European surveillance for enterovirus D68 during the emerging North-American outbreak in 2014

Randy Poelman a,*, Isabelle Schuffenecker b, Coretta Van Leer-Buter a, Laurence Josset b, c, Hubert G.M. Nieters a, Bruno Lina b, c, on behalf of the ESCV-ECDC EV-D68 study group 1

a The University of Groningen, University Medical Center Groningen, Department of Medical Microbiology, Division of Clinical Virology, Groningen, The Netherlands.
b National Enterovirus Reference Centre, Laboratoire de Virologie, Centre de Biologie Est des Hospices Civils de Lyon, Bron, France.
c Virpath Lab, EA4610, Faculté de Médecine Lyon Est, Université Claude Bernard Lyon1, Université de Lyon, Lyon, France.

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A B S T R A C T

Background: In August and September 2014, unexpected clusters of enterovirus-D68 (EV-D68) infections associated with severe respiratory disease emerged from North-America. In September, the European Centre for Disease Prevention and Control (ECDC) asked European countries to strengthen respiratory sample screening for enterovirus detection and typing in cases with severe respiratory presentations. Objectives: To provide a detailed picture of EV-D68 epidemiology in Europe by conducting a retrospective and prospective laboratory analysis of clinical specimens. Study design: An initiative supported by the European Society for Clinical Virology (ESCV) and ECDC was launched to screen for EV-D68 in respiratory specimens between July 1st and December 1st 2014 in Europe and to sequence the VP1 region of detected viruses for phylogenetic analytic purposes. Results: Forty-two institutes, representing 51 laboratories from 17 European countries, analyzed 17,248 specimens yielding 389 EV-D68 positive samples (2.26%) in 14 countries. The proportion of positive samples ranged between 0 and 25% per country. These infections resulted primarily in mild respiratory disease, mainly detected in young children presenting with wheezing and in immuno-compromised adults. The viruses detected in Europe are genetically very similar to those of the North-American epidemic and the majority (83%) could be assigned to clade B. Except for 3 acute flaccid paralysis (AFP) cases, one death and limited ICU admissions, no severe cases were reported. Conclusions: The European study showed that EV-D68 circulated in Europe during summer and fall of 2014 with a moderate disease burden and different pathogenic profile compared to the North-American epidemic. © 2015 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Background

Enterovirus D68 (EV-D68), formerly classified as rhinovirus 87 (RV-87) [14], has a mixture of enterovirus (EV) and rhinovirus (RV) features and can induce severe respiratory symptoms, mostly in children [15,16]. In addition, it has been detected in respiratory samples from patients who developed acute flaccid paralysis (AFP) or acute flaccid myelitis [12], but no definite link between EV-D68 infection and neurological disease has yet been established [13].

To date, EV-D68 has never posed a serious threat to the general public health [7–11]. Only limited clusters (in magnitude and in geographic distribution) have been reported in different regions of the world. In most of these instances, the virus was detected in less than 10% of the EV or RV-related reported cases, and was typically co-circulating with other rhinoviruses or enteroviruses [17–21].

Though, between mid-August and December 2014, an apparent upsurge of severe respiratory tract infections was observed in the United States and Canada, especially among children with underlying chronic pulmonary illness presenting to hospital emergency departments [1–3]. Health authorities in 49 States and the District of Columbia reported 1153 cases of laboratory-confirmed EV-D68 infections by the 15th of January 2015, including 14 fatal cases in young children, and several severe neurological presentations.

* Corresponding author at: University Medical Center Groningen, Department of Medical Microbiology, Hanzeplein 1, P.O. Box 30 001, 9700 RB Groningen, The Netherlands.
E-mail address: r.poelaman@umcg.nl (R. Poelaman).
1 The list of participants of ESCV-ECDC EV-D68 study group along with their affiliation are listed in Appendix A.

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Assessment

2. (cdc.gov/non-polio-enterovirus/about/ev-d68.html#outbreak). At
the same time, though two European countries reported the
circulation of this respiratory virus, no further upsurge of
severe respiratory tract infections was reported in Europe
([4–6]; ecdc.europa.eu/en/publications/Publications/Enterovirus-
D68-United-States-Canada.pdf).

