Enterovirus D68 (EV-D68) was detected in 93 patients from five European countries between 1 January 2019 and 15 January 2020, a season with expected low circulation. Patients were primarily children (n = 67, median age: 4 years), 59 patients required hospitalisation and five had severe neurologic manifestations. Phylogenetic analysis revealed two clusters in the B3 subclade and subclade A2/D. This circulation of EV-D68 associated with neurological manifestations stresses the importance of surveillance and diagnostics beyond expected peak years.

Enterovirus D68 (EV-D68) is primarily a respiratory virus. Previously published data have suggested circulation with a biennial epidemic cycle in Europe and North America [1-3], but surveillance is not consistent. The Danish enterovirus surveillance detected two cases of EV-D68 infection in August 2019 and a further case in early September. Colleagues in other European countries were contacted, and France, Germany, the Netherlands and Sweden responded that they had also seen cases. Here we report on the start of seasonal circulation of EV-D68 in five European countries, a circulation which is still ongoing with further cases detected since the initial submission of this report.

Epidemiological trend and description of cases
A total of 93 EV-D68 infections were reported between 1 January 2019 and 15 January 2020 (Denmark n = 21, France n = 6, Germany n = 36, the Netherlands n = 19, Sweden n = 11). Epidemiological and clinical information was collected for cases where possible (Table 1). As surveillance samples and data are collected retrospectively, data for December 2019 and January 2020 are not complete.

Cases were identified through enterovirus surveillance (n = 31), rhinovirus surveillance (n = 3), influenza and respiratory infection community surveillance (n = 12) or hospital-based diagnostics of respiratory infections (n = 47). Following diagnostic testing of respiratory samples for enterovirus and/or rhinovirus using commercial or in-house assays, EV-D68 cases were identified by either EV-D68-specific real-time PCR or partial sequencing of the VP1 and/or VP4/VP2 region of the genome [9-11]. Most of the cases were identified during the usual enterovirus season starting in late summer (August n = 5, September n = 14, October n = 17, November n = 36 and December n = 15; Figure 2).
Of the Dutch cases, two became ill in Turkey and were diagnosed with EV-D68 after being hospitalised for respiratory support upon their return to the Netherlands. One Swedish patient had a recent travel history to East Asia and one French patient had travelled to Portugal. Most of the cases were children (n = 67), with a median age of 4 years (range: 16 days–89 years). Forty-six patients were female.

Clinical manifestations of cases

Five patients, from Denmark, France and Germany, presented with severe neurological symptoms. Of these, one was a 1 year-old, previously healthy boy who presented with acute flaccid myelitis (AFM) following a febrile respiratory infection. The paralysis was asymmetric, included all four limbs and the torso, with severe paraesthesia in affected limbs. The second patient was a 15-month-old boy who presented with AFM following a digestive prodromal illness. The third patient was a 29-year-old woman who presented with cranial nerve palsy. She underwent a caesarean section in week 40 of pregnancy because of the acute neurological symptoms. The fourth patient was a 6-year-old girl who presented with paralysis of both legs and the bladder. The fifth patient was a 16-year-old girl who presented with loss of balance and coordination, double vision and loss of sensation. In addition to the severe neurological cases, one further patient, a 27-year-old man, presented with a discrete unilateral paresis of the right leg. Two patients suffered seizures, most likely related to underlying conditions.

The clinical manifestations for the remaining patients ranged from mild cold-like symptoms of the upper respiratory tract to severe pneumonia requiring continuous positive airway pressure and respirator support (Table 1). Fifty-nine patients (among the 73 for whom this information was available) required hospitalisation, either because of severity of symptoms or underlying medical conditions. Twenty-one patients had underlying medical conditions, including asthma, cancer, non-HIV-related immunodeficiency, epilepsy and trisomy 14 mosaicism with growth retardation, cognitive impairment and multiple malformations. For one Dutch case, EV-D68 was identified in bronchoalveolar lavage and this patient also had a pneumococci-positive antigen test in urine. The patient died after 11 days of hospitalisation from bilateral pneumonia.

Phylogenetic analysis of enterovirus D68 strains

Full- or nearly full-length genome sequencing was successfully carried out for seven strains (Denmark and the Netherlands: in-house protocols, Sweden [12]). Sequences which were available up to and including 6 December 2019 are available on GenBank (Table 2).

