Epidemiological and clinical characteristics of patients infected with enterovirus D68, France, July to December 2014

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In 2014, the United States (US) experienced a nationwide outbreak of enterovirus D68 (EV-D68) infection with 1,152 cases reported mainly in hospitalised children with severe asthma or bronchiolitis. Following the US alert, 11 laboratories of the French enterovirus (EV) surveillance network participated in an EV-D68 survey. A total of 6,229 respiratory samples, collected from 1 July to 31 December 2014, were screened for EV-D68 resulting in 212 EV-D68-positive samples. These 212 samples corresponded to 200 EV-D68 cases. The overall EV-D68 positivity rates among respiratory samples were of 5% (184/3,645) and 1.1% (28/2,584) in hospitalised children and adults respectively. The maximum weekly EV-D68 positivity rates were of 16.1% for children (n = 24/149; week 43) and 2.6% for adults (n = 3/115; week 42). Of 173 children with EV-D68 infection alone, the main symptoms were asthma (n = 83; 48.0%) and bronchiolitis (n = 37; 21.4%). One child developed acute flaccid paralysis (AFP) following EV-D68-associated pneumonia. Although there was no significant increase in severe respiratory tract infections reported to the French public health authorities, 10.7% (19/177) of the EV-D68 infected children and 14.3% (3/21) of the EV-D68 infected adults were hospitalised in intensive care units. Phylogenetic analysis of the viral protein 1 (VP1) sequences of 179 EV-D68 cases, revealed that 117 sequences (65.4%), including that of the case of AFP, belonged to the B2 variant of clade B viruses. Continuous surveillance of EV-D68 infections is warranted and could benefit from existing influenza-like illness and EV surveillance networks.

Introduction

Enterovirus D68 (EV-D68) was first identified in the United States (US) in 1962 in four paediatric patients with acute respiratory infections (ARI) [1-11]. Until 2014, only sporadic cases of infection with this virus as well as small outbreaks (10 publications during 2006–2011) were reported in Asia, Europe and the US [1-11], with disease manifestations mainly ranging from mild respiratory symptoms to severe ARI requiring intensive care and mechanical ventilation.

In 2014, the US experienced a nationwide outbreak of EV-D68 infection associated with an upsurge of severe respiratory cases admitted to emergency departments. Between mid-August and mid-December, 1,152 EV-D68 cases were reported by the Centers for Disease Control and Prevention (CDC) in 49 states, mainly in hospitalised children with severe asthma or bronchiolitis and occasionally in children with acute flaccid myelitis [12]. The overall disease burden was however, probably
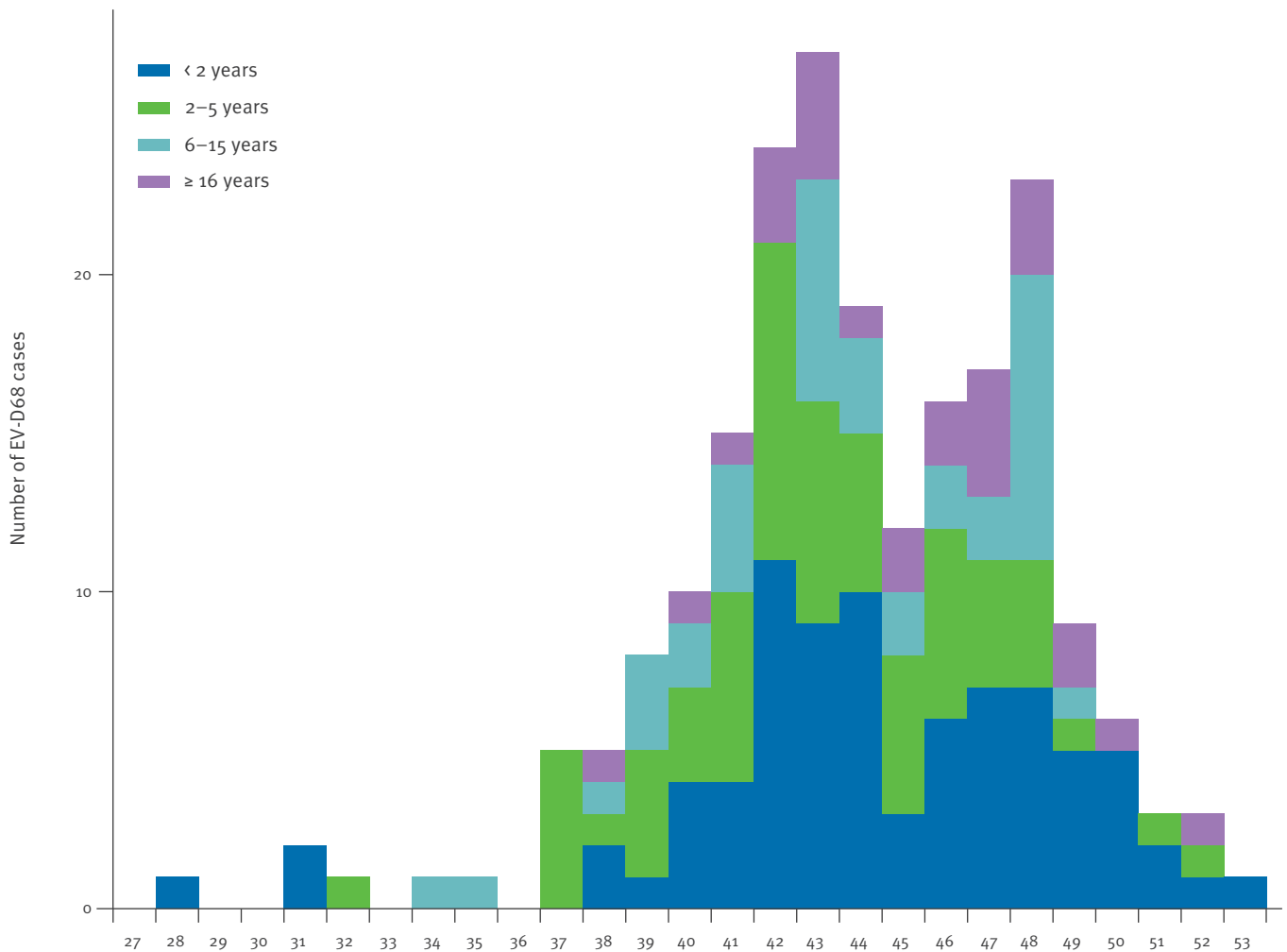
much higher [13,14]. During the autumn, European countries did not report a global increase in hospital admissions for severe respiratory infections or a significant upsurge of ARI [15]. However, reports from Norway and the Netherlands suggested that EV-D68 circulation might have increased [16,17].

