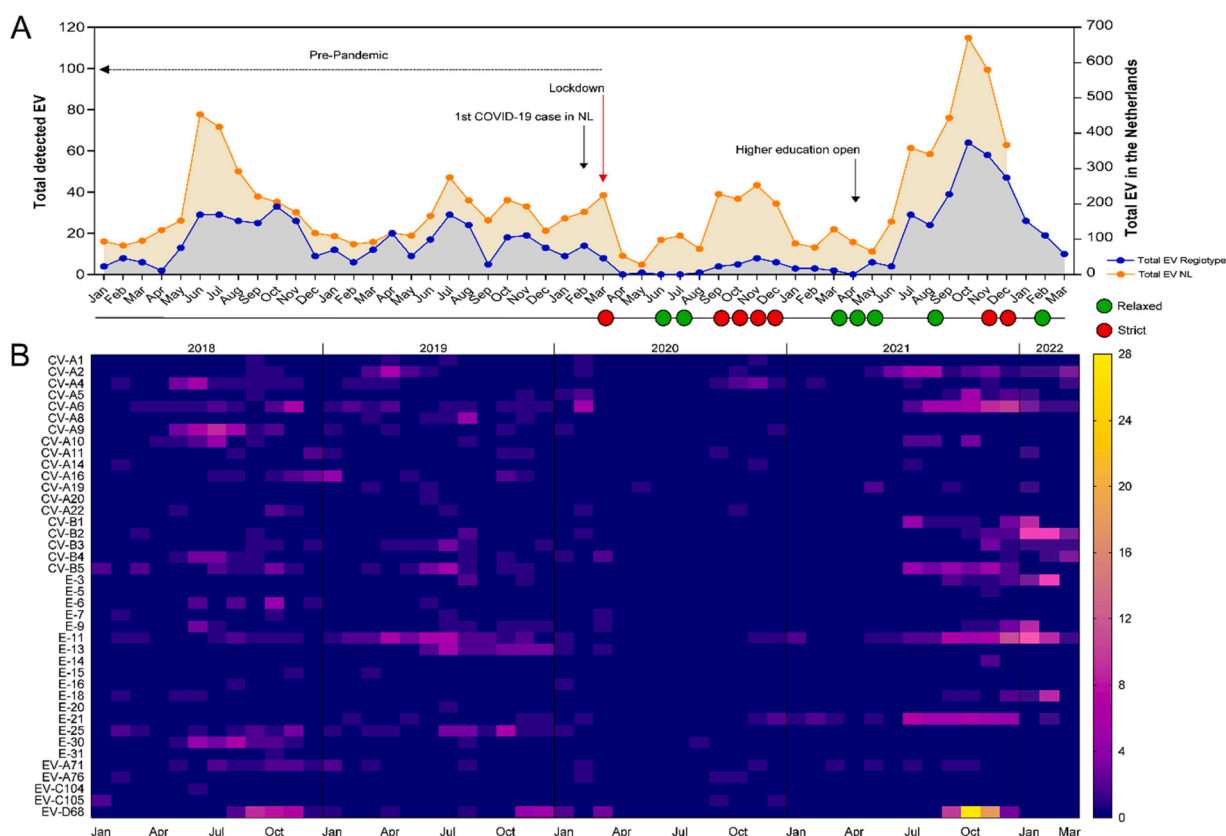

Fig. 2. Graph (2a) and heatmap (2b) showing when enterovirus infections were detected between 2018 and April 2021.
2a: Periods of strickt lockdown and relaxation are indicated in the graph. The graph also shows detecions in the region included in the study (blue line) as well as in the Netherlands (yellow line).
2b: Colors indicate the frequency of detection of each EV-gentype.

Table 1
Number of detected enterovirus genotypes per year, in the Northern part of the Netherlands between January 2018- March 2022.

	2018	2019	2020	2021	2022	Total
CV-A1	1	2	2			5
CV-A2	2	11	2	22	4	41
CV-A4	14	3	7	3	1	28
CV-A5	1	1	3	9	1	15
CV-A6	15	11	5	37	4	72
CV-A8		8				8
CV-A9	26	3	2			31
CV-A10	9	1		7		17
CV-A11	3	3	1		1	8
CV-A14	1	8	1	1		11
CV-A16	8	2				10
CV-A19			1	3	1	5
CV-A20		1				1
CV-A22	4	1	2			7
CV-B1				10	3	13
CV-B2	2	2	1	5	10	20
CV-B3	3	8		4	3	18
CV-B4	9	2	3	1	3	18
CV-B5	12	13		21		46
E-3		3		6	6	15
E-5				1		1
E-6	9	1				10
E-7	2	1	1			4
E-9	4	5	1	5	3	18
E-11	8	33	3	37	10	91
E-13		20	2			22
E-14				2		2
E-15	1	1				2
E-16	1		1			2
E-18	3			4	4	11
E-20		1				1
EV-A76	1		3			4
E-21		4	4	37	1	46
E-25	11	18	1			30
E-30	19	1	1			21
E-31	1					1
EV-A71	10	4	2	5		21
EV-C104	1					1
EV-C105	2		2			4
EV-D68	27	12	5	59		103
Total	210	184	56	279	55	784

times (31 %), neonatal myocarditis three times (23 %), and respiratory insufficiency in a child of less than one year of age once. CV-B1 was considered an incidental finding in feces five times (38 %), including in one adult and one child over the age of one year. CV-B1 affected small infants primarily, as 12 of 13 samples were from children (92 %) with a median age of 25 days (IQR 9–232 days) (Table 2). The CV-B1 sequences obtained during this study period showed similarity, and all belonged to the GIII subclade, which was previously circulating in Asia and Africa (Fig. 3a).

