Continued seasonal circulation of enterovirus D68 in the Netherlands, 2011–2014

A Meijer (Adam.Meijer@rivm.nl)[1], K S Benschop[1], G A Donker[2], H G van der Avoort[1]
1. Centre for Infectious Disease Research, Diagnostics and Screening, National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands
2. NIVEL Primary Care Database, Sentinel Practices, Utrecht, The Netherlands

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Enterovirus D68 (EV-D68) continued to circulate in a seasonal pattern in the Netherlands, after the outbreak in 2010. Outpatient EV-D68 cases, mainly in the under 20 and 50–59 years age groups, presented with relatively mild respiratory disease. Hospital-based enterovirus surveillance identified more severe cases, mainly in children under 10 years of age. Dutch partial VP1 genomic region sequences from 2012 through 2014 were distributed over three sublineages similar to EV-D68 from the outbreak in the US in 2014.

After the 2010 outbreak, enterovirus D68 (EV-D68) continued to circulate in a seasonal pattern in the Netherlands. Here, we report the results of the monitoring of EV-D68 circulation in the Netherlands from week 1 2011 through week 40 2014.

EV-D68 has been sporadically detected since its first description in 1962, up to 2008 [1,2]. From 2008 onwards, EV-D68 outbreaks occurred worldwide, including in 2010 in the Netherlands [2–5]. The largest outbreak is currently occurring in Northern America, causing substantial hospitalisation of children with severe respiratory disease in the United States (US) [3,6]. Many of these children have underlying disease, such as asthma [3,6]. Previous outbreaks described in the literature reported mainly on hospitalised patients [3].

In the Netherlands, retrospective analysis of enteroviruses detected from the general practitioner (GP) sentinel surveillance of influenza-like illness (ILI) and other acute respiratory infections (ARI) showed that circulation of EV-D68 occurred at least since 1996 up to the upsurge of 2010 [5]. EV-D68 cases had significantly more dyspnoea and bronchiolitis compared to EV-D68-negative patients with ILI or ARI notified in the same week [5]. In the Dutch national enterovirus surveillance aimed at exclusion of poliovirus circulation, EV-D68 was rarely detected, mainly because the focus has been on enteroviruses detected in stool specimens [7]. Since 2010, we continued to monitor EV-D68 circulation in the Netherlands through both surveillance schemes.

Specimen collection

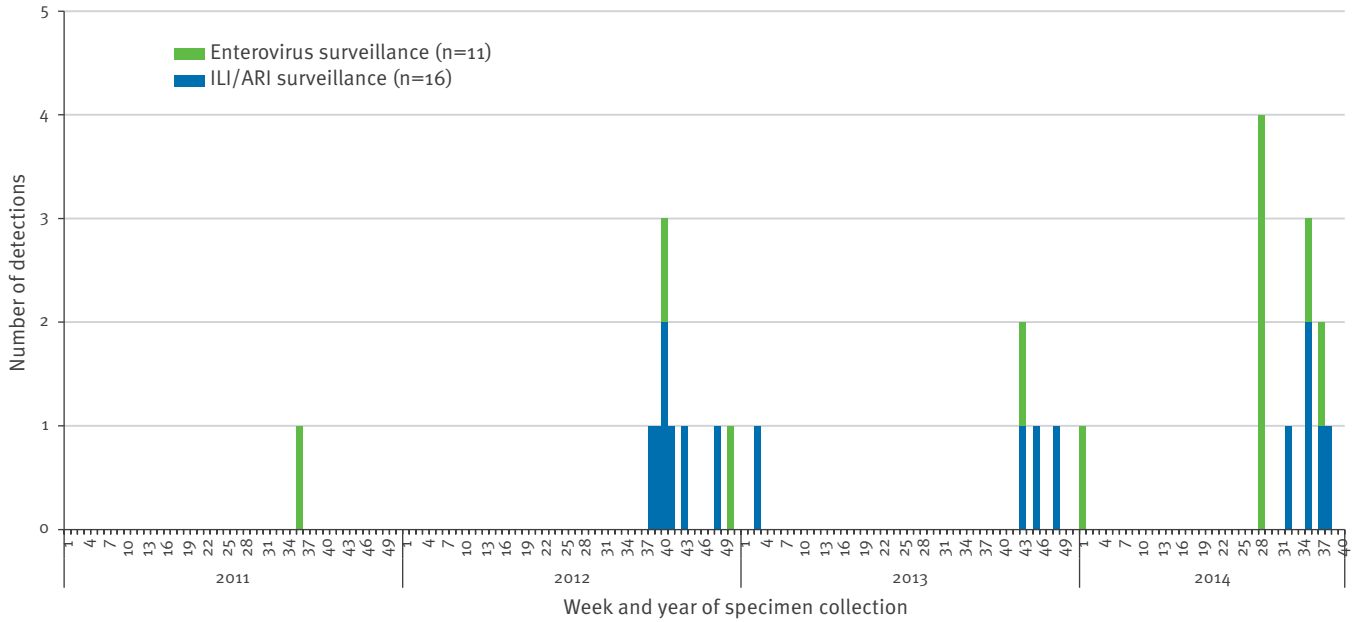
The methods used for specimen collection and for enterovirus detection and VP1 genomic region sequence analysis have been described [5,7,8]. For phylogenetic analysis using MEGA6 [9] all available VP1 sequences (covering nucleotides 132 through 471 relative to the VP1 gene of the Fermon strain) as of 12 October 2014 were downloaded from GenBank. The phylogeny was reconstructed using maximum likelihood and 1,000 bootstrap iterations with new Dutch sequences included (GenBank accession numbers KM975324-KM975350). Numbering of the major clusters (1, 2 and 3) has been described [5] and is synonymous to major clusters B, C and A respectively described by Tokarz et al. [10].