In France, enterovirus (EV) surveillance and molecular typing involve a network of hospital virology laboratories and focus mainly on EV neurological infections in hospitalised patients [18]. In hospitalised patients with respiratory infections, human rhinoviruses and enteroviruses (HRV/EV) infections have been more systematically investigated since early 2010, due to the recent development of HRV/EV and commercial multiplex reverse-transcription polymerase chain reaction (RT-PCR) assays, but they remain underdiagnosed. In addition, no routine typing of EV and HRV is performed, even in severe respiratory cases. In late September 2014, a French child developed severe acute flaccid paralysis (AFP) following EV-D68 pneumonia [19]. Taking all these factors into account, the National Institute of Public Health encouraged the French EV

surveillance network to conduct a systematic analysis of respiratory samples collected from hospitalised patients to evaluate both the level of EV-D68 circulation and its clinical impact.

Methods

French enterovirus surveillance network

EV surveillance in France involves 34 virology/microbiology laboratories in university and general hospitals, including the two EV National Reference Laboratories (NRLs) (based in Lyon and Clermont-Ferrand). Each laboratory reports monthly on a specific website (http://cnr.chu-clermontferrand.fr/CNR) the number and type of samples analysed for EV, the relevant clinical data and EV serotype (when available). Throughout the year, EV-positive samples including mainly cerebrospinal fluid (CSF) specimens are genotyped in nine laboratories of the EV surveillance network (including the two NRLs) [18]. On 9 October 2014, the French EV surveillance laboratories were contacted by the Lyon NRL to take part in a national EV-D68 surveillance study. Participation in the French EV-D68 project was

Distribution of human rhinovirus/enterovirus-and enterovirus D68-positive samples per week, France, July–December 2014 (n=6,229 respiratory samples)
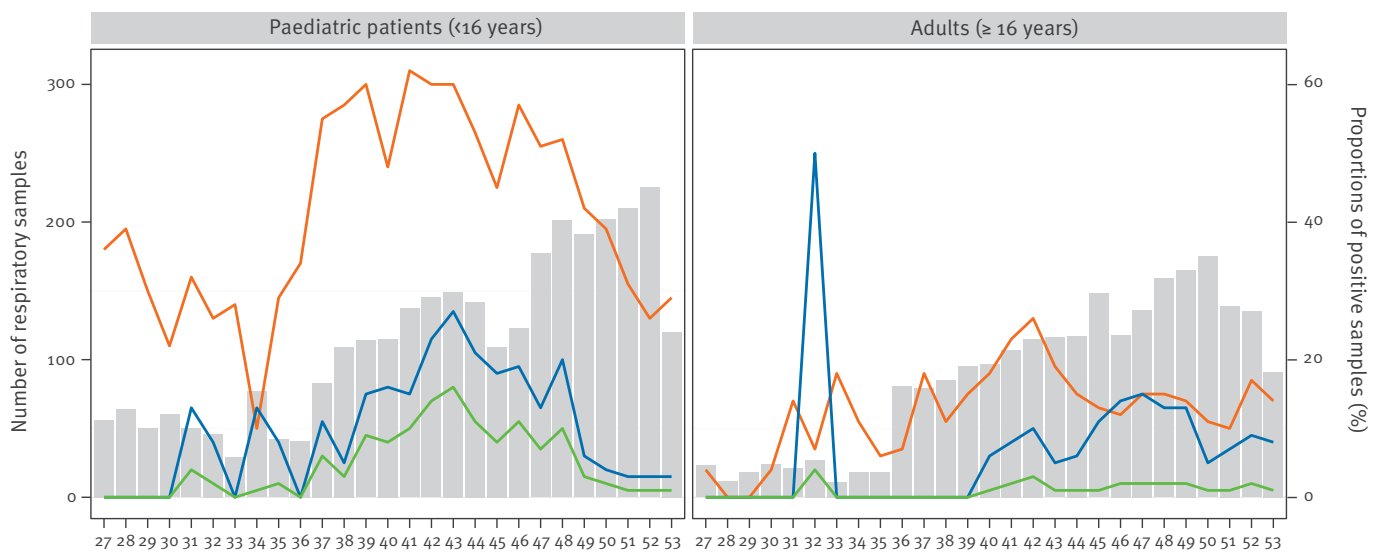
EV: enterovirus; HRV/EV: human rhinovirus/enterovirus.

Orange line: proportion of HRV/EV-positive samples among respiratory samples; green line: proportion of EV-D68-positive samples among respiratory samples; blue line: proportion of EV-D68 among HRV/EV-positive samples; grey histogram: number of respiratory samples tested.

voluntary. Some of the virological data (available as of 1 December, 2014) were also included in a European-wide EV-D68 surveillance study [20].

Screening of respiratory samples for enterovirus D68

Each participating laboratory was requested to test all the respiratory tract specimens collected from 1 July to 31 December 2014 from children (<16 years of age) and adults (≥16 years of age) admitted to or visiting the emergency unit of hospitals or university hospitals. Respiratory tract samples were systematically tested for HRV/EV by the RT-PCR assays routinely used at each participating laboratory. EV or HRV/EV-positive samples were thereafter tested for EV-D68 either by a specific EV-D68 real-time RT-PCR assay [17] or by sequencing of the partial viral protein (VP)4–VP2 sequences [21]. The sensitivity of the HRV/EV and the EV-D68 assays was initially evaluated in each laboratory with a titrated aliquot of the Fermon strain provided by the Lyon NRL. Detection of HRV/EV and EV-D68 in samples was performed either in the participating laboratories, or at the NRLs. Besides HRV/EV screening, all other viral and bacteriological tests were performed according to the physicians' requests.