Arguably, EV-D68 infections are insufficiently detected owing
to a lack of targeted viral diagnostics and clinical awareness [10].
The EV surveillance carried out in Europe, North America and other
parts of the world, provides limited information on EV-related
respiratory illness. The focus for the worldwide EV surveillance
network is primarily on neurological presentation (such as AFP),
to support poliovirus eradication programmes. The monitoring of
respiratory diseases, carried out by for instance influenza surveil-
lance networks by using pathogen-specific PCRs, is mainly directed
towards influenza virus and respiratory syncytial virus during
winter. In addition, most hospital-based laboratories are using PCR
detection methods which target RV, and may fail to detect EV types
[22]. Thus, most of the diagnostic tools used for respiratory virus
detection in either in- or out-patients may not detect respiratory
EV and, when an RV or an EV is detected by generic PCR assays, no
discrimination between these two groups is possible such that the
detected viruses are frequently not genotyped. In addition, informa-
tion about circulating rhinovirus genotypes has been scarcely
documented ([23,24], B. Lina personal communication).

2. Objectives

In order to provide a detailed picture of the EV-D68 circula-
tion in Europe, we conducted a European-wide retrospective and
prospective laboratory analysis of clinical specimens between July
and December 2014, in collaboration with the European Society
for Clinical Virology (ESCV) and the European Centre for Disease
Prevention and Control (ECDC).

3. Study design

3.1. Enrolment of the laboratories

An ad hoc meeting was held at the annual ESCV conference in
Prague (end of September 2014), to launch the European EV-D68
network. Subsequently, the ESCV board invited the members of
the scientific community to join this initiative. The ECDC agreed to
support in early October and invited all European Union/European
Economic Area member states to participate through National
Microbiology Focal Points. These efforts resulted in the involve-
ment of a large number of participating laboratories. Also, Quality
Control for Molecular Diagnostics was involved as External Quality
Assessment Program organization (QCMD EQA, Glasgow, United
Kingdom), providing data on performances of commercial assays
and laboratory developed tests (LDT’s). Forty-two European instit-
tutes which represent a total of 51 laboratories from 17 countries
(Wales and Scotland representing the United Kingdom) responded
to this ESCV/ECDC EV-D68 initiative, of which 35 provided the full
set of data (number of specimens received, number of RV-EV posi-
tives and number of EV-D68 positives, Table 1). Seven laboratories
provided the number of RV-EV positives and number of EV-D68
positives only.

3.2. Detection of EV-D68 in specimens throughout Europe

In September 2014, each participating laboratory was asked to
test their respiratory specimens collected between the 1st of July
and 1st of December 2014 from children younger than 16 years
of age admitted to or visiting the hospital with any respiratory
symptoms. Where possible, laboratories were permitted to
broaden their retrospective surveillance to before July 1st (i.e.,
January 2014) and to include samples from adults. Full reporting
of the results also had to be completed by December 1st, 2014.
For the rapid detection of EV-D68, a specific real-time PCR was
developed and proposed by the laboratory in Groningen (NLD) [4].
Each laboratory could use this specific PCR as needed.

3.3. Sequencing of EV-D68 VP1 in positive specimens and sequence analysis

Participating laboratories carried out the partial sequencing of
VP1 of either EV-D68 positive or EV/RV positive samples as
described by Nix et al. [25]. The ~350 to 400 base pairs VP1 par-
tial sequences obtained were assigned to EV-D68 clades according
to Tokarz et al. [26]. Where needed, laboratories could send their
material to the Groningen or Lyon laboratories for sequencing.

Sequence data were analyzed with Sequencing Analysis (ver-
sion 5.3; ABI) using BioNumerics Software 6.6 (Applied Maths,
Sint-Martens-Latem, Belgium). Phylogenetic trees (maximum parsimony) were obtained according to BioNumerics standard pro-
cedures.

The sequences obtained from the network were aligned using
ClustalW in MEGA5 and compared using R bioconductor. Raw
distances between pair-wise sequences were calculated using
pair-wise deletion method. Amino acids that differentiate each
genogroup were defined using custom scripts. Many of the available
sequences were deposited in GenBank. The accession numbers are
as follows: Denmark (KP729103–KP729109), Finland (KP728259)
France (LN626610; LN681316–330; KP196362–378; KP307990; KP406467–496), Germany (KP745729–43), Ireland (KP739245),
Italy (KP153538–KP153546), Netherlands (KM887894–
KM887906/KM924544–KM924547/KM975346–KM975350/
KM975330–KM975335/KP830119–128/KP830131–135), Norway
(KP744824–KP744839), Spain (KP009456–KP009459/KP122208),
Sweden (KP745744–50).

3.4. Collection of clinical data

In addition to the number of specimens processed for this
study, each laboratory was asked to provide the number of EV- or
RV-detected in-respiratory specimens and the number of EV-D68
positives amongst these specimens.