Phylogenetic analysis of VP1 sequence data was carried out using the Nextstrain augur pipeline [13]. We included samples from this study with ≥ 300 bp in VP1 (Table 2), alongside all available VP1 sequences in GenBank of ≥ 700 bp, randomly down-sampled to 20 samples per country per month to avoid sampling bias and overrepresentation of some countries (particularly during the 2014 and 2016 epidemics; no 2019 samples were down-sampled). The code is available at https://github.com/enterovirus-phylo/evd68-2019; the analysis can be viewed at https://nextstrain.org/community/enterovirus-phylo/evd68-2019/vp1-300. This analysis will be updated with new sequence data as this becomes available. EV-D68 has been characterised into the major clades A, B, and C, with A and B divided into the subclades A1–A2, and B1–B3. Some studies designate A2 as D and subdivide it into D1 and D2 (Figure 3).

The dominant subclades have varied between years of upsurge: the 2014 outbreak was dominated by B1 strains, whereas the 2016 and 2018 outbreaks consisted only of the B3 and A2/D subclades. Some clades and subclades may no longer be circulating, as evidenced by the lack of clade C strains since 2010 and the absence of A1, B1 and B2 strains since 2014/15. In the current study, there was a close genetic relationship between 15 strains from all five countries (Figure 4A). These formed a distinct cluster within clade B3 (EU19 in Figures 2 and 3), which did not include previously detected B3 strains from these countries and was not well represented in the 2016 or 2018 epidemic (see Nextstrain analysis, https://nextstrain.org/community/enterovirus-phylo/evd68-2019/vp1-300). Fourteen of the sequences clustered closely, with an estimated most recent common ancestor (MRCA) in September 2018, suggesting that this cluster may have circulated in Europe during the 2018 season. Four German strains clustered within this group in the VP4/VP2 region (https://nextstrain.org/community/enterovirus-phylo/evd68-2019/vp4vp2). NL-03 (infection presumably acquired in Turkey) was more distant, with
## Table 1a
Cases of enterovirus D68 in five European countries, 1 January 2019–15 January 2020 (n = 93)