Molecular typing of enterovirus D68-positive samples and phylogenetic analyses

Complete VP1 sequences of EV-D68 strains were amplified using EV-D68-specific in-house primers and sequenced using the Sanger method. When a complete VP1 sequence could not be obtained, a partial VP1 or VP4–VP2 sequence was determined [21-23]. All the sequences were generated by the EV NRLs and deposited into the GenBank database under accession numbers KP196362–78, KP307989–92, KP406467–96, KT220441–6, KT220448–505, LN681318–38, and LN874222–53.

A nucleotide (nt) alignment (340 nt, n=391) including all the EV-D68 VP1 sequences available from GenBank (as of 4 June, 2015) and those determined in this study was compiled. Redundant sequences (sharing 100% nt homology) were discarded. Phylogenetic relationships between sequences were inferred using a Bayesian method implemented in the Bayesian Evolutionary Analysis Sampling Trees (BEAST) package (v1.7) (http://beast.bio.ed.ac.uk) [24]. The uncorrelated lognormal molecular clock was employed with a flexible Bayesian skyline plot coalescent prior (15 piece-wise constant groups) and the generalised time reversible (GTR) model of nt substitution. The Markov chains Monte Carlo (MCMC) were run for 200 million generations, with subsampling every 10,000 iterations. Maximum Clade Credibility trees were calculated with the TreeAnnotator programme (v1.5.4). Topological support was assessed by estimating the values of the posterior probability (pp) density of each node.

Patients and clinical characteristics

For each EV-D68-infected patient, a review of the medical chart was carried out retrospectively to document the following data: age and sex; symptoms including fever (≥38.5 °C), cough, rhinitis, pharyngitis, bronchitis

FIGURE 3

Phylogeny of partial viral protein 1 (VP1) coding sequences of enterovirus D68 (n=391 sequences)
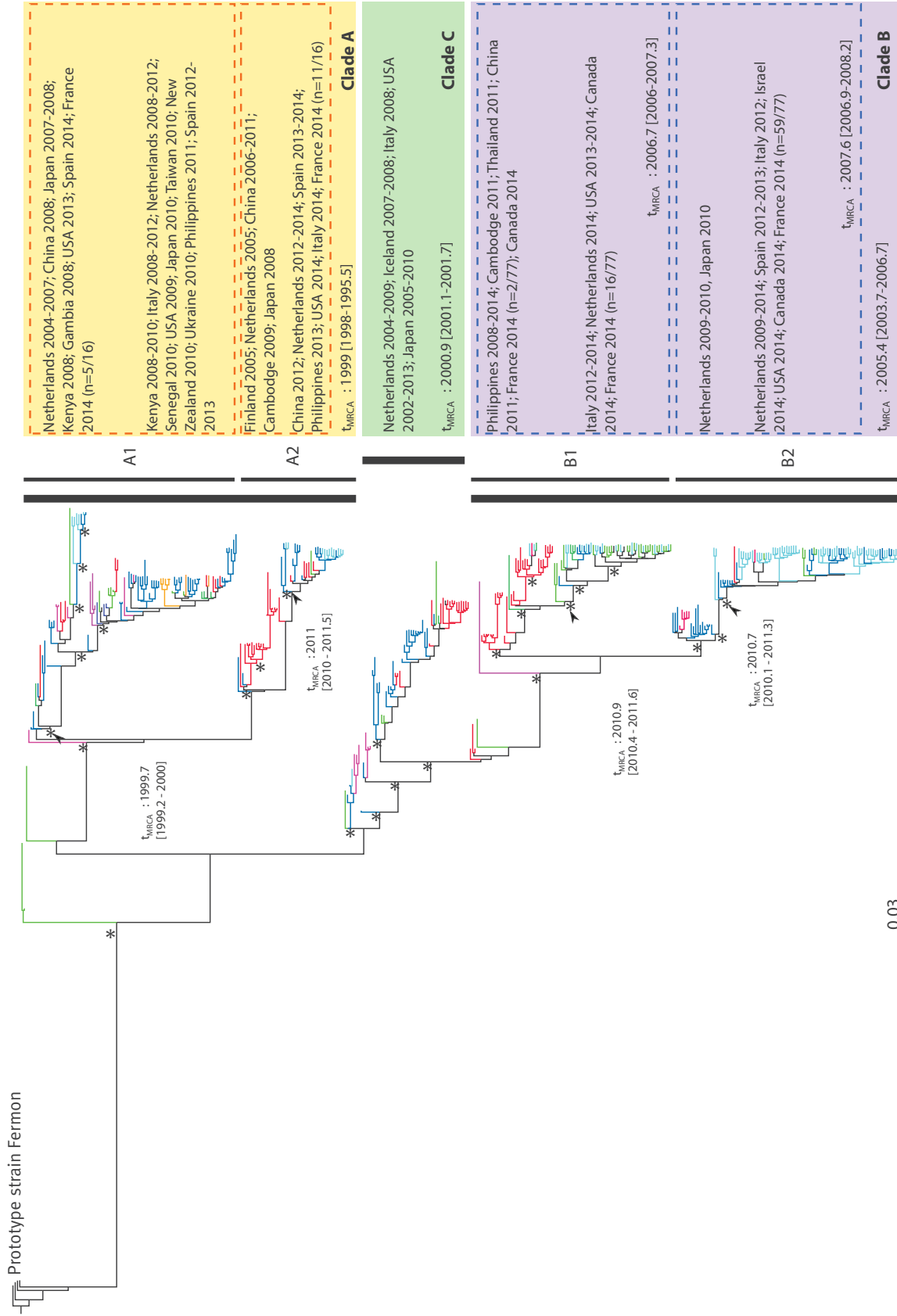
The maximum credibility tree is inferred with the partial VP1 sequence (340nt, position 2,521–2,859 relative to the Fermon EV-D68 strain). The phylogenetic relationships were inferred with a Bayesian method using a relaxed molecular clock model. The tree was reconstructed using Figtree (v1.4.2). For clarity, the sequence names are not included in the tree (but are shown on Suppl. Figure 2). Asterisks indicate key nodes with posterior probability values >0.90. Each tip branch represents a sampled virus sequence. Times of the most recent common ancestor (tMRCA) with the 95 % highest probability density (HPD) are indicated. The continents where the virus strains were sampled are indicated by different colours on the tree branches: Europe, blue; France, light blue; North America, green; Asia, red; Africa, Pink; Oceania, orange.

or bronchiolitis, acute respiratory distress, pneumonia, meningitis, polyradiculoneuritis; severity criteria [25,26] at admission such as need for intensive care and/or need for oxygen; length of hospitalisation including in intensive care unit (ICU); final diagnosis; presence or absence of underlying asthma or wheezing, prematurity, atopy, and chronic respiratory disease. Informed consent was not required for this surveillance study. A standardised Excel sheet including all the items was specifically designed for the present study and completed by each participating laboratory. The Lyon NRL compiled and analysed all the anonymised data.