Enterovirus genotype E-21, with 46 detections during the study period, was one of the few viruses continuing to cause infections during the lockdown period. Out of 46 detections, 13 were from febrile

Table 2
Clinical characteristics of patients with CV-B1 and E-21.

	CV-B1 (n = 13)	E-21 (n = 46)
Median age in children	25 (9-232) days	4.6 (1.5–24.7) months
Under 1	10 (77 %)	28 (61 %)
Adults (16 and older)	1 (7.7 %)	2 (4.3 %)
Male	8 (62 %)	26 (56 %)
Clinical presentation		
Neonatal fever	4 (31 %)	13 (28 %)
Myocarditis	3 (23 %)	
Neurological infection		5 (11 %)
Respiratory insufficiency	1 (7.7 %)	1 (2 %)
Incidental	5 (38 %)	25 (54 %)
Unknown		1 (2 %)

neonates (28 %), five were from neurological infections (11 %), one was a respiratory infection (2 %), and 25 were incidental findings in feces (54 %). Of the children in whom E-21 was detected, sixteen were over the age of one year (35 %), and 28 were infants (61 %). The children had a median age of 4.6 months (IQR 1.5–24.7 months) (Table 2). The E-21 sequences of all four years clustered together in clade B, most closely related to sequences from Russia in 2019 (Fig. 3b).

3.4. Clinical characteristics and phylogenetic features of EV-D68, E-11, CV-A6, CV-B5 and CV-A2

Enterovirus genotype -D68 was the most frequently detected EV genotype both before (n = 41) and after lockdown measures (n = 61). Interestingly, prior to the pandemic, 20 out of 41 samples were taken from adults (49 %). After the lockdown, only 8 out of 61 positive detections came from adults (13 %). The median age of children with EV-D68 was comparable before and after the lockdown (median 3.08 years (IQR 1.12–5.39 years versus 2.65 years (IQR 0.75–4.70 years) $p = 0.40$)). There were no cases of AFM in either of the two study periods. In addition, CSF was the source of one EV-D68 detection in the pre-lockdown period (Table 3).

E-11 (n = 91) was the second most prevalent genotype detected in our study, followed by CV-A6 (n = 72), and CV-B5 (n = 46). The median age increased for children with E-11 and CV-B5 infections. In E-11 cases the median age before the lockdown was 1.4 months (IQR 0.6–14 months), compared to 10 months (IQR 1.7–23 months) ($p < 0.02$) afterward. For CV-B5 the median age rose from 26 days (0.84 months, IQR 0.21–25 months) to 17 months (IQR 7.2–38 months) after the lockdown ($p < 0.02$) (Table 3). Likewise, for both viruses the percentage of infants under the age of one year with these infections decreased, but this was only statistically significant for CV-B5 [64 % to 24 % ($p < 0.02$)]. Children with CV-A6 and CV-A2 detections were of similar age before and after the lockdown. CSF and plasma samples (Table 3) accounted for 24 % of the E-11 detected before the lockdown, suggesting severe clinical illness (three and seven samples respectively). After the lockdown the virus was only once detected in CSF, and in plasma twice, representing a decrease in detections from these sources ($p < 0.02$). The percentage of detections in fecal samples did not change.

For enterovirus genotypes CV-B5, CV-A6 and CV-A2, there were no significant changes in the samples in which the virus was detected. Fecal samples were the most frequent source from which these three EV-genotypes were detected during the two observation periods. Enterovirus CV-A6 was a prominent cause of hand-foot-mouth disease, as it was detected from vesicular fluid in 37 % and 36 % of cases pre-and post-lockdown, respectively (Table 3).

3.5. Phylogenetic analysis

Enterovirus -D68 sequences from the pre-lockdown period, show a clustering in the B3 (n = 25) and the A2 clades (n = 16 (Fig. 4a). After the lockdown, nearly all detections cluster together in the B3 subclade, except two detections which cluster elsewhere in B3, and one which clusters in A2 subclade, different from the A2 subclade sequences detected before the lockdown. The sequences seen in the large cluster in the B3 subclade display great similarity of the fragments, with many (near) identical sequences (Fig. 4a). These B3-clade sequences were compared with sequences recently reported in the USA, where an association was found between high oxygen requirement and presence of four polymorphisms in the BC and the DE loop of theVP-1 sequence (95T and 98A in the BC loop, and 143 N and 146A in the DE loop) [11]. None of the sequences in our study contained all four polymorphism, however, 37 (36 %) had 95T and 143A, and nine (9 %) had 98A and 143 N. The detections with these polymorphisms occurred both before and after the lockdown (figure S2).