| Case number | Sample date (month-year) | Age (years) | Sex | Hospitalised | Pre-existing disease | Travel abroad < 2 weeks before sampling | Fever | Enteric symptoms | Respiratory symptoms | Neurological symptoms | Dermatological symptoms | Co-infections |
|-------------|--------------------------|-------------|-----|--------------|---------------------|------------------------------------------|-------|-----------------|---------------------|---------------------|----------------------|--------------|
| DK-01       | Aug-19                   | 15          | F   | Y            | N                   | N                                        | N     | Y               | None                | Bronchiolitis        | N                    | N            |
| DK-02       | Aug-19                   | 2           | F   | N            | N                   | Y                                        | N     | None            | Common cold         | N                   | N                    | N            |
| DK-03       | Sep-19                   | 3           | F   | Y            | Y                   | N                                        | N     | None            | Acute bronchitis    | N                   | N                    | N            |
| DK-04       | Sep-19                   | 1           | M   | Y            | N                   | N                                        | N     | None            | ILI                 | AFM                 | NA                   | Adenovirus, parechovirus |
| DK-05       | Sep-19                   | 2           | M   | Y            | Y                   | N                                        | N     | None            | Obstructive bronchitis | N                   | Rash                 | Adenovirus |
| DK-06       | Sep-19                   | 29          | F   | Y            | Y                   | N                                        | N     | None            | Common cold         | Cranial nerve palsies, dysphagia, dysphoria | N                    | N            |
| DK-07       | Sep-19                   | 0           | F   | N            | N                   | NA                                       | N     | None            | ILI                 | N                   | N                    | Rash         |
| DK-08       | Sep-19                   | 0           | F   | N            | Y                   | None                                     | N     | None            | Diarrhoea, rumbling stomach | Common cold         | N                    | Bordetella pertussis |
| DK-09       | Oct-19                   | 0           | F   | Y            | Y                   | N                                        | N     | None            | Pneumonia           | Reduced strength in right leg, attenuated patellar and plantar reflexes | N                   | Suspected bacterial infection |
| DK-10       | Oct-19                   | 27          | M   | Y            | Y                   | N                                        | N     | None            | Pneumonia           | Respiratory distress | N                    | N            |
| DK-11       | Oct-19                   | 50          | F   | N            | NA                  | NA                                       | N     | None            | Common cold         | N                   | N                    | N            |
| DK-12       | Oct-19                   | 30          | M   | N            | N                   | Y                                        | N     | None            | ILI                 | N                   | N                    | N            |
| DK-13       | Oct-19                   | 0           | F   | Y            | N                   | N                                        | N     | None            | Pneumonia           | N                   | Na                   | Haemophilus influenzae, Moraxella catarrhalis |
| DK-14       | Nov-19                   | 2           | M   | Y            | Y                   | N                                        | N     | None            | Pneumonia           | N                   | N                    | N            |
| DK-15       | Nov-19                   | 2           | F   | Y            | Y                   | N                                        | N     | None            | Obstructive bronchitis | N                   | N                    | Parechovirus, adenovirus, Haemophilus influenzae |
| DK-16       | Nov-19                   | 1           | M   | Y            | N                   | NA                                       | N     | Diarrhoea        | N                   | N                   | N                    | N            |
| DK-17       | Nov-19                   | 16          | F   | Y            | NA                  | NA                                       | NA    | Nausea, vomiting | Y                   | Ataxic cerebral palsy, diplopia, hypoaesthesia | N                   | N            |
| DK-18       | Nov-19                   | 21          | F   | Y            | N                   | NA                                       | N     | None            | Common cold         | N                   | N                    | Haemophilus influenzae, Pseudomonas aeruginosa |
| DK-19       | Nov-19                   | 2           | M   | Y            | Y                   | NA                                       | N     | None            | Respiratory distress | N                   | N                    | N            |
| DK-20       | Dec-19                   | 2           | F   | Y            | Y                   | NA                                       | N     | NA              | Bilateral pneumonia  | N                   | Pneumococcus         | N            |
| DK-21       | Dec-19                   | 1           | M   | N            | Y                   | NA                                       | NA    | NA              | Obstructive bronchitis | N                   | N                    | Rhinovirus C22, Haemophilus influenzae |
| NL-01       | Jan-19                   | 61          | M   | NA           | NA                  | Y                                        | NA    | NA              | NA                  | NA                  | NA                   | NA            |
| NL-02       | Jan-19                   | 65          | F   | N            | Y                   | N                                        | NA    | Yes             | NA                  | NA                  | NA                   | NA            |
| NL-03       | Apr-19                   | 1           | F   | Y            | NA                  | NA                                       | NA    | NA              | Respiratory insufficiency | NA                  | NA                   | NA            |
| NL-04       | Aug-19                   | 5           | F   | Y            | NA                  | NA                                       | NA    | NA              | Respiratory insufficiency | NA                  | NA                   | NA            |
| NL-05       | Aug-19                   | 75          | F   | NA           | NA                  | NA                                       | NA    | NA              | Pneumonia           | NA                  | NA                   | NA            |
| NL-06       | Sep-19                   | 46          | M   | N            | N                   | N                                        | N     | NA              | Diarrhoea           | ILI                 | NA                   | NA            |
| NL-07       | Oct-19                   | 61          | F   | N            | N                   | N                                        | N     | Na              | ARI, dyspnoea        | NA                  | NA                   | NA            |
| NL-08       | Nov-19                   | 1           | F   | NA           | NA                  | NA                                       | NA    | NA              | Common cold, dyspnoea | NA                  | NA                   | NA            |
| NL-09       | Nov-19                   | 1           | M   | NA           | NA                  | N                                        | N     | NA              | ARI, dyspnoea        | NA                  | NA                   | Respiratory syncytial virus type A |

AFM: acute flaccid myelitis; ARI: acute respiratory infection; DK: Denmark; FR: France; DE: Germany; ILI: influenza-like illness; N: no; NA: not available; NL: the Netherlands; SE: Sweden; URTI: upper respiratory infection; Y: yes.
### Table 1b: Cases of enterovirus D68 in five European countries, 1 January 2019–15 January 2020 (n = 93)