Results

Figure 1 and Table 1 summarise EV-D68 detections through the GP-based sentinel ILI and other ARI surveillance and the national enterovirus surveillance in the Netherlands, in specimens with collection dates from week 1 2011 through week 40 2014. Over the whole period, 27 EV-D68 cases were identified in a seasonal pattern; one in autumn 2011, 10 in autumn-winter period 2011/12, five in autumn-winter period 2012/13, and 11 since summer 2014 (Figure 1). The start of detections in 2014 was earlier compared to the start of detections in 2012 (12 and six weeks earlier in the enterovirus and ILI/ARI surveillance respectively) and in 2013 (15 and 11 weeks earlier in the enterovirus and ILI/ARI surveillance respectively) (Figure 1). By year, the proportion EV-D68 among enteroviruses analysed was much higher (median 25%; range 0–38%) in the ILI/ARI surveillance compared to the enterovirus surveillance (median 0.5%; range 0.3–1.4% (Table 1). However, by year, the percentage of enterovirus detections among ILI/ARI cases was low, on average 1.7% (range 1.4–2.1%) (Table 1).

Figure 1

Enterovirus D68 detections by source, the Netherlands, week 1 2011–week 40 2014



ARI: acute respiratory infections; ILI: influenza-like illness.

Due to increased awareness of the importance of enteroviruses in respiratory infections, laboratories participating in the Dutch national enterovirus surveillance also submitted enteroviruses associated with respiratory illness for typing after 2010; all 11 EV-D68 detections were in respiratory specimens. The age distribution in outpatients over the whole period was not different from that reported before, over the period 1996 through 2010 [5]; cases occurred mainly in the under 20 and in the 50–59 years age groups (Table 2). The male/female ratio was 1.3 (Table 2). In the national enterovirus surveillance, however, EV-D68 was mainly detected in the under 10 years age group and the male/female ratio was 0.8 (Table 2).

The age distribution in 2014 was similar to that for the whole period for both surveillance schemes (data not shown). EV-D68 positive outpatients presented with ILI as well as other ARI, with most prominent symptoms being fever and cough (Table 2). Similar to the situation in Northern America in 2014, the hospitalised cases experienced severe respiratory disease (Table 2).