The enterovirus E-11 sequences obtained before and after the lockdown belonged to the D5 genotype. Before the lockdown the sequences
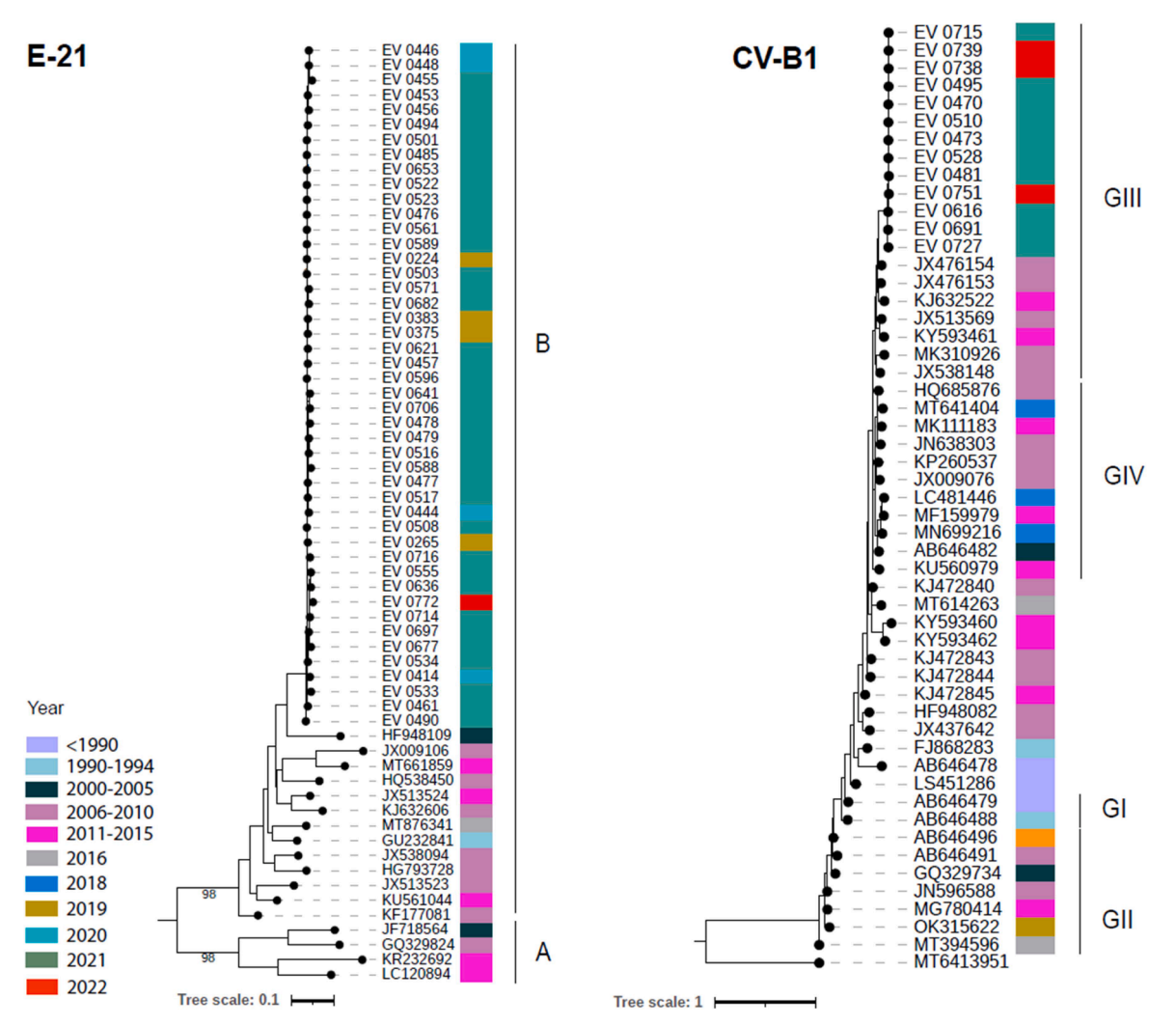
Fig. 3. Maximum likelihood phylogenetic tree of CV-B1 and E21, showing the phylogenetic relationship between the detections found in this present study to detections reported before.