For the EV-D68 positive cases, the laboratories provided the
clinical information blindly, wherever possible, by filling in a short
predefined questionnaire. Specifically, participants were requested
to report the date of specimen collection, age of the patients, clin-
Table 1
European EV-D68 detection data by country and laboratory in Europe (July 1st–December 1st 2014), including 916 specimens from a non-screening strategy.

<table>
<thead>
<tr>
<th>Country</th>
<th>Lab and/or city</th>
<th>Tested</th>
<th>Genus (EV and/or HRV)</th>
<th>EV-D68</th>
<th>Total/Country</th>
<th>EV68/EV Genus (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norway</td>
<td>Oslo NIPH</td>
<td>66</td>
<td>25</td>
<td>9</td>
<td>9</td>
<td>24.9%</td>
</tr>
<tr>
<td></td>
<td>Oslo Ullevål</td>
<td>354</td>
<td>71</td>
<td>34</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Trondheim</td>
<td>157</td>
<td>101</td>
<td>6</td>
<td>49</td>
<td>23.3%</td>
</tr>
<tr>
<td>Sweden</td>
<td>Stockholm Karolinska/PHI/</td>
<td>30†</td>
<td>30</td>
<td>7</td>
<td>7</td>
<td>23.3%</td>
</tr>
<tr>
<td>Finland</td>
<td>Turku UTU</td>
<td>700</td>
<td>140</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Helsinki HUS</td>
<td>109</td>
<td>15</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Helsinki THL</td>
<td>91</td>
<td>29</td>
<td>3</td>
<td>17</td>
<td>9.2%</td>
</tr>
<tr>
<td>Scotland</td>
<td>Glasgow</td>
<td>488‡</td>
<td>488</td>
<td>22</td>
<td>22</td>
<td>4.5%</td>
</tr>
<tr>
<td>Ireland</td>
<td>NVRL Dublin</td>
<td>1010</td>
<td>51</td>
<td>4</td>
<td>4</td>
<td>7.8%</td>
</tr>
<tr>
<td>Wales</td>
<td>Cardiff NHS</td>
<td>604</td>
<td>62</td>
<td>9</td>
<td>9</td>
<td>14.5%</td>
</tr>
<tr>
<td>Netherlands</td>
<td>Erasmus MC Rotterdam</td>
<td>198</td>
<td>46</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leiden UMC</td>
<td>780</td>
<td>185</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Radboud UMC</td>
<td>270</td>
<td>6</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Regional health service Haarlem</td>
<td>252</td>
<td>62</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>UMC Groningen</td>
<td>2397</td>
<td>531</td>
<td>19</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>RIVM Biltbouven NH</td>
<td>370</td>
<td>130</td>
<td>22</td>
<td>56</td>
<td>5.8%</td>
</tr>
<tr>
<td>Denmark</td>
<td>Statens serum institut SSI</td>
<td>252</td>
<td>50</td>
<td>8</td>
<td>8</td>
<td>16.0%</td>
</tr>
<tr>
<td>Germany</td>
<td>Bonn</td>
<td>351</td>
<td>35</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Freiburg</td>
<td>136‡</td>
<td>136</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leipzig, Robert Koch Institut†</td>
<td>1658</td>
<td>58</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leipzig, Institute of Virology</td>
<td>364</td>
<td>93</td>
<td>9</td>
<td>31</td>
<td>9.6%</td>
</tr>
<tr>
<td>Poland</td>
<td>Warsaw NIH</td>
<td>144</td>
<td>22</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Romenia</td>
<td>Bucharest</td>
<td>56</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Austria</td>
<td>Vienna</td>
<td>778</td>
<td>47</td>
<td>1</td>
<td>1</td>
<td>2.1%</td>
</tr>
<tr>
<td>Slovenia</td>
<td>Ljubljana</td>
<td>702</td>
<td>234</td>
<td>48</td>
<td>48</td>
<td>20.5%</td>
</tr>
<tr>
<td>Italy</td>
<td>Milan</td>
<td>176</td>
<td>97</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pavia</td>
<td>195‡</td>
<td>195</td>
<td>9</td>
<td>13</td>
<td>4.5%</td>
</tr>
<tr>
<td>Luxembourg</td>
<td>Luxembourg</td>
<td>19‡</td>
<td>19</td>
<td>1</td>
<td>1</td>
<td>5.3%</td>
</tr>
<tr>
<td>France</td>
<td>Lyon/Nav ref centre EV</td>
<td>1589</td>
<td>439</td>
<td>39</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clermont-Ferrand/Nav ref centre EV</td>
<td>710</td>
<td>191</td>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Brest</td>
<td>199</td>
<td>86</td>
<td>9</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Caen</td>
<td>367</td>
<td>175</td>
<td>22</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dijon</td>
<td>159</td>
<td>37</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Paris</td>
<td>49</td>
<td>25</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Saint-Etienne</td>
<td>104</td>
<td>36</td>
<td>6</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Strasbourg</td>
<td>203</td>
<td>102</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Versailles</td>
<td>109</td>
<td>34</td>
<td>2</td>
<td>117</td>
<td>10.4%</td>
</tr>
<tr>
<td>Spain</td>
<td>Barcelona</td>
<td>529</td>
<td>53</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Valencia</td>
<td>458</td>
<td>89</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Santander</td>
<td>15‡</td>
<td>15</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Valladolid</td>
<td>33‡</td>
<td>33</td>
<td>1</td>
<td>6</td>
<td>3.2%</td>
</tr>
<tr>
<td>Portugal</td>
<td>Lisbon</td>
<td>17</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total number of countries: 52
Total number of labs: 17