Table 1

Detections of enterovirus D68 in general practitioner sentinel influenza-like illness and other acute respiratory infection surveillance and in enterovirus surveillance, the Netherlands, week 1 2011–week 40 2014

Year	Number of clinical specimens tested	Number of enterovirus positive specimens (% of specimens tested)[a]	Number of enterovirus D68 positive specimens (% of enterovirus positive specimens)
ILI/ARI surveillance			
2011	1,369	19 (1.4)	0
2012	1,126	24 (2.1)	7 (29)
2013	1,292	19 (1.5)	4 (21)
2014 (through week 40)	792	13 (1.6)	5 (38)
Enterovirus surveillance			
2011	Unknown	362	1 (0.3)
2012	Unknown	498	2 (0.4)
2013	Unknown	309	2 (0.6)
2014 (through week 40)	Unknown	414	6 (1.4)

ARI: acute respiratory infection; ILI: influenza-like illness.

[a] In enterovirus surveillance the number of enterovirus isolates or enterovirus positive clinical specimens submitted to the National Institute for Public Health and the Environment (RIVM) for VP1 typing is represented.

Table 2

Demographic and clinical characteristics of enterovirus D68 positive patients from the general practitioner sentinel influenza-like illness and other acute respiratory infection surveillance and from the national enterovirus surveillance, the Netherlands, week 1 2011–week 40 2014

Parameter	ILI/ARI surveillance (N = 16) n	Enterovirus surveillance (N = 11) n
Age groups (years)		
< 10	5	8
10–19	3	0
20–29	1	2
30–39	0	0
40–49	1	0
50–59	4	1
60–69	1	0
70–79	1	0
≥ 80	0	0
Sex		
Female	7	6
Male	9	5
Diagnosis[a]		
ILI	8	0
Bronchitis	4	2
Common cold	3	0
Tonsillitis	1	0
Pneumonia	0	2
Symptoms[a]		
Acute[b]	14	0
Cough	13	1
Fever	13	0
Rhinorrhoea	8	0
Sore throat	8	0
Fatigue	6	0
Headache	5	0
Myalgia	4	0
Dyspnoea	4	2
Diarrhoea	1	0
Underlying disease[c]	5	2
No clinical data reported	0	5

ARI: acute respiratory infection; ILI: influenza-like illness.

[a] In ILI/ARI surveillance, diagnosis and symptoms are checkable items on the specimen form; in the enterovirus surveillance they are reported in a free text item.

[b] A prodromal stage of three or four days.

[c] In ILI/ARI and enterovirus surveillance, underlying disease is reported in a free text item on the specimen form.

EV-D68 cases were detected all over the country; no localised outbreak was detected. Phylogenetic analysis of the VP1 genomic region showed that the Dutch EV-D68 from 2014, and from 2012 and 2013 as well, clustered with the US 2014 outbreak sequences in major group 1 in two sublineages and in major group 3 in one of the sublineages (Figure 2). Other Dutch EV-D68 from 2011 through 2014 clustered in other sublineages of major group 3. The sequences in the two sublineages of major group 1 had highly similar amino acid signatures, whereas sequences in the sublineages of major group 3 had clearly different amino acid signatures, with most differences located in the immunogenic BC and DE loops (Figure 3).