Table 3
Clinical characteristics before and after the lockdown, of cases of EV-D68, E-11, CV-A6, CV-B5 and CV-A2.

|	EV-D68 Before (n = 41)	EV-D68 After (n = 61)	E-11 before (n = 42)	E-11 after (n = 49)	CV-A6 before (n = 27)	CV-A6 after (n = 45)	CV-B5 before (n = 25)	CV-B5 after (n = 21)	CV-A2 before (n = 14)	CV-A2 after (n = 27)
Median age (IQR) in children	3.1 (1.12–5.39) years	2.65 (0.75–4.7) years	1.4 (0.6–14) months	10 (1.7–23) months	1.73 (0.91–2.74) years	1.20 (0.38–2.26) years	0.84 (0.21–25) months	17 (7.2–38) months	1.20 (0.33–2.76) years	1.47 (0.55–1.95) years
Under 1 n (%)	5 (12 %)	16 (26 %)	29 (69 %)	25 (51 %)	4 (15 %)	12 (27 %)	16 (64 %)	5 (24 %)	5 (35 %)	8 (30 %)
Adults (16 and older) n (%)	20 (49 %)	8 (13 %)	1 (2.3 %)	1 (2.0 %)	7 (26 %)	9 (20 %)	3 (12 %)	6 (29 %)		
Male n (%)	24(59 %)	37 (60 %)	24 (57 %)	27 (49 %)	21 (78 %)	22 (49 %)	12 (48 %)	9 (43 %)	9 (64 %)	18 (67 %)
Sample type										
CSF n (%)	1 (2.4 %)		3 (7.1 %)	1 (2.0 %)			4 (16 %)	2 (9.5 %)		
Plasma n (%)			7 (17 %)	2 (4.1 %)	3 (11 %)	1 (2.2 %)	4 (16 %)	1 (4.8 %)		
Vesicles n (%)				1 (2.0 %)	10 (37 %)	16 (36 %)				
Respiratory n (%)	35 (85 %)	57 (93 %)	1 (2.3 %)	6 (12 %)	4 (15 %)	8 (18 %)		4 (19 %)		3 (11 %)
Feces n (%)	5 (12 %)	4 (6.5 %)	31 (74 %)	36 (73 %)	10 (37 %)	20 (44 %)	17 (68 %)	14 (67 %)	14 (100 %)	24 (89 %)
Unknown n (%)				3 (6.1 %)						
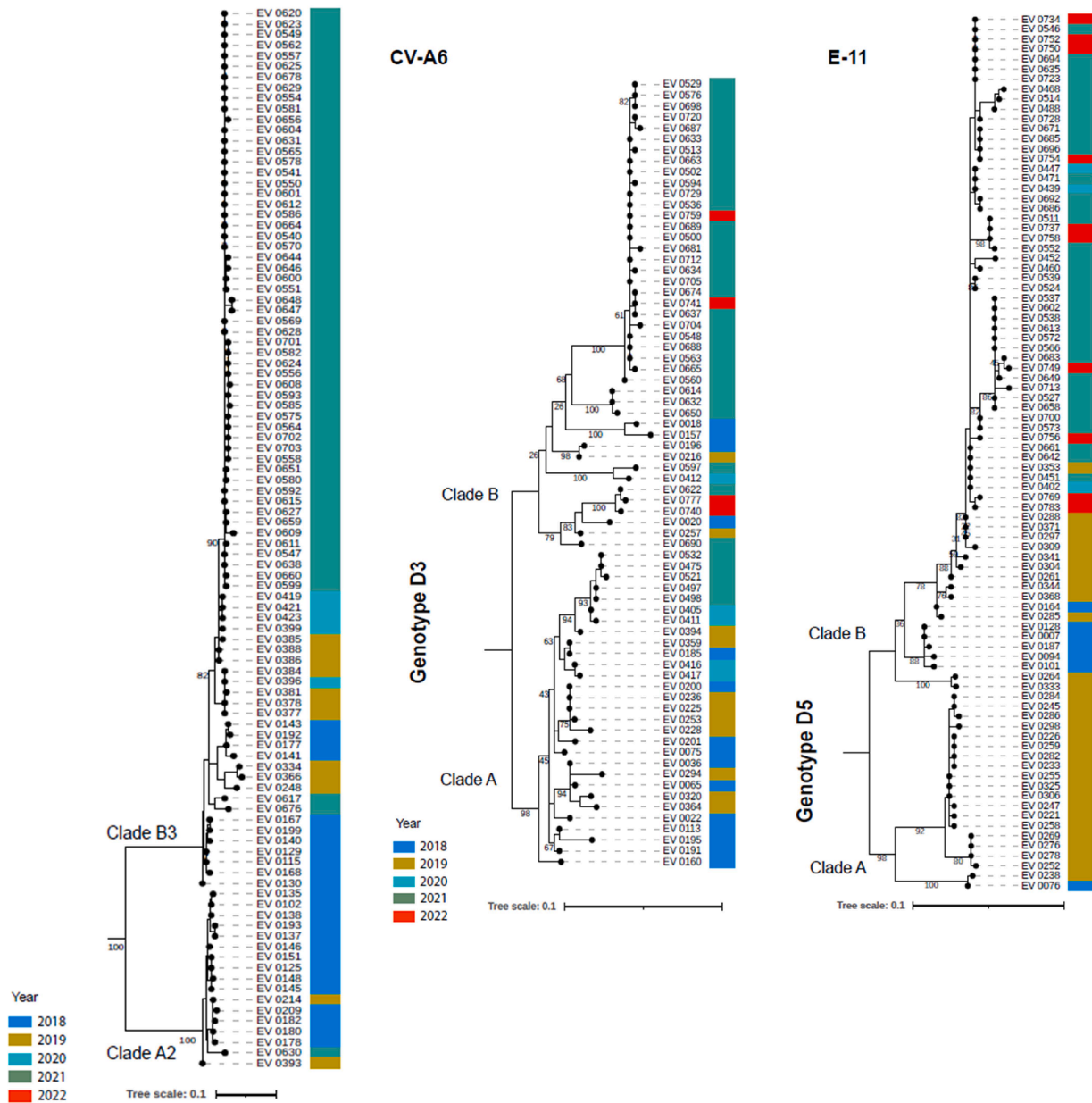
Fig. 4. Maximum likelihood phylogenetic tree of EV-D68 (a), E-11 (b), CV-A6 (c), CV-B5 (d), and CV-A2 (e) detected two years prior to the SARS-CoV-2 pandemic, and immediately after the lockdown measures were relaxed. All genotypes show reduced variability post-lockdown. EV-D68, CV-A6, CV-B5 and CV-A2 show a number of detections clustering together with very high sequence homology.
figure 4a
Fig. 4b and c
Fig. 4d and e.