| Case number | Sample date (month-year) | Age (years) | Sex | Hospitalised | Pre-existing disease | Travel abroad < 2 weeks before sampling | Fever | Enteric symptoms | Respiratory symptoms | Neurological symptoms | Dermatological symptoms | Co-infections |
|-------------|--------------------------|-------------|-----|--------------|---------------------|----------------------------------------|-------|------------------|---------------------|----------------------|------------------------|---------------|
| NL-10       | Nov-19                   | 89          | M   | NA           | NA                  | NA                                     | NA    | NA               | NA                  | NA                   | NA                     | NA            |
| NL-11       | Nov-19                   | 4           | F   | NA           | NA                  | Dyspnoea                               | NA    | NA               | NA                  | NA                   | NA                     | NA            |
| NL-12       | Nov-19                   | 2           | F   | NA           | NA                  | ARI, dyspnoea                          | NA    | NA               | NA                  | NA                   | NA                     | NA            |
| NL-13       | Nov-19                   | 0           | M   | NA           | NA                  | NA                                     | NA    | NA               | NA                  | NA                   | NA                     | NA            |
| NL-14       | Nov-19                   | 2           | F   | NA           | NA                  | NA                                     | NA    | NA               | Pneumonia and bronchial hyperreactivity | NA                   | NA                     | NA            |
| NL-15       | Dec-19                   | 4           | M   | NA           | NA                  | NA                                     | NA    | NA               | NA                  | NA                   | NA                     | NA            |
| NL-16       | Dec-19                   | 53          | M   | NA           | N                   | Y                                      | N     | N                | N                   | N                    | N                      | N             |
| NL-17       | Dec-19                   | 7           | F   | NA           | N                   | N                                      | N     | N                | ARI                 | NA                   | NA                     | NA            |
| NL-18       | Dec-19                   | 22          | M   | NA           | N                   | N                                      | N     | N                | N                   | N                    | N                      | N             |
| NL-19       | Mar-19                   | 60          | M   | N            | N                   | Y                                      | N     | ILL              | Dyspnoea            | NA                   | NA                     | NA            |
| SE-01       | Aug-19                   | 70          | F   | NA           | NA                  | NA                                     | NA    | NA               | NA                  | NA                   | NA                     | NA            |
| SE-02       | Sep-19                   | 2           | M   | NA           | NA                  | NA                                     | NA    | NA               | NA                  | NA                   | NA                     | NA            |
| SE-03       | Sep-19                   | 0           | F   | N            | N                   | N                                      | Y     | N                | Y                   | NA                   | NA                     | NA            |
| SE-04       | Sep-19                   | 5           | F   | NA           | NA                  | NA                                     | NA    | NA               | NA                  | NA                   | NA                     | NA            |
| SE-05       | Oct-19                   | 61          | M   | N            | N                   | Y                                      | Y     | N                | ILL                 | NA                   | NA                     | NA            |
| SE-06       | Oct-19                   | 2           | M   | NA           | NA                  | NA                                     | NA    | NA               | NA                  | NA                   | NA                     | NA            |
| SE-07       | Oct-19                   | 4           | F   | Y            | N                   | N                                      | N     | N                | Y                   | NA                   | NA                     | NA            |
| SE-08       | Oct-19                   | 2           | M   | Y            | N                   | N                                      | Y     | N                | N                   | NA                   | Adenovirus, Rhinovirus, Haemophilus influenzae | NA            |
| SE-09       | Nov-19                   | 2           | M   | NA           | NA                  | NA                                     | NA    | NA               | NA                  | NA                   | NA                     | NA            |
| SE-10       | Nov-19                   | 5           | M   | NA           | NA                  | NA                                     | NA    | NA               | NA                  | NA                   | NA                     | NA            |
| SE-11       | Jan-20                   | 69          | M   | NA           | NA                  | NA                                     | NA    | NA               | NA                  | NA                   | NA                     | NA            |
| FR-01       | Oct-19                   | 0           | F   | Y            | N                   | N                                      | N     | N                | Bronchiolitis        | NA                   | NA                     | NA            |
| FR-02       | Oct-19                   | 33          | M   | Y            | Y                   | N                                      | N     | N                | Pneumonia with acute respiratory distress | N                   | Steptococcus pneumoniae | NA            |
| FR-03       | Nov-19                   | 1           | M   | Y            | N                   | N                                      | N     | Y                | Diarrhoea            | AFM                  | NA                     | NA            |
| FR-04       | Nov-19                   | 0           | F   | Y            | N                   | N                                      | Y     | Y                | Diarrhoea            | Bronchiolitis        | NA                     | NA            |
| FR-05       | Dec-19                   | 51          | F   | Y            | N                   | Y                                      | Y     | N                | Bronchitis           | N                   | N                      | N             |
| FR-06       | Dec-19                   | 66          | M   | Y            | N                   | N                                      | Y     | N                | Respiratory distress | N                   | N                      | N             |
| DE-01       | Jul-19                   | 1           | M   | Y            | N                   | Y                                      | N     | N                | Obstructive bronchitis | N                   | N                      | N             |
| DE-02       | Sep-19                   | 1           | M   | Y            | N                   | Y                                      | N     | N                | Obstructive bronchitis | N                   | Rhinovirus C17          | N             |
| DE-03       | Sep-19                   | 20          | F   | Y            | Y                   | N                                      | Y     | N                | Y                   | Multiple bacterial and viral infections | Na            |
| DE-04       | Sep-19                   | 9           | M   | Y            | Y                   | N                                      | Y     | N                | Asthma exacerbation   | N                   | N                      | N             |
| DE-05       | Sep-19                   | 1           | M   | Y            | N                   | N                                      | Y     | N                | Obstructive bronchitis | N                   | Rhinovirus A49          | N             |