Discussion

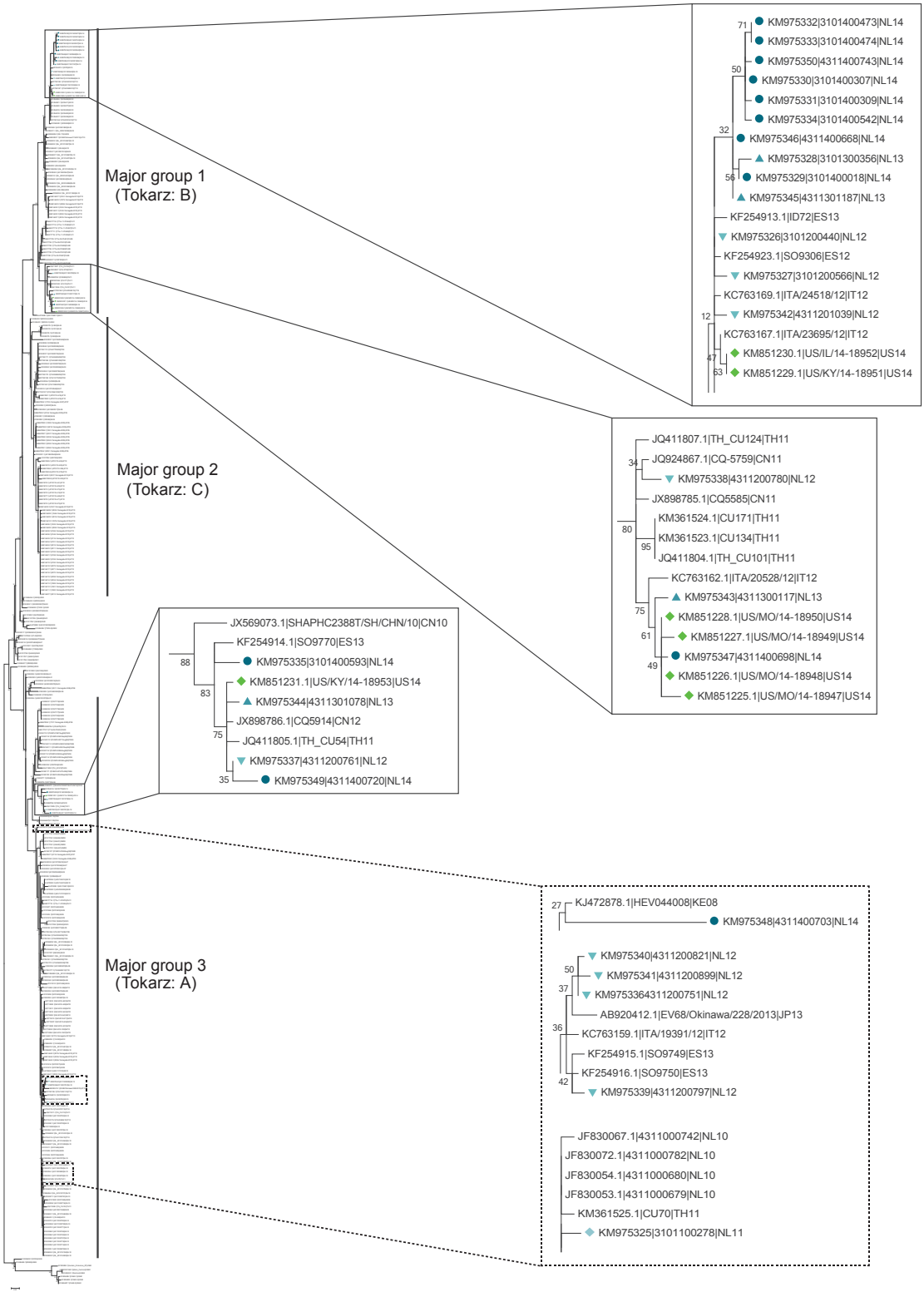
Although rarely detected worldwide, our combined previous and current results over the period 1996–2014 show that EV-D68 seems to circulate every year in a seasonal pattern in the northern hemisphere in predominantly the autumn through early winter period, causing relatively mild respiratory illness in a number of individuals large enough to be picked up by the GP ILI/ARI surveillance [5]. The national enterovirus surveillance shows that a number of EV-D68 cases are admitted to hospital each year with more severe respiratory disease. Clinical presentation ranging from mild to severe respiratory disease is in line with our previous findings, and has been described before [1–6].
None of the patients described in this paper had symptoms of neurological disease or paralysis. A causative link between EV-D68 infection and paralysis has not been established to date [11]. Given the acute flaccid paralysis rate (AFP) indicator used by the World Health Organization for optimal polio surveillance (1–2 cases per 100,000 children below 15 years of age) one can expect that during a large EV-D68 outbreak, also several AFP patients will be shedding EV-D68. The present outbreak in Northern America provides an opportunity to investigate the link.

The difference in age distribution of EV-D68 cases between the ILI/ARI surveillance and the national enterovirus surveillance in our dataset is biased by the fact that 95% of enteroviruses identified by enterovirus surveillance are from children [7]. The male/female ratio of 1.3 among EV-D68 cases in the ILI/ARI surveillance was slightly lower compared to 1.5 over the period 1996 through 2010, but showing the usual male predominance among enterovirus infected persons [5]. Hence, the female predominance among EV-D68 cases in the national enterovirus surveillance is unusual, but likely the result of the low number of cases.