Statistical analysis

Categorical variables with two or more than two levels (e.g. main diagnosis) were analysed using Fisher's exact test and G-test, respectively. The association between explanatory variables and severity was analysed using univariate logistic regression. Continuous variables (e.g. hospitalisation duration) were treated as binary variables and classified according to their median value. Statistical analysis was conducted using R software.

Results

Detection and distribution of enterovirus D68 cases

Eleven laboratories of the French EV network (including the Lyon and Clermont-Ferrand NRLs) participated in the EV-D68 enhanced surveillance. These laboratories were located in eight administrative regions (Table 1). Two of the laboratories analysed only specimens collected from patients under 16 years of age. Performances of the HRV/EV assays and the EV-D68 real-time RT-PCR were comparable among the participating laboratories, as tested on dilutions of a titrated EV-D68 Fermon strain (data not shown).

A total of 6,229 respiratory samples were systematically screened, including 3,645 from children and 2,584 from adults (Table 1). Among the respiratory samples collected from children, 1,501 (41.2%) were HRV/EV positive, of which 184 (12.3%) were positive for EV-D68. Among the respiratory samples collected from adults, 368 (14.2%) were HRV/EV positive, of which 28 (7.6%) were positive for EV-D68. The overall EV-D68 positivity rates among the respiratory samples tested were of 5.0% and of 1.1% in children and adults, respectively (Table 1). Overall the EV-D68-positive respiratory samples (n=212) corresponded to 200 EV-D68 cases including 178 children and 22 adults (Table 1).

While routinely genotyping EV-positive clinical samples that had been detected in laboratories not involved in the EV-D68 study, the NRLs identified nine additional cases (5 children and 4 adults) during the study period. Seven of these were hospitalised patients and two lived in an elderly nursing home. The nine cases were considered in the overall epidemiological analysis, which therefore comprised a total of 209 cases.

Overall, the first EV-D68 case was detected on 11 July 2014 (Figure 1; week 28). The majority (179/209; 85.6%) of the EV-D68 cases were detected from weeks 39 to 49 and two peaks could be observed, one in October (week 43) and one in November (week 48).

The samples of the nine cases, which were detected through routine analysis, were not taken into account to calculate positivity rates, which were based on the total of 212 systematically screened respiratory samples. At week 43, in children, the EV-D68-positive samples represented up to 16.1% (n=24/149) of the respiratory samples tested in that week and 26.7% (n=24/90) of the HRV/EV-positive-samples (Figure 2). At week 42, in adults, the EV-D68-positive samples represented up to 2.6% (n=3/115) of the respiratory samples tested and 10% (n=3/30) of the HRV/EV-positive samples (Figure 2). Circulation of the virus persisted until at least the end of December 2014.

EV-D68 infections were detected in all the regions covered by the participating laboratories, i.e. eight of the 22 French administrative regions (Suppl. Figure 1, available from: http://cnr-chu-clermontferrand.fr/CNR/Pages/Accueil/Publis.aspx).

Clinical characteristics of patients infected by enterovirus D68

EV-D68 infections were detected in both children and adults (Figure 1). Based on medical chart review and final diagnosis, a bacterium or a parasite was likely to be responsible for the symptoms of six children and five adults. The six paediatric patients presented with arthritis due to *Kingella kingae* (1 case); pyelonephritis due to *Escherichia coli* (1 case); gastroenteritis due to norovirus and conjunctivitis due to *Haemophilus influenzae* (1 case); sepsis due to *Streptococcus parasanguis* (1 case); febrile syndrome due to *Plasmodium falciparum* (1 case); meningitis-like syndrome due to *Haemophilus influenzae* (1 case). The five adult patients had either severe sepsis and acute respiratory distress syndrome (ARDS) due to *Pneumocystis carinii* or pneumopathy due to *Pneumocystis jirovecii* (2 cases), *Streptococcoccus pneumoniae* (1 case) or *Escherichia coli* (1 case). Detailed clinical characteristics of the 11 patients are available upon request. These patients were excluded from the 209 previously described patients, when considering the overall description of clinical characteristics, which thus comprised 198 patients, including 177 children and 21 adults (Table 2). The 11 EV-D68 co-infected patients were also not considered in the univariate analyses that were performed to determine if certain characteristics were associated with disease severity.

Paediatric patients

In the 177 children taken into account to investigate the clinical characteristics, EV-D68 was detected in all age

TABLE 1

Detection of human rhinovirus/enterovirus and enterovirus D68 through systematic screening of respiratory samples, France, July–December 2014 (n=6,229 respiratory samples)