clustered in two different clades (designated A and B shown in Fig. 4b). After the lockdown all sequences belonged to the D5B clade, showing some diversity and clustering with different detections from the first observation period (Fig. 4b).

The CV-A6 sequences from before and after the lockdown are grouped in the D3 genotype. Some post-lockdown CV-A6 sequences form a cluster of similar sequences. Both the A and B clades are represented in the pre and post lockdown periods (Fig. 4c).

Phylogenetic analysis of CV-B5 shows that in the years before the lockdown, detections belonged to the B2E and B2F subclades of the B-genotype, whereas after the lockdown nearly all sequences are closely related, and all cluster within the B2F subclade (Fig. 4d).

Prior to the lockdown, the CV-A2 sequences belonged to genotypes C

and D, more specifically subgenotypes D2 and C1, whereas after the lockdown there were no sequences within the D genotype. The post-lockdown sequences show similarity, all belonging to the C3 sub-genotype (Fig. 4e).

4. Discussion

Viral respiratory infections were strongly reduced during the most stringent public health measures aimed at controlling transmission of SARS-CoV-2 in 2020. The efforts to diminish person to person contact and saw reduction of distance traveled (68 %) and frequency of trips (55 %) and contact/visits to other people (90 %) compared to 2019 and decrease of public transport use (90 %) [19] during lockdown in 2020.
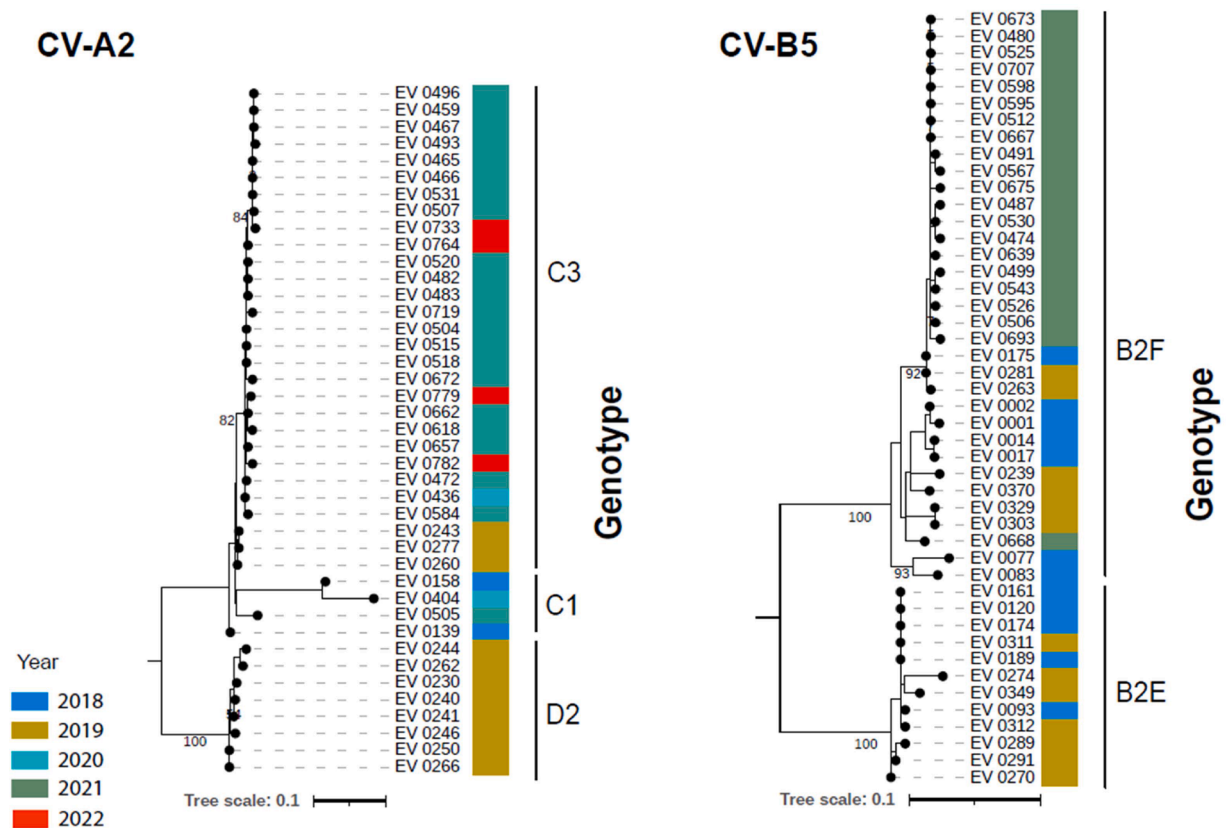
Fig. 4. (*continued*).