AFM: acute flaccid myelitis; ARI: acute respiratory infection; DK: Denmark; FR: France; DE: Germany; ILL: influenza-like illness; N: no; NA: not available; NL: the Netherlands; SE: Sweden; URTI: upper respiratory infection; Y: yes.
| Case number | Sample date (month-year) | Age (years) | Sex | Hospitalised | Pre-existing disease | Travel abroad < 2 weeks before sampling | Fever | Enteric symptoms | Respiratory symptoms | Neurological symptoms | Dermatological symptoms | Co-infections |
|-------------|--------------------------|-------------|-----|--------------|---------------------|---------------------------------------|-------|-----------------|----------------------|----------------------|---------------------|---------------|
| DE-06       | Oct-19                   | 0           | M   | Y            | N                   | N                                     | N     | N               | Obstructive bronchitis | N                   | N                   | N             |
| DE-07       | Oct-19                   | 0           | F   | Y            | N                   | N                                     | Y     | N               | Y                    | N                   | N                   | N             |
| DE-08       | Oct-19                   | 6           | F   | Y            | N                   | N                                     | N     | N               | Y                    | Bilateral lower limb paralysis | N               | N             |
| DE-09       | Oct-19                   | 12          | M   | Y            | N                   | NA                                    | Y     | NA              | NA                   | NA                  | NA                  | N             |
| DE-10       | Nov-19                   | 2           | M   | Y            | N                   | N                                     | Y     | N               | Obstructive bronchitis | N                   | N                   | N             |
| DE-11       | Nov-19                   | 7           | F   | Y            | Y                   | N                                     | Y     | N               | Asthma exacerbation   | N                   | N                   | N             |
| DE-12       | Nov-19                   | 2           | M   | Y            | N                   | Y                                     | N     | Y               | Obstructive bronchitis | N                   | N                   | N             |
| DE-13       | Nov-19                   | 5           | F   | Y            | Y                   | Y                                     | N     | N               | Acute bronchitis      | Seizures            | N                   | Clostridioides difficile |
| DE-14       | Nov-19                   | 22          | F   | Y            | N                   | Y                                     | N     | N               | Obstructive bronchitis | N                   | N                   | N             |
| DE-15       | Nov-19                   | 1           | F   | Y            | N                   | Y                                     | N     | N               | Acute bronchitis      | N                   | N                   | N             |
| DE-16       | Nov-19                   | 8           | F   | Y            | Y                   | NA                                    | NA   | N               | Y                    | NA                  | NA                  | Adenovirus     |
| DE-17       | Nov-19                   | 28          | M   | Y            | N                   | N                                     | N     | N               | Y                    | N                   | N                   | N             |
| DE-18       | Nov-19                   | 8           | M   | Y            | N                   | NA                                    | NA   | N               | Y                    | NA                  | N                   | N             |
| DE-19       | Nov-19                   | 0           | F   | Y            | Y                   | N                                     | Y     | N               | Y                    | N                   | N                   | N             |
| DE-20       | Nov-19                   | 5           | F   | Y            | N                   | N                                     | N     | N               | Obstructive bronchitis | N                   | N                   | N             |
| DE-21       | Nov-19                   | 6           | F   | Y            | Y                   | Y                                     | Y     | N               | Y                    | N                   | N                   | N             |
| DE-22       | Nov-19                   | 2           | M   | Y            | NA                   | NA                                    | Y     | NA              | NA                   | NA                  | N                   | N             |
| DE-23       | Nov-19                   | 29          | M   | Y            | Y                   | N                                     | N     | N               | Y                    | N                   | N                   | N             |
| DE-24       | Nov-19                   | 7           | M   | Y            | NA                   | NA                                    | NA   | N               | N                    | NA                  | N                   | N             |
| DE-25       | Oct-19                   | 74          | F   | Y            | Y                   | N                                     | N     | N               | Y                    | N                   | NA                  | N             |
| DE-26       | Nov-19                   | 71          | M   | N            | Y                   | Y                                     | N     | N               | N                    | N                   | N                   | N             |
| DE-27       | Nov-19                   | 1           | M   | Y            | N                   | N                                     | N     | N               | Obstructive bronchitis | N                   | N                   | N             |
| DE-28       | Nov-19                   | 3           | M   | Y            | N                   | NA                                    | Y     | N               | Y                    | N                   | N                   | N             |
| DE-29       | Nov-19                   | 40          | F   | N            | N                   | NA                                    | Y     | NA              | Y                    | NA                  | N                   | N             |
| DE-30       | Dec-19                   | 2           | F   | Y            | N                   | N                                     | N     | N               | Obstructive bronchitis | N                   | N                   | N             |
| DE-31       | Dec-19                   | 1           | F   | Y            | N                   | Y                                     | N     | N               | Obstructive bronchitis | N                   | N                   | N             |
| DE-32       | Dec-19                   | 6           | M   | Y            | N                   | N                                     | Y     | N               | Pneumonia             | N                   | N                   | N             |
| DE-33       | Dec-19                   | 2           | M   | Y            | N                   | N                                     | Y     | N               | Bronchitis and pneumonia | N                   | N                   | Rhinovirus C55 |
| DE-34       | Dec-19                   | 1           | M   | Y            | N                   | N                                     | Y     | Pneumonia       | N                    | N                   | N                   | Bocavirus 1    |
| DE-35       | Dec-19                   | 5           | F   | Y            | Y                   | N                                     | N     | N               | Obstructive bronchitis | Seizures            | N                   | Respiratory syncytial virus |
| DE-36       | Dec-19                   | 2           | M   | Y            | N                   | N                                     | Y     | URTI            | N                    | N                   | N                   | N             |