EV-QCMD Quality Control panel. This information was correlated with the in silico self-evaluation provided by the participating labs.

3.6. Statistical analysis

Statistical analysis was performed by using the Chi-Square Test (CI 95%) and Fisher’s exact test (p-value < 0.05).

4. Results

4.1. EV-D68 detection and distribution in Europe

A total of 17,248 (mostly respiratory) samples were tested, mainly collected between the 1st of July and the 1st of December (Table 1). Overall, a picornavirus (EV or RV) was detected in 4273 specimens, of which 389 were identified as EV-D68. The European mean EV/RV positivity rate (calculated from the 35 labs) in respiratory specimens was 20.5% (3357/16,332), of which 10.21% (343/3357) were EV-D68 positive, yielding an EV-D68 positivity rate of 2.1% (343/16,332) in respiratory samples.

The virus was detected in each participating country across Europe, except for three: Poland, Portugal and Romania; but in these countries only a limited number of samples were tested. The proportion of EV-D68 positive samples among the total number of respiratory samples tested differed between countries, ranging from 0% to 25% (Table 1, Fig. 1). EV-D68 was detected throughout the five months of the study, with a peak in October and a clear
epidemic trend (Fig. 2). A North–South gradient was observed with a higher level of detection in Northern (Norway, Sweden, Denmark) than in Southern European countries (Italy, Spain, Portugal: Fig. 1; Table 1). Only 6 cases were detected in Spain, 13 in Italy and none in Portugal.

4.2. Sequencing

Of the 389 EV-D68 strains that were detected, 205 (52.7%) were sequenced and subsequently assigned to the subgroups described by Tokarz et al. [26]. All the EV-D68 viruses belonged to clades A (n = 35; 17.1%) and B (n = 170; 82.9%); no clade C virus has been detected. Viruses from the clades A and B were co-detected in 12 countries, while Spain reported only clade A viruses and Slovenia and Norway only clade B viruses. The Blast analysis and the maximum parsimony tree show high nucleotide homology between the US and European viruses (Fig. 3, Suppl. Figure 1). Identical sequences were obtained from viruses detected in different countries (Fig. 3, Suppl. Figure 1). Both figures show a segregation of the clade A viruses into two sub-clusters, A1 (n = 11; 31.4%) and A2 (n = 24; 68.6%) with an average raw nucleotide distance of 11% between the two sub-clusters (Fig. 3). The significance of this segregation needs to be confirmed by further Bayesian analysis. The nucleotide divergence between the whole clade A and clade B was 13.8%. All 2014 VP1 sequences of clade A viruses had a deletion of one amino acid in position 140, while 9 other amino-acids could differentiate at least one clade from another (Suppl. Table 1). More than 30 amino acids at the end of the VP1 coding sequence were found to be specific to the 2014 sequences when compared to previous sequences (data not shown). These changes are observed in regions where neither the impact on the immunogenicity of the virus nor that on binding to sialic acids was known [27]. Finally, there were no absolute differences in the BC and DE loops of VP1 between the US and the European 2014 viruses (Suppl. Table 2). However, when comparing the frequencies of amino-acids at each position, two positions in the BC and DE loops, at residues 97 and 143, respectively, showed significant differences between the USA and European strains in Clade B (Fisher’s exact test; p < 0.05). As there were only a few viral sequences from the USA outbreak, further analysis must be done before it can be concluded that the strains circulating in the USA and Europe in 2014 were different. Such a comparison could not be performed for viruses of the sub-clade A1 for which no sequence from the USA outbreak was available.