Enteroviruses reappeared rapidly after these measures were eased, causing a wave of infections.

We determined the EV genotypes in our region between January 2018 until April 2022, to investigate if epidemiological differences could be observed, potentially caused by a funnel effect, resulting from the temporarily interrupted transmission. Several differences were noticed when comparing the circulating genotypes pre- and post- lockdown.

Some of the EV-serotypes that circulated in our area before the lockdown, did not return in the period following the lockdown (CV-A16, CV-A9, E-13, E-25 and E-30). This absence of these highly prevalent EVs may be coincidental. It is known that EV serotypes do not circulate every year, as some genotypes may not be detected for years and suddenly make a reappearance for unknown reasons. In our study, this was exemplified by CV-B1, which emerged in the months after the lockdown. CV-B1 is characterized by increases in circulation, usually lasting for a few years, and occurring with irregular intervals of sometimes more than a decade [20]. In our region, CV-B1 was only detected twice between 2008 and 2020, both in samples from severely ill neonates [17]. In contrast, this genotype was detected thirteen times in 2021 and 2022. These cases appear similar to previous reports, both clinically and phylogenetically. Clinically, CV-B1 is known to cause severe neonatal infections, and that is what we found in seven of thirteen detections. Phylogenetically, the detections of our study cluster with sequences from recent years. [21–22].

Enterovirus E-21 was seen sporadically before the pandemic and as an upsurge between June 2021 and the beginning of 2022. This virus was an incidental finding in fecal samples in more than half of the cases. However, a significant number of samples came from febrile neonates and from patients with neurological findings, showing the pathogenic potential of E-21. The pathology caused by this virus is known to be most serious in very young infants, which is also what we found. Also, the phylogenetic features of the sequences in our study appear similar those reported before [23].

The five enterovirus-genotypes (EV-D68, E-11, CV-B5, CV-A6 and CV-A2) which circulated significantly during both the pre- and the post-lockdown periods, showed reduced genetic variability after the lockdown. Moreover, in the case of EV-D68, CV-B5, CV-A6 and CV-A2, but not E-11, a substantial number of the post-lockdown detections showed a high sequence homology, resembling outbreaks. Nevertheless, the infections were not outbreak-associated and occurred over a period of weeks to months in different geographical locations and health care settings.

The reduction in genetic variability is most notable for EV-D68, the most prevalent EV-genotype in the past years in Northern Netherlands. This EV causes severe respiratory infections, as well as AFM in a small percentage of infected individuals [9,10,24]. An increased number of cases of EV-D68 has recently been reported in the USA, describing infections occurring in 2021 and 2022. The sequences belonged to the B3 subclade, and a significant number of detections displayed amino acid changes in the BC loop of the VP-1 part of the genome, which correlated clinically with higher O_2-requirement in patients [11]. We did not observe EV-D68 sequences simultaneously containing all four of the described polymorphisms in our study, although we did see the polymorphisms occurring in detections before and after the lockdown.

The most notable changes observed for E-11 and CV-B5 in our study concerned the shift in reported age of the affected children. These two EV-genotypes mainly caused infections in infants both prior to and after the lockdown, however an increase in age was observed for both E-11 and CV-B5 in the second observation period. Based on the sample types which were the source of the detections, we have no reason to believe that these viruses caused more severe illness after the lockdown. In fact, for E-11 the number of detections in CSF and plasma decreased.

Our study is the first to investigate epidemiological and clinical differences in enterovirus infections, comparing the period right before the pandemic, with the period immediately after lockdown measures were relaxed. Our study has some limitations however, which warrant

some caution. Firstly, the phylogenetic analysis was based on a partial VP1 sequence which is frequently used for this purpose. Even though we show high sequence homology between many of the detections of each genotype (with the exception of E-11), the fragment we used is relatively short and does not imply that the entire genome displays the same degree of similarity. Moreover, the sequencing method we used results in a high percentage of non-typeable EV detections. This percentage was relatively constant during the study period and was largely due to untypeable EV detections in fecal samples, as this was the material in which EV were detected most. As a result of this, the possibility that untypeable genotypes circulated has to be taken into consideration. However, since the percentage of untypeable EV-detections before and after the pandemic is similar, there is no indication that the introduction of one or more new clades or genotypes occurred during the study period. In addition, the number of detections of each virus is relatively low, so additional studies with larger numbers would be valuable to confirm if changes have happened as a result of the lockdown measures or by chance or natural occurrence. More importantly, whether or not a patient was tested for EVs, depended on the treating physician. Although the health-care providers in our region did not report changing testing protocols during the study period, we cannot exclude changing health-care-seeking behavior of patients or altered testing practices of health-care providers.