Town of the laboratory, administrative region	Screening period	RT–PCR assay used for HRV/EV detection	Samples tested for HRV/EV n	HRV/EV-positive samples n (%)	EV-D68-positive samples among HRV/EV positive samples n (%)	EV-D68-positive samples among samples tested for HRV/EV n (%)	EV-D68-positive patients n
Paediatric patients (<16 years)							
Amiens, Picardie	1 Jul–31 Dec	Luminex xTAG RVP FAST	397	125 (31.5)	18 (14.4)	18 (4.5)	18
Brest, Bretagne	1 Jul–14 Dec	RespiFinder SMART 22 FAST v2	142	75 (52.8)	7 (9.3)	7 (4.9)	7
Caen, Normandie	1 Sep–31 Dec	RespiFinder SMART 22 FAST v2	614	353 (57.5)	50 (14.2)	50 (8.1)	48
Clermont-Ferrand, Auvergne	1 Jul–31 Dec	Rhino and EV/Cc r–gene	289	121 (41.9)	24 (19.8)	24 (8.3)	23
Dijon, Bourgogne	1 Jul–31 Dec	Rhino and EV/Cc r–gene	115	36 (31.3)	6 (16.7)	6 (5.2)	5
Lyon, Rhône-Alpes	1 Jul–31 Dec	Rhino and EV/Cc r–gene	1,060	349 (32.9)	35 (10.0)	35 (3.3)	33
Paris, Ile de France (Saint Louis)	1 Jul–7 Dec	RespiFinder SMART 22 FAST v2	77	35 (45.5)	0 (0.0)	0 (0.0)	0
Paris, Ile de France (Paul Brousse)	1 Jul–31 Dec	Rhino and EV/Cc r–gene	321	122 (38.0)	6 (4.9)	6 (1.9)	6
Saint–Etienne, Rhône-Alpes	10 Oct–31 Dec	Rhino and EV/Cc r–gene	204	80 (39.2)	14 (17.5)	14 (6.9)	14
Strasbourg, Alsace	19 Sep–31 Dec	Luminex xTAG RVP FAST	304	147 (48.4)	11 (7.5)	11 (3.6)	11
Versailles, Ile de France	1 Jul–31 Dec	Rhino and EV/Cc r–gene	122	58 (47.5)	13 (22.4)	13 (10.7)	13
Total	–	–	**3,645**	**1,501 (41.2)**	**184 (12.3)**	**184 (5.0)**	**178**
Adult patients (≥16 years)							
Amiens, Picardie	1 Jul–31 Dec	Luminex xTAG RVP FAST	216	36 (16.7)	7 (19.4)	7 (3.2)	4
Brest, Bretagne	1 Jul–14 Dec	RespiFinder SMART 22 FAST v2	130	29 (22.3)	4 (13.8)	4 (3.1)	4
Caen, Normandie	1 Sep–31 Dec	RespiFinder SMART 22 FAST v2	416	78 (18.8)	1 (1.3)	1 (0.2)	1
Clermont–Ferrand, Auvergne	1 Jul–31 Dec	Rhino and EV/Cc r–gene	367	54 (14.7)	4 (7.4)	4 (1.1)	3
Dijon, Bourgogne	1 Jul–31 Dec	Rhino and EV/Cc r–gene	214	25 (11.7)	1 (4.0)	1 (0.5)	1
Lyon, Rhône–Alpes	1 Sep–31 Dec	Rhino and EV/Cc r–gene	1,036	123 (11.9)	11 (8.9)	11 (1.1)	9
Paris, Ile de France (Paul Brousse)	1 Jul–31 Dec	Rhino and EV/Cc r–gene	40	7 (17.5)	0 (0.0)	0 (0.0)	0
Saint–Etienne, Rhône Alpes	10 Oct–31 Dec	Rhino and EV/Cc r–gene	41	1 (2.4)	0 (0.0)	0 (0.0)	0
Versailles, Ile de France	1 Jul–31 Dec	Rhino and EV/Cc r–gene	124	15 (12.1)	0 (0.0)	0 (0.0)	0
Total	–	–	**2,584**	**368 (14.2)**	**28 (7.6)**	**28 (1.1)**	**22**

EV: enterovirus; HRV/EV: human rhinovirus/enterovirus; RT-PCR: reverse-transcription polymerase chain reaction.

The study involved 11 voluntary laboratories of the 34 in the EV surveillance network (including two different virology laboratories from the Paris area). A total of 212 EV-D68-positive samples corresponding to 200 EV-D68 cases were detected by the systematic screening of respiratory tract samples collected from children (<16 years-old) and adults (≥16 years-old) admitted to or visiting emergency units of hospitals or university hospitals.

groups and the most affected age group was <2 years (<2 years: 76 patients, including ≤28 days: 6 patients; 2–5 years: 73 patients; 6–15 years: 28 patients). The median age of the patients was 2.33 years (range: 3 days–13.5 years). Information on hospitalisation was available for 174 patients. A total of 160/174 (92.0%) patients were hospitalised and 14/174 (8.0%) were outpatients (short stay at the emergency unit but no overnight hospitalisation). A final diagnosis was available for 173 (97.7%) patients and a total of 166/173 (96.0%) presented with acute respiratory infections. The main diagnoses were asthma (n=83; 48.0%) and bronchiolitis (n=37; 21.4%). Other diagnoses are summarised in Table 2. Among the children hospitalised for asthma (82/83; Table 2), 64 (78.0%) had a previous history of asthma or wheezing. In univariate analysis however, the history of asthma or wheezing as a determinant of severity or hospitalisation in ICU was not statistically significant (Table 3).

Four patients (2.3%) presented with neurological signs (Table 2). One four-year-old patient developed AFP following EV-D68 associated pneumonia; CSF showed pleocytosis with normal protein and glucose levels and spinal magnetic resonance imagery showed gadolinium enhancement of the ventral nerve roots of the cauda equine [19]. One patient aged 20 months developed meningitis-like symptoms. Two infants with underlying epilepsy developed severe seizures in a context of bronchiolitis or pneumonia. Three children presented with isolated neonatal fever, one with a severe sepsis syndrome and one with hypotonia. One EV-D68 infection was diagnosed in the context of a sudden infant death syndrome (SIDS) in a two-month-old girl; detection of EV-D68 in blood was negative and no other pathogen was detected.

Nineteen children (10.7%) were hospitalised in ICUs (median duration: 3 days; range: 1–137 days) (Table 2). Of these, two ex-premature babies with bronchopulmonary dysplasia were infected by EV-D68 while already in neonatal ICU and developed severe respiratory decompensation. Among the 17 remaining patients (see clinical presentation in Table 2), 15 had pre-existing chronic conditions (prematurity: 4; asthma/wheezing: 9; pulmonary vein atresia: 1, ventricular septal defect: 1, drepanocytosis: 1; epilepsy: 2) and two patients, who presented with pneumothorax (without asthma) or AFP, had no underlying disease. All but one patient hospitalised in ICU had favourable outcomes. The patient who developed AFP was extubated after 4.5 months in ICU, but still showed severe sequelae of right upper limb after 12 months. No death could be directly imputed to EV-D68.