AFM: acute flaccid myelitis; ARI: acute respiratory infection; DK: Denmark; FR: France; DE: Germany; ILI: influenza-like illness; N: no; NA: not available; NL: the Netherlands; SE: Sweden; URTI: upper respiratory infection; Y: yes.
an estimated MRCA with the larger 2019 European B3 subclade of January 2016.

Seventeen other strains (US18 in Figures 2 and 3) formed two further clusters within clade B3, one cluster with five strains and an MRCA of May 2019, the other with 12 strains and an MRCA of September 2018 (Figure 4B). These two clusters had a common MRCA in August 2018. This common ancestor fell within a large American cluster circulating in 2018, which previously contained only three non-American sequences. Strains NL-09, DK-09 and four German strains also fell within this American cluster; NL-09 separately from the other 2019 VP1 samples and the other five in the VP4/VP2 region (Figure 2, sequence data not shown). Samples DE-01 and DE-12 formed a pair and were descended from a cluster of B3 strains that circulated in Europe in 2018, including one of the Dutch cases detected in January (NL-02; cluster EU18 in Figures 2 and 3). The other Dutch case from January (NL-07; cluster A2/D in Figures 2 and 3), along with five other strains, belonged to clade A2/D (Figure 4C) and rooted, although separately, in a cluster circulating in Europe in autumn 2018. Five and 21 sequences, respectively, were long enough to be included in the Nextstrain full-length (≥ 6,000 bp) and VP1 (≥ 700 bp) builds; they can be viewed at https://nextstrain.org/enterovirus/d68/
Table 2A
GenBank accession numbers and available sequence for phylogenetic analysis, enterovirus D68 strains from five European countries, 1 January–6 December 2019 (n = 67)