The number of hospitalised EV-D68 cases identified through the national enterovirus surveillance in the Netherlands is likely underestimated. When first described, EV-D68 was found to be relatively acid resistant and was distinguished from the acid-sensitive human rhinovirus type 87 (HRV87) on this basis [12]. However, in 2002, HRV87 was reclassified as EV-D68 based on phylogenetic analysis [13]. Many RT-PCR diagnostic tests for enteroviruses as well as rhinoviruses are targeted at the 5' untranslated region of the genome [8]. Many of these tests are capable of detecting EV-D68 despite mismatches in primers and probes with the EV-D68 target sites, although with varying sensitivity depending on reagents and equipment used for RT-PCR [8]. This might also result in a false negative or a false rhinovirus-positive result [8]. Performed

Figure 2

Phylogenetic analysis of partial VP1 genomic region sequences of enterovirus D68, nucleotides 132 through 471 relative to the VP1 genomic region of the Fermon strain[a], covering the BC and DE immunogenic loops in the VP1 protein[b]



[a] GenBank ID: AF081348.1.

[b] Figure 3.

[c] One enterovirus D68 from 2013 could only be identified by sequencing of the 5' untranslated region diagnostic RT-PCR product and is therefore not included in Figures 2 and 3.

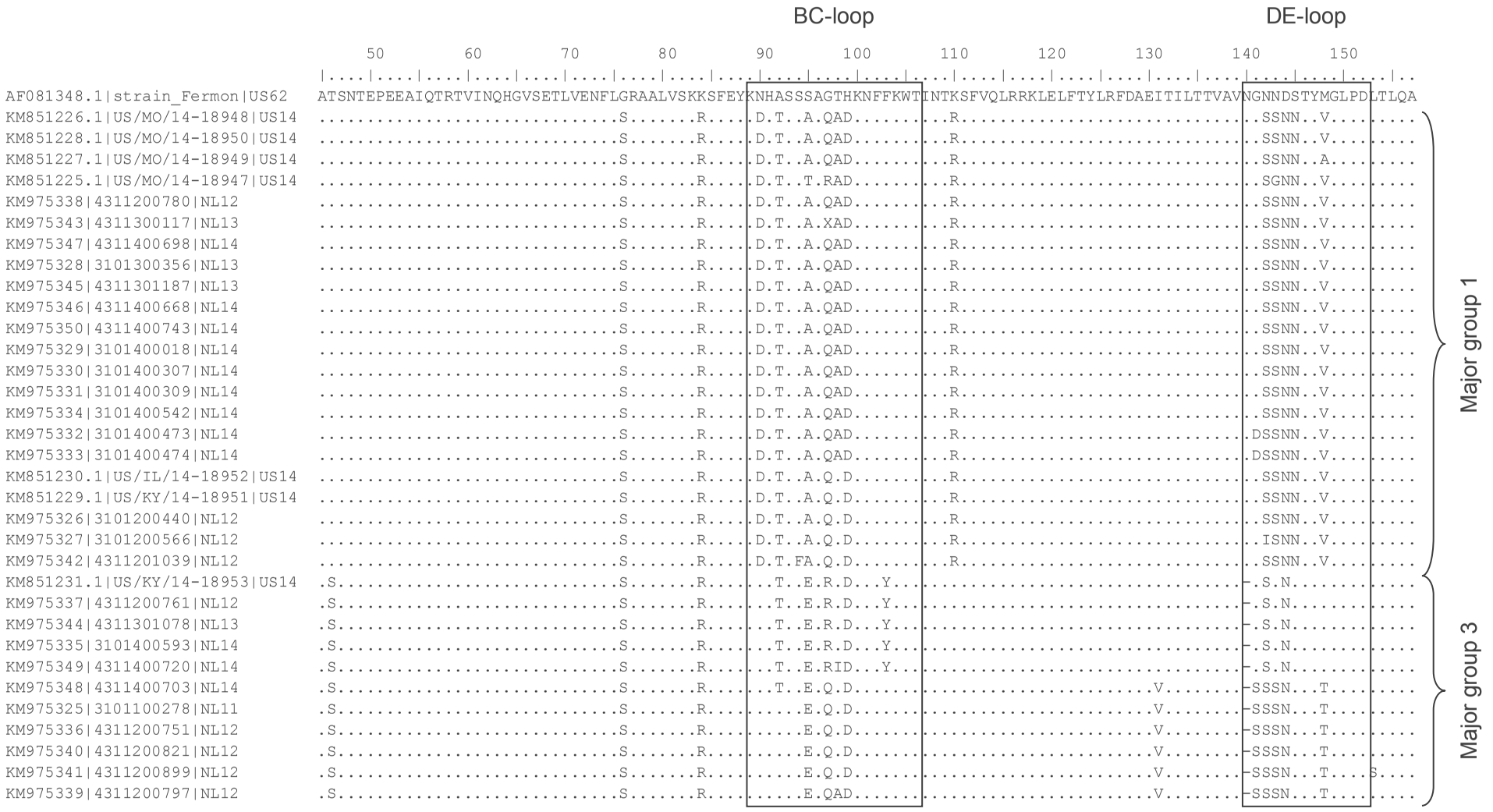
The maximum likelihood tree is shown with the percentage bootstrap support for branching events after 1,000 iterations indicated at the nodes. Major phylogenetic groups as described in references 5 and 10 are indicated on the right of the tree. Dutch sequences covering the period 2011–2014 and sequences from the 2014 outbreak in the US are enlarged.