Adult patients
The median age of the 21 adult patients was 36.7 years (range: 17.2–98.9 years). Fourteen were hospitalised and five were outpatients (2 patients not documented) (Table 2). A diagnosis and clinical signs were available for 17 patients. The diagnoses were as follows:

asthma (n=4; all with underlying history of asthma); pneumonia (n=4), chronic obstructive pulmonary disease (COPD) exacerbation (n=3; all with stage III COPD), upper respiratory tract infection (n=2), bronchitis (n=1), influenza-like illness (n=1) and pneumothorax (n=1). One patient was asymptomatic (allograft follow-up).

Three patients were hospitalised in ICU for two, three and six days, respectively; two of them presented with pneumonia: a 25 year-old patient who developed a severe respiratory distress without underlying risk factors during the week 29 of gestation and a 23 year-old patient with underlying Duchenne muscular dystrophy; the third patient presented with exacerbation of COPD. All the adult cases had favourable outcomes.

Enterovirus D68 sequencing and phylogenetic analysis
EV-D68 was tentatively sequenced in 207 of 209 patients. Among these 207, EV-D68 infection was confirmed in 201 patients either by VP1 sequencing (n=179) or by VP4–VP2 sequencing (n=22). In six patients, the virus could not be sequenced, probably because of the low viral load (cycle thresholds of EV-D68 real-time RT-PCRs were between 39.3 and 40.7). A total of 178/201 (88.6%) EV-D68 viruses belonged to clade B and 23/201 (11.4%) belonged to clade A [27]. Of the 159 clade B viruses for which the VP1 sequence was obtained, 42 (26.4%) and 117 (73.6%) were assigned to the sublineage B1 and B2, respectively (data not shown). Clade A and B viruses were identified throughout the screening period and the proportion of A and B viruses per week did not vary significantly (data not shown). Clade A viruses were detected more frequently in adults (10/23, 43.5%) than in children (13/178, 7.3%) (p<0.001). Proportions of A and B viruses did not differ significantly between patients hospitalised in ICU and patients not hospitalised in ICU.

To investigate a large sample drawn from different geographical origins, a Bayesian analysis was performed on partial VP1 sequences, including those of 93 viruses from France and 298 viruses from other geographical regions (Figure 3 and Suppl. Figure 2, available from: http://cnr-chu-clermontferrand.fr/CNR/Pages/Accueil/Publis.aspx). The results suggested that all the recent EV-D68 strains formed one genogroup which could be further divided in two major lineages: the first corresponded to clade A lineage while the second included clades B and C [27]. This phylogenetic topology was confirmed by a Bayesian analysis on complete VP1 sequences (data not shown) and was concordant with the topology described by Lauinger et al. [11]. Sixteen French strains fell within the clade A and clustered in two highly supported lineages (posterior probability, pp>0.97) designated A1 (n=5 strains) and A2 (n=11 strains). The French A1 viruses clustered with strains collected in 2013–2014 in the US, Spain and the Netherlands. The French A2 viruses clustered with viruses recovered between 2012 and 2014 from three

Clinical characteristics of enterovirus D68 cases, France, July–December 2014 (n = 198 patients)[a]

Characteristic	Paediatric patients (<16 years)			Adult patients (≥16 years)		
	Patients N	Hospitalised patients N	Patients in ICU N	Patients N	Hospitalised patients N	Patients in ICU N
Sex-ratio (M/F)	1.39 (103/74)			0.62 (8/13)		
Median hospitalisation duration	4 days (range: 1–172 days) (n = 147)[b]			3 days (2–18) (n = 12)[b]		
Median hospitalisation duration in ICU	3 days (range: 1–132 days) (n = 17)[b]			3 days (2–6) (n = 3)		
Number of patients with oxygen therapy	78 (n = 171)[b,c]			6 (n = 17)[b,d]		
Number of patients with history of asthma/wheezing	85 (n = 168)[b]			4 (n = 10)[b]		
Respiratory presentation						
Asthma	83[e]	82[e]	8	4	4	0
Severe asthma	*24[e]*	*24[e]*	*8*	*1*	*1*	*0*
Bronchiolitis	37[e]	34[e]	4[f]	0	0	0
Severe bronchiolitis	*4[e]*	*4[e]*	*4[f]*	*0*	*0*	*0*
COPD exacerbation	0	0	0	3	3	1
Respiratory distress only	4	4	3	0	0	0
Pneumonia	11[e]	11[e]	1[f]	4	4	2
Upper respiratory tract infection	26	18	0	2	0	0
Other	7[g]	7	2	3[h]	2	0
Neurological presentation						
Acute flaccid paralysis	1	1	1	0	0	0
Seizures	2[e]	2[e]	2[f]	0	0	0
Other	1 (meningitis-like)	1	0	0	0	0
Other presentation						
Hypotonia	1	1	0	0	0	0
Neonate fever (≥38.5 °C)	3	2	0	0	0	0
Other	2[i]	1	0	0	0	0
Asymptomatic	0	0	0	1[j]	0	0
Not documented	4	1	0	4	1	0
Total	177	160	19	21	14	3

COPD: chronic obstructive pulmonary disease; EV: enterovirus; F: female; ICU: intensive care unit; M: male.

A given patient could have more than one clinical characteristic.

[a] We excluded six paediatric and five adult patients for whom the clinical signs were likely to be due to a bacterium or a parasite, from the total of 209 cases.

[b] The number of patients for whom the information was available is indicated in parentheses.

[c] Two ex-premature babies with bronchodysplasia were already under continuous oxygen therapy.

[d] Four patients with underlying COPD (n=3) or Duchenne muscular dystrophy (n=1) were already under continuous oxygen therapy.

[e] Three patients presented with asthma and pneumonia; one patient with bronchiolitis and seizures; one with pneumonia and seizures.

[f] One patient presented with bronchiolitis and seizures; one with pneumonia and seizures.

[g] Pneumothorax (n=1); acute thoracic syndrome (n=1); bronchitis (n=5).

[h] Influenza-like illness (n=1); pneumothorax (n=1); bronchitis (n=1).

[i] Infant sepsis (n=1); sudden infant death syndrome (n=1).