| Case number | GenBank accession number | Sequence           |
|-------------|--------------------------|--------------------|
| DK-01       | MN896974                 | Partial genome     |
| DK-02       | MN896975                 | Partial genome     |
| DK-03       | NA                       | NA                 |
| DK-04       | MN896976                 | Partial VP1        |
| DK-05       | NA                       | NA                 |
| DK-06       | MN896977                 | Partial VP1        |
| DK-07       | MN896978                 | Full genome        |
| DK-08       | NA                       | NA                 |
| DK-09       | MN896979                 | Partial VP4/VP2    |
| DK-10       | MN896980                 | Partial VP1        |
| DK-11       | NA                       | NA                 |
| DK-12       | MN896981                 | Partial VP1        |
| DK-13       | MN896982                 | Partial VP1        |
| DK-14       | MN896983                 | Partial VP1        |
| DK-15       | MN896985                 | Partial VP1        |
| DK-16       | MN896984                 | Partial VP1        |
| DK-17       | NA                       | NA                 |
| DK-18       | MN896986                 | Partial VP1        |
| NL-01       | MN764886                 | Partial VP1        |
| NL-02       | MN764887                 | Partial VP1        |
| NL-03       | MN764888                 | Partial VP1        |
| NL-04       | MN764889                 | Partial VP1        |
| NL-05       | MN726800                 | Full genome        |
| NL-06       | MN726801                 | Full genome        |
| NL-07       | MN726798                 | Full genome        |
| NL-08       | MN726799                 | Partial VP1        |
| NL-09       | MN809623                 | Complete VP1       |
| NL-10       | MN809624                 | Partial VP1        |
| NL-11       | MN809625                 | Partial VP1        |
| NL-12       | MN809626                 | Partial VP1        |
| SE-01       | MN935869                 | Full genome        |
| SE-02       | NA                       | NA                 |
| SE-03       | NA                       | NA                 |
| SE-04       | MN935870                 | Full genome        |
| SE-05       | NA                       | NA                 |
| SE-06       | NA                       | NA                 |
| SE-07       | NA                       | NA                 |
| SE-08       | NA                       | NA                 |
| SE-09       | NA                       | NA                 |
| FR-01       | LR743438                 | Complete VP1       |
| FR-02       | LR743439                 | Complete VP1       |
| FR-03       | LR743440                 | Complete VP1       |
| FR-04       | LR743441                 | Complete VP1       |
| DE-01       | MN814240                 | Partial VP1        |
| DE-02       | MN814241                 | Partial VP1        |
| DE-03       | MN814242                 | Partial VP1        |
| DE-04       | MN814243                 | Partial VP1        |
| DE-05       | NA                       | NA                 |
| DE-06       | MN814244                 | Partial VP1        |

NA: not available.
Discussion

Here we report the detection of EV-D68 infections associated with both respiratory and neurological manifestations in five European countries in the autumn of 2019. Following its first identification in 1962, only 699 cases of EV-D68 were reported in scientific literature before 2014 [14]. From 2014 onwards, the epidemiology appears to have changed, and EV-D68 has increasingly been associated with outbreaks of respiratory infections and a concurrent upsurge of AFM [15,16] with poor long-term prognosis [17,18]. We identified 15 sequences, collected from five countries, which formed a distinct cluster within subclade B3 not previously described in Europe, illustrating the rapid evolution and spread of EV-D68. The sequences most closely related to this novel B3 cluster were sampled in India in 2017 and from sewage in the United Kingdom in 2018. This would suggest either a very low circulation of strains of this cluster or a lack of sustained global surveillance for EV-D68 and/or submission of sequences in GenBank, or both.

The EV-D68 cases were detected through routine surveillance networks in each country, with alerts to physicians posted in Denmark and the Netherlands. The number of samples processed in each country was within the expected range for the enterovirus season, but the detection rate of EV-D68 was higher than expected in Denmark, Germany and Sweden, where the number of detections for the 2018 season had been unexpectedly low. Detection rates in the other countries were similar to those seen in previous years with low circulation. The rate of hospitalisation among cases was high, however, current surveillance systems primarily allow the detection of severe infections. In this study, 12 of 93 cases were detected through community-based surveillance and a further three cases through sequencing of rhinovirus-positive samples, highlighting the importance of including such surveillance when monitoring infections such as EV-D68. The detection of EV-D68 associated disease indicates a continuous, and most likely underestimated, global circulation of this enterovirus type that was recognised after the large outbreak in the United States in 2014 had sparked increased testing. This justifies the need for reinforced enterovirus surveillance based on a more systematic screening of respiratory samples, collected in hospitalised patients or through the surveillance of respiratory infections (such as influenza community surveillance). Our report also highlights the heterogeneity of the surveillance of EV-D68 in Europe, which may hamper both the comparison of the epidemiological pattern between countries and, more importantly, the early detection of an outbreak. Continuous surveillance will add to our understanding of how EV-D68

N/A: not available.