The results of this study cannot be generalized. At this time, there is no data indicating that a decrease in sequence diversity of circulating EVs has occurred elsewhere in the world. Based on our study alone we cannot conclude if a funnel effect was present, or if the contraction in genetic diversity we describe is coincidental. Moreover, this study alone does not imply that some EV clades and genotypes have disappeared indefinitely. Even so, the possibility that the pandemic lockdown measures may have had lasting effects on virus or clade circulation of EVs, has to be considered. With recent research showing that some clades or variants may be linked with severity of disease, we recommend continued surveillance of enteroviruses, especially as these viruses reemerge after relaxation of the lockdown measures world-wide.

Declaration 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.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcv.2023.105617.

References

[1] M. Redlberger-Fritz, M. Kundi, S.W. Aberle, Puchhammer-Stöckl E. Significant impact of nationwide SARS-CoV-2 lockdown measures on the circulation of other respiratory virus infections in Austria, J Clin Virol (2021), https://doi.org/10.1016/j.jcv.2021.104795.

[2] J.W. Tang, S. Bialasiewicz, D.E. Dwyer, M. Dilcher, R. Tellier, J. Taylor, H. Hua, L. Jennings, J. Kok, A. Levy, D. Smith, I.G. Barr, S.G. Sullivan, Where have all the viruses gone? Disappearance of seasonal respiratory viruses during the COVID-19 pandemic, J Med Virol 93 (7) (2021) 4099–4101, https://doi.org/10.1002/jmv.26964. Jul.

[3] P.K. Knudsen, A. Lind, I. Klundby, S. Dudman, The incidence of infectious diseases and viruses other than SARS-CoV-2 amongst hospitalised children in Oslo, Norway during the Covid-19 pandemic 2020-2021, J Clin Virol Plus 2 (1) (2022), 100060, https://doi.org/10.1016/j.jcvp.2021.100060. Feb.

[4] K.L. Laurie, S. Rockman, Which influenza viruses will emerge following the SARS-CoV-2 pandemic? Influenza Other Respir Viruses 15 (5) (2021) 573–576, https://doi.org/10.1111/irv.12866. Sep.

[5] F. Yasmin, S.H. Ali, I. Ullah, Norovirus outbreak amid COVID-19 in the United Kingdom; priorities for achieving control, J Med Virol 94 (3) (2022) 1232–1235, https://doi.org/10.1002/jmv.27426. Mar.

[6] S. Fratty Ilana, Reznik-Balter Shira, Nemet Ital, Atari Nofar, Kliker Limor, Sherbany Hilda, Keller Nathan, Stein Michal, Mendelson Ella, Mandelboim Michal, Outbreak of influenza and other respiratory viruses in hospitalized patients alongside the SARS-CoV-2 pandemic, Front Microbiol 13 (2022), https://doi.org/10.3389/fmicb.2022.902476.

[7] V. Dhanasekaran, S. Sullivan, K.M. Edwards, R. Xie, A. Khvorov, S.A. Valkenburg, B.J. Cowling, I.G. Barr, Human seasonal influenza under COVID-19 and the potential consequences of influenza lineage elimination, Nat Commun 13 (1) (2022 Mar 31) 1721, https://doi.org/10.1038/s41467-022-29402-5.

[8] D. Casas-Alba, M.F. de Sevilla, A. Valero-Rello, C. Fortuny, J.-J. García-García, C. Ortez, J. Muchart, T. Armangué, I. Jordan, C. Luaces, I. Barrabeig, R. González-Sanz, M. Cabrerizo, C. Muñoz-Almagro, C. Launes, Outbreak of brainstem encephalitis associated with enterovirus-A71 in Catalonia, Spain (2016): a clinical observational study in a children's reference centre in Catalonia, Clin Microbiol Infect 23 (11) (2017) 874–881, https://doi.org/10.1016/j.cmi.2017.03.016.

[9] K. Messacar, E.J. Asturias, A.M. Hixon, C. Van Leer-Buter, H.G.M. Niesters, K.L. Tyler, M.J. Abzug, S.R. Dominguez, Enterovirus D68 and acute flaccid myelitis-evaluating the evidence for causality, Lancet Infect Dis 18 (8) (2018) e239–e247, https://doi.org/10.1016/S1473-3099(18)30094-X. Aug.