[j] Allograft follow-up.

different continents. The remaining 77 French strains belonged to two lineages designated B1 (pp=0.94; n=18 strains) and B2 (pp=1; n=59) within the clade B. The B1 lineage included most of the strains sampled in 2014 in the US and 18 French strains, while the B2 lineage was almost exclusively composed of strains recovered in Europe and comprised the majority of strains detected in France in 2014 (59/93, 63.4%). The AFP case was associated with a B2 strain [19]. The EV-D68 sequences detected in Europe between 2012 and 2014 were closely related to those from viruses detected in 2014 in Israel (n=2), US (n=4) and Canada (n=1).

Discussion

From mid-August 2014 until the end of December, EV-D68 caused a geographically widespread outbreak of respiratory disease of unprecedented magnitude in the US, leading to substantial hospitalisation for

TABLE 3

Univariate analysis of potential factors for severe disease in children infected with enterovirus D68, France, July–December 2014 (n=177)

Characteristic	Severity[a]				ICU admission				Oxygen therapy				Hospitalisation duration[b]			
	No	Yes	OR (95% CI)	P	No	Yes	OR (95% CI)	P	No	Yes	OR (95% CI)	p	≤4 days	>4 days	OR (95% CI)	P
Sex																
Male	75[a]	23[a]	0.90 (0.44–1.85)	0.7781	90	12	1.22 (0.46–3.43)	0.6937	54	45	0.98 (0.54–1.82)	0.9608	55	30	1.06 (0.54–2.14)	0.8579
Female	53[a]	18[a]			64	7			39	33			41	21		
Prematurity																
Yes	18	6	0.99 (0.34–2.57)	0.9850	20	4	1.72 (0.46–5.32)	0.3755	15	9	0.65 (0.26–1.57)	0.3476	8	8	2.07 (0.72–6.02)	0.1729
No	104	35			129	15			74	68			85	41		
History of asthma or wheezing																
Yes	59	22	1.30 (0.64–2.65)	0.4733	76	8	0.72 (0.26–1.87)	0.5005	32	51	3.48 (1.86–6.65)	**0.0001**	50	26	0.98 (0.49–1.94)	0.9423
No	66	19			75	11			59	27			45	24		
History of atopy																
Yes	18	8	1.49 (0.57–3.67)	0.3970	27	2	0.60 (0.09–2.31)	0.5171	13	16	1.59 (0.71–3.6)	0.2630	21	8	0.65 (0.25–1.55)	0.3494
No	104	31			122	15			76	59			70	41		
History of chronic respiratory insufficiency																
Yes	3	4	4.29 (0.91–22.6)	0.0642	5	2	3.39 (0.46–17.12)	0.1632	4	3	0.86 (0.17–4.03)	0.8484	0	3	NA (NA)	NA
No	119	37			144	17			85	74			92	47		
EV-D68 clade																
A	9	1	3.03 (0.54–56.72)	0.3009	10	1	1.32 (0.23–25)	0.7949	5	6	0.71 (0.2–2.46)	0.5870	8	1	4.38 (0.77–82.55)	0.1699
B	113	38			136	18			82	70			84	46		
Age																
‹2 years	59	15	Ref	Ref	64	10	Ref	Ref	40	33	Ref	Ref	35	28	Ref	Ref
2–5 years	44	15	1.34 (0.59–3.05)	0.4806	56	5	0.57 (0.17–1.71)	0.3325	30	31	1.25 (0.63–2.48)	0.5173	41	13	0.4 (0.17–0.87)	**0.0230**
›5 years	25	11	1.73 (0.69–4.29)	0.2363	34	4	0.75 (0.19–2.44)	0.6516	23	14	0.74 (0.32–1.64)	0.4611	20	10	0.63 (0.25–1.53)	0.3100

CI: confidence interval; EV: enterovirus; ICU: intensive care unit; NA: not applicable because of the small number of reports; OR: odds ratio; P: p-value; Ref: reference.

[a] Severity criteria were defined as elsewhere [25,26] and included the need for intensive care and need for oxygen. Severity criteria were only known for 169 cases.

[b] Dichotomised according to median value. Median hospitalisation time (for inpatients) was four days.

severe respiratory disease. In the context of the US alert, a systematic screening of EV-D68 was performed by 11 voluntary hospital laboratories of the French EV surveillance network on 6,229 respiratory samples collected between 1 July and 31 December 2014.

This report concerns the largest number of EV-D68 cases ever documented for France. Due to the implementation of systematic screening of EV-D68, a total of 200 EV-D68 infections were diagnosed and EV-D68 was detected in all the administrative regions from where the participating laboratories were involved (i.e. 8 of the 22 administrative regions), suggesting that EV-D68 might have circulated even more widely throughout the country. Previously, two small clusters of cases had been reported in 2008 (19 cases; Oct–Nov; Basse-Normandie region) and 2009 (10 cases; Sep–Nov;

Champagne-Ardennes region), respectively [8,9] and only 66 EV-D68 cases were reported to the National Institute for Public Health between 2006 and 2013. However, during the 2007 to 2013 period, EV-D68 infections were probably underestimated, because HRV/EV screening in ARI was restricted to a limited number of laboratories (particularly before 2010), genotyping of HRV/EV-positive samples was rarely performed and the specific detection of EV-D68 by real-time RT-PCR was unavailable. On the other hand, no EV-D68 case was detected by systematic screening of respiratory samples collected in Lyon from September until December 2013 (data not shown), whereas 42 cases were identified between July and December 2014. This suggests that the circulation level of EV-D68 was higher in 2014 than in 2013, at least in the Lyon area and possibly elsewhere in France. In this respect, surveillance studies in

the Philippines [28], Italy [10] and the Netherlands [7] showed that EV-D68 may follow a cyclic pattern of circulation with a two-year interval.

The overall EV-D68 detection rate that we observed in a hospital-based setting between July and December 2014 in France (3.4%; maximum 8.4% on week 43) was similar to that observed in a European-wide survey (2.1% [20]) conducted on 17,384 respiratory samples from 17 countries collected mainly from hospitalised patients between July and November 2014 – and in which the virological results for 117 French patients, available as of 1 December, 2014, were included. It was much lower than that reported by the CDC during the August to December period (36% of 2,600 respiratory samples) (http://www.cdc.gov/non-polio-enterovirus/about/EV-D68.html). However, the proportion reported by the CDC was calculated mainly from severe cases, which may hamper comparisons. Comparison between findings in France and the US may also be hampered by increased public/physician awareness and more active case finding in the US.