Supplementary Fig. 1. Tables 1 and 2 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jcv.2015.07.296

4.3. Clinical analysis

EV-D68 was detected in all age groups (Table 2). From the 389 documented cases, three AFP cases (two in Norway and one in France) and one death, of a 14-year-old Italian patient with severe neurological underlying disease, have already been reported [40]. Underlying conditions that predisposes to the development of severe respiratory infections were frequent in all age groups (Table 2, Suppl. Table 3). Data on underlying conditions were
available in only 91/120 (76%) children below the age of 2. Of these 20/91 (21.9%) had chronic or recurrent respiratory problems, 10 (11%) had other underlying disease, 3 (3.3%) where immuno-compromised, whereas the majority (58/91, 63.7%) did not have any recognized underlying disease (Table 2, Suppl. Table 3). In the age group 2–5 years information was missing from 63 individuals (46.7%). When documented, 44/72 (61.1%) were known to have underlying conditions, the largest group having a chronic or recurrent pulmonary disease (n = 27/72, 37.5%). In 197 documented children of all age groups (<17), immuno-compromised conditions were present in only 8 of EV-D68 cases (4.1%). This was different for the adults (>17) in question, where immuno-compromised patients (following transplantation or cancer treatment) formed a significantly larger proportion of the cohort than with the children (37.5% vs 4.1%; Chi-square test). Nonetheless, although the difference is not significant, only 9/45 documented adults (20%) had underlying chronic respiratory diseases as compared to 63/197 documented children (32%).

Supplementary Table 3 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jcv.2015.07.296

In the four age groups, similar proportions of patients admitted to the ICU amongst the hospitalized cases were observed (15/110, 11/121, 5/45 and 3/38 for 0–1 year, 2–5 year, 6–16 and 17 and over, respectively). None of the clades was associated with clinical severity or age-distribution.

4.4. Performance of commercial test kits and (specific) real-time PCR

Overall, 29 laboratories used LDT’s, 18 used commercial assays and 3 used both in-house and commercial assays. One laboratory did not provide details of the assay used. Briefly, the retrospective analysis of the performance of the commercially available kits confirmed that the SEEGENE Anyplex II RV16 detection assay (Korea) that is used by two laboratories did not detect EV-D68, while this assay preferentially targeting only EV. The Cepheid Smartcycler (Cepheid, Sunnyvale, USA), the Fast-Track Diagnostics (Sliema, Malta) and the Diagenode human EV test (Liege, Belgium), did detect EV-D68. In addition, the most commonly used commercial assays, such as the xTAG Respiratory Viral Panel (Luminex Europe, Den Bosch, The Netherlands) Respifinder Smart 22 fast v2 (Pathfinder, Maastricht, The Netherlands), Rhino&Entero/Ccr-gene (bioMerieux/Argene, Marcy l’Etoile, France), were not able to discriminate between RV

Table 2

Underlying diseases categories in EV-D68-positive cases by age group in the 14 EU/EEA countries (July 1st–December 1st 2014).

<table>
<thead>
<tr>
<th>Age</th>
<th>EV68 positive</th>
<th>Underlying disease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Chronic respiratory illness</td>
</tr>
<tr>
<td>0–1</td>
<td>120</td>
<td>20 (16.7%)</td>
</tr>
<tr>
<td>2–5</td>
<td>135</td>
<td>27 (20.0%)</td>
</tr>
<tr>
<td>6–16</td>
<td>56</td>
<td>16 (28.6%)</td>
</tr>
<tr>
<td>≥17</td>
<td>78</td>
<td>9 (11.5%)</td>
</tr>
<tr>
<td>Total</td>
<td>389</td>
<td>72 (18.5%)</td>
</tr>
</tbody>
</table>
and EV, but detected EV-68. Specific mentioning that such kits may or may not detect EV-D68 is not included in their product information insert, but testing the Fermon strain did indicate the detection potential of some of these assays. Besides, a total of 31 laboratories implemented one of the available specific EV-D68 real-time PCR’s [4,6].