[10] M. Knoester, J. Helfferich, R. Poelman, C. Van Leer-Buter, O.F. Brouwer, H.G.M. Niesters, 2016 EV-D68 AFM Working Group. Twenty-nine cases of enterovirus-D68-associated acute flaccid myelitis in Europe 2016: a case series and epidemiologic overview, in: Pediatr Infect Dis J., 38, 2019 Jan, pp. 16–21, https://doi.org/10.1097/INF.0000000000002188.

[11] Amary Fall, Lijie Han, Omar Abdullah, Julie M. Norton, Raghda E. Eldesouki, Michael Forman, C. Paul Morris, Eili Klein, Heba H. Mostafa, An increase in enterovirus D68 circulation and viral evolution during a period of increased influenza like illness, The Johns Hopkins Health System, USA, 2022, J Clin Virol 160 (2023). doi:10.1016/j.jcv.2023.105379.

[12] Launes, Outbreak of brainstem encephalitis associated with enterovirus-A71 in Catalonia, Spain (2016): a clinical observational study in a children's reference centre in Catalonia, Clin Microbiol Infect, Volume 23, Issue 11, 2017, Pages 874-881, ISSN 1198-743X, https://doi.org/10.1016/j.cmi.2017.03.016.

[13] S.S. Wong, C.C. Yip, S.K. Lau, K.Y. Yuen, Human enterovirus 71 and hand, foot and mouth disease, Epidemiol Infect 138 (8) (2010) 1071–1089, https://doi.org/10.1017/S0950268809991555. Aug.

[14] W.A. Nix, M.S. Oberste, M.A. Pallansch, Sensitive, seminested PCR amplification of VP1 sequences for direct identification of all enterovirus serotypes from original clinical specimens, J Clin Microbiol 44 (2006) 2698–2704, https://doi.org/10.1128/JCM.00542-06.

[15] Randy Poelman, Elisabeth H. Schölvinck, Renze Borger, Hubert G.M. Niesters, Coretta van Leer-Buter, The emergence of enterovirus D68 in a Dutch University medical center and the necessity for routinely screening for respiratory viruses, J Clin Virol 62 (2015). doi:10.1016/j.jcv.2014.11.011.

[16] M.S. Oberste, K. Maher, D.R. Kilpatrick, M.R. Flemister, B.A. Brown, M.A. Pallansch, Typing of human enteroviruses by partial sequencing of VP1, J Clin Microbiol 37 (5) (1999) 1288–1293. May10.1128/JCM.37.5.1288-1293.1999.

[17] C.C. Van Leer-Buter, R. Poelman, R. Borger, H.G. Niesters, Newly identified enterovirus C genotypes, identified in the Netherlands through routine sequencing of all enteroviruses detected in clinical materials from 2008 to 2015, J Clin Microbiol 54 (9) (2016) 2306–2314, https://doi.org/10.1128/JCM.00207-16. Sep.

[18] Kroneman A., Vennema H., Deforche K., v d Avoort H., Peñaranda S., Oberste M.S., Vinjé J., Koopmans M. An automated genotyping tool for enteroviruses and noroviruses. J Clin Virol. 2011 Jun;51(2):121–5. doi: 10.1016/j.jcv.2011.03.006.

[19] M. de Haas, R. Faber, M. Hamersma, How COVID-19 and the Dutch 'intelligent lockdown' change activities, work and travel behaviour: evidence from longitudinal data in the Netherlands, Transp Res Interdiscip Perspect (2020), https://doi.org/10.1016/j.trip.2020.100150, 6, 100150.

[20] Khetsuriani N., Lamonte-Fowlkes A., Oberst S., Pallansch M.A.; Centers for disease control and prevention. Enterovirus Surveillance–United States, 1970–2005. MMWR Surveill Summ. 2006 Sep 15;55(8):1–20. PMID: 16971890.

[21] M. Isacsohn, A.I. Eidelman, M. Kaplan, A. Goren, B. Rudensky, R. Handsher, Y. Barak, Neonatal coxsackievirus group B infections: experience of a single department of neonatology, Isr J Med Sci 30 (5–6) (1994) 371–374. May-JunPMID:7518424.

[22] N.A. Verma, X.T. Zheng, M.U. Harris, S.B. Cadichon, H. Melin-Aldana, N. Khetsuriani, M.S. Oberste, S.T. Shulman, Outbreak of life-threatening coxsackievirus B1 myocarditis in neonates, Clin Infect Dis 49 (5) (2009 Sep 1) 759–763, https://doi.org/10.1086/605089.

[23] N. Khetsuriani, A. Lamonte, M.S. Oberste, M. Pallansch, Neonatal enterovirus infections reported to the national enterovirus surveillance system in the United States, 1983-2003, Pediatr Infect Dis J 25 (10) (2006) 889–893, https://doi.org/10.1097/01.inf.0000237798.07462.32. Oct.

[24] Hixon A.M., Frost J., Rudy M.J., Messacar K., Clarke P., Tyler K.L. Understanding enterovirus D68-induced neurologic disease: a basic science review. Viruses. 2019 Sep 4;11(9):821. doi: 10.3390/v11090821.