At the time of the US alert, and despite existing surveillance systems for respiratory tract illness (RTI) or influenza-like illness [29,30], no upsurge of the number of hospitalisations for RTI, or of the number of HRV/EV positive respiratory samples, was reported in France. This suggests that the impact of the circulation of EV-D68 on public health was more limited in France and Europe than in the US and may explain why only rare alerts were reported in Europe [15-17].

Our longitudinal study provided a comprehensive description of the epidemiological and clinical characteristics of EV-D68 infections in hospitalised patients during the entire study period. Most cases (87.5%) were detected in children, as observed in the US [14]. The EV-68 detection rate in respiratory samples from children was of 9.7% (n = 100/1,035) in the September to October period and was similar to that observed at the same period in hospitalised children from the Oslo area [16]. Most children (93%) with an EV-D68 infection presented with respiratory symptoms, mainly asthma and bronchiolitis, as described in hospitalised patients in the 2014 US outbreak, an outbreak in Canada in the same year, and in previous reports [6,8,13,14,31]. EV-D68 could also be associated with respiratory distress without underlying asthma or bronchiolitis, especially in ex-premature babies with bronchopulmonary dysplasia. Among the children who were hospitalised for asthma, 78% had a history of asthma or wheezing, consistent with US reports. In our study, underlying asthma or wheezing was not identified as a risk factor for developing more severe asthma or being hospitalised in ICU, however statistical power may have been limited by the sample size.

Viral factors may also contribute to the disease. Even though identical VP1 sequences were detected in both mild and severe RTI cases, full length analysis of viral genomes is warranted to determine whether specific mutations in coding or non-coding regions influence severity, as observed for poliovirus or EV-A71 [32,33].

Neurological signs were observed in four patients. Only one AFP case was reported during this survey [19] and no increase in AFP cases was reported to the public health authorities during the EV-D68 circulation period. For the three remaining cases of patients with meningitis-like symptoms or with seizures, although such disease manifestations have not been previously described with EV-D68, they are frequently associated with EV infections particularly in young children. However, we cannot rule out the possibility that other viral or bacterial infection could have contributed to these neurological signs. Of note, in 2014, no EV-D68 was detected in 1,197 CSF specimens genotyped throughout the EV national surveillance. So, apart from the AFP-associated case, the spectrum of illnesses associated with EV-D68 was similar to that of rhinoviruses, as previously reported [1,3-10,13,14,16,17,19,31]. Although no significant increase in severe respiratory disease was reported to the French national public health authorities in autumn 2014, the present study showed that EV-D68 did have a clear clinical impact, with 10.7% of the paediatric cases and 14.3% of the adult cases being hospitalised in ICUs. Moreover, its implication in nosocomial infections should be considered [17,34]. This highlights the need for clinical laboratories to take EV-D68 in account in the differential diagnosis of patients with severe respiratory symptoms, including in adult patients.

EV-D68 infections in France in 2014 were mainly associated with the B2 variant, as in other European countries [20]. However, it was not possible to determine whether the B2 variant was circulating in France before 2014 because the molecular characterisation of EV/HRV-positive respiratory samples is not routinely performed, as exemplified by our finding of only one French EV-D68 VP1 sequence in GenBank from prior to 2014 (sequence from genogroup C; 1999). In the Netherlands, virus surveillance between 2004 and 2014 provided evidence of the successive replacement of the major lineage by another lineage in each period of increased virus reporting. While clade C predominated until 2008, an outbreak in 2010 was mainly associated with the circulation of clade A strains [7]. The B2 viruses also circulated in 2010 but to a lesser extent, [6,7] and became predominant in 2014 [17]. This type of circulation pattern – the replacement of an earlier variant during periods of low virus incidence – is reminiscent of that observed for EV-A71 [35,36]. The succession of predominant lineages could be driven by the immunity of the general population. In this respect, Imamura et al. [37] showed that there were antigenic differences between the recent lineages of EV-D68 circulating strains. Finally, the different lineages were present simultaneously over several countries and continents. The close genetic relatedness between EV-D68 strains sampled from distant countries suggests that

this virus is subject to frequent geographical turnover. Further studies based on larger samples of complete VP1 sequences are needed to investigate the dynamics of EV-D68 geographical transportation between countries and over continents.

This study comprised some limitations. The screening was not population-based as it depended on the voluntary participation of only about one-third of EV network laboratories in France. We also lacked historical EV-D68 screening data at a national level for comparison, and the sample size was limited in terms of the statistical power support in univariate analyses. Moreover, we cannot exclude that respiratory samples may have been collected for viral screening more frequently from children than from adults and that EV-D68 positivity rate may have been underestimated in adults. Our data were however likely not biased towards more severe infections as they were based on testing results of respiratory samples collected for routine viral screening of respiratory infections.

The autumn of 2014 was marked by increased EV-D68 detection in many parts of the world [12-17,31], associated, at least in parts of the US and Canada, with a significant upsurge of severe respiratory infections, sometimes followed by neurological signs. A similar outbreak may possibly also occur in Europe in the future, and the results of our study show that in France, a number of EV-D68 infections had a clinical impact. This justifies the need for continuous surveillance of EV-D68 infections in Europe. The surveillance could rely on existing and effective surveillance programmes such as the influenza and influenza-like illness surveillance systems, the EV surveillance networks and the surveillance of AFP cases. The increasing awareness of HRV/EV as major respiratory pathogens and the development of commercial molecular assays for these viruses has allowed the implementation of HRV/EV diagnosis in an increasing number of virology laboratories [33,38]. Moreover, virus characterisation should be encouraged, at least in the event of severe respiratory signs.

Conflict of interest

None declared.

Authors' contributions

IS designed the study and coordinated the laboratory network involved in this study, together with AM. IS, AM, DH, LP, JPL, CM, JL, CD, SP, QL, JMM and SMJ provided respiratory samples and collected epidemiological and clinical data. IS compiled and analysed the clinical data. LJ performed the statistical analyses. AM performed the phylogenetic analyses. IS, AM and LJ wrote the first draft of the paper. All the authors, including BL, CH, DA and HPL, reviewed the manuscript critically.

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