5. Discussion

Between 1963 and 2014, EV-D68 upsurges involving children with underlying respiratory diseases have been reported in different countries, albeit with a quite limited number of cases [8,18,28,29]. In contrast to the rapidly accumulating EV-D68 cases observed in North America in 2014 [1,11], no sudden increase of EV-D68 was reported in Europe, except for Norway and the Netherlands [4–6]. The initiative launched by the European Society for Clinical Virology (ESCV) and supported by the European Centre for Disease prevention and Control (ECDC) was primarily aimed at a rapid European-wide screening for the detection of the possibly emerging EV-D68.

Following the launch of the initiative, EV-D68 detection protocols were formulated and implemented to screen 17,248 respiratory specimens collected between July and November 2014, allowing a rapid response within a two-month period (October–December 2014). EV-D68 was detected in all participating countries, except for three (Fig. 1, Table 1), though two analyzed a limited number of specimens (17 and 56, respectively) and the other used an assay which may have been the cause for missing (a low level of circulation of) the virus. The 2014 instance was the largest European EV-D68 epidemic described since the isolation of the Fermon strain in 1962.

The trend of detection (though somewhat biased by the implementation of the survey itself) showed a clear epidemic curve, with a peak of cases in October (Fig. 2) and local epidemics reported [4–6]. The number of cases diminished in December, as would be expected for an EV epidemic, particularly where RSV and influenza epidemics come into play [30].

A European North–South gradient was observed, revealing discrepancies in the levels of virus detection. In some instances, discrepancies were observed between laboratories in the same country (Table 1). This could be partly explained by local epidemics or clusters (as in Norway and Slovenia) or by local differences in testing periods (the Netherlands). This divergence in observed results raised questions about the capacity of some assays to detect EV-D68. Thanks to the recent EV-RV EQA panels provided by QCMD, which included EV-D68, we could confirm that most of the participating laboratories used kits appropriate for EV-D68 detection. This was further attested when they used the EV-D68-specific detection protocols. However, a few laboratories did not; the Seegene Anyplex II RV16 detection assay failed to detect EV-D68.

Still, relatively few clinical diagnostic laboratories employ systematic EV detection followed by genotyping in the routine screening strategy of respiratory materials, both in Europe and the USA [11,16,29]. Hence, clusters of EV infections may remain undocumented until a significant EV-related clinical burden arises.

The clinical picture that we describe is a reflection of the underlying hospitalized population, rather than a representation of the community or of the primary healthcare level. Consistently about 10% of the cases in all age groups required intensive care respiratory support. Of the EV-D68 cases recorded in our study, pre-existing respiratory conditions were frequent in young children, whereas underlying immune-compromising conditions were frequent in adults (Table 2). This finding has been reported elsewhere [31].

In contrast to the USA, only one death was reported in Europe during this EV-D68 survey and only 3 children were diagnosed with EV-D68 related AFP [2,6,36]. This difference in clinical impact was also noted in the recent retrospective study conducted in the USA. Greninger et al. reported that EV-D68 of clade B similar to those detected in the European survey was frequently detected in the respiratory secretions of patients with acute flaccid myelitis [12]. No such cases have been observed in Europe to date. This absence is not attributable either to a lack of detection capabilities or of appropriate sample collection. Indeed, most European countries have active surveillance programmes for poliovirus infections in which EV-associated neurological presentations are reported and documented by reference laboratories [22]. Three cases of EV-D68 related AFP have been reported (two in Norway and one in France), showing the capacity of the system to detect AFP-related cases [36]. Even if no link is established between AFP and EV-D68 infection, more surveillance is needed to better assess its putative role in severe neurological presentations [12,13,37].

As part of our study, 205 isolates were sequenced to investigate their relationship with the circulating EV-D68 virus types in North America and to determine if severity could be correlated to a given clade of the virus [26]. Our sequence data confirms that A and B clades were circulating, just as in the USA and Canada [2]. The European and North American EV-D68 viruses were genetically closely related, but a specific virus genetic signature was not associated with virulence.

EV-D68 can be easily transmitted through droplets and is the most frequently encountered group D EV (although this is a small group) [11,14,15]. It has been demonstrated that this virus binds to the sialic acid α2,6, as do other respiratory viruses, as a result of adaptations in the BC and DE loops of the VP1 capsid region [27]. Despite ongoing research, there is no antiviral treatment available [32,33]. However, Liu et al. recently suggested that Plecanaril could be effective in treating the latest EV-D68 infections because of its excellent binding into the hydrophobic canyon of the capsid; this canyon being narrower than those in most other RVs and EVs [34]. Though this drug is not commercially available, it may be useful to evaluate its contribution to the management of severe EV-D68 infections, and the alleviation of outbreaks such as those occurring in nursing homes [24,35].