◆ Sequences from the US, 2014

Blue labels indicate sequences from the Netherlands:
◆ 2011 (n=1)
▼ 2012 (n=9)
▲ 2013 (n=4[c])
● 2014 (n=12)

Figure 3

Analysis of partial VP1 amino acid sequences of enterovirus D68 in the Netherlands covering the period 2011–2014 and of enterovirus D68 from the 2014 outbreak in the United States



X: mixed Q/R amino acids.

Major groups as identified in Figure 2 are indicated on the right of the alignment. Numbering of amino acid residues is relative to the start of the VP1 reading frame of the Fermon strain. Amino acids common to the Fermon strain are indicated with a dot in the alignment. The putative BC and DE loops are indicated by boxes on the aligned amino acid sequences.

One enterovirus D68 from 2013 could only be identified by sequencing of the 5' untranslated region diagnostic RT-PCR product and is therefore not included in Figures 2 and 3.

on respiratory specimens, these tests might therefore wrongly identify an EV-D68 virus as a rhinovirus, and further investigation by typing in the national enterovirus surveillance protocol will not be performed [7,8]. Furthermore, a number of Dutch laboratories have started to type enteroviruses themselves and share data through the national enterovirus surveillance, although this is done with delay and infrequently. These laboratories participate in VIRO-TypeNed (formerly called TYPENED) [14] to provide a year-round surveillance and current efforts are directed at updating VIRO-TypeNed with EV-D68 detections.

Previous work has indicated that co-circulation of the different phylogenetic lineages of EV-D68 is the result of increased variability of the VP1 genomic region, i.e. the BC and DE loops, leading to reduced cross-neutralising antibodies raised against viruses of the major groups [5,10,15]. Variation of the highly conserved internal ribosome entry site in the 5' untranslated region, present in major group 1 and 2 viruses, has been suggested to be associated with increased virulence [10]. However, the US 2014 outbreak viruses are located in major groups 1 and 3, and similar viruses have been detected in the Netherlands, but associated with mild disease. Nevertheless, underlying disease like asthma seems to be an important factor for development of severe disease following EV-D68 infection [6]. Further in depth analysis of the EV-D68 full genomes from mild and severe cases and linked virological, clinical and epidemiological information should provide further insight in the factors determining severity of EV-D68 infection.

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Conflict of interest

None declared.

Authors' contributions

Adam Meijer, Harrie van der Avoort and Kimberley Benschop collected data. Gé Donker coordinated the sentinel GP network collecting specimens. Adam Meijer performed the analysis of the data and wrote the first draft of the paper. All other authors reviewed the manuscript critically, and comments and suggestions were incorporated in the final version by Adam Meijer.

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