| Case number | GenBank accession number | Sequence            |
|-------------|-------------------------|---------------------|
| DE-07       | MN832475                | Partial VP4/VP2     |
| DE-08       | MN812202                | Partial VP1         |
| DE-09       | MN832476                | Partial VP4/VP2     |
| DE-10       | MN814245                | Partial VP1         |
| DE-11       | MN814246                | Partial VP1         |
| DE-12       | MN814247                | Partial VP1         |
| DE-13       | MN814248                | Partial VP1         |
| DE-14       | MN814249                | Partial VP1         |
| DE-15       | MN814250                | Partial VP1         |
| DE-16       | MN832477                | Partial VP4/VP2     |
| DE-17       | MN832478                | Partial VP4/VP2     |
| DE-18       | MN832479                | Partial VP4/VP2     |
| DE-19       | MN832480                | Partial VP4/VP2     |
| DE-20       | MN814251                | Partial VP1         |
| DE-21       | MN832481                | Partial VP4/VP2     |
| DE-22       | MN832482                | Partial VP4/VP2     |
| DE-23       | MN832483                | Partial VP4/VP2     |
| DE-24       | MN832484                | Partial VP4/VP2     |

Table 2b
GenBank accession numbers and available sequence for phylogenetic analysis, enterovirus D68 strains from five European countries, 1 January–6 December 2019 (n = 67)
Figure 3
Phylogenetic analysis of enterovirus D68 with Nextstrain using partial VP1 sequences (n = 1,693)

EU18: 2019 sequences with most recent common ancestor in large B3 cluster of European sequences from 2018; EU19: novel B3 cluster identified in this study; US18: 2019 sequences with most recent common ancestor in large B3 cluster predominantly containing sequences from the United States in 2018.

For sequences with a sample date up to and including 2018, VP1 fragments of ≥ 700 bp in length were included, for sequences from 2019, fragments of ≥ 300 bp were included.

Divergence between major subclade divisions is shown, sequences are colour-coded according to date of sampling. Brackets identify where sequences reported in this study cluster with previous samples.
Figure 4
Phylogenetic analysis of three clusters of partial VP1 sequences of enterovirus D68 strains using Nextstrain, five European countries, 1 January–6 December 2019 (n = 36)

A. Cluster B3 EU19

Country
- France
- Germany
- Denmark
- Netherlands
- Sweden
- United Kingdom
- India
- Senegal
- United States

Neurological Symptom
- AFM: acute flaccid myelitis
- Cranial nerve palsy
- Limb paralysis
- Limb weakness

Estimated substitution rate
- 4.58 x 10⁻³ subs/site/yr

B. B3 US18 (partial)

C. A2/D (partial)

AFM: acute flaccid myelitis.

Zoomed in view of three phylogenetic clusters containing 2019 EV-D68 sequences. Nodes are coloured by country; the colour legend is the same for all three panels. Date estimate confidence intervals are shown as light grey bars for nodes leading to 2019 strains. Years are marked with dashed vertical lines. Red dots: sequences from this study; diamond shapes: neurological symptoms for 2019 cases.

Panel A shows a novel 2019 B3 cluster which is poorly represented in previous years. Panel B shows two 2019 clusters which descend from a large, United States-dominated, 2018 B3 cluster. The partial VP1 sequences of the upper seven strains with nodes having wide date estimate confidence intervals are identical. Panel C shows 2019 A2/D strains clustering with strains that circulated in France and Senegal in 2018.
Evolves outside of outbreak periods and whether low-prevalence years vary in geographic or demographic distribution. The detection of EV-D68 in a presumed year of low activity and the presence of patients with severe neurologic symptoms, stresses the importance of continuous and systematic surveillance and availability of diagnostics for workup of clinical cases.

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Conflict of interest

None declared.

Authors’ contributions

SEM: Initiated the study, wrote the first draft, edited subsequent drafts and prepared the final manuscript.

KB: Provided data, critically reviewed and edited all drafts of the manuscript.

RD: Provided data, critically reviewed and edited all drafts of the manuscript.

AMir: Provided data, critically reviewed and edited all drafts of the manuscript.

JLB: Critically reviewed and edited the manuscript.

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CH: Critically reviewed and edited the manuscript.

MP: Provided data, critically reviewed and edited the manuscript.

SteB: Provided data, critically reviewed and edited the manuscript.

EVJ: Carried out the phylogenetic analysis, critically reviewed and edited all drafts of the manuscript.

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