However, more sequences from the US outbreak are required to determine whether specific residues in the BC and DE loops of VP1 might have different amino acid proportions in US versus European sequences [2]. Despite this apparent lack of difference in the severity of the diseases, some evolution was observed in the BC loop which reinforces the argument in favour of distinguishing between clades A and B [38,39].

In conclusion, this collaborative venture highlights the merits of working together on cross-border studies of infectious disease epidemics using established networks. A timely response is essential to evaluate the circulation and relevance of an emerging virus. Therefore the global or at least continental networks, such as the ECDC and ESCV could provide, need to be equipped to facilitate and streamline proper procedures for a rapid and efficient handling of an emerging virus, whenever it occurs. Additionally, long-term studies are needed to adequately collect clinical data for a better understanding of the pathogenesis.

Conflict of interest

The authors declare that there is no conflict of interest.

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Ethical approval

None required.

Appendix A. List of participants ESCV-ECDC Enterovirus
D68 ad-hoc study group

A.1. Austria

Theresia Popow-Kraupp, Stephan W. Aberle – (Department of Virology, Medical University of Vienna).

A.2. Denmark:

Thea Kølsen Fischer – (Virus Surveillance and Research Unit, Department of Microbiological Diagnostics and Virology, Statens Serum Institut and Clinical Institute, University of Southern Denmark).

Sofie Midgley – (National WHO Polio Reference Laboratory at Statens Serum Institut).

Claus Bohn Christiansen – (Virology Unit, Department of Clinical Microbiology at Rigshospitalet).

A.3. Finland

Matt Waris, Riikka Österback, Tytti Vuorinen – (Department of Virology, University of Turku).


Maija Lappalainen, Anne Jääskeläinen, Teemu Smura – (Department of Virology and Immunology, University of Helsinki and Helsinki University Hospital).

A.4. France

Léa Pilorge, Marie-Christine Legrand-Quillien, Christopher Payan – (Microbiology laboratory, University hospital of Brest).

Joëlle Petitjean, Astrid Vabret, Mélanie Ribault – (Virology laboratory, University hospital of Caen).

Audrey Mirand, Hélène Peigue-Lafeuille, Cécile Henquell – (National Reference Center for Enteroviruses and Parechoviruses, University Hospital of Clermont-Ferrand).

Catherine Manoha, Jean-Baptiste Bour, Magali Darniot – (Virology laboratory, University hospital of Dijon).

Isabelle Schuffenecker, Laurence Josset, Bruno Lina – (Virology laboratory, National Reference Center for Enteroviruses and Parechoviruses, Hospices Civils de Lyon).

Jérôme Le Goff, Séverine Mercier-Delarue, Catherine Scieux – (Université Paris Diderot, Sorbonne Paris Cité, Microbiology laboratory, Hôpital Saint-Louis, APHP, Paris).

Sylvie Pillet, Bruno Pozzetto – (Laboratory of infectious agents and hygiene, University Hospital of Saint-Etienne, Saint-Etienne).

Quentin Lepiller, Samira Fall-Kremer, Françoise Stoll-Keller – (Virology institute, University hospital of Strasbourg).

Stéphanie Marque-Juillet; Aymeric Coutard, Marlène Amara – (Microbiology laboratory, Hospital of Versailles).

A.5. Germany

Sindy Böttcher, Sabine Diedrich – (Berlin, Robert Koch Institute, National Reference Centre for Poliomyelitis and Enteroviruses); The Robert Koch Institute collated data from the following Departments and Institutes: Institute of Virology and Immunobiology, University Hospital of Würzburg, Christiane Priftert, Benedikt Weißbrich; Institute of Virology, University Hospital of Düsseldorf, Ortwin Adams; Department of Virology, Labor Berlin – Charite Vivantes GmbH), Angela Stein, Jörg Hofmann; Department of Infectious Diseases, Virology, University Hospital Heidelberg, Paus Schnitzler.

Anna Maria Eis-Hübinger, Souhaib Aldabbagh, Ulrike Reber – (Institute of Virology, University of Bonn Medical Centre).

Marcus Panning, Daniela Huzly, Sibylle Bierbaum – (Institute of Virology, Albert-Ludwigs University Freiburg).

Uwe G. Liebert, Melanie Maier – (Institute of Virology, University of Leipzig).

A.6. Ireland

Michael J. Carr, Gráinne Tuite, Jorge Abboud Guerra, Joanne O’Gorman, Cillian De Gascun – (National Virus Reference Laboratory, University College Dublin).

A.7. Italy

Fausto Baldanti – (University of Pavia, Fondazione IRCCS Policlinico San Matteo, Pavia).

Antonio Piralla – (Fondazione IRCCS Policlinico San Matteo, Pavia).

Susanna Esposito, Nicola Principi, Luca Ruggiero – (Milan, Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico, Pediatric High Intensive Care Unit).

A.8. Luxembourg

Matthias Opp – (Dudelange, Laboratoire National de Santé).

A.9. Netherlands

Gé Donker – (Utrecht, Netherlands institute for health services research).

Adam Meijer, Harrie van der Avoort, Kimberley Benshop – (Bilthoven, Centre for Infectious Disease Research, Diagnostics and Screening, National Institute of Public Health and the Environment).

Marit de Lange – (Bilthoven, Centre for Infectious Diseases, Epidemiology and Surveillance, National Institute of Public Health).

Hubert Niesters, Randy Poelman, Coretta Van Leer-Buter, Renze Borger, Liesbeth Scholvinck – (University Medical Center Groningen).

Wil van der Reijden, Dick Veenendaal – (Haarlem, Regional Laboratory for Public Health Kennemerland).

Eric C.J. Claas, Ann C.T.M. Vossen – (Department of Medical Microbiology, Leiden University Medical Center Leiden).

Janette Rahamat-Langendoom, Willem J.G. Melchers – (Nijmegen, Department of Medical Microbiology, Radboud University Medical Center).

Marion P.G. Koopmans, Annemiek A. van der Eijck, Suzan D. Pas – (Rotterdam, ErasmusMC, Department of Viroscience).

A.10. Norway

Anne-Martre Bakken Kran – (Department of Medical Microbiology, Oslo University Hospital).

Karoline Bragstad, Susanne Gjeruldsen Dudman – (Oslo, Norwegian Institute of Public Health).

Andreas Christensen, Sidsel Krookstad – (Trondheim, Department of Medical Microbiology, St. Olavs University Hospital).
A.11. Poland
A.12. Portugal
Raquel Guimaraes, Pedro Pechiraga, Paula Cristovao, Ines Costa – (Lisbon, National Influenza Reference Laboratory).
A.13. Romania
Cristina Tecu, Emilia Lupulescu, Carmen Cherciu – (Bucharest, Cantacuzino National Research and Development Institute for Microbiology and Immunology).
A.14. Scotland
Emily Goldstein, Susan Bennett, Amanda Bradley-Stewart, Rory Gunson – (West of Scotland Specialist Virology Centre Glasgow).
A.15. Slovenia
Natasa Berginc, Katarina Prosen – (Ljubljana, Laboratory for Public Health Virology, National Laboratory of Health, Environment and Food).
A.16. Spain
Magda Camps, Laura Gimferrer, Andres Anton – (Respiratory Virus Unit/Virology Section, University Hospital Vall d’Hebron, Barcelona).
F. Xavier Lopez-Labrador; Laura Cano Perez – (Virology Laboratory, Genomics and Health Area, FISABIO-Public Health, Generalitat Valenciana, Valencia/CIBERESP, Instituto de Salud Carlos III).
Concepción Gimeno Cardona, M Dolores Ocate Mochón – (Microbiology Service, General Hospital of Valenciana).
Javier Buesa, David Navarro – (Microbiology Service, Hospital Clínico de Valencia).
R. Ortiz de Lejarazu Leonardo, Iván Sanz Muñoz, Silvia Rojo – (University Hospital of Valladolid).
Mónica Gozalo-Margullió, Jesús Agüero Balbín – (Service of Microbiology, University Hospital Marqués de Valdecilla-IDIVAL, Santander).
A.17. Sweden
Jan Albert, Agnetta Samuelson, Maria Rotzen Östlund, Robert Dyrud – (Stockholm, Department of Clinical Microbiology, Karolinska University Hospital).
Mia Brytting, Elenor Haunzenberger, Katherina Zakikhany – (Stockholm, Public Health Agency of Sweden).
Margareta Eriksson – (Stockholm, Astrid Lindgren Children’s Hospital).
A.18. Wales
Catherine Moore – (Public Health Wales Microbiology Cardiff, University Hospital of Wales).
A.19. ECDC
Eeva Broberg, Pasi Penttinen – European Centre for Disease Prevention and Control (Stockholm Sweden).
A.20. QCMD
Elaine McCulloch, Caterina Di Lorenzo, Paul Wallace, Anton Van Loon – Quality Control for Molecular Diagnostics (Scotland/United Kingdom).

References


